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Annual Review

OVERVIEW OF DATA AND CONCEPTUAL APPROACHES FOR DERIVATION OF QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS FOR ECOTOXICOLOGICAL EFFECTS OF ORGANIC CHEMICALS

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Abstract—The use of quantitative structure–activity relationships (QSARs) in assessing potential toxic effects of organic chemicals on aquatic organisms continues to evolve as computational efficiency and toxicological understanding advance. With the ever-increasing production of new chemicals, and the need to optimize resources to assess thousands of existing chemicals in commerce, regulatory agencies have turned to QSARs as essential tools to help prioritize tiered risk assessments when empirical data are not available to evaluate toxicological effects. Progress in designing scientifically credible QSARs is intimately associated with the development of empirically derived databases of well-defined and quantified toxicity endpoints, which are based on a strategic evaluation of diverse sets of chemical structures, modes of toxic action, and species. This review provides a brief overview of four databases created for the purpose of developing QSARs for estimating toxicity of chemicals to aquatic organisms. The evolution of QSARs based initially on general chemical classification schemes, to models founded on modes of toxic action that range from nonspecific partitioning into hydrophobic cellular membranes to receptor-mediated mechanisms is summarized. Finally, an overview of expert systems that integrate chemical-specific mode of action classification and associated QSAR selection for estimating potential toxicological effects of organic chemicals is presented.

Keywords—Quantitative structure–activity relationships Ecological risk assessment Toxic action modes Aquatic toxicology Industrial organic chemicals

INTRODUCTION

Evaluating the potential hazard posed by thousands of untested industrial organic chemicals is a challenge confronting national and international regulatory agencies including the U.S. Environmental Protection Agency (U.S. EPA), Canadian Ministry of the Environment, and the European Union [1–3]. Because of time and funding constraints, conducting toxicity tests on the tens of thousands of new and existing chemicals released into the environment is not feasible. To maximize efficiency and consistency in evaluating and prioritizing those chemicals that are empirically assessed for adverse effects, quantitative structure–activity relationships (QSARs) can be employed as scientifically credible tools for predicting acute toxicity, when few or no empirical data are available [1].

The field of QSAR research is first noted in the literature at the beginning of the 20th century with the publication of the Meyer–Overton rule. This rule states that the potency of anesthetics, or narcotics of the central nervous system, is correlated to chemical lipophilicity (see reviews by Lipnick [4,5]). Over the last 100 years, hypotheses concerning the modes of action for narcotics have evolved from general perturbation of cellular membranes due to a nonspecific partitioning of xenobiotics, to mechanisms that invoke partitioning into specific membrane microsites or hydrophobic pockets of membrane-bound proteins (see review by Bradbury et al. [6]). Regardless of the actual site of narcotic action, the Meyer–Overton rule, with subsequent refinements by Miller and Ferguson in the 1930s, provides the foundation for the QSARs used today to estimate toxicity of narcotic industrial organic chemicals [6].

The notion that the potency of chemicals that act through mechanisms other than narcosis is, in part, related to their partitioning into hydrophobic biological compartments also is a fundamental principle for QSARs that were developed to address a wide range of agrochemicals and pharmaceuticals. As summarized by Martin [7], Hansch adopted the log of the octanol–water partition coefficient as a modeling parameter to represent the partitioning of chemicals into biolipids [8–11]. For modes of action where additional interactions with biological macromolecules occur, partitioning (P) will explain part of the observed response, but mechanistically plausible steric properties (S), electronic factors (El), or both may be required to more adequately relate toxicity response (C, expressed as mol/L) to chemical attributes (Eqn. 1). Empirically derived constants (a, b, c, and x) are obtained by fitting the empirical toxicity or efficacy data to the equation.

$$\log(\mathrm{C}) = x + a(\mathrm{El}) + b(\mathrm{P}) + c(\mathrm{S}) \quad (1)$$

Consistent with the concepts put forth by Hansch, the fundamental assumption in QSAR development for application in aquatic toxicology is that toxic potency is correlated to chemical concentration at the site of action. When fish or aquatic invertebrates are exposed to a xenobiotic in solution, tissue concentrations of the chemical will come to a steady-state level proportional to the compound's hydrophobicity, assuming that

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the aqueous concentration of the chemical remains constant during the exposure. Consequently, octanol–water partition coefficients are typically employed in QSARs as the chemical descriptor that captures that aspect of variability in toxicity across chemicals that is solely attributable to varying degrees of hydrophobicity, and uptake, of xenobiotics.

As is true for development and application of QSARs for drug and agrochemical design and discovery, QSARs for predicting adverse effects of xenobiotics in ecological risk assessments require empirical toxicity data of high quality and models developed from a known or hypothesized mode of toxic action [12]. This review will focus on QSARs derived from four of the larger data sets that were created specifically for predicting adverse effects to aquatic organisms. These databases contain toxicity results for acute effects in the fathead minnow (*Pimephales promelas*), the guppy (*Poecilia reticulata*), the ciliate *Tetrahymena pyriformis*, and a luminescent bacterium, *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*) [13–19]. The data sets each originated from a single research facility that used standard test protocols and are based on a consistently derived endpoint (e.g., median effective concentration or median lethal concentration [LC50]) when using a suite of chemicals that include a wide range of modes of toxic action and chemical classes.

ECOTOXICITY DATA FOR QSAR DEVELOPMENT

The development of QSARs necessitates the availability of reliable toxicity data sets based on well-documented and uniform testing protocols and well-defined and readily quantifiable toxic endpoints. Four extensive databases, developed specifically for use in deriving QSARs to predict the toxicity of industrial organic chemicals to aquatic organisms, have been established. These data sets, derived from the fathead minnow, the guppy, a ciliate (*T. pyriformis*), and the luminescent bacterium *V. fischeri*, continue to assist in expanding the knowledge of acute modes of toxic action and have resulted in the development of most QSARs in use today for predicting toxic effects to aquatic organisms.

Fathead minnow database

This U.S. EPA database consists of 753 flow-through bioassays conducted with juvenile fathead minnows on 617 chemicals selected from a cross section of the Toxic Substances Control Act Inventory of industrial organic chemicals [13,20]. All studies employ 28- to 36-d-old animals, are 96 h in duration, and consist of multiple treatment levels (typically five effect concentrations and a control) and a single dilution water source [21,22]. All aqueous chemical exposure concentrations are quantified and meet a minimum set of well-defined quality assurance measures [13]. The 96-h mortality responses are analyzed with the trimmed Spearman–Karber method to obtain an LC50 and 95% confidence interval, where possible [23]. For a more complete description of the database, see Russom et al. [13].

Guppy database

The University of Utrecht (Utrecht, The Netherlands) has a database of acute toxicity results for 180 organic chemicals tested in static renewal bioassays with two- to three-month-old guppies [14,24–30]. Bioassay durations range from 7 to 14 d and incorporate a range of chemical concentrations. To quantify lethal potency as LC50s, methods described by Litchfield and Wilcoxon [31] are employed. A more complete description of the methods used to generate this toxicity database are presented in Könemann [29].

Tetrahymena *database*

The *Tetrahymena* toxicity database, Tetratox, includes more than 2,500 static bioassays tested on approximately 1,400 organic chemicals to measure population growth inhibition of the freshwater ciliate *T. pyriformis* [15]. The bioassays vary in duration from 40 to 72 h, but the method is now standardized to 40 h. Each bioassay includes a minimum of five effect concentrations and one control flask, each in duplicate. Treatment flasks are inoculated with late log-growth-phase ciliates at an initial density of approximately 2,500 cells/ml. At the end of the study, population density is measured spectrophotometrically. By using Probit analysis procedures [32], the percent control-normalized absorbance is used to estimate the 50% growth inhibition concentration and associated 95% fiducial intervals. For a more complete description of these methods see Schultz [15].

Vibrio fischeri *database*

Kaiser and coworkers [19] have a database of more than 1,350 Microtox™ (AZUR Environmental, Newark, DE, USA) tests for approximately 1,300 organic chemicals with the luminescent marine bacterium *V. fischeri*. The Microtox test measures the decrease in light output of a luminescent bacterium as the toxic response. This database includes results at 5-, 15-, and 30-min durations. Because the bacterial studies are conducted in a cost- and time-effective manner, an important objective in creating this database is the establishment of correlations between the Microtox response and acute toxicity in aquatic organisms, to estimate the potency of chemicals to invertebrates and fish.

QSAR DEVELOPMENT

The evolution of environmental QSARs from chemical classification-based approaches to more toxicologically oriented classifications has led to challenges in developing rules or techniques to assign chemicals to modes of action and to then develop and apply appropriate QSARs to predict adverse effects. Various techniques have been proposed for developing and selecting models for estimating toxicity. These approaches include expert systems that utilize chemical substructures or fragments or various physical and chemical parameters, such as octanol–water partition coefficient, ionization potential, and redox potential, to identify chemicals acting via specific modes of action [13–15]. In addition, feed-forward neural networks, combined with statistical linear correlations, have been proposed for this purpose [33–37].

Models based on chemical analogue classification

Initial research in the field of QSARs for ecological risk assessments was predicated on the assumption that chemicals from the same chemical class should behave in a toxicologically similar manner. Consequently, homologous series of chemicals were used to develop structure–toxicity relationships and the assumption was made that toxic effects were imparted by common structural components used in chemical class assignments. Further, the assumption was made that potency varied with chemical uptake, which correlated with the hydrophobicity of substituent moieties within the chemical class.

Table 1 summarizes some representative QSARs based on

Table 1. Representative quantitative structure–activity relationships developed for specific chemical classes by using the octanol–water partition coefficient to estimate toxicity (log mol/L). Endpoints include median lethal concentration (LC50) for the guppy (*Poecilia reticulata*) and fathead minnow (*Pimephales promelas*) and 50% growth inhibition concentrations (IGC50) for the ciliate *Tetrahymena pyriformis*

Chemical group	Organism	Endpoint	Slope	Intercept	r^2	n	Reference
Benzenes	Guppy	LC50	0.845	4.63	0.987	12	[29]
Aliphatic alcohols	Ciliate	IGC50	0.80	2.04	0.982	34	[18]
Chlorinated phenols	Guppy	LC50	0.58	3.20	0.954	11	[30]
Esters	Fathead minnow	LC50	0.535	2.75	0.828	25	[112]
Isothiocyanates	Ciliate	IGC50	0.037	1.59	0.847	5	[16]
Acrylates	Fathead minnow	LC50	0.194	4.45	0.957	5	[113]

commonly defined chemical classes. Consistent with previous comments, octanol–water partition coefficients have been used to predict toxic responses. Consequently, slopes in these regressions should be near unity if partitioning was the only process responsible for differences in potency across the chemicals with a defined class. As seen in Table 1, models for chlorinated phenols, esters, isothiocyanates, and acrylates have slopes that are much less than unity. These chemical group QSARs may require electronic or steric parameters to more fully explain the variability in the observed toxicity, under the assumption that the chemicals' adverse effects and potency are related to additional toxicodynamic or toxicokinetic processes common to all the chemicals in the training set. Alternatively, regressions with slopes less than unity could reflect that chemicals within the indicated classes elicit toxic effects through different modes of toxic action.

Models based on mode of toxic action classification

Research completed over the past several years addressing the joint toxic action of chemicals and toxicodynamic responses observed in fish has challenged the notion that QSARs are reliably based on typical chemical classification schemes [38–44]. Furthermore, an evaluation of the fathead minnow database by Russom et al. [13] illustrates that toxicological classifications based on typically used chemical classes can be problematic (see Fig. 1). Many chemical classes historically associated with a baseline narcosis QSAR, such as ethers, alcohols, ketones, esters, and benzenes, include chemicals that act via a baseline narcosis mode of action, as well as chemicals that act through an electrophile-based mode of action [29,45–48]. Conversely, chemical classes not usually identified as acting by a baseline narcosis mode of action, such as the phenols, include chemicals that act through baseline narcosis, polar narcosis, oxidative phosphorylation uncoupling, or electrophile-based modes of action. Subsequently, the development and application of QSARs have evolved from a chemical class perspective to one that is more consistent with assumptions regarding modes of toxic action [13,14,49]. Therefore, the use of mode of action-based QSARs requires an appreciation of both toxic mechanisms and the critical structural characteristics and properties of a chemical that govern its action by a specific mechanism. As described by Bradbury [12], a mode of action domain must be clearly defined in terms of the biological model, endpoint, and exposure scenario (dosimetry). The necessity to carefully define the endpoint and to select the appropriate biological model, including the selection of in

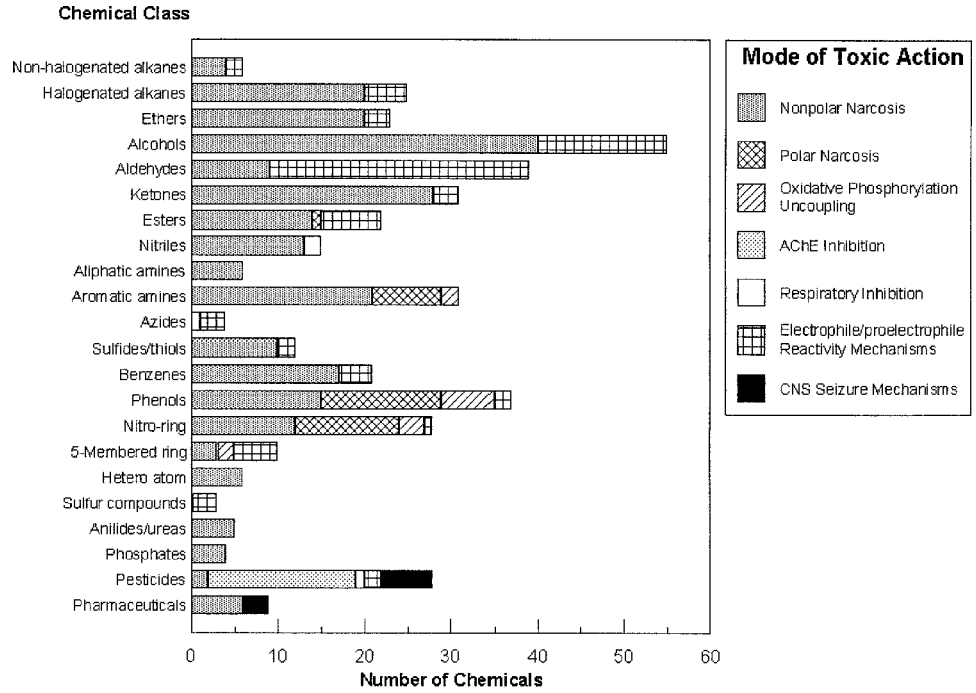

Fig. 1. Observed modes of toxic action associated with fathead minnow 96-h median lethal concentration (LC50) values as a function of chemical classes. CNS = central nervous system. Adapted from Russom et al. [13].

Table 2. Representative baseline narcosis quantitative structure activity relationships for estimating acute median lethal concentration (LC50) to the guppy (*Poecilia reticulata*), fathead minnow (*Pimephales promelas*), carp (*Cyprinus* sp.), goldfish (*Carassius auratus*), and the ciliate *Tetrahymena pyriformis.* Toxicity (log mol/L) is estimated from the octanol–water partition coefficient

Organism	Endpoint	Slope	Intercept	r^2	n	Reference
Guppy	LC50	0.871	1.13	0.976	50	[29]
Fathead minnow	LC50	0.94	1.25	0.94	60	[13, 47]
Carp	LC50	0.919	0.967	0.97	5	[8]
Goldfish	LC50	0.881	0.989	0.918	5	[8]
Ciliate	LC50	0.929	2.639	0.986	20	[15]

vivo or in vitro systems and an exposure scenario to distinguish modes of action, also are highlighted in Nendza and Muller [50].

Nonpolar, baseline narcosis QSARs

Research reported by Könemann [29] and Veith et al. [48] in the early 1980s described minimum, or baseline, toxicity as defined by general narcosis QSARs. These models, and related QSARs, are reliable for estimating the potential toxicity of neutral narcotic chemicals (see Table 2). Nearly 70% of all industrial organic chemicals are estimated to act via baseline and polar narcosis modes of action in acute (e.g., 4- to 14-d) exposures (see below) [13,49].

Narcosis has been defined as a reversible state of arrested activity of protoplasmic structures resulting from exposure to the xenobiotic. In the context of the intact organism, narcosis and general anesthesia are terms commonly used interchangeably. The exact narcosis mechanism remains an area of active research with hypotheses centering around lipid membrane perturbations or binding to specific lipid proteins [4,5,51,52]. The assumption is made that aqueous concentrations of narcotic chemicals are proportional to the concentrations at the site of action when the xenobiotic blood concentration is at equilibrium with the aqueous exposure concentration. A slope approaching unity provides evidence of this relationship (see Table 2). Consistent with this assumption, several studies have documented that the internal concentration observed for narcotic chemicals at lethality is reasonably constant [53–56].

Polar narcosis QSARs

Some industrial organic chemicals exhibit reversible responses, similar to baseline narcotics, but are thought to act through a different mechanism. In general, polar narcotics include those anilines, phenols, and pyridines that do not have substituents associated with reactive or uncoupling modes of action (see *Oxidative phosphorylation uncoupling QSARs* and *Reactive modes of toxic action* sections in this article). Broderius and coworkers [40,41,44] demonstrated that these chemicals are not strictly additive with baseline narcotics, such as 1-octanol. In addition, fish acute toxicity syndrome studies conducted by Bradbury and coworkers [39] demonstrated that the physiological responses observed in rainbow trout (*Oncorhynchus mykiss*) vary significantly from those observed with classic baseline narcotics [57,58]. In general, fish exposed to baseline narcotics are hypoactive, with a significantly reduced startle response and a general slowing of all respiratory functions [57]. The response of fish exposed to polar narcotics differs, with the onset of clonic seizures initiated by a cough response, which is a major discriminating symptom [39].

Several QSARs for estimating the toxicity of polar narcotics to aquatic organisms are presented in Table 3. As indicated previously, it has been widely assumed that polar narcotics act through a different molecular mechanism than baseline narcotics. Because polar narcotics can readily form hydrogen bonds, they have been speculated to interact with a different set of receptors in the nervous system. Kamlet et al. [59] proposed that the mechanism of action for polar narcotics is attributed to their dipolarity, hydrogen bond donor acidity, or both. Veith and Broderius [44] observed that as the octanol–water partition coefficient increased beyond 2.7, the effect of hydrogen bonding affinity was moderated for polar narcotics. Consequently, in some QSARs, the acid dissociation constant (pK_a) is used to explain toxicity associated with hydrogen bond acidity. Other researchers have used molecular orbital descriptors to characterize the hydrogen bonding of polar narcotics. For instance, Ramos et al. [60] developed QSARs to estimate acute and chronic effects on population growth of an algae (*Chlorella pyrenoidosa*) exposed to a series of anilines and phenols. By using a partial least squares regression method [61], the octanol–water partition coefficient was employed to account for partitioning of the chemicals into the organism and the most negative partial charge on a nonhydrogen atom, most positive partial charge on a hydrogen atom, energy of the highest occupied molecular orbital (E_{HOMO}), and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) were employed to describe hydrogen bonding capability.

Note that some workers [62] have suggested that the apparent difference in toxic potency between nonpolar and polar narcotics relative to octanol–water–based LC50 predictions may be due to the inability of octanol to reflect biological membranes. When using L-α-dimyristoyl phosphatidylcholine–water partition coefficients for toxicity predictions, a sin-

Table 3. Representative polar narcosis quantitative structure–activity relationships for estimating acute median lethal concentration (LC50) to the guppy (*Poecilia reticulata*) and fathead minnow (*Pimephales promelas*), and 50% growth inhibition concentration (IGC50) to the ciliate *Tetrahymena pyriformis.* Toxicity (log mol/L) is estimated by using octanol–water partition coefficient (log K_{OW}), the acid dissociation constant (pK_a), or both

		Slope					
Organism	Endpoint	Log K_{OW}	pK_a	Intercept	r^2	n	Reference
Guppy	LC50	0.46	—	3.04	0.824	11	[30]
Guppy	LC50	1.12	0.43	8.35	0.962	11	[30]
Fathead minnow	LC50	0.65	—	2.29	0.90	39	[44]
Ciliate	IGC50	0.5744	—	0.8652	0.756	30	[113]
Ciliate	IGC50	0.6577	0.3171	1.986	0.912	30	[113]

Table 4. Representative quantitative structure–activity relationships for estimating acute median lethal concentration (log LC50 in mol/L) of oxidative phosphorylation uncouplers to the guppy (*Poecilia reticulata*) and fathead minnow (*Pimephales promelas*), 50% growth inhibition concentration (IGC50 in mol/L) to the ciliate *Tetrahymena pyriformis*, and toxic potency measured as decrease in luminescence (log pT30 as 30-min toxic potency) in the bacterium *Vibrio fischeri*

Species	Endpoint	Slope	Intercept	r^2	n	Reference
Fathead minnow	LC50	0.67	−2.95	0.82	12	[13]
Bacteria	pT30	0.489	0.126	0.848	16	[115]
Fathead minnow	LC50	0.526	0.408	0.858	8	[115]
Ciliate	IGC50	0.401	0.189	0.824	12	[115]

gle QSAR for both classes of narcotics was reported. Although this relationship does not provide a biological explanation for the clear differences in toxicological symptoms noted for the two classes of narcotics [39], the observation that different surrogates for hydrophobic environments within nerve membranes would provide different correlations is consistent with the notion that multiple receptor proteins (e.g., ion channels), protein–lipid microenvironments, or both control action potentials and their propagation [6].

Oxidative phosphorylation uncoupling QSARs

Uncouplers of oxidative phosphorylation inhibit the synthesis of adenosine triphosphate within the mitochondria by short-circuiting hydrogen ion transport and phosphorylation [63,64]. These chemicals are typically weak acids and are typified by phenols, anilines, and pyridines that, unlike polar narcotics, include multiple electronegative groups (i.e., more than one nitro substituents, more than three halogen substituents, or both bonded to the aromatic ring). Rainbow trout exposed to respiratory uncouplers exhibited a significant increase in ventilation volume and total oxygen consumed, without a concurrent increase in ventilation rate [42,57]. Joint toxic action studies demonstrated that these chemicals are strictly additive with the known oxidative phosphorylation uncoupler, 2,4-dinitrophenol [41]. Slopes in regression models for oxidative phosphorylation uncoupling demonstrate that partitioning accounts for only a portion of the toxicity of these chemicals (see Table 4). The addition of a parameter that captures the ionization potential of these chemicals may improve these QSARs.

Reactive modes of toxic action

In recent years, finding means of characterizing toxic modes of action for reactive toxicants has been a major research challenge. These chemicals, or their activated metabolites, react covalently with nucleophilic sites in cellular biomacromolecules (e.g., through nucleophilic substitution, Michael-type addition, or Schiff-base reactions) or elicit oxidative stress through redox cycling to elicit toxic effects. Several reviews have proposed two-dimensional (2-D) substructural fragments that are associated with the electrophilic modes of action [13,14,65]. Various attempts also have been made to define quantum chemical descriptors that would be useful in describing these nonpecific reactive modes of action [66–80]. Although these studies have established mechanistically plausible approaches to predict the toxicity of reactive toxicants, the lack of sufficient and consistently derived toxicity data sets has hampered the means to fully develop and evaluate these QSARs for risk assessment application. However, recent studies are providing the initial in vitro biological models, appropriate exposure techniques, and quantifiable endpoints to begin classifying reactive compounds within predominate toxicological pathways based on a variety of alkylation reactions and oxidative stress [50,81–84]. The classification of a compound as a reactive toxicant also is complicated by the potential role of metabolic activation. Research addressing principles underlying the means of estimating routes and rates of metabolic activation from chemical structure provides a range of potential approaches to improve this aspect of predicting modes of toxic action [85–88].

In recent years, an enhanced understanding of reactive modes of toxic action has resulted in more elaborate models for predicting the ultraviolet-enhanced toxicity of polycyclic aromatic hydrocarbons (PAHs) and related chemicals [89–92]. The primary mode of action for phototoxic PAHs, particularly in short-term exposures, is thought to arise from oxidative stress (for reviews, see [93–95]). With respect specifically to PAH phototoxicity to aquatic organisms, Mekenyan and coworkers [91,92] described a QSAR based upon calculated values of the gap between the highest-occupied and lowest-unoccupied molecular orbitals (HOMO–LUMO gap) of PAHs. This model describes a relationship between the energy of light absorbed, stability of unactivated and activated states of the PAH molecules, and subsequent potential to form the reactive singlet oxygen species. By using the toxicity data generated by Newsted and Giesy [96], the model established a phototoxicity window that reflects relevant molecular properties of PAHs (as depicted by the HOMO–LUMO gap), and the occurrence of environmentally realistic ultraviolet light spectra. Further modeling of a set of substituted PAHs indicated that most substituents did not greatly alter the HOMO–LUMO gap, suggesting that knowledge of the base PAH structure is sufficient to predict potential for photoinduced toxicity [97–99]. Although the initial models were developed with PAH training sets, evaluation of the model with other phototoxic chemicals (e.g., alpha-terthienyls) suggests that the basic conceptual approach can be valid for predicting potential phototoxicity independent of chemical class [100].

QSAR DECISION SUPPORT SYSTEMS

A primary uncertainty in the use of QSARs is the selection of appropriate models for the chemicals of interest. As previously mentioned, many QSARs have been developed based on traditional chemical classification systems, but a chemical could be classified in more than one group (e.g., whether 4-aminophenol is a phenol or an aniline). As discussed previously, many traditional chemical classes clearly can incorporate multiple modes of action. As a consequence, QSAR support systems have increasingly converted from a chemical class perspective to one that is more consistent with assumptions regarding modes of toxic action [12–14,101,102].

Assessment tools for the evaluation of risk (ASTER) is an expert system developed by the U.S. EPA that selects QSARs based on the predicted mode of action of chemicals [13,103]. To this end, the previously described U.S. EPA fathead minnow database includes an assessment of the mode of toxic action for each chemical based on additional data gathered during the 96-h toxicity tests, such as dose–response relationships and behavioral responses and, for a subset of the chemicals in the database, results of joint toxic action studies with fathead minnows or acute toxicity syndrome studies observed

in rainbow trout [38–44,57–58,104,105]. These data were supplemented with information gleaned from the toxicological literature specific to the issue of toxicodynamic classifications [13]. Major modes of toxic action classifications include baseline narcosis, polar narcosis, oxidative phosphorylation uncoupling, respiratory inhibition, electrophile–proelectrophile reactivity, acetylcholinesterase inhibition, and several mechanisms associated with central nervous system seizure responses (excluding acetylcholinesterase inhibition). Because complete data sets were not always available for every chemical, a confidence level was assigned to each mode of action designation. After mode of action determinations for each chemical in the data set, unique structural fragments, or combinations of structural fragments, were identified for each mode of action group.

Based on the mode of action assessments, a heuristic model that used conditional statements and Boolean logic was developed that searches for various substructure fragments associated with each mode of action in an unknown chemical. Mode of action determinations are subsequently linked to the appropriate QSAR models, if available within the ASTER system. If structural fragments satisfy requirements for more than one mode of action, the mode of action resulting in the lowest LC50, as predicted from the corresponding QSAR, is selected as the default. The other possible modes of action are identified and provided to the user, who can override the mode of action selection made by the expert system, thereby invoking an alternate QSAR. To validate the mode of action assignments and the predictability of associated QSAR models, fathead minnow 96-h flow-through LC50 data, based on measured water concentrations, were retrieved from the ECOTOX (ecotoxicology) database ($n = 739$) [106]. After excluding chemicals in the U.S. EPA fathead minnow data set ($n = 642$), a validation set of 97 chemicals was obtained. The correlation coefficient of predicted toxicity values, compared to the empirical data found in the ECOTOX database, was 0.95.

Similar rule-based systems have been developed for the guppy and ciliate databases. The University of Utrecht has identified four separate classes of chemical activity: inert or nonpolar narcosis, polar narcosis, nonspecific reactivity (chemicals that react with nonspecific biomolecules and chemicals that are bioactivated), and specifically acting chemicals (e.g., chemicals that specifically bind to a known biological receptor) [14,101,102,107]. As with the ASTER system, substructures are identified for each chemical activity and QSARs are subsequently assigned based on this classification method. Schultz and coworkers [15] have proposed a similar rule-based system for classifying potential hazards of substances with data from the Tetratox database.

Neural networks, in theory, eliminate the need for professional judgement in relating chemical substructures to modes of action. Kaiser and coworkers [34] used neural networks and pattern recognition techniques to explain the toxic potency of chemicals exclusively by molecular structure, rather than surrogate physicochemical parameters. In modeling the fathead minnow, ciliate, and bacterial luminescence data, mixed models were used where the nonlinear component of the relationship was managed through one or more feed-forward neural networks, combined with statistical linear corrections based on the errors generated by the networks [33–37].

DISCUSSION

In the field of environmental risk assessment, structure–activity relationships are increasingly used to predict the ecological fate and effects of chemicals when few or no empirical data are available. Coupled with the use of these models are analogue selection techniques in which data associated with structurally similar chemicals are used to estimate risk levels of chemicals for which no data are available [46,108–110]. The proper application and continued acceptance of these techniques to predict toxicological effects require credible methods or models to systematically assign chemicals to appropriate QSARs and analogues.

As summarized in this review, the selection of structural analogues or QSARs historically was based on the assumption that chemicals from the same chemical class should behave in a toxicologically similar manner. However, in practice, the delineation of chemical classes has proved problematic, and mechanistic research has established that typical chemical classification schemes do not necessarily reflect any similarity in modes of toxic action. Consequently, QSAR development and application increasingly include a deliberate integration of mechanistic toxicology and computational chemistry to identify critical structural characteristics and properties that govern modes of toxic action and inherent xenobiotic potency.

To develop a credible approach for predicting mode of toxic action and potency from chemical structure requires establishment of knowledge bases that contain training sets of chemicals whose modes of action and potency are well defined. In developing predictive models, it is essential that the mode of action domain for a specific application be clearly defined in terms of the exposure regime, biological model and endpoint, and range of chemical properties and attributes. Failure to adequately specify a knowledge base across the variables associated with each of the three conceptual dimensions of the mode of action domain can lead to toxicologically meaningless information and statistically inadequate mode of action prediction schemes and associated QSARs. Consistent with this approach of developing QSAR decision support systems, data sets associated with acute toxicity of industrial organic chemicals to the fathead minnow, the guppy, *Tetrahymena*, and *V. fischeri* were specifically established to formulate mode of action expert systems and neural networks, as well as associated mechanism-specific QSARs.

To date, the majority of mode of action expert systems and associated QSARs have been based on 2-D representations of chemical structures and associated properties. These 2-D techniques have permitted the development of mechanistically reasonable toxicity models that can assess the adverse effects of numbers of chemicals in a computationally efficient manner, which is essential given the large number of chemicals in the world's industrial chemical inventories for which no ecotoxicological data are available. Through the use of these modeling techniques, the acute toxicity of approximately 70% of the discrete chemicals in these inventories can be predicted with an error rate commensurate with experimental variability.

Remaining challenges facing regulatory agencies, and the associated toxicology and computational chemistry research communities, are the need to predict the potential acute and chronic effects of chemicals whose toxicity are elicited through covalent binding to critical nucleophilic sites in DNA, RNA, peptides and proteins; the disruption of cellular redox balance by the generation of reactive oxygen through redox cycling; and noncovalent interactions with specific regulatory receptor proteins (e.g., hormones). Because of the very nature of these toxicological mechanisms, 2-D QSAR techniques generally are inadequate to predict potency. The means to relate chemical

structure to these toxicological processes will require the development and application of a new generation of QSAR techniques that permit the rapid estimation of effects based on three-dimensional (3-D) representations of chemical structure, and associated electronic and steric properties. Preliminary work on use of 3-D modeling to discriminate mode of toxic action from in vitro test data has been conducted by Nendza and coworkers [50].

Although ab initio and semiempirical quantum chemical and force-field methods are widely used in the drug and agrochemical discovery and design fields, these computational techniques have not been incorporated within the toolbox of risk assessment approaches used to evaluate industrial chemicals for adverse effects. In the past, meeting this challenge rested, in part, on the development of efficient computational software, and associated hardware, to permit real-time desktop 3-D calculations. In the last several years, the capability of calculating 3-D structures and associated semiempirical parameters, sufficient for most modeling applications, has evolved to the point that daily application of 3-D QSARs is feasible.

With the challenge of efficiently calculating 3-D structures and properties met, the means to develop and apply 3-D computational techniques is no longer a limiting factor in bringing the next generation of QSAR techniques to the risk assessment field. As summarized in this review, exploratory QSAR techniques to predict toxicity due to a variety of electrophilic mechanisms and oxidative stress have been developed. Schmieder et al. describe how related 3-D computational techniques have been developed to predict relative binding affinity of industrial chemicals to a variety of hormone receptors. These recent successes in 3-D QSAR research establish that the fundamental challenge to bring the next generation of QSAR techniques to the risk assessment community rests on the ability of the toxicology and computational chemistry research communities to build the required mechanistically sound and well-defined toxic effect knowledge bases. This challenge is no different than that faced by the QSAR research groups of 20 years ago, whose ability to create multidisciplinary teams of toxicologists, chemists, mathematicians, and computer specialists established the predictive techniques widely used today across regulatory programs in North America and Europe. Creation of this next generation of knowledge bases will not only support new predictive models, but will undoubtedly lead to new insights on toxicological processes at the molecular and cellular level, as well as the development of novel approaches to measure and quantify the biochemical, morphological, and physiological effects of xenobiotics by using a wide range of in vitro models.

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