Application of the Activity Framework for Assessing Aquatic Ecotoxicology Data for Organic Chemicals

Paul Thomas,[†] James Dawick,[‡] Mark Lampi,[§] Philippe Lemaire,[∥] Shaun Presow,[⊥] Roger van Egmond,[#] Jon A. Arnot,[∇] Donald Mackay,[○] Philipp Mayer,[◆] and Malyka Galay Burgos*,[¶]

[†]CEHTRA/KREATiS, ZAC de Saint Hubert, 23 rue du Creuzat, 38080 L'Isle d'Abeau, France

[‡]Shell Health, Brabazon House, Concord Business Park, Manchester, Greater Manchester, M22 0RR, United Kingdom

[§]ExxonMobil Biomedical Sciences, Inc., 1545 US 22 East, Annandale, New Jersey 08801, United States

[∥]Total Fluides, 24 Cours Michelet — La Défense 10, F-92069, Paris La Défense Cedex, France

[⊥]Euro Chlor, Avenue E. Van Nieuwenhuyse 4, B-1160 Brussels, Belgium

[#]Unilever, Colworth Science Park, Sharnbrook, Bedford, Bedfordshire MK44 1LQ, United Kingdom

[∇]ARC Arnot Research & Consulting Inc., 36 Sproat Avenue, Toronto, ON M4M 1W4, Canada

[○]Trent University, 1600 West Bank Drive, Peterborough, Ontario K9J 7B8, Canada

[◆]Technical University of Denmark, Department of Environmental Engineering, Lyngby, Denmark

[¶]European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Avenue E. Van Nieuwenhuyse 2, B-1160 Brussels, Belgium

ABSTRACT: Toxicological research in the 1930s gave the first indications of the link between narcotic toxicity and the chemical activity of organic chemicals. More recently, chemical activity has been proposed as a novel exposure parameter that describes the fraction of saturation and that quantifies the potential for partitioning and diffusive uptake. In the present study, more than 2000 acute and chronic algal, aquatic invertebrates and fish toxicity data, as well as water solubility and melting point values, were collected from a series of sources. The data were critically reviewed and grouped by mode of action (MoA). We considered 660 toxicity data to be of acceptable quality. The 328 data which applied to the 72 substances identified as MoA 1 were then evaluated within the activity-toxicity framework: EC50 and LC50 values for all three taxa correlated generally well with (subcooled) liquid solubilities. Acute toxicity was typically exerted within the chemical activity range of 0.01−0.1, whereas chronic toxicity was exerted in the range of 0.001−0.01. These results confirm that chemical activity has the potential to contribute to the determination, interpretation and prediction of toxicity to aquatic organisms. It also has the potential to enhance regulation of organic chemicals by linking results from laboratory tests, monitoring and modeling programs. The framework can provide an additional line of evidence for assessing aquatic toxicity, for improving the design of toxicity tests, reducing animal usage and addressing chemical mixtures.
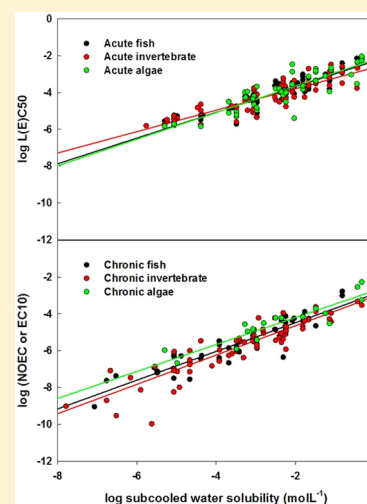
INTRODUCTION

In risk assessments the likelihood of adverse effects of organic chemicals on aquatic organisms is evaluated by comparing predicted exposure in the environment with the exposure that is required to exert toxic effects. The effects assessments are generally based on data obtained from a range of standardized toxicity tests of varying duration and employing a range of relevant species.

Exposure to aquatic organisms can occur both from the water phase and the diet; however, current guidelines for determination of toxic effects largely derive effects end points solely from water-born exposure.[1−5] The concentration in the test medium (water) is generally used to quantify the effect (toxicity) end point (e.g., Mackay et al.[6]); however, this exposure medium is only a surrogate for the amount of toxicant that actually reaches the site of toxic action in the organism resulting in the toxic effect at the assessment end point. It is generally accepted that the toxic effect is directly attributable to the delivered amount of chemical to a target within the organism and only indirectly to the external exposure.[7]

McCarty and Mackay[8] proposed the use of critical body residues (CBRs) for use in ecological risk assessment, where exceedance of an effect threshold leads to an observed biological response that is largely proportional to the amount of the chemical at the sites of toxic action. The usefulness of

this approach is highlighted by the recognition of a number of toxic modes of action (MoA). In a series of papers, Verhaar et al.[9,10] proposed a framework for the identification of four classes of compounds with different modes of action. The classes include two for narcosis with nonpolar narcosis (MoA1) defined as baseline toxicity (inert substances) and polar narcosis (MoA2) less inert chemicals, more toxic than predicted by baseline toxicity estimations, which are commonly identified as possessing a hydrogen bond donor. Two further non-narcosis classes are defined where MoA 3 refers to those substances containing a reactive group, which can react in a nonspecific manner with biomolecules, leading to higher toxicity and MoA 4 substances are those that interact with specific receptors within an organism causing toxicity. For the purpose of this study, substances which could not be assigned to any of the above modes of action were disregarded. Other studies[11] have demonstrated a relationship between the octanol−water partition coefficient (K_{ow}) and nonpolar narcosis. The concept has been further developed by evaluating larger data sets (e.g., Russom et al.[12]) and approaches that use the Abraham polyparameter Linear Free Energy Relationships (ppLFERs) to identify nonpolar and polar narcotics[13] instead of K_{ow}. The K_{ow} and ppLFER approaches seek to characterize the same underlying behavior of chemical partitioning from the aqueous exposure medium to hypothesized target sites in the body, that is, toxicokinetics.

The link between activity and toxicity first proposed by Ferguson[14] for baseline narcotics has been explored more recently.[13,15−18] Precise control of chemical activity in toxicity tests can be achieved by passive dosing techniques using a polymer loaded with the test substance as a partitioning donor as demonstrated by Schmidt et al.[17] Hydrophobic substances are also suitable for passive dosing. These authors also showed that the toxicity of mixtures can be assessed by addition of activities, since lethality from exposures to individual chemicals and mixtures occurred to terrestrial and aquatic invertebrates at a sum of chemical activity of 0.01−0.1.[17,19] Potential additional advantages of expressing toxicity using the activity framework are that it can be applied to air-breathing and water-respiring animals, it avoids the variability in CBR attributable to lipid content differences and it enables measured activities causing baseline toxicity in laboratory studies to be compared with activities that are measured or predicted in the environment.[20,21]

In this study, the relationships between chemical activity and adverse effects and no effect level estimates on aquatic organisms were further explored by reviewing and analyzing toxicity data for various aquatic taxa using the mode of action (MoA) scheme as outlined above. In the first step, a database of critically reviewed data in the form of lethal, effect and no-effect concentrations (LCX, ECX, or NOECs) in water corresponding to acute and chronic effect test end points was compiled. The chemicals were assigned into groups according to MoA[22] and the aqueous solubility data was then converted into a format suitable for establishing chemical activities. Only MoA 1 data are reported in this work. The resulting relationships between LCX, ECX, NOECs, solubilities and activities are then analyzed and discussed.

The general aim was to apply the activity framework and evaluate the contribution to more effective risk assessment by integrating information on chemical structure and properties, MoA, acute and chronic effects for a range of aquatic organisms. In doing so the observed variability in activity

levels corresponding to toxicity and time to steady state and equilibrium, and how activity may assist in the assignment of toxic MoAs were addressed. If successful, the activity concept or hypothesis could be applied in the regulatory process as a weight of evidence component for toxicity evaluation and eventually applied predictively to reduce the number and cost of acute and chronic toxicity studies and animal usage in a regulatory context. In the interests of clarity, recent related studies and the thermodynamic relationships between activity and aqueous concentrations that are used to test the activity hypothesis have been reviewed.

ACTIVITY, AQUEOUS CONCENTRATIONS AND TOXICITY

Ferguson[14] demonstrated in 1939 that chemical activity could be used as a metric which would allow insight into exposure, the inherent assumption being that at equilibrium the activity in the organism will approach the activity in the exposure medium. Fundamentally, equilibrium partitioning of a substance between two phases occurs when the chemical potential of the substance is equal in both phases.[23] More convenient criteria of equilibrium are the related quantities of chemical activity and fugacity that are linearly related to concentrations (at least at low concentrations), and can also be applied to air, water, soils, sediments, and biota. Fugacity is essentially the chemical's partial pressure (Pa) and can range from zero to a maximum of the substance's liquid state vapor pressure. The chemical activity (a) quantifies the energetic level of a substance relative to saturation. The subcooled water solubility of the substance (where $a = 1$) serves as the reference state, and thus chemical activity is defined as between 0 and 1.[16,23]

Narcotic toxicity has been proposed to initiate within a relatively narrow range of chemical activities, whereas external concentrations required to cause baseline toxicity are known to vary by orders of magnitude.[16] The working hypothesis of the study was that acute narcotic toxicity occurs 1−2 orders of magnitude below saturation (i.e., chemical activity 0.01−0.1), whereas chronic toxicity might be exerted at somewhat lower activities.

The test of the hypothesis is that the highly variable effect concentrations for a diverse set of chemical substances will correspond to a relatively narrow range of activities. Rather than calculate the activities corresponding to the LCs and ECs, it is more convenient to plot these metrics of toxicity against solubility of the liquid state chemical. The 1:1 line ($Y = X$) represents then a chemical activity of unity, and parallel lines can easily be drawn to represent chemical activities of 0.01 and 0.1.

When the chemical is a solid, that is, the melting point (T_M; units K) exceeds ambient temperature, it is necessary to use the subcooled liquid state properties to estimate chemical activity. In a solution at low concentration the chemical behaves as if its saturation condition or reference state is that of the subcooled liquid state vapor pressure or solubility, not the solid state that is additionally influenced by crystalline interactions in the solid. The vapor pressure and solubility of the solid substance are thus lower than that of the hypothetical subcooled liquid by a factor termed the fugacity ratio (F). The fugacity ratio can be estimated at the ambient temperature (T; units K) from the substance's T_M and the entropy of fusion at the melting point (ΔS; units $J/mol\ K$). A value of 56.5 J/(mol K) can be assumed in some cases to estimate ΔS and thus F can be calculated as $\exp(−6.79(T_M/T − 1))$, where $6.79 = 56.5/8.314$, that is, the

estimate for ΔS divided by the gas constant (R; units 8.314 J/mol K).

An example is solid naphthalene with a molar mass of 128 g/mol, MP of 80 °C (353 K) a solid vapor pressure of 10.9 Pa and aqueous solubility of 33 mg L^{-1}. At 25 °C, F is 0.286, thus the corresponding liquid state values are 38.1 Pa and 115.4 mg L^{-1} or 0.90 mol/m^3 and 0.00090 mol L^{-1}. At a low concentration in air and water the effective reference or saturation state is that of the liquid, thus at 1% of saturation the fugacity or partial pressure of naphthalene is 0.381 Pa, the concentration in water is 1.154 mg L^{-1} and the activity is 0.01. The activity corresponding to the solid state vapor pressure and solubility is 0.286. This is the fugacity ratio. An implication is that naphthalene is unlikely to exist in solution in air or water at conditions exceeding an activity of 0.286 because at higher activities solid naphthalene will phase separate or "precipitate" from solution. High melting point solids such as hexachlorobenzene may be unable to achieve concentrations and activities necessary to cause toxic effects.[16,24] This constraint does not necessarily apply to liquid mixtures of high melting point solids such as commercial PCBs, crude oils and petroleum products.[13,19]

Data on the solid or liquid solubility of the chemicals in water, melting point and molecular weight of each substance were collected at the test temperature. For liquids, the fugacity ratio F, as previously defined, is equal to 1.0 and the liquid solubility was used directly. For solids, F was calculated and the higher subcooled liquid solubility calculated as the solid solubility divided by F. The solubilities, typically reported in mg L^{-1} were converted into mol L^{-1}. For substances that are miscible with water, a hypothetical solubility of 55.5 mol L^{-1} was used as reported by Mackay,[25] that is, the reciprocal of the molar volume of water.

DATA COMPILATION AND DATA QUALITY ASSESSMENT

The critical first step of the analysis was to obtain quality data for a large number of substances. Various sources were used, including the recently disseminated European Chemicals Agency (ECHA) database (http://www.echa.europa.eu, retrieved June–December 2011). The REACH regulation[26] in Europe, required the submission by industry of large amounts of toxicology data to ECHA. Data for large volume chemicals (manufactured/imported >1000 tonnes per year) were submitted in December 2010 and disseminated in a reduced format to the public in 2011. As a part of this evaluation and registration, industry was required to perform a literature review and perform quality/reliability assessments on each study available for each of the substances registered. This provided a large resource of reviewed toxicological and ecotoxicological data. The studies submitted for these registration dossiers had been classified for scientific reliability in accordance with the Klimisch rating.[27] The collection of these data for a large number of chemicals provides an opportunity to extract information from this ECHA database. Given that the REACH dossiers also report physicochemical properties such as water solubility, this allows the calculation of activity through the liquid solubility in water and direct comparison with toxicity results for a wide variety of substances.

Where possible, the REACH registration dossiers of a series of selected organic substances were examined. The submitted data on acute and chronic toxicity to fish, invertebrates and algae, as well as solubility, were reviewed. As an initial screening exercise, only data rated Klimisch 1 (reliable without restrictions) or Klimisch 2 (reliable with restrictions) were used. It should be noted that this screening depended on the Klimisch rating assigned by the REACH registrants. Quantitative structure–activity relationship (QSAR) data for toxicity were not used in our analysis. Where a REACH registration dossier was not available for a particular substance, data from other dossiers were used. For example, Euro Chlor has published risk assessments for a number of chlorinated substances, including some that are no longer produced (http://www.eurochlor.org/download-centre/marine-risk-assessments.aspx, retrieved October 2011). Additionally, since pesticides and plant protection products were not registered under REACH, data for these substances were largely obtained from the U.S. EPA Ecotox Database (http://cfpub.epa.gov/ecotox/, retrieved October 2011). Other data were taken directly from peer-reviewed publications;[22,28] Thomas et al.[29] and other sources.[30–37]

After collection of the study details, the data were further reviewed for experimental errors and nonstandard conditions, such as open systems for volatile substances. Studies with a reported NOEC (no observed effect concentration) or E/LC50 value higher than the reported water solubility were excluded. For acute end points, only standard durations as defined in the OECD guidelines for each trophic level were deemed appropriate. Subchronic results were rejected as unsuitable for meeting the chronic toxicity end point. In addition, nonstandard regulatory effects end points were not accepted. Studies that reported only nominal concentrations for volatile or unstable substances were also excluded. Data were preferentially obtained from a single dossier when multiple dossiers exist for a single substance. The data from sources other than ECHA dossiers were also reviewed for nonstandard conditions as part of the data reliability assessment. The intent of the data quality analysis employed here was to identify and set-aside data points that were subject to error and therefore highly uncertain; however, despite these efforts it is recognized that error and uncertainty in the selected data still remain.

VERIFICATION OF BASELINE NARCOTIC MODE OF ACTION

The substances selected were divided into four groups,[22] according to the Verhaar and modified Verhaar classifications.[9,10,38] Mode of action was established using the Toxtree software;[39] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree, retrieved August 2011). Both the original and modified methods were used to verify the data. Some differences in the two methods were noted. For example, the original Verhaar method classification for certain compounds indicates a nonpolar narcosis mode of action while the modified Verhaar classification assigns them to a specific mode of action, which was not justified by the effective chemical activity of these substances as they fell within the expected limits of activity for classification as MoA 1. Only MoA 1 substances are addressed in this publication.

The collected data were compiled into a single data set in Excel for screening and are available from ECETOC[22] as part of the Supporting Information.

■ RESULTS AND DISCUSSION

New Data and Analysis Supporting the Toxicity-Activity Hypothesis. Over 2000 individual acute and chronic data for fish, aquatic invertebrates and algae were extracted from the various data sources and evaluated. After screening, approximately 660 measurements for 123 substances met the quality criteria. When the MoA was assigned, there were 328 data for 72 substances classified as exhibiting MoA 1 type toxicity. This limited data screening exercise highlights the paucity of reliable data that may be useful for critical analyses, including chemical risk assessment.

As described above in the section on "Activity, Aqueous Concentrations and Toxicity", narcotic toxicity has been proposed to occur within a narrow range of chemical activities, with acute narcotic toxicity occurring 1−2 orders of magnitude below saturation (i.e., chemical activity 0.01−0.1), whereas chronic toxicity might be exerted at somewhat lower activities. Figure 1 confirms this general trend, with the bulk of fish and
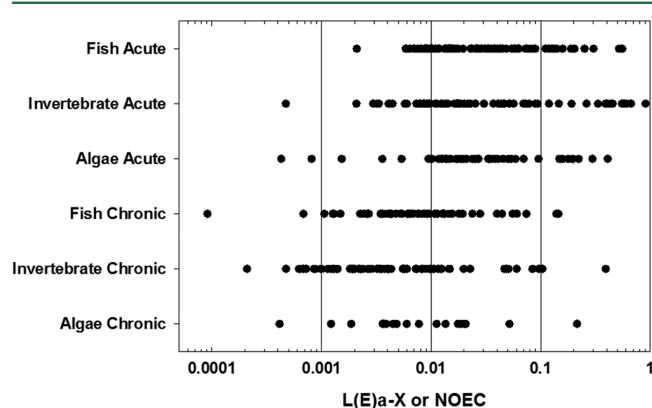

Figure 1. L(E)a-X values (NOEC) for the six data sets examined.

invertebrate acute effects observed in this range, and chronic effects trending to lower activities. Interestingly, the algae acute data fit well with the other acute values while the algae chronic data also fit well with long-term studies from invertebrates and fish.

Figures 2−4 show acute effect (A) and chronic effect/no-effect (B) toxicity data measured for fish (Figure 2), aquatic invertebrates (Figure 3) and algae (Figure 4) as a function of the measured or estimated water solubility limits (or subcooled liquid solubility for solids) for the test chemicals. The data plotted in each figure were subjected to linear regression, and the slope, intercept and coefficient of determination (R^2) are presented in Table 1.

The acute toxicity data for fish generally fall between activities of 0.01 and 0.1 (Figure 1, 2A), supporting existing literature. Chronic activity data for fish largely ranged from 0.001 to 0.01 (Figure 1, 2B), a factor of approximately 10 lower than the acute data, as expected. The slope deviates from one, or perfection, as may be expected in imperfect biological systems. This may be an artifact of biotransformation, as has been proposed by Mackay et al.[18] It could also be because, in the case of poorly water-soluble substances, that the test compound had not reached true aqueous solubility. Other possible reasons include differences in fish size, lipid content and growth rate during experimentation.

The acute and chronic invertebrate toxicity data (Figure 1, 3A, B) are similar but more scattered perhaps reflecting the
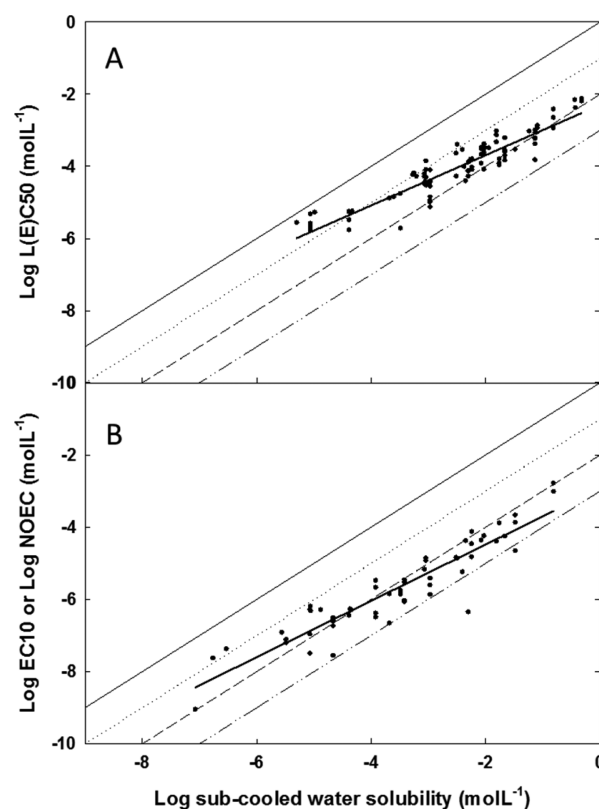

Figure 2. Acute (A) and Chronic (B) toxicity data for fish. Regression coefficients are found in Table 1. Solid line: a = 1; dotted line: a = 0.1; dashed line: a = 0.01; mixed line: a = 0.001.

experimental challenges in maintaining constant aqueous concentrations in invertebrate tests. The algal toxicity data (Figure 1, 4A, B) also show a greater spread in activities, potentially due to the difficulty in measuring truly dissolved concentrations of the test substance in the algal system given the high amount of organic carbon that accompanies this assay.

In general, the results are consistent with those of Reichenberg and Mayer[16] and Mackay et al.,[18] but they are derived from a more rigorous evaluation of a larger and more diverse compilation of data, including a greater range of aquatic species covering both acute and chronic effects. Using the activity framework decreased the wide range of exposures from concentrations of 10^{-9} to 10^2 molL^{-1} to a narrow range of activities from approximately 10^{-3} to 10^{-1} and even to a factor of 10 if acute and chronic data are considered separately.

Data Quality. Considerable effort was made to validate the data set by using, for the most part, values classified in the available databases as Klimisch 1 and 2.[27] These data were then further scrutinized and were considered fit for purpose although in a few cases significant and unexplained differences between end point values exist for the same substance. An example of this is 1,4-dichlorobenzene for which only seven studies out of 15 on fish were judged valid by the authors and the results nevertheless, varied by a factor of 10 (from 1.12 mg L^{-1} for a study on *O. mykiss* to 11.7 mg L^{-1} for *P. promelas*). Correcting for temperature used in the studies in this case does not improve the result. Fortunately, such wide variability within a trophic level data set was the exception rather than the rule. The physicochemical parameters, both (subcooled) solubility and melting point were also subject to variation when multiple values were available. These properties are not used as a
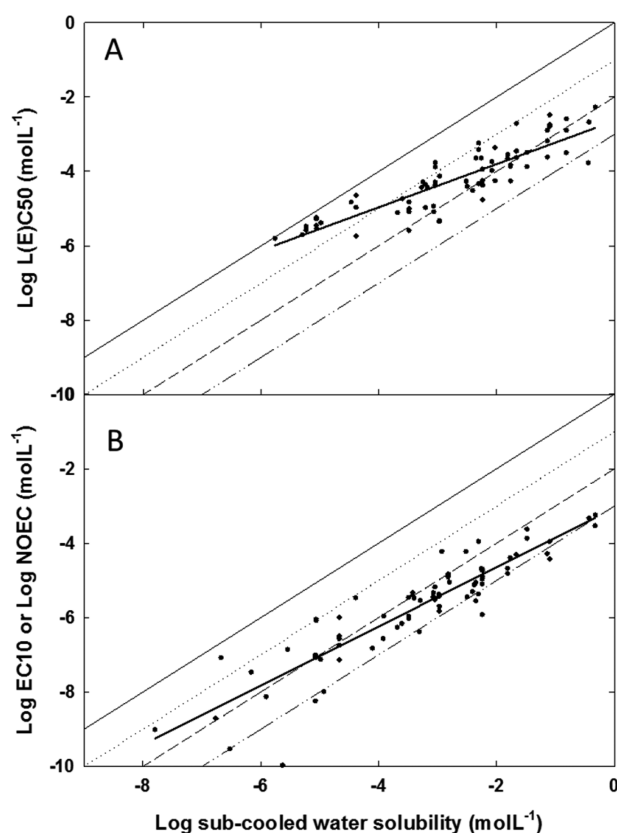
Figure 3. Acute (A) and Chronic (B) toxicity data for invertebrates. Regression coefficients are found in Table 1. Solid line: a = 1; dotted line: a = 0.1; dashed line: a = 0.01; mixed line: a = 0.001.

Figure 4. Acute (A) and Chronic (B) toxicity data for algae. Regression coefficients are found in Table 1. Solid line: a = 1; dotted line: a = 0.1; dashed line: a = 0.01; mixed line: a = 0.001.

regulatory threshold for classification and labeling or risk assessment under current practice of EU risk assessment and thus the attention to quality may be less than required for accurate activity determination and the physicochemical property data used in the present analysis were not scrutinized to the same extent as the reported toxicity data. Despite these drawbacks, the physicochemical property data used in this study are considered to be generally acceptable, allowing an in-depth assessment. Clearly, the development of high quality toxicity data as a training or validation set for activity calculations is the only way to achieve certainty in predictions based on activities.

Approximately two-thirds of the collated data were assessed as not fit for purpose (all the studies in the ECHA disseminated data set which did not meet Klimisch 1 or 2 and 30% of the data which apparently attained these Klimisch scores but were still found to have methodological difficulties for the purpose of this study). Nevertheless, the remaining data were of sufficient quality for inclusion in the database.[22] While there is still variability in the results of standard studies, it seems that well executed aquatic toxicity tests in most cases (at solubilities that are >0.1 mmol L^{-1}) fit well with the concept of activity for MoA 1 substances.

Passive dosing methods employing a loaded polymer as partitioning donor are currently being used more frequently[17,19] and have made significant improvements in terms of controlling chemical activities in toxicological research and testing. These techniques thus allow chemical activity and toxicity to be linked experimentally, while avoiding the error associated with standard testing and to conversions from
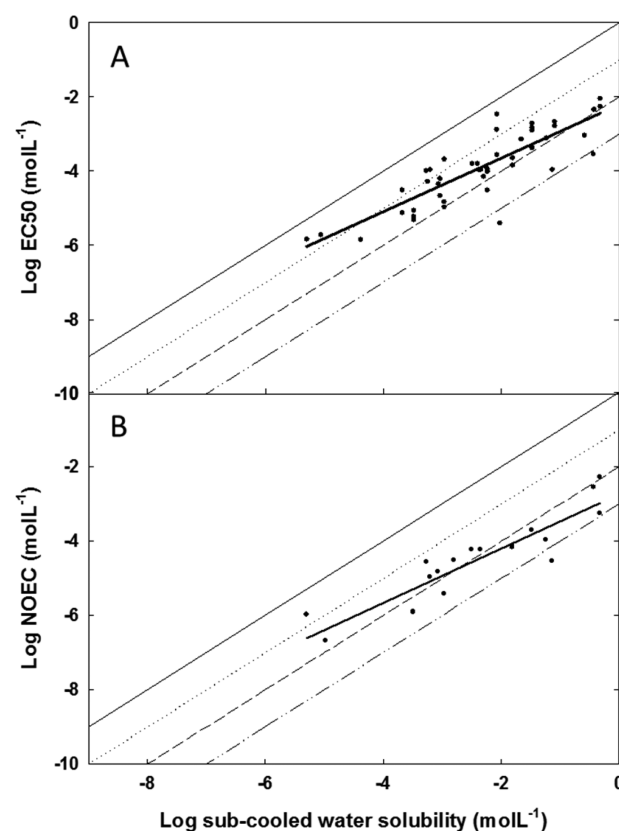
Table 1. Summary of Regression Data from Plots in Figures 2−4

data	MoA	slope	intercept	R^2
fish acute	1	0.697	−2.30	0.86
invertebrate acute	1	0.581	−2.64	0.79
algae acute	1	0.722	−2.21	0.73
fish chronic	1	0.780	−2.92	0.86
invertebrate chronic	1	0.795	−3.06	0.82
algae chronic	1	0.731	−2.74	0.83

concentrations to activity. Consequently, it might be feasible to study activity-toxicity relationships with even better precision, accuracy and thus detail. Additionally, such passive dosing techniques allow simple toxicity testing exactly at the saturation level, which facilitates limit testing for screening purposes and thus experimental reductions.[19]

Further confirmation of narcotic MoA was obtained by comparisons with classification schemes.[9,10,38] For the majority of data analyzed in the current study, chemical activity was consistent with mode of action from these schemes. In certain cases, data points from the current analysis deviated from those predicted by the existing classification schemes (detailed explanation in ECETOC[22]). Thus, it appears that the activity framework provides a further tool to improve our ability to accurately classify, and confirm MoA 1.

Equilibrium, Steady State and Variability within the Data Set. According to Mackay et al.[18] the ratio between effective concentrations and liquid solubility is in the range of 0.01−0.1 for more soluble chemicals, increasing from 0.1 to 1 for more hydrophobic substances. Thus, the slope is not 1 as

predicted by a constant activity hypothesis but closer to 0.8 (Figures 2−4). This suggests that more hydrophobic substances appear to require higher activities and so are less toxic than predicted. Several explanations are provided by these authors to account for this difference: metabolic biotransformation rates reducing body burdens, reduced bioavailability with increasing hydrophobicity, cosolvents introducing confounding factors, inherent sensitivity of different species, increased activity coefficient of large hydrophobic molecules in the lipid phase or the fact that the tests may not reach equilibrium within the allotted study duration.

Further variability in the experimental data could be due to several sources, including lack of reliable water solubility, melting point and/or effects data. The use of NOECs rather than statistically derived values (e.g., EC10) could also impact the variability of chronic effects data.

Data Analysis. Linear regression coefficients from the data in Figures 2−4 are presented in Table 1. Using the method proposed in OECD 305,[40] time to steady state was calculated, and in the case of the fish MoA 1 plot, three fish studies are not expected to have reached equilibrium ($S_L < 10^{-4}$ mol L^{-1}). Removal of these three studies from the plot nominally increased the slope (data not shown).

The chronic fish regression slope (0.78) is slightly lower than that found by Mackay et al.[18] The time to steady state for those studies was calculated (data not shown), and overall it would seem that the organisms had reached steady state, within the time frame of the chronic studies.

It might be expected that equilibrium has not been reached in acute studies by the end of the study period for highly hydrophobic substances. However, as the toxicity data for algae are based on assays on unicellular organisms, we would expect steady state to be reached over the 72−96 h study duration. The situation with algae is nevertheless more complex as algae have a doubling time that is much shorter than the duration of the test, that is, 6−8 h.

When the acute invertebrate data are filtered so that data points for substances with an aqueous solubility lower than $10^{-4.4}$ mol L^{-1} (i.e., highly hydrophobic substances) are removed, the slope increases from 0.59 to 0.73. The new slope is similar to that observed for the chronic invertebrate data at 0.7 and is also closer to the acute fish and algae slopes. This indicates that at lower aqueous solubilities ($<10^{-4.4}$ mol L^{-1}), experimental factors such as the use of cosolvents, and lack of steady state could be playing an increasing role.

For algae, variability within the data set is slightly greater and validity of the studies more difficult to conclude upon (due to static exposure regime, substance loss at 72 h and open and agitated test vessels). It is nonetheless reassuring that the slopes for EC50 and NOEC data for MoA 1 chemicals are similar as the time to equilibrium will not change for these two end points and the slopes for both end points at 0.72 and 0.73 respectively, were not far from the values found for fish and invertebrates.

An important concept to consider in applying the activity concept and evaluating existing laboratory data are data points for which activity is greater than one. While theoretically possible due to supersaturation of the substance, this is more likely an indicator of the use of cosolvents, or the presence of undissolved substance (physical effects), which increases the measured concentration in the test media.

In general, the data fit quite well to the activity framework, despite the variability and scarcity of high quality test data. This is encouraging as these points can be addressed through careful experimental design, and advances in dosing techniques, particularly for poorly water-soluble substances.[19,41] This analysis shows that MoA 1 chemicals behave, in general, as expected, with nearly all data points falling above the 0.001 activity line. In addition, there was a reasonably good correlation between the water solubility and E/LC50 data, suggesting that the changes in toxicity were largely explained by changes in water solubility.

It is important to emphasize that 30% of the toxicity data considered valid (Klimisch 1 and 2) in ECHA disseminated dossiers were found in this study to be flawed and therefore were not used. This highlights the fact that limitations around data quality are still quite prevalent. The need for high quality data applies to water solubility and melting point data, as well as the toxicity data, since all of these parameters are critical for establishing the activity-toxicity relationships. Due to inherent variability between and within laboratories, care must be taken in the development of these data. However, as high correlations for MoA 1 chemicals were observed for this study, the development of such data is possible. The similarities between the Russom et al.,[12] Verhaar et al.[9] acute fish toxicity slopes and the slopes derived in this study support this. It is recommended that the development of a high quality ecotoxicity database using purpose-built study methodologies accounting for time to steady state, and measurement of concentrations within the test organism as well as the exposure medium be considered.

The activity concept has not yet been applied in chemical risk assessment. The results of this work are in line with those of previous work.[18] The toxicity data were generally within the expected chemical activity ranges for acute narcotic toxicity (0.01−0.1) and slightly lower, for chronic effects (0.001−0.01). Similarities in slopes were observed for acute and chronic data, and between the trophic levels examined, which indicates that species-specific characteristics (e.g., behavior and biology) play a minor role in the chemical activity framework for MoA 1 substances. Acute and chronic activity data generally followed theory, with most of the acute data falling between activities of 0.01 and 0.1, while chronic data was lower falling between 0.001 and 0.01 with a greater spread but over a wider range of solubility (Figures 1−4). The data also indicate the importance of using modern, advanced methods to maintain constant concentrations as near to aqueous solubility as possible, without exceeding it, for poorly soluble substances to generate data of the highest quality possible.

Future work could include a detailed examination of MoA 2 (and other MoA) substances. An initial analysis was completed in ECETOC,[22] but further discussion of these data was beyond the scope of the present publication.

The authors are of the opinion that this work demonstrates proof of concept for application in the development of QSARs to predict acute and chronic toxicity for substances that exhibit baseline toxicity or MoA 1. Ultimately these QSARs could reduce both the costs and animal usage in acute and chronic experimental studies in a regulatory context and assist in the design of more effective tests.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: malyka.galay-burgos@ecetoc.org.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) OECD. *OECD Guideline for the Testing of Chemicals. Test No. 203: Fish, acute toxicity test*; Organisation for Economic Co-operation and Development: Paris, 1992.

(2) OECD. *OECD Guideline for the Testing of Chemicals. Test No. 202: Daphnia sp. acute immobilisation test*; Organisation for Economic Co-operation and Development: Paris, 2004.

(3) OECD. *OECD Guideline for the Testing of Chemicals. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*; Organisation for Economic Co-operation and Development: Paris, 2011.

(4) OECD. *OECD Guideline for the Testing of Chemicals. Test No. 210: Fish, Early-Life Stage Toxicity Test*; Organisation for Economic Co-operation and Development: Paris, 2013.

(5) OECD. *OECD Guideline for the Testing of Chemicals. Test No. 211: Daphnia magna Reproduction Test*; Organisation for Economic Co-operation and Development: Paris, 2012.

(6) Mackay, D.; Puig, H.; McCarty, L. S. An equation describing the time course and variability in uptake and toxicity of narcotic chemicals to fish. *Environ. Toxicol. Chem.* **1992**, *11*, 941−951.

(7) Escher, B. I.; Hermens, J. L. M. Modes of action in ecotoxicology: Their role in body burdens, species sensitivity, QSARs, and mixture effects. *Environ. Sci. Technol.* **2002**, *36*, 4201−4217.

(8) McCarty, L. S.; Mackay, D. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Technol.* **1993**, *27*, 1719−1728.

(9) Verhaar, H. J. M.; van Leeuwen, C. J.; Hermens, J. L. M. Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* **1992**, *25*, 471−491.

(10) Verhaar, H. J. M.; Solbé, J. F.; Speksnijder, J.; van Leeuwen, C. J.; Hermens, J. L. M. Classifying environmental pollutants: Part 3. External validation of the classification system. *Chemosphere* **2000**, *40*, 875−883.

(11) Veith, G. D.; Call, D. J.; Brooke, L. T. Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. *Can. J. Fish. Aquat. Sci.* **1983**, *40*, 743−748.

(12) Russom, C. L.; Bradbury, S. P.; Broderius, S. J.; Hammermeister, D. E.; Drummond, R. A. Predicting modes of toxic action from chemical structure: Acute toxicity in the Fathead minnow (*Pimephales Promelas*). *Environ. Toxicol. Chem.* **1997**, *16*, 948−967.

(13) Kipka, U.; Di Toro, D. M. Technical basis for polar and nonpolar narcotic chemicals and polycyclic aromatic hydrocarbon criteria. III. A polyparameter model for target lipid partitioning. *Environ. Toxicol. Chem.* **2009**, *28*, 1429−1438.

(14) Ferguson, J. The use of chemical potentials as indices of toxicity. *Proc. R. Soc. London, Ser. B* **1939**, *127*, 387−404.

(15) Mayer, P.; Reichenberg, F. Can highly hydrophobic organic substances cause aquatic baseline toxicity and can they contribute to mixture toxicity? *Environ. Toxicol. Chem.* **2006**, *25*, 2639−2644.

(16) Reichenberg, F.; Mayer, P. Two complementary sides of bioavailability: Accessibility and chemical activity of organic contaminants in sediments and soils. *Environ. Toxicol. Chem.* **2006**, *25*, 1239−1245.

(17) Schmidt, S. N.; Holmstrup, M.; Smith, K. E. C.; Mayer, P. Passive dosing of Polycyclic Aromatic Hydrocarbon (PAH) mixtures to terrestrial springtails: Linking mixture toxicity to chemical activities, equilibrium lipid concentrations, and toxic units. *Environ. Sci. Technol.* **2013**, *47*, 7020−7027.

(18) Mackay, D.; Arnot, J. A.; Petkova, E. P.; Wallace, K. B.; Call, D. J.; Brooke, L. T.; Veith, G. D. The physicochemical basis of QSARs for baseline toxicity. *SAR QSAR Environ. Res.* **2009**, *20*, 393−414.

(19) Smith, K. E. C.; Dom, N.; Schmidt, S. N.; Blust, R.; Holmstrup, M.; Mayer, P. Baseline toxic mixtures of non-toxic chemicals −

Solubility addition increases exposure for solid hydrophobic chemicals. *Environ. Sci. Tech.* **2013**, *47*, 2026−2033.

(20) Mackay, D.; Arnot, J. A. The application of fugacity and activity to simulating the environmental fate of organic contaminants. *J. Chem. Eng. Data* **2011**, *56*, 1348−1355.

(21) Mackay, D.; Arnot, J. A.; Wania, F.; Bailey, R. E. Chemical activity as an integrating concept in environmental assessment and management of contaminants. *Integr. Environ. Assess. Manage.* **2011**, *7*, 248−255.

(22) ECETOC. *Activity-Based Relationships for Aquatic Ecotoxicology Data: Use of the Activity Approach to Strengthen MoA Predictions*, Technical Report No. 120; European Centre for Ecotoxicology and Toxicology of Chemicals: Brussels, Belgium, 2013.

(23) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; 2nd ed.; Wiley-Interscience: New York, NY, 2003.

(24) Lipnick, R. L. Narcosis: Fundamental and baseline toxicity mechanism for nonelectrolyte organic chemicals. In *Practical Applications of Quantitative Structure−Activity Relationships (QSAR) in Environmental Chemistry and Toxicology*; Karcher, W., Devillers, J., Eds.; Kluwer: Dordrecht, The Netherlands, 1990; pp 129−144.

(25) Mackay, D. *Multimedia Environmental Models: The Fugacity Approach*; 2nd ed.; Lewis Publishers, CRC Press: Boca Raton, FL, 2001; p 272.

(26) EC. *Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18th December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94*; European Commission, 2006.

(27) Klimisch, H. J.; Andreae, M.; Tillmann, U. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul. Toxicol. Pharmacol.* **1997**, *25*, 1−5.

(28) McGrath, J. A.; Parkerton, T. F.; Hellweger, F. L.; Di Toro, D. M. Validation of the narcosis target lipid model for petroleum products: Gasoline as a case study. *Environ. Toxicol. Chem.* **2005**, *24*, 2382−2394.

(29) Thomas, P.; Holt, M.; Lemaire, P.; Malcomber, I.; Salvito, D.; Thompson, R. A weight-of-evidence (WOE) approach for determining mode of action: An ECETOC case study. *Integr. Environ. Assess. Manage.* **2008**, *4*, 374−375.

(30) Toxnet Hazardous Substances Data Bank (HSDB). http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

(31) Mackay, D.; Shiu, W. Y.; Ma, K. C. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. In *Volatile Organic Chemicals*; Lewis Publishers, CRC Press: Boca Raton, FL, 1993; Vol. *III*.

(32) *CRC Handbook of Chemistry and Physics*, 95th ed. (Internet Version 2015); Haynes, W. M., Ed.; CRC Press: Boca Raton, FL, 2014.

(33) Banerjee, S.; Yalkowsky, S. H.; Valvani, C. Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility-partition coefficient correlation. *Environ. Sci. Technol.* **1980**, *14*, 1227−1229.

(34) Horvath, A. L. *Halogenated Hydrocarbons: Solubility-Miscibility with Water*; Marcel Dekker Inc., NY, 1982.

(35) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 82nd ed.; CRC Press: Boca Raton, FL, 2001−2002.

(36) WHO. CICAD. World Health Organisation Concise International Chemical Assessment Documents website. http://www.who.int/ipcs/publications/cicad/en/.

(37) OECD. SIDS. Organisation for Economic Co-operation and Development Existing Chemicals Database website. http://webnet.oecd.org/hpv/ui/Default.aspx.

(38) Enoch, S. J.; Hewitt, M.; Cronin, M. T.; Azam, S.; Madden, J. C. Classification of chemicals according to mechanism of aquatic toxicity: An evaluation of the implementation of the Verhaar scheme in Toxtree. *Chemosphere* **2008**, *73*, 243−248.

(39) Patlewicz, G.; Jeliazkova, N.; Safford, R. J.; Worth, A. P.; Aleksiev, B. An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. *SAR QSAR Environ. Res.* **2008**, *19*, 495−524.

(40) OECD. *OECD Guideline for the Testing of Chemicals. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure*; Organisation for Economic Co-operation and Development: Paris, 2012.

(41) Butler, J. D.; Parkerton, T. F.; Letinski, D. J.; Bragin, G. E.; Lampi, M. A.; Cooper, K. R. A novel passive dosing system for determining the toxicity of phenanthrene to early life stages of zebrafish. *Sci. Total Environ.* **2013**, *463−464*, 952−958.