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PREDICTING MODES OF TOXIC ACTION FROM CHEMICAL STRUCTURE: ACUTE TOXICITY IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*)

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Abstract—In the field of aquatic toxicology, quantitative structure–activity relationships (QSARs) have developed as scientifically credible models for predicting the toxicity of chemicals when little or no empirical data are available. In recent years, 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. The objective of this research was to develop procedures that relate modes of acute toxic action in the fathead minnow (*Pimephales promelas*) to chemical structures and properties. An empirically derived database for diverse chemical structures of acute toxicity and corresponding modes of toxic action was developed through joint toxic action studies, the establishment of toxicodynamic profiles, and behavioral and dose–response interpretation of 96-h LC50 tests. Using the results from these efforts, as well as principles in the toxicological literature, approximately 600 chemicals were classified as narcotics (three distinct groups), oxidative phosphorylation uncouplers, respiratory inhibitors, electrophiles/proelectrophiles, acetylcholinesterase inhibitors, or central nervous system seizure agents. Using this data set, a computer-based expert system has been established whereby chemical structures are associated with likely modes of toxic action and, when available, corresponding QSARs.

Keywords—Quantitative structure–activity relationships Expert systems Toxic action mode Aquatic toxicology *Pimephales promela*

INTRODUCTION

In the field of aquatic toxicology, first-generation quantitative structure–activity relationships (QSARs) have developed as scientifically credible tools for predicting the acute toxicity of chemicals when little or no empirical data are available [1]. In part, the success in establishing these QSARs is dependent upon well-defined and quantifiable toxicity endpoints, such as the 96-h 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 adverse effects. The proper application and continued acceptance of these predictive toxicology techniques, therefore, require methods to systematically assign chemicals to appropriate QSARs or analogues. This critical process in the use of predictive techniques represents a major area of uncertainty in ecological risk assessments for chemical stressors [2,3], where errors in QSAR selections can result in 10- to 1,000-fold errors in toxic potency estimates.

Traditionally, the selection of structural analogues or QSARs has been based on the assumption that compounds from the same ''chemical class'' should behave in a toxicologically similar manner. Although this working hypothesis seems reasonable, the identification 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 [4–12]. 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 [13,14]. The use of mode of action-based QSARs, therefore, requires an appreciation of both toxic mechanisms and the critical structural characteristics and properties of a chemical that governs its action by a specific mechanism.

Establishment of toxicologically credible 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 [13]. A typical endpoint used in initial effect assessments for aquatic organisms is the 96-h LC50 value for the fathead minnow. Collaborative research undertaken through our laboratory has established a database for this endpoint that contains values for approximately 600 chemicals [15–19] and which serves as a foundation for the development of QSARs. The chemical set chosen for study was based on an assessment of the U.S. industrial chemical inventory of discrete organic chemicals [20].

Using this chemical data set, we describe an investigation that relates modes of acute toxic action in the fathead minnow to chemical structures and properties. An empirically derived database of chemical structures and corresponding modes of toxic action was developed through joint toxic action studies, the establishment of toxicodynamic profiles, and behavioral

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Electronic copies of the data sets used in this study are available upon request. Mention of trade names, models, or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

and dose–response interpretation of 96-h LC50 tests. Using the results from these efforts, as well as principles in the toxicological literature, the chemicals were classified as either narcotics, oxidative phosphorylation uncouplers, respiratory inhibitors, electrophiles/proelectrophiles, acetylcholinesterase (AChE) inhibitors, or central nervous system (CNS) seizure agents. Using this data set, a computer-based expert system has been established whereby chemical structures are associated with likely modes of toxic action and, when there are sufficient data, corresponding QSARs.

MATERIALS AND METHODS

To develop an expert system to predict acute mode of toxic action from chemical structure first requires a knowledge base from which rules can be derived. In this study, the knowledge base was derived from analyses of the chemicals in the fathead minnow acute toxicity database [15–19]. Based on empirical mode of action assessments, a knowledge base was established from which substructural fragments of chemicals were associated with modes of toxic action. In turn, these rules were written in Fortran and linked to mode of action-specific QSARs, when available.

Each chemical was classified into one of eight modes of action: base-line narcosis or narcosis I [21], polar narcosis or narcosis II [12], ester narcosis or narcosis III [11,22], oxidative phosphorylation uncoupling [23], respiratory inhibition [24], electrophile/proelectrophile reactivity [25,26], AChE inhibition [24], or several mechanisms of CNS seizure responses [27]. For the purpose of this paper, mode of toxic action should not necessarily be construed to impart the sense of distinct molecular mechanisms. For example, CNS seizure agents and respiratory inhibitors can act through a variety of receptors [24,27] and electrophiles/proelectrophiles [26] can bind to a spectrum of cellular nucleophiles through a number of potential reactions (e.g., nucleophilic substitution, Schiff-base formation, Michael-type addition, etc.). As referenced throughout the subsequent sections, reference toxicants, whose modes of action are generally accepted through multiple lines of evidence, are used to facilitate the assessment of unknown compounds.

Empirical mode of toxic action assessments

Chemicals in the fathead minnow database were evaluated through analyses of dose–response relationships and behavioral responses [28,29] associated with 96-h LC50 bioassays. These assessments were further supported by the results of joint toxic action studies [7,8,11,30] and fish acute toxicity syndrome (FATS) investigations [4,5,9,10] on chemical subsets from the database. Finally, the mode of action assignments were further evaluated through an examination of toxicological literature specific to the issue of toxicodynamic classifications [6]. Based on the amount of available information for a given compound, a level of confidence was assigned to a mode of action determination. The mode of action assessments are described in more detail in the following subsections.

Fathead minnow acute toxicity database. Ninety-six-hour flow-through exposures using 28- to 36-d-old juvenile fathead minnows were conducted on 617 chemicals [15–19]. Compounds were selected for testing from the Toxic Substances Control Act inventory of chemicals to represent a cross-section of industrial organic chemicals [20]. For quality assurance evaluations, 94 chemicals were selected for retesting, including reference toxicants such as octanol, pentachlorophenol, phenol, and carbaryl, which results in a database of 753 tests.

A detailed description of the biological and chemical test protocols used for these exposures has been published [15,16]. Briefly, all tests were conducted using Lake Superior water at 25 ± 1°C. Aqueous toxicant concentrations were measured in all tests with quality assurance criteria requiring 80% agreement between duplicate samples and 90 to 110% spike recovery. Flow-through exposures were conducted using cycling proportional [31], modified Benoit [32], or electronic [16] diluters. Tests conducted on the Benoit and electronic diluters did not have replicate tank exposures. Median lethal concentrations (LC50s) were calculated using the Trimmed Spearman–Karber Method, with 95% confidence intervals being calculated when possible [33].

Dose–response assessments. The change of LC50 values over time (LC50 ratio) and the ratio of measured 96-h LC50 values to those predicted from a baseline narcosis (narcosis I) QSAR [21] were used as supportive data for assessing potential modes of action. To characterize time until death, a ratio of the 24-h LC50 to the 96-h LC50 was calculated for each 96-h fathead minnow exposure using Equation 1:

$$\text{LC50 ratio} = \text{24 h LC50/96 h LC50} \quad (1)$$

For exposures where an LC50 did not occur in the first 24 h, ratios of the 48- to 96- or 72- to 96-h values were used. Instances where an LC50 was not obtained until 96 h were noted. Ratios that were approximately 1.0 were considered indicative of narcosis I, whereas ratios greater than 2 or cases where LC50s were not achieved in the first 24 h were generally considered indicative of a different mode of action. It was noted, however, that compounds with a high log octanol : water partition coefficient (log P) may increase the LC50 ratio due to an increased time for the compound to reach equilibrium between the water and biophases.

Excess toxicity (Te) values [14,21,26,34] were calculated by dividing predicted narcosis I LC50 values by the observed values as indicated in Equation 2:

$$\text{Excess toxicity (Te)} = \text{predicted LC50/observed LC50} \quad (2)$$

Excess toxicity values that were greater than 10 were considered indicative of compounds not acting by narcosis I. The narcosis I QSAR used in deriving LC50 ratio and Te values is given by Equation 3 [21]:

$$\log \text{molar LC50} = -0.94 \log P + 0.94 \log(0.000068P + 1) - 1.25 \quad (3)$$

where P is the octanol : water partition coefficient.

Behavioral assessments. In conjunction with 96-h flow-through tests conducted at our laboratory after 1983, changes in behavior and morphology were systematically recorded using methods described by Drummond and co-workers [28,29]. Behavioral signs of stress were identified for fathead minnows exposed to reference toxicants and used to classify chemicals into three behavioral syndromes. As reported previously, fish displaying type I behavior had depressed locomotor activity with little or no response to outside stimuli. Body coloration became darker with most fish dying within 24 h. Fish exhibiting a type II behavior syndrome were hyperactive and usually overreactive to outside stimuli. Death tended to occur over several days of exposure. With the type III behavior syndrome, fish elicited spontaneous locomotor activity with a high incidence of convulsion, spasms, tetany, scoliosis, lordosis, and/

or hemorrhaging in the vertebral column. Modes of action (and associated reference toxicants) typically associated with type I, type II, and type III syndromes were narcosis I (octanol, MS-222); narcosis II (phenol) and oxidative phosphorylation uncoupling (pentachlorophenol, 2,4-dinitrophenol); and AChE inhibition (malathion, carbaryl) and electrophile reactivity (gill irritation; acrolein, benzaldehyde), respectively. For chemical exposures conducted prior to development of the behavior syndrome classification technique, comments made by the researcher regarding fish behavioral responses were compared to definitions of behavior syndromes and an estimate of a behavior syndrome was made.

Joint toxic action studies. Standard flow-through acute toxicity tests using juvenile fathead minnows were conducted using equitoxic binary, multiple proportion binary, and multiple equitoxic mixtures using methods previously described by Broderius and Kahl [7]. Each mixture bioassay included one reference compound for a specific mode of action. The following modes of action have been investigated: narcosis I [7,8,30], narcosis II [8,11,30], oxidative phosphorylation uncoupling [8,30], and respiratory inhibition [8,30]. To assign modes of toxic action to unknown chemical(s), mixture toxicity indices (MTIs) were calculated as described in Equation 4:

$$\text{MTI} = 1 - (\log \text{TU}/\log N) \quad (4)$$

where TU is the toxic units, i.e., the sum of the ratios of the test concentration for each chemical in a mixture at the standard acute response to its 96-h LC50 value and N is the number of compounds in the mixture. A mode of action was assigned to a chemical when it was determined to be additive with a reference compound (i.e., an MTI of 0.750–1.25). Thus far, 125 chemicals from the fathead minnow acute toxicity database have been retested in a total of 186 acute joint action bioassays. Based on this testing, four specific mode of action groups have been identified using reference toxicants. These modes of action include narcosis I, narcosis II, oxidative phosphorylation uncoupling, and respiratory inhibition.

FATS. The experimental approach to assess the physiological response of rainbow trout (*Oncorhynchus mykiss*) to intoxication by selected chemicals has been described previously [4,5,9,10]. Briefly, spinally transected trout (0.6–1.0 kg) are exposed to lethal concentrations of the selected toxicant in specially designed respirometer-metabolism chambers and a series of respiratory-cardiovascular variables are monitored until death. Those variables typically measured include heart rate, ventilation rate, cough rate, arterial blood oxygen, hematocrit, ventilation volume, oxygen utilization, arterial blood pH, arterial blood carbon dioxide, and total oxygen consumption. By using discriminant function analysis, the complex data sets derived from these studies can be simplified and the best response variables for classifying a chemical within a specific FATS can be determined. A subset of chemicals from the fathead minnow database were assessed to evaluate further the reliability of mode of action determinations based on behavioral syndrome and joint toxic action analyses. Concordance between narcosis I [9] and narcosis II [5] agents, oxidative phosphorylation uncouplers [9], respiratory inhibitors [35], AChE inhibitors [4,10], CNS seizure agents [4], and direct-acting electrophiles (gill irritants) [10] and specific FATS has been established.

Mode of action assignments. Using data sets described in the previous sections, as well as supportive information in the literature [6], chemicals within the fathead minnow database were assigned to modes of toxic action. Major modes of toxic action included narcosis I, narcosis II, narcosis III, oxidative phosphorylation uncoupling, respiratory inhibition, electrophile/proelectrophile reactivity, AChE inhibition, and several mechanisms of CNS seizure responses (excluding AChE inhibition).

Because complete data sets were not always available for every chemical, a confidence level was assigned to each mode of action designation. To attain the highest level of confidence (A level) in a mode of action classification, a FATS, joint toxic action determination, or chemical-specific literature information was required. Information required for a level B determination included behavior syndrome, LC50 ratio, and Te value data that were consistent with that observed for structurally similar compounds whose mode of action assignment was based on A level information. In some instances, a B level of certainty was assigned if the LC50 ratios and Te values were consistent with prototypical compounds in a mode of action group when comparisons of behavior syndromes were inconclusive. A C level of certainty was attained when there were less than three level B components, but information such as the concentration/response slope, behavior comments, and/or chemical similarity to prototypical compounds was available to support the assessment. A level D certainty was indicated when there was no confidence in a mode of action classification due to insufficient data. At times, conflicts between data were noted when more than one mode of action was suggested. In these instances multiple mode of action assignments were made.

As a panel, the authors assessed each chemical in the database based on the above criteria. For example, 1-pentanol was additive with octanol in a joint toxic action study and fish elicited a type I behavior syndrome during the acute exposure (A level of certainty). In addition, the Te value and LC50 ratio approached 1.0 (B level of certainty). Based on these data, a narcosis I mode of action was assigned to 1-pentanol with an A level of certainty. After an initial analysis, the database was reviewed a second time with special attention given to compounds originally identified as having conflicting data, as well as to confirm that the selection guidelines had been consistently followed.

Expert system

Using the knowledge base described above, an expert system was developed that assigns acute modes of toxic action to compounds based on topological (two-dimensional), substructural ''rules'' and subsequently invokes a corresponding QSAR to provide toxic potency predictions. The algorithms and QSARs are contained within ASTER (ASsessment Tools for the Evaluation of Risk), which is a VAX-based system [36]. Development of the substructural rules and associated QSARs for each mode of action are described below.

Substructural mode of action rules. Through an examination of the knowledge base, unique structural fragments, or combinations of structural fragments, were identified for each mode of action classification. For classes of pesticides that had not been extensively tested, structural fragments were derived through an analysis of registered active compounds and the literature [37,38]. The electrophile/proelectrophile fragments were also compared to toxicophores identified in the literature [25]. Appendix 1 summarizes the substructural fragments assigned to modes of action.

Fragments were subsequently coded using Simplified Input

Table 1. The QSAR models for modes of toxic action identified in a database of fathead minnow 96-h LC50 values [15–19]

Eqn. no.	Mode of action	QSAR Slope	QSAR Intercept	n	R^2 (%)	s
3[a]	Narcosis I	−0.94	−1.25	60	94[b]	0.34[b]
5[c]	Narcosis II	−0.65	−2.29	39	90	0.27
6[d]	Narcosis III	−0.71	−2.43	7	83	0.36
7[e]	Uncoupler of oxidative phosphorylation	−0.67	−2.95	12	82	0.39

QSARs generated using log molar LC50 values with log P as independent variable.
[a] Equation from Veith et al. [21].
[b] Value not reported by Veith et al. [21], but recalculated for the present study.
[c] Equation from Veith and Broderius [11].
[d] Modified from Veith et al. [22]. See Appendix 2 for chemicals used in deriving the equation.
[e] All chemicals in Appendix 2 identified as acting by an oxidative phosphorylation uncoupling mode of action were used to derive Equation 7.

Language for Chemists (SILC) [39], which is an extended version of SMILES (Simplified Molecular Line Entry System) notation [40–42]. SILC facilitates searching for two-dimensional substructures within a chemical, or database of chemicals, with substructures defined by atom and bond types. To implement SILC similarity search algorithms, the SMILES string for the entire structure must undergo a canonicalization procedure, which results in a unique identifier for each atom within a connection table.

A heuristic model, written in Fortran, was developed that identified the various substructure fragments associated with each mode of action using conditional statements and Boolean logic; i.e., if a given substructure is present, then a given mode of toxic action is assigned to the compound. For instance, one query in the program is: if a chemical has an oxygen attached to an aromatic ring (i.e., if the chemical is a phenol) and the number of halogens attached to the aromatic ring is less than or equal to 2, then the likely mode of action is narcosis II. Mode of action determinations are subsequently linked to the appropriate QSAR, if available. 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 identified are provided, however, and the user can override the mode of action selection made by the expert system and thereby invoke an alternate QSAR.

QSARs. Table 1 lists the QSARs that were linked to the expert system and used to predict fathead minnow 96-h LC50 values. The QSARs based on narcosis I (Eqn. 3) [21] and narcosis II (Eqn. 5) [11] were previously published. The QSAR for narcosis III (Eqn. 6 in Table 1) was modified from that reported by Veith et al. [22] to only include monoesters because empirical mode of action assessments indicated that diesters seemingly acted through a different mode of action. Chemicals used in the narcosis III QSAR (Table 1) are identified in Appendix 2. The QSAR derived for predicting the toxicity of oxidative phosphorylation uncouplers has not been reported previously but is similar to models previously published for the fathead minnow [43,44]. All oxidative phosphorylation uncouplers identified in Appendix 2 were used to derive the uncoupler QSAR in Table 1. Calculated and measured log P values used to estimate toxicity (Appendix 2) are from the CLOGP™ program version 3.4 and STARLIST database, respectively, within the UDRIVE system version 3.53, 1988, from Pomona College Medicinal Chemistry Project, Claremont, California, USA. All regressions were conducted using MINITAB software release 10.2 for Windows, 1994, Minitab, Inc., State College, Pennsylvania, USA.

Although a number of QSARs for predicting the toxicity of electrophilic/proelectrophilic compounds have been reported, these relationships are typically based on small sets of congeneric chemicals [45–47], and there remains significant uncertainty concerning the proper selection of these models as a function of specific molecular mechanisms and associated two-dimensional [25,48] versus stereoelectronic (i.e., three-dimensional) structure [49–53]. Consequently, these QSARs were not employed in the evaluation of observed and predicted 96-h LC50 values because their use would likely have confounded the resulting interpretation.

RESULTS

Empirical mode of toxic action assessments

As presented in the Materials and Methods, a number of toxicodynamic responses were used to determine modes of toxic action associated with 96-h exposures to the fathead minnow. The information most heavily relied upon in making assessments included the results from joint toxic action bioassays and FATS studies, as well as evaluations of behavioral syndromes, LC50 ratios, and Te values from chemical-specific 96-h LC50 tests. Using prototypical compounds for mode of action groups, data derived from joint toxic action bioassays, FATS studies, and behavioral assessments formed the basis for many determinations and guided toxicodynamic interpretations for topologically similar (i.e., two-dimensional similarity) chemicals whose mode of action assessments were based on LC50 ratios and Te values.

Based on joint toxic action bioassays, 72 compounds (Appendix 2) were identified as strictly additive with a single reference toxicant; 33 additive with octanol (i.e., narcosis I), 29 additive with phenol (i.e., narcosis II), 8 additive with 2,4-dinitrophenol (i.e., oxidative phosphorylation uncoupling), and 2 additive with cyanide (respiratory inhibition). Seven aniline and phenol derivatives, with log P values >2.7, were additive with both phenol and octanol. In the present study, phenol and aniline derivatives that were additive with phenol and octanol, having a log $P > 2.7$ were categorized as acting by a narcosis I mode of action, consistent with the proposal of Veith and Broderius [12] that as log P increases beyond 2.7 the effect of hydrogen bonding in narcosis II is moderated for these compounds. Fish acute toxicity syndromes, based on exposures to prototypical compounds identified through joint

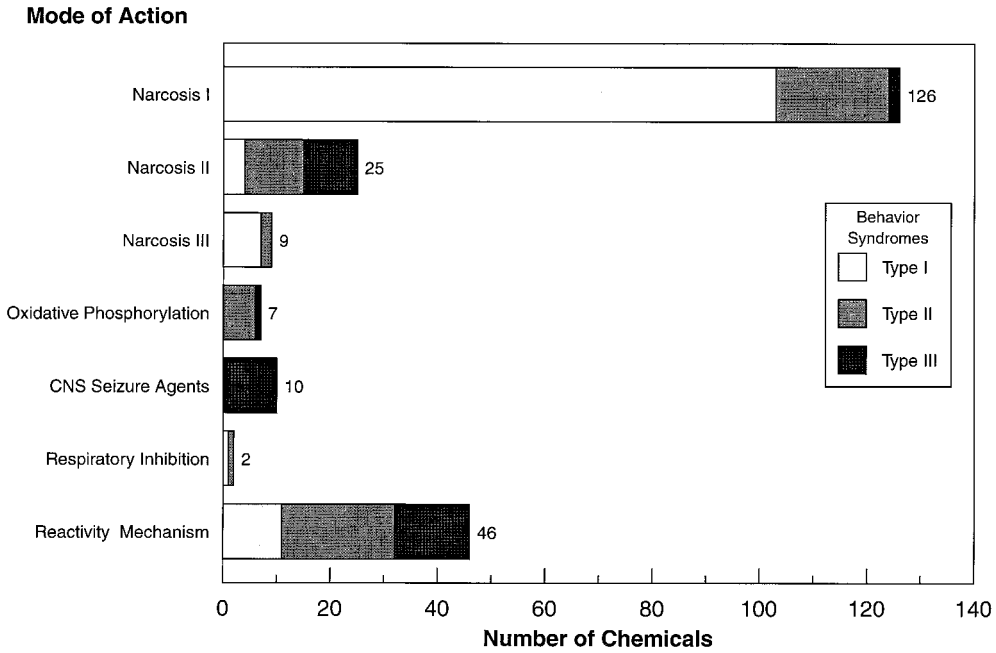

Fig. 1. Behavior syndromes [28] for 225 chemicals in a database of fathead minnow 96-h LC50 values grouped by mode of toxic action. See text and Appendix 2 for definitions of behavior syndromes and procedure used to determine the acute mode of toxic action.

toxic action studies or as reported in the literature, were identified for 17 chemicals, which represented modes of action associated with narcosis I, narcosis II, oxidative phosphorylation uncoupling, electrophile reactivity (presumably through a Schiff-base mechanism) [25,53], AChE inhibition, and several CNS seizure mechanisms (Appendix 2). Behavior syndromes were assigned to 225 of the chemicals in the database (Fig. 1). The type I syndrome was the predominant behavior observed in fish exposed to narcosis I chemicals; however, all three behavior syndromes were observed within some modes of action (e.g., narcosis II and reactive).

Figure 2 summarizes LC50 ratios for chemicals identified as acting by narcosis I, narcosis II, or narcosis III, electrophile/proelectrophile reactivity, and CNS seizure mechanisms (including AChE inhibition). Chemicals associated with electrophile/proelectrophile mechanisms of action tended to have higher LC50 ratios, when compared to narcotics. The percentage of chemicals within a mode of action that did not elicit sufficient mortality for an LC50 in the first 24 h (mean of the respective log *P* values in parenthesis) was 7% (3.27), 40% (1.38), 20% (1.40), 25% (3.99), 25% (−0.50), 36% (1.79), 25% (1.35), and 30% (6.18) for narcosis I, narcosis II, narcosis III, oxidative phosphorylation uncoupling, respiratory inhibition, electrophile/proelectrophile reactivity, AChE inhibition, and CNS seizure mechanisms, respectively. Log excess toxicity values associated with compounds identified as acting by a narcosis I, narcosis II or III, reactive electrophile/proelectrophile, and CNS seizure mechanisms (including AChE inhibition) ranged (means in parentheses) from −0.70 to 0.53 (0.07), −0.05 to 2.08 (0.77), 1.50 to 5.20 (3.36), and −0.15 to 3.62 (1.88), respectively (see Fig. 3). Verhaar et al. [14] reported that less inert (i.e., narcosis II and III) and reactive (i.e., electrophiles/proelectrophiles) and specific (i.e., AChE inhibitors and CNS seizure agents) toxicants were 0.70 to 1.0 and 1.0 to 4.0 times more toxic to the guppy (*Poecilia reticulata*) than would be predicted from a baseline narcosis QSAR [54], which is consistent with the findings reported here for the fathead minnow.

Using the spectrum of toxicodynamic responses described above, 461 of the 617 chemicals in the acute toxicity database were assigned a mode of toxic action (Appendix 2). Of these 461 compounds, 105, 170, 165, and 21 were assigned modes of action with an A, B, C, or D level of confidence, respectively, with 260, 36, 26, 12, 4, 17, 97, and 9 acting by a narcosis I, narcosis II, narcosis III, oxidative phosphorylation uncoupling, respiratory inhibition, AChE inhibition, electrophile/proelectrophile reactivity, and CNS seizure mechanisms, respectively. An examination of the empirical mode of action database illustrates that toxicological classifications based on typically used chemical classes can be problematic (Fig. 4). Many chemical classes usually associated with a narcosis I QSAR [21,54], such as ethers, alcohols, ketones, esters, and benzenes, include compounds acting by narcosis I as well as compounds acting through an electrophilic-based mode of action. Conversely, chemical classes not usually identified as acting by a narcosis I mode of action, such as the phenols, included compounds determined to act either through narcosis I, narcosis II, oxidative phosphorylation uncoupling, or electrophilic-based modes of action. The 21 chemicals assigned modes of action with a D level of certainty, included amides, diketones, acetophenones, and nitro-substituted acetophenones, benzenes, phenols, and aldehydes.

Of the 156 chemicals for which an assessment was not possible, 36 were not sufficiently toxic to provide a 96-h LC50. Modes of action could not be assessed for the remaining 120 compounds because of conflicting or insufficient data. Chemical groups that could not be classified by mode of action using data from the present study included aliphatic caged structures, carboxylic acids, aliphatic amines, sulfides, piperazines, quarternary ammonium compounds, and organotin derivatives. In addition, several substituted benzenes elicited nonmonitonical patterns of death, suggesting that these and structurally similar

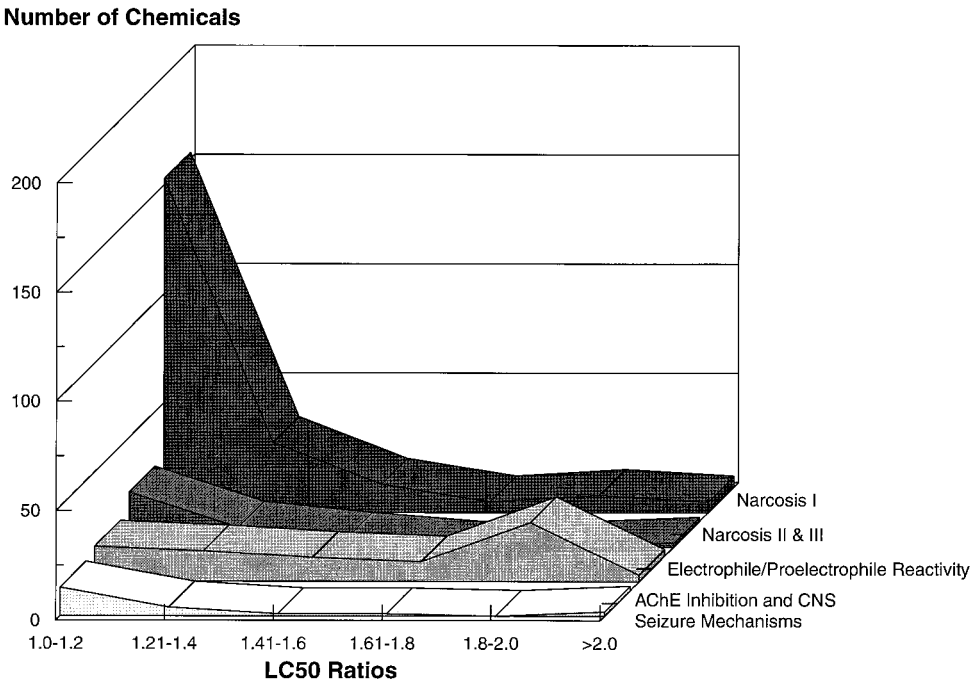

Fig. 2. The ratio of the 24- to 96-h fathead minnow LC50 values grouped by mode of toxic action.

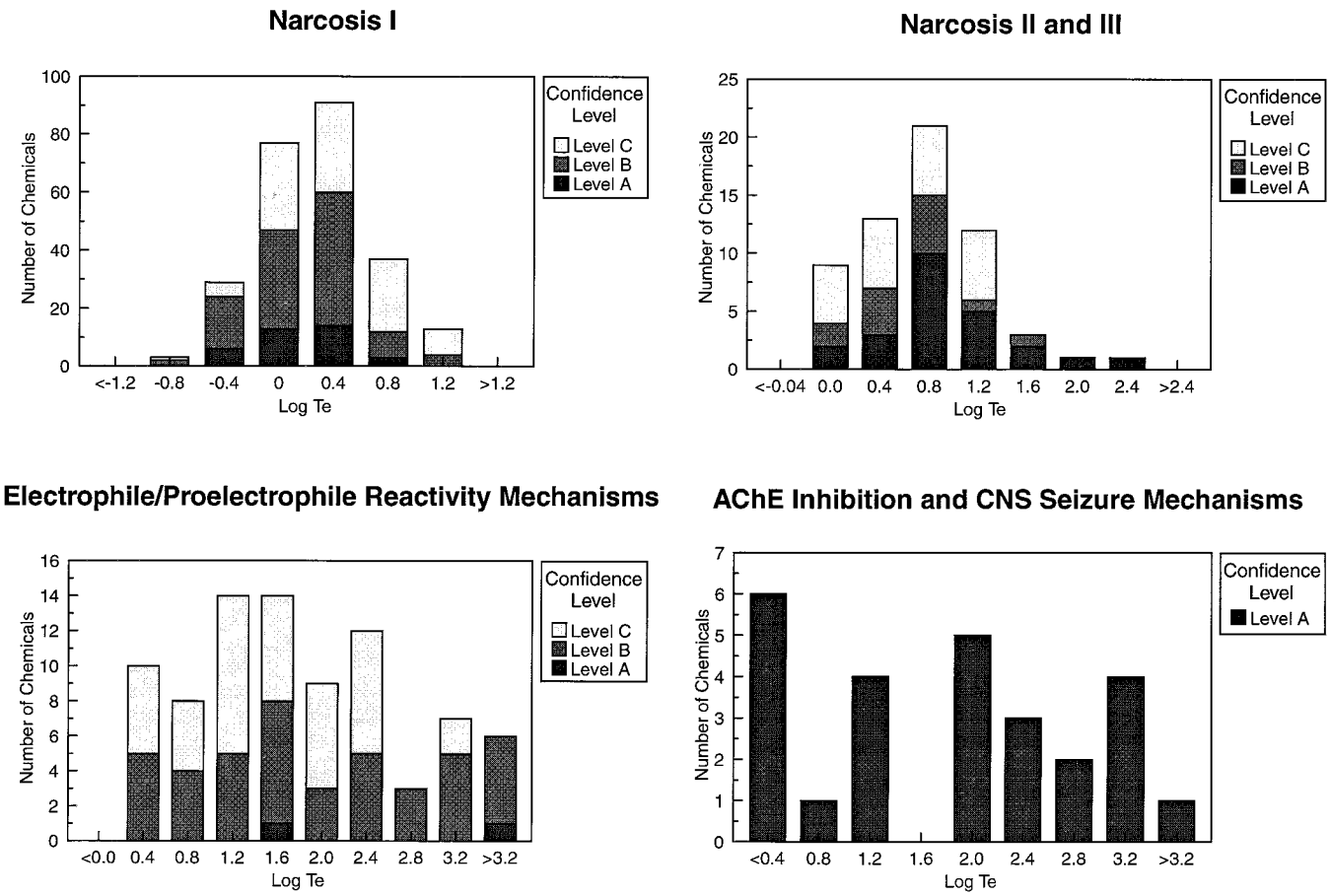

Fig. 3. The log of the ratio of the predicted 96-h fathead minnow LC50, based on a narcosis I QSAR [21] to the observed 96-h LC50 (i.e., the log Te) grouped by mode of toxic action. See the text and Appendix 2 for a definition of confidence levels associated with observed modes of toxic action.

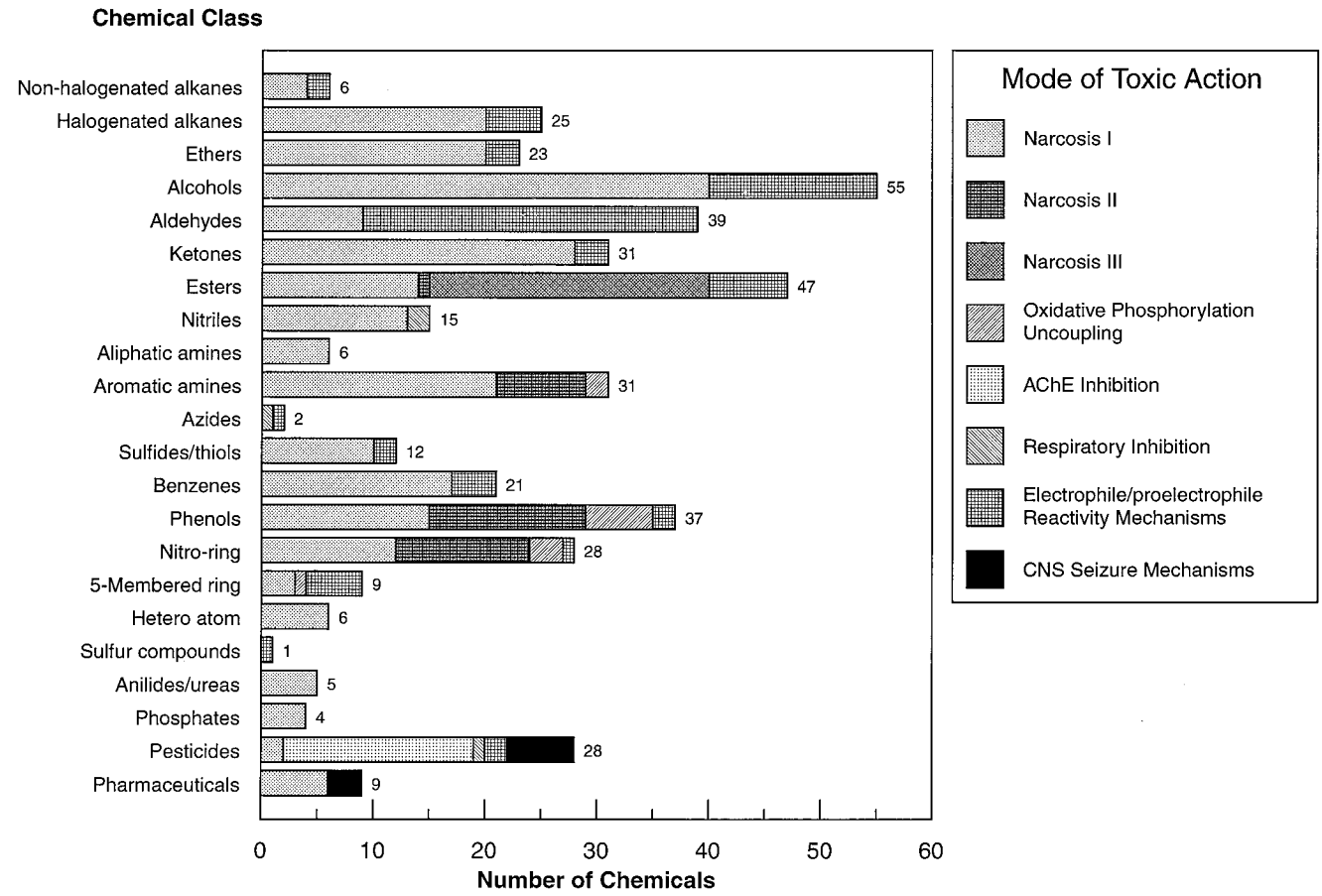

Fig. 4. Observed modes of toxic action associated with fathead minnow 96-h LC50 values (see Appendix 2) as a function of chemical classes.

compounds may be associated with more than one mode of action across the exposure concentrations used in the 96-h bioassay (see Discussion).

Expert system

As described above, joint toxic action responses, FATS, and behavioral syndromes, in combination with LC50 ratios and Te values, were used to identify groups of compounds hypothesized to act through common modes of toxic action. An analysis of the structures within the mode of action groups (excluding those compounds whose mode of action assignment was associated with a D level of confidence) was then undertaken to identify unique substructures associated with each mode of action. These substructures were subsequently used to establish a rule-based expert system to predict mode of action from structure (Appendix 1).

Mode of action predictions. To evaluate the extent to which the substructural rules captured the information in the knowledge base, compounds with A, B, and C levels of confidence were assessed by the expert system and predicted modes of action were compared to those observed. Of the 440 compounds evaluated, 378 (86%) mode of action predictions were consistent with those observed. Within modes of action, the percentage of compounds correctly predicted by the expert system was greater than 80% with the exception of CNS seizure mechanisms and respiratory inhibitors, where the expert system predicted correctly for 55% and 50% of the chemicals, respectively. The limitations of the expert system to predict correctly the mode of action for CNS seizure mechanisms and respiratory inhibitors is most likely due to the limited data sets available for these modes of action. Eight of the 62 compounds for which there were discrepancies between predicted and observed modes of action had an A level of confidence in the knowledge base (Table 2). As explained in the Discussion, six of these eight discrepancies (i.e., four CNS seizure agents and two distinct respiratory inhibitors, see Table 2) resulted from a deliberate decision not to specify complex two-dimensional rules for substructures associated with specific receptor-based mechanisms of action. The remaining two discrepancies were associated with naphthol and 4-nitrobenzamide, which were predicted by the expert system to act through a narcosis I mode of action. In the knowledge base, both compounds were assumed to act through a narcosis II mode of action, based on their additivity with phenol, as reported by Broderius et al. [8]. Broderius et al. [8] indicated, however, that as a chemical group, amides could not be readily classified by a single mode of action, with some compounds (i.e., *m*-bromobenzamide and *p*-tert-butyl benzamide) being additive with octanol (i.e., narcosis I), whereas others were not additive with either phenol or octanol (e.g., anthranilamide, 2-hydroxybenzamide). As a consequence, the expert system was derived under the assumption that amides act through narcosis I. In essence, uncertainty in the toxicodynamic knowledge base concerning amides is reflected in the associated substructural rules and potential errors in mode of action predictions. Finally, naphthol was the only fused-ring compound tested in a joint toxic action study [8]. Based on limited information available for this class of structures, the rule that associates a hy-

droxy substituent on a six-membered aromatic ring with narcosis II was not expanded to include fused-ring aromatic compounds.

The remaining compounds ($N = 54$) with a discrepancy between predicted and observed modes of action were most commonly associated with those chemicals empirically defined as acting through a narcosis-type mode of action ($N = 37$) or electrophile/proelectrophile reactivity ($N = 17$; see Table 2). In that the observed modes of action associated with many of the discrepancies were associated with B and C levels of confidence, some of the differences may be associated with errors in the interpretation of the empirical data, rather than errors in the substructure rules. For the 32 compounds empirically determined to act via narcosis I, but predicted to act through other modes of action, the substructural fragments identified by the expert system did correctly predict the mode of action for topologically similar compounds. Sixteen of the 17 chemicals empirically identified as acting by an electrophilic/proelectrophilic mode of action were predicted to act via a narcosis I or II mode of action (Table 2). Usually the topology of these compounds was unique compared to the other reactive compounds in the knowledge base and they also did not appear to have substructures that were typically associated with reactive mechanisms reported in the literature [14,25,26]. The empirical mode of action assignments for these compounds were not based on data with a high level of confidence (A level). Generally, the assignments were based on log Te values that were typical of those observed for known narcotics, ranging from 0.18 to 0.72, except for 2,2′-methylene-bis-(4-chlorophenol), 2,2,2-trifluoroethanol, 2,4,5-tribromoimidazole, and 1,3-dibromopropane, which had log Te values of 1.09, 1.29, 1.72, and 1.80, respectively.

Ninety-six-hour LC50 predictions. Based on narcosis I, narcosis II, narcosis III, and oxidative phosphorylation uncoupling modes of action, 96-h LC50 values could be predicted for 286 of the 440 chemical structures in the fathead minnow knowledge base. The correlation between predicted and observed log molar LC50s was 0.94 ($R^2 = 0.88$; see Fig. 5). Inspection of the relationship between observed and predicted LC50 values did not reveal any systematic error associated with specific modes of action or chemical classes.

Data from the AQUIRE (AQUatic toxicity Information and REtrieval) database [55] were subsequently used to evaluate further the mode of action expert system and associated QSARs. A search of AQUIRE was conducted for all fathead minnow 96-h LC50 tests. This search resulted in 2,585 96-h LC50 bioassays for 996 chemicals. A single data point was selected for each chemical, with data from flow-through bioassays having measured water concentrations preferentially selected. If more than two data values existed with similar test protocols, the median value was selected. If only two data records were available for a particular chemical, the lowest LC50 was selected. Of the 996 compounds, SMILES strings were available for 739 chemicals. Using the mode of action/QSAR restrictions described previously and an additional restriction to only predict LC50s for chemicals with log P values within the QSAR equation ranges of -1 to 6, 96-h LC50 values could be estimated for 454 chemicals. Chemicals used in developing the expert system were excluded from this analysis, resulting in a data set of 97 compounds. The correlation coefficient for the predicted toxicity values (i.e., mode of action linked QSAR-based predictions) to those observed for these 97 compounds was 0.78 ($R^2 = 0.61$; see Fig. 6). As previously mentioned, because of insufficient or incomplete toxicodynamic data, modes of action for several chemical classes were not readily resolved in the knowledge base (e.g., carboxylic acids and amines), whereas in other instances substructural rules for some chemical classes (e.g., some classes of pesticides) were deliberately not incorporated in the expert system. As a consequence, toxicity estimates for these compounds were based on narcosis I predictions and were outliers in the above correlation. Elimination of these compounds ($N = 9$) from the data set resulted in a correlation coefficient of 0.95 ($R^2 = 0.91$).

DISCUSSION

Using a broad base of toxicodynamic information that included the results of joint toxic action bioassays, FATS, behavioral syndromes, LC50 ratios, and Te values, empirically defined modes of toxic action associated with 96-h LC50 tests with the fathead minnow were established. In turn, this knowledge base was used to develop an expert system, based on two-dimensional substructural rules, for predicting modes of toxic action, which included narcosis I, narcosis II, narcosis III, oxidative phosphorylation uncoupling, respiratory inhibitors, electrophile/proelectrophile reactivity, AChE inhibition, and a limited number of CNS seizure mechanisms. This analysis incorporated the results of 617 96-h LC50 bioassays [15–19] with 225 associated behavioral assessments [28,29], 72 joint toxic action experiments [7,8], and FATS studies with 17 compounds [4,5,9,10].

It is important to note that the exercise described here was based on a database that is biased toward industrial organic chemicals, which are not overtly designed to have biological activity, and therefore it should not be used as a primary resource to estimate modes of action for several chemical groups (e.g., alkaloids, herbicides, organometallics). In addition, several chemical classes were difficult to assess due to limited data sets (e.g., carboxylic acids, amines). As a consequence, the expert system derived from this data set was deliberately designed not to include substructures more commonly associated with pharmaceutical agents and naturally derived compounds. Clearly, this limitation also significantly reduces the complexity and inefficiency of the rule-based system. However, the storage of Chemical Abstract Service (CAS) numbers and SMILES strings for registered pharmaceuticals and pesticides in the expert system permits a linkage to ecotoxicological data reported in the literature [36].

The identification of substructures associated with specific modes of acute toxic action in small aquarium fish have been discussed previously [12,13,25,56]. In addition, a classification scheme was recently developed by Verhaar et al. [14], based on an examination of Te values for 116 chemicals tested with the guppy, to identify inert (i.e., narcosis I), less than inert (i.e., narcosis II and narcosis III), reactive (i.e., electrophiles/proelectrophiles), and specifically acting compounds (i.e., neurotoxic insecticides). However, the knowledge base described in this study provides the largest chemical data set and suite of toxicodynamic data reported to date that can be used in developing an expert system for assessing modes of toxic action in fish.

Although the knowledge base described here provides one of the broadest data sets for assessing modes of toxic action in fish, there are significant issues in the empirical interpretation of the data that can ultimately lead to discrepancies with predictions obtained from the associated expert system. Clearly, the proper selection of prototypical compounds for devel-

Table 2. Chemicals with discrepancies between observed modes of toxic action and those predicted by an expert system

Name	Mode of action knowledge base[a]	Predicted mode of action[b]
Tributyl phosphate	Narcosis I (B)[c]	Acetylcholinesterase inhibition
Diethyl benzylphosphonate	Narcosis I (B)[c]	Acetylcholinesterase inhibition
2-Amino-4-chloro-6-methylpyrimidine	Narcosis I (B)[c]	Narcosis II
3-Hydroxy-2-nitropyridine	Narcosis I (B)[c]	Narcosis II
2-Dimethylaminopyridine	Narcosis I (B)[c]	Narcosis II
α,α,α-4-Tetrafluoro-*m*-toluidine	Narcosis I (B)[c]	Narcosis II
α,α,α-4-Tetrafluoro-*o*-toluidine	Narcosis I (B)[c]	Narcosis II
γ-Decanolactone	Narcosis I (B)[c]	Narcosis III
Di-*n*-Butylisophthalate	Narcosis I (B)[c]	Reactive electrophile/proelectrophile
4-(Diethylamino) benzaldehyde	Narcosis I (B)[c]	Reactive electrophile/proelectrophile
4-(Diethylamino)salicylaldehyde	Narcosis I (B)[c]	Reactive electrophile/proelectrophile
3-(4-Tert-butylphenoxy)benzaldehyde	Narcosis I (B)[c]	Reactive electrophile/proelectrophile
3-(3,4-Dichlorophenoxy)benzaldehyde	Narcosis I (B)[c]	Reactive electrophile/proelectrophile
Tris(2-butoxyethyl) phosphate	Narcosis I (C)[c]	Acetylcholinesterase inhibition
2-Amino-5-chlorobenzonitrile	Narcosis I (C)[c]	Narcosis II
Tert-butyl acetate	Narcosis I (C)[c]	Narcosis III
Methyl 4-cyanobenzoate	Narcosis I (C)[c]	Narcosis III
p-Dimethylaminobenzaldehyde	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
3-Ethoxy-4-hydroxybenzaldehyde	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
p-Isopropyl benzaldehyde	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
Di-*n*-butylterephthalate	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
o-Tolualdehyde	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
Dimethyl aminoterephthalate	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
p-Phenoxybenzaldehyde	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
Diethyl phthalate	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
Di-*n*-butylorthophthalate	Narcosis I (C)[c]	Reactive electrophile/proelectrophile
3,5-Dichloro-4-hydroxybenzonitrile	Narcosis I (C)[c]	Oxidative phosphorylation uncoupling
2,3,4,5,6-Pentafluoroaniline	Narcosis I (C)[c]	Oxidative phosphorylation uncoupling
2,3,4,5-Tetrachlorophenol	Narcosis I (C)[c]	Oxidative phosphorylation uncoupling
4,4′-Isopropylidenebis(2,6-dichlorophenol)	Narcosis I (C)[c]	Oxidative phosphorylation uncoupling
3,5-Diiodo-4-hydroxybenzonitrile	Narcosis I (C)[c]	Oxidative phosphorylation uncoupling
2,3,4,6-Tetrachlorophenol	Narcosis I (C)[c]	Oxidative phosphorylation uncoupling
4-Nitrobenzamide	Narcosis II (A)[c]	Narcosis I
1-Naphthol	Narcosis II (A)[c]	Oxidative phosphorylation uncoupling
3-Trifluoromethyl-4-nitrophenol	Narcosis II (B)[c]	Oxidative phosphorylation uncoupling
3-Amino-5,6-dimethyl-1,2,4-triazine	Narcosis II (C)[c]	Narcosis I
Dibutyl fumarate	Narcosis III (C)[c]	Reactive electrophile/proelectrophile
Dibutyl adipate	Narcosis III (C)[c]	Reactive electrophile/proelectrophile
Dibutyl succinate	Narcosis III (C)[c]	Reactive electrophile/proelectrophile
1,3-Dibromopropane	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
N-vinylcarbazole	Reactive electrophile/proelectrophile (B)[c]	Narcosis I

Table 2. Continued

Name	Mode of action knowledge base[a]	Predicted mode of action[b]
1,9-Decadiene	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
2,3-Benzofuran	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
2,3-Dimethyl-1,3-butadiene	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
2-Methylimidazole	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
3-Methylindole	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
3-Bromothiophene	Reactive electrophile/proelectrophile (B)[c]	Narcosis I
p-(Tert-butyl)-phenyl-*N*-methylcarbamate	Reactive electrophile/proelectrophile (C)[c]	Acetylcholinesterase inhibition
Furan	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
2,4,5-Tribromoimidazole	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
o-Methoxybenzamide	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
2-Chloroethyl-*N*-cyclohexylcarbamate	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
p-Fluorophenyl ether	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
2,2,2-Trifluoroethanol	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
2-Acetyl-1-methylpyrrole	Reactive electrophile/proelectrophile (C)[c]	Narcosis I
2,2′-Methylene-bis-(4-chlorophenol)	Reactive electrophile/proelectrophile (C)[c]	Narcosis II
Caffeine	Central nervous system seizure mechanisms (A)[c]	Narcosis I
Amphetamine sulfate	Central nervous system seizure mechanisms (A)[c]	Narcosis I
Strychnine hemisulfate salt	Central nervous system seizure mechanisms (A)[c]	Narcosis I
Nicotine sulfate	Central nervous system seizure mechanisms (A)[c]	Narcosis II
Rotenone	Respiratory inhibition (A)[c]	Narcosis I
Sodium azide	Respiratory inhibition (A)[c]	Narcosis I

[a] See Appendix 2 for a complete list of chemicals in the mode of action knowledge base.
[b] Mode of action predictions based on an expert system [36]. Rules are summarized in Appendix 1.
[c] Level of confidence associated with an observed mode of action. See Appendix 2 and text for a definition for the levels.

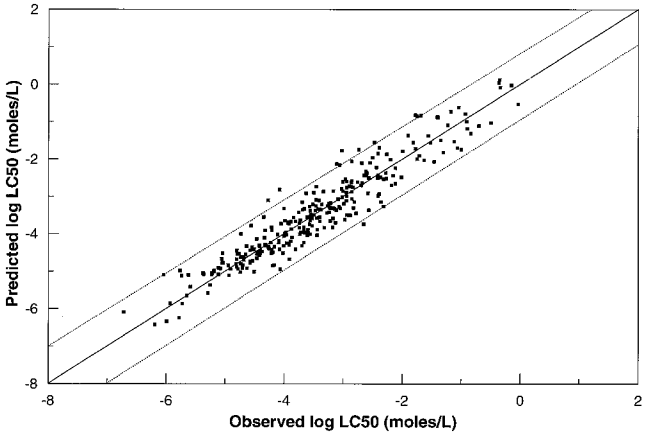

Fig. 5. Comparison of observed log molar fathead minnow 96-h LC50 values (see text and Appendix 2) to a mode of action expert system and associated log molar 96-h LC50 values (N = 286) as predicted by narcosis I, narcosis II, narcosis III, and oxidative phosphorylation uncoupling QSARs (see Appendix 2). Solid line represents unity. Dotted lines represent plus or minus one log unit from unity.

oping the joint toxic action, FATS, and behavioral syndrome data sets is critical. However, the use of well-studied prototypical compounds and the consistency in results obtained across the joint toxic action, FATS, and behavioral assays suggest these compounds were reasonably representative for the specific modes of action. In cases where joint toxic action, FATS, or behavioral assays were not available, mode of action evaluations were based primarily on LC50 ratios and Te values. When interpreting LC50 ratios, it must be remembered that delayed toxicity observed for some chemicals may be due to high lipophilicity and slow chemical uptake (e.g., see LC50 ratios for narcosis I, oxidative phosphorylation uncoupling, and CNS convulsant modes of action in Appendix 2 and Fig. 2).

An insufficient bioassay length for some modes of action could also be an explanation for some discrepancies between observed and predicted modes of action. For instance, substructural rules to identify electrophile/proelectrophile moieties associated with specific mechanisms are well-defined [25]; however, in some cases a 96-h exposure may be insufficient for the toxic responses associated with a reactivity-based

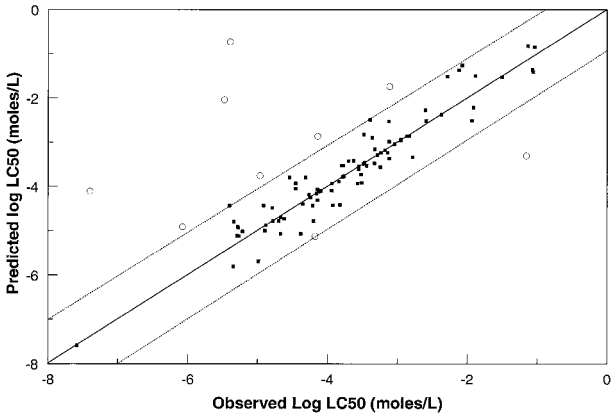

Fig. 6. Comparison of the observed log molar fathead minnow 96-h LC50 values obtained from the AQUIRE database [55] to log molar 96-h LC50 values (N = 97) predicted by a mode of action expert system and associated narcosis I, narcosis II, narcosis III, and oxidative phosphorylation uncoupling QSARs (see Table 1). Outliers (⊗) were associated with chemical classes for which insufficient data were available to predict a mode of action (e.g., carboxylic acids and amines) or which substructural rules were deliberately not incorporated in the expert system (e.g., some classes of pesticides). Outliers were assumed to act via narcosis I by the expert system. Solid line represents unity. Dotted lines represent plus or minus one log unit from unity.

mechanism to be expressed. As a consequence, a narcosis I or indeterminant mode of action response can be observed. For example, although the guppy and fathead minnow are similar in species sensitivity when exposed to narcotic chemicals [21], a 4-d LC50 for 4-toluidine using the fathead minnow was 160 mg/L (Appendix 2) and a 14-d LC50 using the guppy was 10.7 mg/L [57]. Longer exposures may be required for sufficient production of reactive metabolites in this case (e.g., *N*-hydroxylation of primary aromatic amines) [58,59]. Additionally, seven chemicals (i.e., 2-adamantanone, benzene, 2,3-benzofuran, chlorobenzene, 2-chlorophenol, phenol, and toluene) in the fathead minnow database described here displayed nonmonitonical patterns of death (i.e., higher mortality in the low toxicant concentrations than in the high toxicant concentrations) during a 96-h exposure. This later observation suggests that at lower doses a narcosis body burden threshold was not attained; however, the 96-h exposure was sufficently long for toxic responses associated with a reactivity-based mode of action to be elicited.

In addition to uncertainties associated with development of a mode of action knowledge base, this and related [14] rule-based systems are also influenced by uncertainties that arise from the subjective bias of the expert(s) in specifying substructural fragments associated with modes of toxic action. In addition, a substructure-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 log P or pK_a, 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 and the basis with which to assess the uncertainty in an analog or QSAR selection [13].

A fragment-based rule system is also potentially limited because it reduces a chemical structure to a specified substructure and ignores the other topological and potential electronic features of the entire compound that may influence its propensity to act under a given mode of toxic action. For example, the current expert system is limited in evaluating reactive toxicants where global and/or local stereoelectronic parameters may be required to assess the propensity of a compound to act as a soft or hard electrophile [49–51] or to undergo redox cycling [60,61]. The need to develop more global techniques to predict modes of toxic action is especially critical for reactive toxicants because these compounds are typically among the most potent industrial chemicals and their identification raises concern with chronic exposures [13]. Over the last several years, a number of studies have been published that describe the use of quantum-chemical descriptors of soft electrophilicity (e.g., average nucleophilic superdelocalizability) as a means to resolve reactive toxicants and uncouplers of oxidative phosphorylation from narcotics in a 114-chemical subset of the mode of action knowledge base described here [49–51]. Although these findings are encouraging, they must be balanced with the need to assess large sets of compounds in a computationally efficient, but toxicologically relevant manner. In this regard, recent studies have explored relationships between substructural fragments and quantum-chemical descriptors, using nucleophilic substitution, Michael-type addition, and Schiff-base formation as representative molecular mechanisms [52,53]. The classification of a compound as a reactive electrophile is also complicated by the potential role of metabolic activation. The current expert system is generally based on the molecular structure of the parent compound, although some compounds in the knowledge base (e.g., acetylenic and allylic alcohols) [34,45,62] clearly elicit their effects through reactive intermediates. In these instances, the substructural rules are indirectly incorporating biotransformation. Recent research addressing principles underlaying the means of estimating routes and rates of metabolic activation from chemical structure [63–67] certainly provide a range of potential approaches to improve this aspect of predicting modes of toxic action.

CONCLUSIONS

The need to establish more toxicologically credible methods and models to systematically assign chemicals to appropriate QSARs and to select analogs has been identified as a major area of uncertainty in prospective ecological risk assessments for chemical stressors in aquatic ecosystems [1–3,6]. Development of the expert system described here, in addition to a similar approach based on a toxicodynamic knowledge base for the guppy [14], represents an attempt to establish a toxicologically based scheme to classify chemicals and associated mode of action-based QSARs. To date, many classification schemes and QSARs have been based on chemical classes of compounds, which can be difficult to defend from a toxicological perspective [13]. As evidenced in this study, compounds that transcend traditional chemical classes can act through the same mode of action and compounds from the same chemical class can act through several different modes. Clearly, an immediate application of improved capabilities to assess modes of action from chemical structure lies in the continued refinement of QSAR and analog selection techniques to predict the toxicity of untested xenobiotics. The advancement of these selection techniques in conjunction with improved understanding of toxic mechanisms are also needed for 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.

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APPENDIX 1

Summary of substructural fragments associated with mode of toxic action identification for chemicals in a database of fathead minnow 96-h LC50 values [15–19], and augmented with supportive toxicological information in the literature [25,37,38]

1.0 Narcosis I[a]
- 1.1 Aldehyde
  - 1.1.1 Carbon atom of aldehyde connected to a quaternary ammonium
- 1.2 Aniline
  - 1.2.1 With acetophenone or benzamide substructures on aniline ring
  - 1.2.3 With one nitro group attached to an aromatic carbon that is not para to the aniline group
- 1.3 Phenol
  - 1.3.1 With acetophenone or benzamide substructures on phenol ring
- 1.4 Pyridine
  - 1.4.1 Connected to one or more benzene or pyridine rings
  - 1.4.2 Aliphatic carbon connected to the pyridine ring ortho to the nitrogen
- 1.5 Any compound that does not contain any of the substructures identified below

2.0 Narcosis II
- 2.1 Benzene ring
  - 2.1.1 Nitro group para to amide group on aromatic ring
- 2.2 Aniline
  - 2.2.1 One nitro group that is para to the aniline
  - 2.2.2 Any aniline that does not contain substructures identified below
- 2.3 Phenol
  - 2.3.1 Amine group on the phenol ring
  - 2.3.2 Pyridinols
  - 2.3.3 pK_a is greater than or equal to 6.0
- 2.4 Pyridines
  - 2.4.1 Any pyridine that does not contain substructures identified under 1.0 or below

3.0 Narcosis III
- 3.1 Any ester that does not contain substructures identified under section 5.0 below

4.0 Oxidative phosphorylation uncouplers
- 4.1 Anilines
  - 4.1.1 Anilines with more than three halogens attached to aromatic carbons
  - 4.1.2 Anilines with more than one nitro group attached to aromatic carbons
- 4.2 Phenols
  - 4.2.1 An azo linkage between an aromatic carbon in a phenol and another aromatic ring
  - 4.2.2 Phenols with more than three halogens attached to aromatic carbons
  - 4.2.3 Phenols with more than one nitro group attached to aromatic carbons
  - 4.2.4 Phenols with pK_a values less than or equal to 6.3; unless substructure(s) has been identified in other modes of action
- 4.3 Pyridines
  - 4.3.1 Pyridines with more than three halogens attached to aromatic carbons

5.0 Reactive electrophiles/proelectrophiles
- 5.1 Benzene rings, without aniline or phenol substructures, that have two nitro groups on one ring
- 5.2 Acetamidophenols
- 5.3 Quinolines
- 5.4 Esters
  - 5.4.1 Chlorodiesters
  - 5.4.2 Acrylates
  - 5.4.3 Diesters
- 5.5 Acylation-based reactivity
  - 5.5.1 Ketenes
  - 5.5.2 Acid halides
  - 5.5.3 Dialkyl carbomyl chlorides
  - 5.5.4 Carboxylic acid anhydrides

APPENDIX 1
Continued

- 5.6 Isocyanates and isothiocyanates
- 5.7 Carbonyl-based reactivity
  - 5.7.1 Lactone (α-, β-, and unsaturated)
  - 5.7.2 Aldehydes with the exception of ones identified above
- 5.8 Epoxides and aziridines
- 5.9 Sulfonic, sulfuric, and phosphoric acid esters
- 5.10 Haloacetamides, haloacetates, haloethyl amines, haloethyl sulfides, and haloethers
- 5.11 Addition to a carbon–carbon double bond
  - 5.11.1 Allylic and propargylic alcohols
  - 5.11.2 Quinones
  - 5.11.3 Unsaturated amides
  - 5.11.4 Allylic cyano, nitro, sulfone, carboxy, and carbonyl group
  - 5.11.5 Styrenes
- 5.12 Allylic and benzylic halides
- 5.13 Diazo compounds
- 5.14 Mustards
- 5.15 Sulfhydryl based
  - 5.15.1 Disulfides
  - 5.15.2 Sulfenyl halides
  - 5.15.3 Peroxides
  - 5.15.4 Thiocyanates
- 5.16 Hydrazines
- 5.17 N-nitroso and C-nitroso compounds
- 5.18 Allylic/propargylic nitriles, or α-halogenated substituted nitriles
- 5.19 Oximes
- 5.20 β-Halogenated alcohols
- 5.21 Halogenated acetophenones
- 5.22 Pyridiniums and quaternary ammonium compounds
- 5.23 Diketones

6.0 Acetylcholinesterase inhibitors
- 6.1 Carbamates
  - 6.1.1 Ortho-aryl substituted alkyl and dialkyl carbamates
  - 6.1.2 Heterocylic dialkyl carbamates
  - 6.1.3 Oxime methylcarbamates
  - 6.1.4 Procarbamates
- 6.2 Organophosphates
  - 6.2.1 Phosphonates
  - 6.2.2 Phosphates, phosphorothionates, phosphorothiolates, phosphorodithioates
  - 6.2.3 Phosphoroamidates
  - 6.2.4 Phosphorohalides and phosphorocyanides

7.0 Central nervous system seizure agents
- 7.1 Organochlorines: chlorinated alicyclics[b]
- 7.2 Organochlorines: dichlorodiphenyl ethanes[b]
- 7.3 Pyrethroids[b]

[a] In addition, the expert system assumes a Narcosis I mode of action if the chemical does not meet substructural requirements identified in sections 2.0 through 7.3.

[b] See Coats [37] for a detailed structural description of these insecticide classes.

APPENDIX 2

Empirical mode of toxic action assessments for 461 chemicals in a database of fathead minnow 96-h LC50 values [15–19]

Chemical Abstracts Services Registry number	Chemical	LC50 (mg/L)	Log *P*[a]	Behavior syndrome[b]	Te value[c]	LC50 ratio[d]
Narcosis I: Level A confidence[e]						
50-06-6	Phenobarbital	484	1.47[f]	Type I	1.1	1.05
57-33-0	Pentobarbital	49.5	2.10[f]	Type I	3.0	1.03
57-43-2	Amobarbital	85.4	2.07[f]	Type I	1.7	1.09
71-73-8	Thiopental, sodium salt	26.2	2.10[f]	Type I	6.1	1.08
71-41-0[g]	1-Pentanol	472	1.56[f]	Type I	0.4	1.02
78-83-1[g]	2-Methyl-1-propanol	1,430	0.76[f]		0.6	1.00
78-87-5[g]	1,2-Dichloropropane	127	1.99		0.7	1.35
79-00-5[g]	1,1,2-Trichloroethane	81.6	2.05		1.1	1.30
80-46-6[h]	*p*-Tert-pentylphenol	2.59	3.98		1.2	1.25
90-43-7[h]	2-Phenylphenol	6.15	3.36		1.3	1.04
91-20-3[g]	Naphthalene	6.14	3.30[f]		1.1	1.27
95-76-1[h]	3,4-Dichloroaniline	7.57	2.69[f]	Type II	4.0	1.29
98-86-2[g]	Acetophenone	162	1.58		1.4	1.01
99-97-8[g]	*N,N*-dimethyl-*p*-toluidine	48.9	2.81[f]	Type I	0.4	1.00
108-10-1[g]	4-Methyl-2-pentanone	522	1.31[f]		0.8	1.10
108-20-3[g]	Isopropyl ether	786	1.52[f]	Type I	0.3	1.00
111-13-7[g]	2-Octanone	36.0	2.37[f]		1.2	1.00
111-27-3[g]	1-Hexanol	97.7	2.03[f]		0.7	1.02
111-70-6[g]	1-Heptanol	34.5	2.72[f]	Type I	1.0	1.00
111-87-5[i]	1-Octanol	13.5	2.97[f]	Type I	0.9	1.02
115-20-8[g]	2,2,2-Trichloroethanol	299	1.42[f]		1.3	1.08
118-79-6[g]	2,4,6-Tribromophenol	6.54	4.02	Type II	0.9	1.46
120-82-1[g]	1,2,4-Trichlorobenzene	2.99	4.02[f]		1.1	1.16
120-83-2[h]	2,4-Dichlorophenol	7.75	3.06[f]		2.3	1.34
121-69-7[g]	*N,N*-dimethylaniline	64.1	2.31[f]	Type I	0.6	1.00
127-18-4[g]	Tetrachloroethylene	16.5	3.40[f]	Type II	0.5	1.20
142-96-1[g]	Butyl ether	32.3	3.21[f]		0.4	ND
143-08-8[g]	1-Nonanol	5.70	4.26[f]		1.0	1.05
150-78-7[g]	*p*-Dimethoxybenzene	117	2.15		0.6	1.15[j]
309-43-3	Secobarbital sodium salt	23.6	1.97[f]	Type I	8.8	1.03
589-16-2[h]	4-Ethylaniline	73.0	1.96[f]	Type I	1.4	1.31
634-67-3[h]	2,3,4-Trichloroaniline	3.64	3.33[f]		2.7	1.42
693-65-2[g]	Pentyl ether	3.14	4.04		0.9	ND
831-82-3[h]	*p*-Phenoxyphenol	4.95	3.75		0.9	1.09
1965-09-9	4,4′-Dihydroxydiphenyl ether	5.78	3.18	Type II	2.5	1.10
2234-16-4[g]	2′,4′-Dichloroacetophenone	11.7	2.84		2.1	1.03
2243-27-8[g]	*n*-Octyl cyanide	5.25	3.12[f]		1.4	1.02
5217-47-0	1,3-Diethyl-2-thiobarbituric acid	4,510	[n]	Type I	ND	1.18
5673-07-4[g]	2,6-Dimethoxytoluene	20.2	2.80		1.0	ND
22726-00-7[g]	*m*-Bromobenzamide	92.7	1.65[f]	Type I	3.4	1.03
24544-04-5[g]	2,6-Diisopropylaniline	15.3	3.18[f]	Type I	0.2	1.00
39905-57-2[g]	4-Hexyloxyaniline	3.01	3.66	Type I	2.0	1.21
56108-12-4[g]	*p*-(Tert-butyl)benzamide	31.9	2.51[f]	Type I	1.4	1.00
Narcosis I: Level B confidence						
[k]	*p*-Chlorophenyl-*o*-nitrophenyl ether	1.92	4.79	Type I	1.4	2.00
[k]	Di-*n*-butylisophthalate	0.90	5.53	Type I	2.9	1.07[j]
[k]	1,1-Diphenyl-2-propyn-1-ol	11.1	2.71	Type I	3.1	1.00
[k]	4,7-Dithiadecane	7.52	3.52	Type I	0.8	1.09[j]
51-79-6	Urethane	5,240	−0.15[f]	Type I	1.4	1.04
59-97-2	Tolazoline hydrochloride	354	2.65[f]	Type I	0.1	1.44
60-29-7	Diethyl ether	2,560	0.89[f]	Type I	0.2	1.10
64-17-5	Ethanol	14,700	−0.31[f]		0.4	1.08
67-56-1	Methanol–rhodamine b	29,400	−0.77[f]		0.3	1.01
67-63-0	2-Propanol	8,680	0.05[f]		0.3	1.06
67-72-1	Hexachloroethane	1.42	4.14[f]		1.8	1.10
70-69-9	4′-Aminopropiophenone	146	1.43	Type I	2.6	1.06
71-23-8	1-Propanol	4,550	0.25[f]		0.4	1.09
71-36-3	1-Butanol	1,730	0.88[f]		0.4	1.01
71-55-6	1,1,1-Trichloroethane	47.3	2.49[f]	Type II	0.7	1.00
75-09-2	Dichloromethane	330	1.25[f]		1.0	1.00
75-65-0	2-Methyl-2-propanol	6,410	0.35[f]	Type I	0.3	1.00
76-01-7	Pentachloroethane	7.53	3.63		0.8	1.00
77-74-7	3-Methyl-3-pentanol	672	1.53	Type I	0.3	1.10
77-75-8	3-Methyl-1-pentyn-3-ol	1,220	0.86	Type II	0.7	1.02
78-27-3	1-Ethynyl-cyclohexanol	256	1.73[f]	Type I	0.6	1.12[j]
78-92-2	2-Butanol	3,670	0.61[f]	Type I	0.3	1.07
79-01-6	Trichloroethylene	44.1	2.42[f]		0.9	1.30
79-34-5	1,1,2,2-Tetrachloroethane	20.3	2.39[f]		1.6	1.10
79-77-6	β-Ionone	5.09	3.96	Type I	0.7	1.00[j]
91-66-7	*N,N*-diethylaniline	16.4	3.31[f]	Type I	0.5	1.00
91-88-3	2-(*N*-ethyl-*m*-toluidino)ethanol	52.9	2.49	Type I	0.9	1.07
96-18-4	1,2,3-Trichloropropane	57.7	1.98	Type I	1.7	1.00
96-80-0	2-(diisopropylamino)-ethanol	201	0.86	Type I	6.3	1.03
100-37-8	*N,N*-diethylethanolamine	1,780	0.32	Type I	1.8	1.35
100-71-0	2-Ethylpyridine	414	1.69[f]	Type I	0.3	1.00
103-05-9	Benzyl-tert-butanol	66.4	2.57	Type I	0.6	1.00
104-76-7	2-Ethyl-1-hexanol	28.2	2.81	Type I	0.6	1.02
104-90-5	5-Ethyl-2-methylpyridine	81.1	2.49	Type I	0.4	1.00
106-94-5	1-Bromopropane	67.3	2.10[f]	Type I	1.1	1.94
107-06-2	1,2-Dichloroethane	136	1.48[f]		1.7	1.22
107-12-0	Propionitrile	1,520	0.16[f]	Type I	1.5	1.00
107-41-5	2-Methyl-2,4-pentanediol	10,700	−0.67		2.6	1.01
108-93-0	Cyclohexanol	704	1.23[f]		0.6	1.06
109-06-8	2-Picoline	897	1.11[f]	Type I	0.5	1.00
109-65-9	1-Bromobutane	36.7	2.75[f]	Type I	0.6	1.00
110-43-0	2-Heptanone	131	1.98[f]	Type I	0.7	1.00
110-56-5	1,4-Dichlorobutane	51.6	2.24		1.1	1.00
111-25-1	1-Bromohexane	3.45	3.80[f]		1.1	1.00
111-46-6	2-Hydroxyethyl ether	75,200	−1.30		1.3	1.00
111-83-1	1-Bromooctane	0.838	4.89[f]		2.4	1.20

APPENDIX 2
Continued

Chemical Abstracts Services Registry number	Chemical	LC50 (mg/L)	Log *P*[a]	Behavior syndrome[b]	Te value[c]	LC50 ratio[d]
111-90-0	2-(2-Ethoxyethoxy)-ethanol	26,500	−0.54[f]		0.4	ND
112-12-9	2-Undecanone	1.50	4.09[f]	Type I	2.2	1.19
112-27-6	Triethylene glycol	68,900	−1.24		2.1	ND
112-30-1	1-Decanol	2.40	4.57[f]		1.2	1.00
115-19-5	2-Methyl-3-butyn-2-ol	3,290	0.28[f]	Type II	0.7	1.02
120-07-0	*N*-Phenyldiethanolamine	735	0.44	Type I	5.3	1.00
120-21-8	4-(Diethylamino)benzaldehyde	23.9	2.94	Type I	0.8	1.03
122-39-4	Diphenylamine	3.79	3.50[f]	Type I	1.4	1.00[l]
122-99-6	2-Phenoxyethanol	344	1.16[f]		1.8	1.01
126-73-8	Tributyl phosphate	9.48	3.53	Type I	0.8	1.00
127-66-2	2-Phenyl-3-butyn-2-ol	113	1.68	Type I	1.9	1.00
142-28-9	1,3-Dichloropropane	111	2.00[f]		0.6	1.00
393-39-5	α,α,α-4-Tetrafluoro-*o*-toluidine	29.6	2.62	Type I	1.2	1.00
447-60-9	α,α,α-Trifluoro-*o*-tolunitrile	42.2	2.46	Type I	1.1	1.32
470-82-6	Cineole	102	2.76	Type II	0.2	1.00
496-16-2	2,3-Dihydrobenzofuran	81.7	2.14[f]	Type I	0.8	1.20
525-82-6	Flavone	3.50	3.56[f]	Type I	2.4	1.04
527-60-6	2,4,6-Trimethylphenol	13.0	3.42	Type I	0.4	1.09
591-78-6	2-Hexanone	428	1.38[f]	Type I	0.7	1.00
592-46-1	2,4-Hexadiene	20.0	2.96	Type II	0.4	1.30
600-36-2	2,4-Dimethyl-3-pentanol	163	1.93	Type I	0.6	1.00
616-86-4	4-Ethoxy-2-nitroaniline	26.0	2.47	Type I	1.9	1.00
621-08-9	Benzyl sulfoxide	80.1	1.96	Type I	2.3	1.11
622-40-2	4-(2-Hydroxyethyl) morpholine	2,710	−0.45	Type I	7.2	1.41
625-86-5	2,5-Dimethylfuran	71.1	2.62	Type II	0.2	1.20
628-76-2	1,5-Dichloropentane	25.3	2.76		0.8	1.13
629-04-9	1-Bromoheptane	1.47	4.36[f]		1.6	1.10
693-93-6	4-Methyloxazole	1,390	0.49	Type I	0.4	1.99
706-14-9	γ-Decanolactone	18.0	2.72[f]	Type I	1.5	1.04
764-13-6	2,5-Dimethyl-2,4-hexadiene	3.78	3.76	Type I	0.7	1.20
821-55-6	2-Nonanone	15.2	3.14[f]	Type I	1.0	1.00
822-86-6	Trans-1,2-dichlorocyclohexane	18.4	3.18[f]		0.5	1.00
920-66-1	1,1,1,3,3,3-Hexafluoro-2-propanol	244	1.66[f]	Type I	1.2	1.09
928-96-1	Cis-3-hexen-1-ol	381	1.34	Type I	0.8	1.06
928-97-2	Trans-3-hexen-1-ol	271	1.34	Type I	1.1	1.00
939-23-1	4-Phenylpyridine	16.1	2.59[f]	Type I	2.1	1.06
945-51-7	Phenyl sulfoxide	87.3	2.06[f]	Type I	1.4	1.00
1080-32-6	Diethyl benzylphosphonate	336	1.59	Type I	1.6	1.02
1126-79-0	Butyl phenyl ether	5.77	3.65	Type I	0.8	1.15
1482-15-1	3,4-Dimethyl-1-pentyn-3-ol	205	1.26	Type II	2.0	1.33
2008-58-4	2,6-Dichlorobenzamide	469	1.25[f]	Type I	1.5	1.00
2216-51-5	1R,2S,5R-(−)-Menthol	18.9	3.23	Type I	0.5	1.00
2357-47-3	α,α,α-4-Tetrafluoro-*m*-toludine	30.1	2.62		1.2	1.21
2362-61-0	Trans-2-phenyl-1-cyclohexanol	44.4	2.82	Type I	0.5	1.00
2894-51-1	2-Amino-4′-chlorobenzophenone	2.12	3.95	Type I	2.1	1.16
4253-89-8	Isopropyl disulfide	8.31	3.42	Type I	0.8	1.00[l]
5395-75-5	3,6-Dithiaoctane	60.2	2.46	Type I	0.7	1.00
5600-21-5	2-Amino-4-chloro-6-methylpyrimidine	141	1.13	Type I	11	1.04
5683-33-0	2-Dimethylaminopyridine	127	1.43[f]	Type I	2.4	1.00
6001-64-5	1,1,1-Trichloro-2-methyl-2-propanol	135	2.03[f]	Type I	0.9	1.00
6175-49-1	2-Dodecanone	1.18	4.49	Type I	1.9	1.19
6921-29-5	Tripropargylamine	296	1.26[f]	Type I	1.5	1.00
6948-86-3	*N,N*-bis(2,2-diethoxyethyl) methylamine	635	1.15	Type I	1.1	1.00
7212-44-4	3-Hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene	1.43	4.4	Type I	2.0	1.27
7250-67-1	1-(2-Chloroethyl)-pyrrolidine	153	1.43	Type I	2.8	1.02
13909-73-4	2′,3′,4′-Trimethoxyacetophenone	199	1.12[f]	Type I	3.7	1.90[l]
14548-45-9	4-Bromophenyl 3-pyridyl ketone	20.4	2.97	Type I	1.3	1.15
15045-43-9	2,2,5,5-Tetramethyltetrahydrofuran	168	2.40	Type II	0.2	1.00
15128-82-2	3-Hydroxy-2-nitropyridine	167	1.01	Type I	5.3	1.31
17754-90-4[g]	4-(Diethylamino)-salicylaldehyde	5.36	3.34	Type I	1.8	1.28
19549-98-5	3,6-Dimethyl-1-heptyn-3-ol	49.0	2.32	Type I	1.1	1.00
20662-84-4	2,4,5-Trimethyloxazole	449	1.79	Type I	0.1	1.00
29553-26-2	2-Methyl-3,3,4,4-tetrafluoro-2-butanol	582	1.03	Type I	1.7	1.00
34723-82-5	2-(Bromomethyl)-tetrahydro-2h-pyran	205	1.61	Type I	1.5	1.80
69770-23-6	3-(4-Tert-butylphenoxy)benzaldehyde	0.37	5.93	Type I	6.4	[m]
79124-76-8	3-(3,4-Dichlorophenoxy)benzaldehyde	0.30	5.49		8.5	1.23
Narcosis I: Level C confidence						
[k]	3′-Chloro-*o*-formotoluidide	46.6	2.27		5.0	1.02
55-21-0	Benzamide	661	0.64[f]		2.6	1.07
58-90-2	2,3,4,6-Tetrachlorophenol	1.03	4.45[f]	Type II	3.0	1.08
67-36-7	*p*-Phenoxybenzaldehyde	4.60	3.96		0.8	1.61
67-64-1	Acetone	7,160	−0.24[f]		0.7	1.09
67-68-5	Methyl sulfoxide	34,000	−1.35[f]		2.6	1.00
75-05-8	Acetonitrile	1,644	−0.34[f]		2.9	1.43
75-97-8	3,3-Dimethyl-2-butanone	87.0	0.97		7.9	1.77
78-51-3	Tris(2-butoxyethyl) phosphate	11.2	4.09	Type II	0.4	1.03
78-93-3	2-Butanone	3,220	0.29[f]		0.7	1.02
79-95-8	4,4′-Isopropylidene-bis-(2,6-dichlorophenol)	1.33	6.44	Type II	2.5	1.21
81-19-6	α,α-2,6-Tetrachlorotoluene	0.97	4.64		2.7	1.65
83-32-9	Acenaphthene	1.73	3.92[f]		1.8	1.06[l]
84-66-2	Diethyl phthalate	31.8	2.47[f]		1.9	1.34
84-74-2	Di-*n*-butyl-ortho-phthalate	1.00	4.72[f]		3.6	1.48
87-17-2	Salicylanilide	3.95	3.27[f]		3.0	1.19
94-62-2	Piperine	7.84	2.70	Type I	6.2	1.31
95-47-6	*o*-Xylene	16.4	3.12[f]		0.5	ND
95-63-6	1,2,4-Trimethylbenzene	7.72	3.78[f]		0.4	1.27
96-22-0	3-Pentanone	1,540	0.79		0.6	1.00
98-54-4	*p*-Tert-butylphenol	5.15	3.31[f]		1.5	1.21
98-95-3	Nitrobenzene	119	1.85[f]		1.1	1.40
99-03-6	*m*-Aminoacetophenone	382	0.90		2.8	[m]
99-08-1	*m*-Nitrotoluene	25.6	2.45[f]		1.5	1.00

APPENDIX 2
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Chemical Abstracts Services Registry number	Chemical	LC50 (mg/L)	Log *P*[a]	Behavior syndrome[b]	Te value[c]	LC50 ratio[d]
100-10-7	*p*-Dimethylaminobenzaldehyde	45.7	1.81[f]		2.2	1.21
100-41-4	Ethylbenzene	10.5	3.15[f]		0.6	1.53
100-61-8	*N*-methylaniline	100	1.66[f]	Type II	1.7	2.06
100-79-8	Solketal	16,700	−0.07		0.5	1.10
101-84-8	Phenyl ether	4.00	4.21[f]		0.6	ND
102-27-2	*N*-ethyl-*m*-toluidine	49.5	2.82		0.4	1.01
104-13-2	4-Butylaniline	10.2	3.15	Type II	1.0	1.21
104-40-5	Nonylphenol (mixed)	0.14	6.36		14.6	1.14[j]
106-42-3	*p*-Xylene	8.87	3.15[f]		0.8	1.60
107-47-1	Tert-butyl sulfide	29.1	3.32		0.3	1.03
108-94-1	Cyclohexanone	621	0.81[f]		1.3	1.07
109-97-7	Pyrrole	210	0.75[f]		3.5	1.05
109-99-9	Tetrahydrofuran	2,160	0.46[f]		0.7	1.20
110-06-5	Tert-butyl disulfide	1.37	4.22	Type I	1.9	1.13[j]
110-12-3	5-Methyl-2-hexanone	159	1.88[f]		1.0	1.00
110-54-3	Hexane	2.50	3.87		0.7	1.10
110-73-6	2-(Ethylamino)ethanol	1,480	−0.46	Type I	9.2	[m]
110-82-7	Cyclohexane	4.53	3.44[f]		0.8	1.20
110-88-3	1,3,5-Trioxane	5,950	−0.43[f]		2.9	1.10
110-93-0	6-Methyl-5-hepten-2-one	85.7	1.70		2.1	1.00
111-42-2	Diethanolamine	47,100	−1.43[f]	Type I	3.0	1.59
111-47-7	*n*-Propyl sulfide	21.7	2.96		0.5	1.00
111-69-3	1,4-Dicyanobutane	1,930	−0.32[f]		7.8	1.00
119-61-9	Benzophenone	14.7	3.18[f]		0.8	1.04
121-32-4	3-Ethoxy-4-hydroxybenzaldehyde	87.6	1.88		1.8	1.12
121-73-3	1-Chloro-3-nitrobenzene	18.8	2.41[f]		2.6	1.00
122-03-2	*p*-Isopropyl benzaldehyde	6.62	3.07		1.8	1.17
123-91-1	1,4-Dioxane	10,300	−0.27[f]		0.9	ND
128-37-0	2,6-Di-tert-butyl-4-methylphenol	0.363	6.07		5.6	3.06
128-44-9	Saccharin sodium salt hydrate	18,300	0.91[f]		0.1	1.06
134-62-3	*N*,*N*-diethyl-*m*-toluamide	110	2.31		0.7	1.03
141-91-3	2,6-Dimethylmorpholine	387	0.32	Type II	8.4	1.66
141-93-5	*m*-Diethylbenzene	4.15	4.5		0.4	1.63
314-40-9	Bromacil	186	2.11[f]		0.8	ND
330-54-1	Diuron	14.2	2.80[f]		2.4	1.64
350-46-9	1-Fluoro-4-nitrobenzene	28.4	1.80[f]		5.7	1.05
368-77-4	α,α,α-Trifluoro-*m*-tolunitrile	47.7	2.46		1.0	1.18
459-59-6	4-Fluoro-*N*-methylaniline	38.4	2.09	Type II	2.0	1.37[j]
502-56-7	5-Nonanone	31.0	2.91		0.5	1.00
529-19-1	*o*-Tolunitrile	44.7	2.21[f]		1.2	1.00
529-20-4	*o*-Tolualdehyde	52.9	2.26[f]		1.0	1.09
538-68-1	Amylbenzene	1.71	4.91		0.9	2.35
540-88-5	Tert-butyl acetate	327	1.38		1.0	1.03
552-41-0	2′-Hydroxy-4′-methoxyacetophenone	69.5	1.98[f]	Type III	1.4	1.18
563-80-4	3-Methyl-2-butanone	864	0.56[f]		1.6	1.49
583-53-9	1,2-Dibromobenzene	4.05	3.64[f]		1.9	1.32
589-09-3	*N*-allylaniline	35.9	2.16		2.0	1.00
593-08-8	2-Tridecanone	0.36	5.02		5.6	1.92
607-00-1	*N*,*N*-diphenylformamide	30.4	[n]		ND	1.44
609-23-4	2,4,6-Triiodophenol	1.21	4.8	Type II	3.8	1.10
620-88-2	4-Nitrophenyl phenyl ether	2.65	4.28		1.1	ND
629-40-3[g]	1,6-Dicyanohexane	528	0.59[f]		3.7	1.00
693-54-9	2-Decanone	4.83	3.73[f]		1.1	1.02
709-98-8	Propanil	8.60	3.07[f]		2.0	1.34
761-65-9	*N*,*N*-dibutylformamide	89.3	2.14		1.0	1.00
771-60-8	2,3,4,5,6-Pentafluoroaniline	37.1	2.22		2.3	1.06
791-28-6	Triphenylphosphine oxide	53.7	2.83[f]	Type II	0.7	1.04
1129-35-7	Methyl 4-cyanobenzoate	46.8	1.72		4.7	1.32
1634-04-4	Tert-butyl methyl ether	672	0.94[f]		0.8	ND
1689-83-4	3,5-Diiodo-4-hydroxybenzonitrile	6.80	3.51		2.0	1.00
1891-95-8	3,5-Dichloro-4-hydroxybenzonitrile	24.3	2.69		1.3	1.02
1962-75-0	Di-*n*-butylterephthalate	0.59	5.53[f]	Type I	4.4	[m]
2416-94-6	2,3,6-Trimethylphenol	8.20	3.42		0.7	1.56
2437-25-4	*n*-Undecyl cyanide	0.43	4.9		4.4	4.16
2460-49-3	4,5-Dichloroguaiacol	4.47	3.26[f]		2.4	1.10
3558-69-8	2,6-Diphenylpyridine	0.21	4.82[f]		11.7	2.05
4901-51-3	2,3,4,5-Tetrachlorophenol	0.41	4.21[f]		5.2	1.08
4916-57-8	1,2-Bis(4-pyridyl)ethane	151	1.93	Type I	1.0	1.04
5331-91-9	5-Chloro-2-mercaptobenzothiazole	3.21	3.61		1.9	1.12
5372-81-6	Dimethyl aminoterephthalate	8.94	2.45	Type I	6.7	1.25
5922-60-1	2-Amino-5-chlorobenzonitrile	28.6	1.91		4.8	[m]
13608-87-2	2′,3′,4′-Trichloroacetophenone	2.00	3.57		3.7	1.90[j]
14548-46-0	4-Benzoylpyridine	103	1.98[f]	Type III	1.4	1.00
15972-60-8	Alachlor	5.00	3.52[f]		1.9	1.98
54576-32-8	3,8-Dithiadecane	6.06	3.11	Type I	2.2	1.83
55792-61-5	2′-(Octyloxy)-acetanilide	0.45	4.41	Type I	7.5	1.49
Narcosis I: Level D confidence						
65-45-2	2-Hydroxybenzamide	101	1.28[f]		480	1.46
88-68-6	Anthranilamide	395	0.35[f]		9.1	1.28
126-81-8	5,5-Dimethyl-1,3-cyclohexanedione	11,500	0.51		0.2	[m]
16245-79-7	4-Octylaniline	0.12	5.27	Type I	16.6	1.58[j]
37529-30-9	4-Decylaniline	0.062	6.32		34.9	[m]
Narcosis II: Level A confidence						
59-50-7[o]	4-Chloro-3-methyl phenol	5.47	3.10[f]	Type II	1.5	2.67
62-53-3[o,p]	Aniline	105	0.90[f]	Type II	5.6	1.67
90-15-3[o]	1-Naphthol	4.63	2.84[f]		4.0	1.65
95-51-2[o,p]	2-Chloroaniline	5.74	1.90[f]	Type II	20.8	[m]
95-57-8[o]	2-Chlorophenol	11.4	2.15[f]	Type II	5.0	2.00[j]
100-02-7[o]	4-Nitrophenol	44.8	1.91[f]		2.2	1.44
100-70-9[o]	2-Cyanopyridine	726	0.50[f]	Type III	2.7	1.45

APPENDIX 2
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Chemical Abstracts Services Registry number	Chemical	LC50 (mg/L)	Log *P*[a]	Behavior syndrome[b]	Te value[c]	LC50 ratio[d]
105-67-9[o,p]	2,4-Dimethylphenol	16.6	2.30[f]		2.9	1.74
106-47-8[o,p]	4-Chloroaniline	31.4	1.83[f]	Type II	4.5	[m]
108-95-2[p]	Phenol	32.7	1.46[f]	Type II	7.8	[m]
110-86-1[o]	Pyridine	99.8	0.65[f]	Type III	10.3	4.51
119-34-6[o]	4-Amino-2-nitrophenol	36.2	0.96		30	1.04
120-80-9[o]	Catechol	9.22	0.88[f]	Type II	119	1.80
121-87-9[o]	2-Chloro-4-nitroaniline	20.1	2.17	Type I	4.7	1.11
150-19-6[o]	3-Methoxyphenol	74.0	1.58[f]		3.1	1.14[l]
615-65-6[o]	2-Chloro-4-methylaniline	35.9	2.58		0.9	1.42
619-80-7[o]	4-Nitrobenzamide	133	0.82[f]		11.9	1.09[l]
1122-54-9[o]	4-Acetylpyridine	168	0.48[f]	Type III	14.4	1.59
16879-02-0[o]	6-Chloro-2-pyridinol	214	1.78	Type I	0.7	1.18[l]
Narcosis II: Level B confidence						
88-30-2[o]	3-Trifluoromethyl-4-nitrophenol	9.14	3.00	Type II	2.1	1.02
95-48-7	*o*-Cresol	14.0	2.12	Type II	4.5	1.05[l]
106-40-1[o]	*p*-Bromoaniline	47.5	2.26[f]		1.6	[m]
106-49-0	4-Toluidine	160	1.39[f]	Type II	2.0	1.70
108-89-4	4-Picoline	403	1.22[f]	Type III	0.9	1.74
150-76-5	4-Methoxyphenol	110	1.34[f]	Type III	3.5	2.56
1072-97-5	2-Amino-5-bromopyridine	177	1.39	Type III	2.7	1.12
Narcosis II: Level C confidence						
88-75-5	2-Nitrophenol	160	1.85		0.9	1.25
106-48-9	4-Chlorophenol	6.11	2.48	Type III	5.7	1.35
108-99-6	3-Picoline	144	1.31	Type I	2.1	1.16[l]
769-28-8	3-Cyano-4,6-dimethyl-2-hydroxypyridine	157	2.03	Type II	0.7	2.11
1484-26-0	3-Benzyloxyaniline	9.14	2.79		3.1	1.09
2859-67-8	3-(3-Pyridyl)-1-propanol	150	0.60[f]		14	2.13[l]
6602-32-0	2-Bromo-3-pyridinol	469	1.65		0.6	1.33[l]
6636-78-8	2-Chloro-3-pyridinol	622	1.50		0.4	1.01[l]
17584-12-2	3-Amino-5,6-dimethyl-1,2,4-triazine	952	−0.21	Type I	11.5	1.41[l]
Narcosis II: Level D confidence						
88-06-2	2,4,6-Trichlorophenol	4.89	3.69[f]	Type III	0.6	1.86
Narcosis III: Level A confidence						
79-20-9[g,q]	Methyl acetate	357	0.18[f]	Type II	8.8	1.25[l]
94-09-7[g]	Ethyl *p*-aminobenzoate	35.7	1.86[f]	Type I	4.6	1.04
111-15-9[g,q]	2-Ethoxyethyl acetate	42.1	0.65		43.2	1.04
886-86-2[g,i]	Ethyl 3-aminobenzoate methanesulfonic acid salt	79.0	1.96	Type I	2.7	1.33
1126-46-1[g]	Methyl *p*-chlorobenzoate	11.0	2.90		1.8	1.34
Narcosis III: Level B confidence						
118-61-6	Ethyl salicylate	20.2	3.14	Type I	0.6	1.01
133-11-9	Phenyl 4-aminosalicylate	4.76	3.15[f]	Type I	3.8	1.12
2370-63-0	2-Ethoxyethyl methacrylate	27.7	1.40	Type I	15.6	2.37
2455-24-5	Tetrahydrofurfuryl methacrylate	34.7	1.3	Type I	16.5	[m]
2495-37-6	Benzyl methacrylate	4.67	2.82	Type I	5.0	1.59
4655-34-9	Isopropyl methacrylate	38.0	2.25[f]	Type I	1.5	1.18[l]
Narcosis III: Level C confidence						
105-75-9	Dibutyl fumarate	0.63	3.91	Type I	9.8	1.19
105-99-7	Dibutyl adipate	3.64	3.82		1.6	1.14
109-60-4[q]	Propyl acetate	60.0	1.2		7.1	1.17
118-55-8	Phenyl salicylate	1.18	4.12		2.9	1.05
123-66-0[q]	Ethyl hexanoate	8.90	2.79		2.3	1.35[l]
123-86-4[q]	Butyl acetate	18.0	1.73		8.6	1.28
141-03-7	Dibutyl succinate	4.46	3.54		1.8	1.07
141-78-6[q]	Ethyl acetate	230	0.73[f]		4.4	1.48
142-92-7[q]	Hexyl acetate	4.40	2.79		4.6	1.39
619-50-1	Methyl *p*-nitrobenzoate	23.8	2.02		5.4	1.01
868-77-9	2-Hydroxyethyl methacrylate	227	0.47[f]	Type II	11.6	2.00[l]
2150-47-2	Methyl 2,4-dihydroxybenzoate	45.8	2.22		1.7	1.08
2905-69-3	Methyl 2,5-dichlorobenzoate	14.0	3.37		0.7	1.04
42087-80-9	Methyl 4-chloro-2-nitrobenzoate	27.7	2.49		2.0	1.08
Narcosis III: Level D confidence						
5292-45-5	Dimethyl nitroterephthalate	6.52	1.92	Type I	32.5	1.09
Oxidative phosphorylation uncoupling: Level A confidence						
51-28-5[r]	2,4-Dinitrophenol	10.9	1.54[f]	Type II	27.8	1.03
87-86-5[r,s]	Pentachlorophenol	0.222	5.12[f]	Type II	11.9	ND
97-02-9[s]	2,4-Dinitroaniline	14.8	1.84		12.4	1.00
534-52-1[s]	4,6-Dinitro-*o*-cresol	1.73	2.56		23.1	1.42
573-56-8[s]	2,6-Dinitrophenol	39.7	1.91	Type II	3.4	1.00
1689-82-3[s]	*p*-Phenylazophenol	1.17	3.18		11.1	1.01[l]
2176-62-7[s]	Pentachloropyridine	0.47	4.34	Type III	7.2	2.55
3481-20-7[s]	2,3,5,6-Tetrachloroaniline	0.27	4.10[f]		14	1.56[l]
101836-92-4[r,s]	2,4-Dinitro-1-naphthol sodium salt	4.24	3.09	Type II	5.3	1.00
Oxidative phosphorylation uncoupling: Level B confidence						
608-71-9	Pentabromophenol	0.093	4.69	Type II	58.2	1.08[l]
1198-55-6	Tetrachlorocatechol	1.27	4.29[f]	Type II	2.7	1.38
Oxidative phosphorylation uncoupling: Level C confidence						
88-85-7	2-Sec-butyl-4,6-dinitrophenol	0.535	3.69[f]		9.4	1.19
Acetylcholinesterase inhibition: Level A confidence						
63-25-2[t]	Carbaryl (sevin)	8.75	2.36[f]	Type III	7.8	1.19
86-50-0	Azinphos-methyl	0.064	2.75[f]		761	ND
114-26-1	Propoxur	8.80	1.45[f]		58.1	1.86
115-90-2	Fensulfothion	43.1	2.23[f]		3.2	ND
116-06-3	Aldicarb	0.861	1.12	Type III	1,100	1.00

APPENDIX 2
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Chemical Abstracts Services Registry number	Chemical	LC50 (mg/L)	Log *P*[a]	Behavior syndrome[b]	Te value[c]	LC50 ratio[d]
121-75-5[t]	Malathion	14.1	2.36[f]	Type III	8.1	1.23[j]
298-04-4	Disulfoton	2.73	4.02[f]		1.2	ND
333-41-5	Diazinon	9.35	3.81[f]	Type III	0.7	1.37
786-19-6	Carbophenothion	0.237	5.33[f]		10.3	ND
1563-66-2	Carbofuran	0.844	2.32[f]		72	1.16
2032-59-9	Aminocarb	1.95	2.16		50	1.15[j]
2104-64-5	*O*-Ethyl-*o*-(*p*-nitrophenol phenyl)-phosphonothioate	0.079	3.85[f]		89.4	2.66
2921-88-2[t]	Chlorpyrifos	0.318	4.96[f]		7.1	ND
8065-48-3	Demeton	16.0	2.20		7.8	ND
13071-79-9	Terbufos	0.0133	4.48[f]		272	ND
16752-77-5	Methomyl	2.11	0.60[f]		1,180	1.25
23135-22-0	Oxamyl	6.78	−0.47		4,130	1.07[j]
Respiratory inhibition: Level A confidence						
83-79-4n[u,v]	Rotenone	0.0052	4.10[f]	Type II	1,430	1.20
26628-22-8[u]	Sodium azide	5.46	[n]	Type I	ND	2.11
Respiratory inhibition: Level C confidence						
107-14-2	Chloroacetonitrile	1.35	0.45[f]		1,190	2.59
109-77-3	Malononitrile	0.56	−0.50[f]		89,000	[m]
Electrophilic/proelectrophilic reactivity: Level A confidence						
100-52-7[w]	Benzaldehyde	9.87	1.48[f]	Type III	31.9	4.60
107-02-8[w]	Acrolein	0.017	−0.01[f]	Type II	161,000	1.25
Electrophilic/proelectrophilic reactivity: Level B confidence						
57-14-7	1,1-Dimethylhydrazine	7.85	−1.50	Type II	11,100	1.60[j]
58-27-5	2-Methyl-1,4-naphthoquinone	0.11	2.20[f]	Type II	762	1.15
66-25-1	Hexanal	17.5	1.78[f]	Type II	8.6	1.97
83-34-1	3-Methylindole	8.84	2.79	Type II	2.1	2.06
87-68-3	Hexachloro-1,3-butadiene	0.09	4.78[f]		27.7	2.40[j]
96-05-9	Allyl methacrylate	0.99	1.57	Type III	240	1.62
106-63-8	Isobutyl acrylate	2.10	2.22[f]	Type III	28.7	[m]
107-07-3	2-Chloroethanol	53.7	0.03[f]		83.9	1.55
107-18-6	Allyl alcohol	0.32	−0.25		17,500	1.97
107-19-7	2-Propyn-1-ol	1.48	−0.37	Type I	4,590	1.81[j]
109-64-8	1,3-Dibromopropane	2.09	1.99	Type II	63.2	2.88
110-62-3	Valeraldehyde	12.9	1.36	Type I	20.6	1.04[j]
110-65-6	2-Butyne-1,4-diol	53.6	−1.83	Type II	4,740	1.31
123-15-9	2-Methylvaleraldehyde	18.8	1.67	Type II	8.1	2.1[j]
127-00-4	1-Chloro-2-propanol	245	0.14		16	1.98[j]
271-89-6	2,3-Benzofuran	14.0	2.67[f]	Type III	1.5	1.6[j]
513-81-5	2,3-Dimethyl-1,3-butadiene	6.91	2.70	Type III	2.0	[m]
542-75-6	1,3-Dichloropropene	0.239	1.60		821	2.38
555-16-8	4-Nitrobenzaldehyde	10.1	1.50	Type II	32.8	[m]
590-86-3	Isovaleraldehyde	3.25	1.23	Type II	104	1.22[j]
629-19-6	Propyl disulfide	2.62	3.86	Type III	1.2	1.53
693-98-1	2-Methylimidazole	286	0.60	Type II	4.4	1.26
760-23-6	3,4-Dichloro-1-butene	8.18	1.97	Type I	10.7	3.00
764-01-2	2-Butyn-1-ol	10.1	0.16	Type I	276	1.48
818-61-1	2-Hydroxyethyl acrylate	4.80	−0.21[f]	Type III	2,145	2.50
818-72-4	1-Octyn-3-ol	0.413	2.05	Type II	205	1.93
872-31-1	3-Bromothiophene	6.19	2.62[f]	Type II	5.3	1.86
882-33-7	Phenyl disulfide	0.11	4.41[f]	Type II	25.4	2.36
924-41-4	1,5-Hexadien-3-ol	38.1	0.57	Type I	42.2	1.86
927-74-2	3-Butyn-1-ol	36.1	−0.50	Type I	322	1.60[j]
999-61-1	2-Hydroxypropyl acrylate	3.34	0.35[f]	Type III	951	*m*
1484-13-5	*N*-Vinylcarbazole	0.0032	[n]	Type II	ND	1.86[j]
1647-16-1	1,9-Decadiene	0.29	4.90	Type III	4.9	1.3
1746-23-2	Tert-butylstyrene	0.49	4.84	Type III	3.4	1.25[j]
1871-57-4	3-Chloro-2-chloromethyl-1-propene	0.19	1.56	Type II	1,270	1.80[j]
2117-11-5	(±)-4-Pentyn-2-ol	35.1	−0.08	Type II	160	1.74
3066-71-5	Cyclohexyl acrylate	1.48	2.78	Type II	15	1.67[j]
4798-44-1	1-Hexen-3-ol	30.4	1.12		16.4	1.34
6203-18-5	4-Dimethylaminocinnamaldehyde	5.90	[n]	Type II	ND	1.33
7383-19-9	1-Heptyn-3-ol	1.76	1.52	Type III	134	1.43
30030-25-2	Chloromethyl styrene	0.31	3.43	Type III	20.4	1.61[j]
65337-13-5	DL-3-Butyn-2-ol	11.7	−0.06	Type I	383	1.12
Electrophilic/proelectrophilic reactivity: Level C confidence						
[k]	2-Chloroethyl-*N*-cyclohexyl carbamate	35.0	2.46	Type II	1.6	1.23[j]
70-30-4	2,2′-Methylene bis-(3,4,6-trichlorophenol)	0.021	7.54[f]		178	1.24
75-07-0	Ethanal	33.8	−0.22		129	1.95
75-89-8	2,2,2-Trifluoroethanol	119	0.41[f]	Type II	19.5	1.37[j]
90-02-8	Salicylaldehyde	2.30	1.81[f]		59.7	1.09
90-59-5	3,5-Dibromosalicylaldehyde	0.85	3.83		7.4	1.88
95-01-2	2,4-Dihydroxybenzaldehyde	13.1	1.71		14.7	1.10
96-13-9	2,3-Dibromopropanol	71.0	0.63		44.2	2.24
96-17-3	2-Methylbutyraldehyde	9.97	1.14	Type I	41.2	2.47
97-23-4	2,2′-Methylenebis(4-chlorophenol)	0.31	4.26[f]		12.4	1.03
104-88-1	4-Chlorobenzaldehyde	2.20	2.10[f]		38.5	1.82
110-00-9	Furan	61.0	1.34[f]		3.5	1.6[j]
121-33-5	Vanillin	83.8	1.21[f]		10.9	1.96
123-54-6	2,4-Pentanedione	135	−0.54		103	ND
123-72-8	Butanal	14.7	0.88[f]		37.8	1.25
148-53-8	*o*-Vanillin	2.40	1.37[f]		170	1.62
330-93-8	*p*-Fluorophenyl ether	1.13	4.74	Type III	1.9	[m]
387-45-1	2-Chloro-6-fluorobenzaldehyde	9.41	2.54		4.0	1.47
446-52-6	*o*-Fluorobenzaldehyde	1.35	1.76		115	2.70[j]
454-89-7	α,α,α-Trifluoro-*m*-tolualdehyde	0.924	2.47[f]	Type I	52	2.28
500-22-1	3-Pyridine-carboxaldehyde	16.4	0.51		122	1.12[j]
552-89-6	*o*-Nitrobenzaldehyde	14.4	1.74[f]		11.9	1.40

APPENDIX 2
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Chemical Abstracts Services Registry number	Chemical	LC50 (mg/L)	Log *P*[a]	Behavior syndrome[b]	Te value[c]	LC50 ratio[d]
613-45-6	2,4-Dimethoxybenzaldehyde	20.1	1.91		7.5	1.87
653-37-2	Pentafluorobenzaldehyde	1.10	2.45		51.1	2.18
683-72-7	2,2-Dichloroacetamide	241	0.09	Type I	24.6	1.69[l]
708-76-9	4,6-Dimethoxy-2-hydroxy benzaldehyde	2.68	2.33		25.1	1.90
874-42-0	2,4-Dichlorobenzaldehyde	1.80	3.11		7.3	2.34[j]
932-16-1	2-Acetyl-1-methylpyrrole	157	1.02		4.8	[m]
1204-21-3	α-Bromo-2′,5′-dimethoxyacetophenone	0.066	2.39		970	1.01
2034-22-2	2,4,5-Tribromoimidazole	6.12	1.96[f]		52.8	1.00
2439-77-2	*o*-Methoxybenzamide	120	0.87[f]	Type III	10.8	1.08[j]
2626-83-7	*p*-(Tert-butyl)-phenyl-*N*-methyl-carbamate	10.0	3.06[f]		1.7	1.43
2973-76-4	5-Bromovanillin	59.7	2.09		2.4	1.15[l]
3698-83-7	1,3-Dichloro-4,6-dinitrobenzene	0.0456	2.49		1,202	1.77
3944-76-1	2,3-Dimethylvaleraldehyde	16.0	2.07	Type I	4.6	1.10[l]
4460-86-0	2,4,5-Trimethoxybenzaldehyde	49.5	1.38		11.3	1.60
6284-83-9	1,3,5-Trichloro-2,4-dinitrobenzene	0.222	2.65		230	2.98
6361-21-3	2-Chloro-5-nitrobenzaldehyde	3.87	2.28		20.1	1.68
10031-82-0	*p*-Ethoxybenzaldehyde	28.1	2.31		2.1	1.40[j]
Electrophilic/proelectrophilic reactivity: Level D confidence						
[k]	4-(Hexyloxy)-*m*-anisaldehyde	2.67	3.99		1.6	[m]
[k]	5-Bromo-2-nitrovanillin	73.3	1.88[f]		3.6	1.00[j]
90-47-1	Xanthone	[x]	2.98	Type III	ND	ND
93-91-4	1-Benzoylacetone	1.10	1.05	Type I	856	1.55
95-52-3	2-Fluorotoluene	19.4	2.93		0.6	[m]
121-14-2	2,4-Dinitrotoluene	24.3	2.00	Type III	4.1	1.49
329-71-5	2,5-Dinitrophenol	3.36	1.75[f]	Type II	70.2	1.20
371-40-4	4-Fluoroaniline	16.9	1.15[f]		30.7	1.93
623-25-6	α,α′-Dichloro-*p*-xylene	0.039	3.27		248	3.59
2138-22-9	4-Chlorocatechol	1.58	1.97	Type II	72.9	2.22
2447-79-2	2,4-Dichlorobenzamide	95.6	1.82		2.2	[m]
3428-24-8	4,5-Dichlorocatechol	0.89	2.90	Type II	22.7	1.38
5465-65-6	4′-Chloro-3′-nitroacetophenone	5.50	1.96		29.6	2.22
13209-15-9	α,α,α′,α′-Tetrabromo-*o*-xylene	0.437	5.17		9.5	1.11[l]
Central nervous system seizure/stimulant mechanisms: Level A confidence						
58-08-2	Caffeine	151	−0.07[f]	Type III	84.1	1.17
60-13-9	Amphetamine sulfate	28.8	1.76[f]	Type III	16	1.54
60-41-3[y]	Strychnine hemisulfate salt	1.11	1.93[f]	Type III	234	1.29
65-30-5	Nicotine sulfate	13.8	1.17[f]	Type III	153	0.15
115-32-2	Dicofol	0.603	6.06		5.7	ND
10453-86-8	Resmethrin	0.006	6.18	Type III	523	2.33[j]
51630-58-1[z]	Fenvalerate	0.0015	6.20[f]	Type III	765	ND
52645-53-1	Permethrin	0.016	6.50[f]		226	ND
70124-77-5	Flucythrinate	0.00019	6.20[f]		16,300	ND

[a] Log of the octanol : water partition coefficient estimated using CLOGP™ program version 3.4 within the UDRIVE system version 3.53, 1988, from Pomona College Medicinal Chemistry Project, Claremont, CA, USA.
[b] Behavior syndromes are behavioral signs of stress identified for fathead minnows exposed to reference toxicants—Type I: depressed activity, underreactive, fish die in first 24 h of exposure; type II: hyperactivity, overreactive to outside stimuli, and delayed mortality; type III: spontaneous locomotor activity, convulsion, spasms, tetany, scoliosis, lordosis, and/or hemorrhaging in the vertebral column [28].
[c] A ratio of the predicted LC50 based on a narcosis I model [21] divided by observed LC50.
[d] The ratio of the 24- and 96-h LC50 values.
[e] Each mode of action classification was assigned a level of confidence based on the type of data used when making the assessment; confidence levels were high (Level A using FATS and joint toxic action data), moderate (Level B using behavior syndrome, LC50 ratios, Te values, and structural similarity to Level A compounds), and low (Level C using behavioral comments, concentration/response slope, and structural similarity within a chemical class). No confidence (Level D) was associated with assessments for which there were insufficient data.
[f] A measured log of the octanol : water partition coefficient from the STARLIST database, the UDRIVE system version 3.53, 1988, from Pomona College Medicinal Chemistry Project, Claremont, CA, USA.
[g] Chemical was additive with 1-octanol, a reference narcosis I toxicant, in joint toxic action studies [8].
[h] Chemical was additive with 1-octanol and phenol, reference narcosis I and narcosis II toxicants, respectively, in separate joint toxic action studies [8].
[i] Chemical identified as eliciting a narcosis I fish acute toxicity syndrome in rainbow trout [9].
[j] A 48- to 96-h LC50 ratio; an LC50 could not be calculated until 48 h of exposure.
[k] Chemical Abstract Service Registry number is not available for this compound.
[l] A 72- to 96-h LC50 ratio; an LC50 could not be calculated until 72 h of exposure.
[m] An LC50 could not be calculated until 96 h of exposure.
[n] Octanol : water partition coefficients were not available.
[o] Chemical was additive with phenol, a reference narcosis II toxicant, in joint toxic action studies [8].
[p] Chemical identified as eliciting a narcosis II fish acute toxicity syndrome in rainbow trout [5].
[q] Chemical used to derive a QSAR for narcosis III mode of action (see Eqn. 6, Table 1).
[r] Chemical identified as eliciting an oxidative phosphorylation uncoupling fish acute toxicity syndrome in rainbow trout [9].
[s] Chemical was additive with 2,4-dinitrophenol, a reference oxidative phosphorylation uncoupler, in joint toxic action studies [8].
[t] Chemical identified as eliciting an acetylcholinesterase inhibition fish acute toxicity syndrome in rainbow trout [4,10].
[u] Chemical was additive with either rotenone or sodium azide, reference respiratory inhibitors, in joint toxic action studies [8].
[v] Chemical identified as eliciting a respiratory inhibition fish acute toxicity syndrome in rainbow trout [35].
[w] Chemical identified as eliciting an electrophilic-based (gill irritation) fish acute toxicity syndrome in rainbow trout [10].
[x] Only 1 of 10 fish exposed to xanthone died in a 96-h exposure, but behavioral effects were observed in the fish.
[y] Strychnine identified as eliciting a unique central nervous system seizure fish acute toxicity syndrome in rainbow trout [4].
[z] Fenvalerate identified as eliciting a unique central nervous system seizure fish acute toxicity syndrome in rainbow trout associated with pyrethroid insecticides [4].
ND, value was not available.