# (Q)SARs to Predict Environmental Toxicities: Current Status and Future Needs

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*Abstract*

The current state of the art of (Quantitative) Structure-Activity Relationships ((Q)SARs) to predict environmental toxicity is assessed along with recommendations to develop these models further. The acute toxicity of compounds acting by the non-polar narcotic mechanism of action can be well predicted, however other approaches, including read-across, may be required for compounds acting by specific mechanisms of action. The chronic toxicity of compounds to environmental species is more difficult to predict from (Q)SARs, with robust data sets and more mechanistic information required. In addition, the toxicity of mixtures is little addressed by (Q)SAR approaches. Developments in environmental toxicology including Adverse Outcome Pathways (AOPs) and omics responses should be utilised to develop better, more mechanistically relevant, (Q)SAR models.

*Introduction*

(Quantitative) Structure-Activity Relationships ((Q)SARs) have been developed for a number of environmental toxicities. The purpose of the (Q)SARs in this field has been to provide rapid assessment of the potential of a chemical to cause lethality or non-lethal adverse effects to environmental species, including both fauna (for which the models are best developed) and flora,[1] as well as being able to predict physico-chemical and fate properties.[2] The models have been developed as a response to different legislation across the globe (e.g. EU REACH, US TSCA etc) as well as to assist in the design of greener chemicals and reduction of animal testing.[35] Whilst it is not intended as a comprehensive review, this perspectives paper brings together the state of the art of (Q)SARs for acute and chronic toxicities and makes recommendations for future work, in the context of advances in what is termed "21st Century Toxicology".[6] It should be noted, in this paper, "(Q)SAR" (with parentheses around the "Q") refers to all quantitative and qualitative (structural alert or grouping) approaches, whilst "QSAR" (without parentheses) refers only to those traditional QSAR models where some form of potency is estimated.

*(Q)SARs for Acute Toxicity*

The linkage between the properties of a molecule and potency in terms of acute toxicity, especially to aquatic species, has been appreciated for well over a century. Solubility was initially seen as a driver of acute toxicity[7] with seminal work from Overton[8] and Meyer[9] developing the use (still very much applied to this day) of partitioning between polar and non-polar phases being a surrogate for uptake and distribution into an organism. As such, there is overwhelming evidence that the ability of small molecules, i.e. molecular weight less than 600Da molecules with a reasonable logarithm of the octanol-water partition coefficient (log P) value (e.g. between 0 and 5), to elicit lethality is due, in part at least, to their ability to reach the active site.[10,11] If no specific mechanism of toxicity is present, then lethality is a function of the distribution of the molecule alone – with the site of action assumed to be in cellular (and other) membranes, although the precise mechanism of action is not fully understood.[11-13]

To comprehend the reason for the good predictions of acute aquatic toxicity (for some chemicals), the experimental methodology must be considered. Experimental evaluation of acute toxicity implies the determination of a concentration that results in endpoints such as lethality, inhibition of growth as well as other effects. This concentration (e.g. the $EC_{50}$ or $LC_{50}$ etc) relies on the capability of the xenobiotic to be absorbed and / or transported to the site of action and the interaction of the xenobiotic at the site of action, in other words toxicokinetics and toxicodynamics. As aquatic toxicity tests will often reach an equilibrium state, toxicity is directly proportional to the uptake of a compound. Bringing toxicokinetics and toxicodynamics together, McFarland[14] proposed the following generic model for toxicity:

$$\text{Log } 1/C = a\,(\text{penetration}) + b\,(\text{interaction}) + c \qquad (1)$$

Where C is the concentration causing a measureable toxic potency (normally a $EC_{50}$ or $LC_{50}$),

a and b are intercepts in the relationship,

c is the constant.

Typically in the practical application of eq. (1), the penetration term is described by log P; interaction is described by a term relating to the (specific) interaction of the xenobiotic with biological molecules e.g. electrophilic toxicants are described by molecular orbital properties.

The potency of compounds that are unreactive is thought to be driven by the ability to reach the site of action alone, thus the interaction term is negligible in eq (1). As a result, the toxicity of such compounds has been well described by log P for decades following the pioneering work of Hansch,[15-16] with perhaps the most widely applied QSAR that of Könemann.[17] The relationship for non-reactive compounds holds across species within reasonable limits of solubility. Thus Könemann demonstrated an excellent relationship between acute toxicity and log P, [17] and this has been demonstrated for many other species[18-19] and has allowed for the definition of the domain of non-polar narcosis.[20] Other approaches to predict toxicity have been put forward. 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, hence providing a means of estimating acute and chronic toxicity (providing a good value of aqueous solubility is available) as well as potentially assigning compounds to mechanisms / modes of action.[21] The use of chemical activity as an overriding principle is undoubtedly founded in a good understanding of physical chemistry, however recent debate suggests that it requires further clarification and elucidation.[22,23]

The toxicity of unreactive compounds has often, and perhaps confusingly, been termed narcosis by environmental toxicologists, and is also referred to as baseline toxicity. The term narcosis was coined with reference to the anaesthetic-like effects *in vivo* of these compounds i.e. a slowing down of physiological function, leading to a comatose state and ultimately death. It is also considered to be reversible, thus if an organism is placed in a clean test system it should recover.[10,18] It is noted that the term “narcosis” with regard to acute toxicity does cause confusion to those more familiar with the use of this term from a pharmacological or (mammalian) toxicological point of view.

Thus, if a compound can be identified as being unreactive, or narcotic, acute toxicity to a variety of species can be predicted accurately from structure alone. As a result, there should be no need to perform acute toxicity tests for well characterised unreactive compounds. A number of schemes have been utilised to assign a chemical to a mechanism of action e.g. Verhaar,[24] Russom[25] and Barron[26] etc. Whilst there is widespread use of these systems, there has been no concerted effort to evaluate them properly and only limited attempts to extend these approaches.[27-28] As a result, there is a clear research need to extend these methods, with particular reference to classifying compounds as non-polar narcotics. It is of interest that the concept of a basal cytotoxicity mechanism is now being taken up in mammalian toxicity[29] as well as being possible for rat and mouse acute lethality.[30]

The classification of compounds as being narcotic is further complicated by the (strong) possibility of more than one mechanism of unreactive, reversible toxicity. There is evidence for the presence of a further significant mechanism, being termed polar narcosis or Class II.[31-32] Whether this is a distinct mechanism of action has been a subject of some contention and debate with some authors considering it to be an artefact of the solvent system used to measure or calculate log P.[33] Other narcotic mechanisms have also been proposed e.g. amine[34] and ester narcosis.[35] Whilst there is no agreement in whether these are truly distinct mechanisms, or simply an issue with regard to log P, it is possible to define the domains for high quality log P derived QSARs, thus enabling their use.

There is also no clear or precise hypothesis for the mechanism of action of narcosis. It has long been described as “membrane perturbation”, however this is an undefined term. Narcotic compounds certainly accumulate within biological membranes, thus this should be considered as the site of action, but the exact cause of toxicity is largely undefined. It is well established that compounds will

accumulate in cellular membranes, it is also possible, although less acknowledged, that accumulation may occur within organelle membranes. There may, of course, be a number of effects at the membrane. Recent evidence suggests that interaction at the calcium receptor,[36] or, with regard to mammalian toxicity, mitochondrial toxicity may be important.[29,37] This is clearly an area where analysis of omics responses, [36] in addition to the Fish Acute Toxicity Syndromes,[38] will play dividends.

Whilst most industrial chemicals as thought to act by a narcotic mechanism of action,[11,31] a number of compounds have specific toxic mechanisms of action, thus their potency is elevated above that of a narcotic response.[19] Crudely speaking, these specific mechanisms of acute toxicity can be described as being electrophilic / nucleophilic (commonly termed reactive)[39] or due to inhibition of specific enzymes e.g. inhibition of acetylcholinesterase, whilst acknowledging other mechanisms do exist e.g. redox cycling, formation of Reactive Oxygen Species. In order to create models for such toxicant using a generic approach such as eq (1), a further term is required to capture the specific nature of the toxicity. For instance, Cronin, Schultz and co-workers developed a series of QSARs for electrophilic toxicants where the electrophilic nature of the compounds was accounted for by molecular orbital properties;[40-42] Bermudez-Saldana and Cronin developed QSARs for the toxicity of organophosphates to fish by including specific terms for the organophosphate group.[43] In some circumstances, given the difficulty of obtaining reliable data sets, read-across may become a practical alternative to QSAR.[44] In this context read-across is suitable where a small number of similar compounds are available, with data for at least one of them. Read-across circumvents, to some extent at least, the data requirements (i.e. high number of data) that may be needed to build a robust QSAR and is seen as being a solution to predicting complex toxicities, such as chronic, development and reproductive effects.[45,46]

Overall, the prediction of acute toxicity has benefitted from the development of relevant databases, notable amongst these are the data compilations for the fathead minnow[25] and *Tetrahymena pyriformis*.[47] These are significant for at least three reasons: chemicals were rationally selected to cover well defined (where possible) mechanisms of action and broad chemical space, they were of high quality (albeit with caveats such as being non-guideline or GLP (those performed according to Good Laboratory Practice criteria) studies, based on nominal concentrations) and measurements made within the same laboratory. These datasets have allowed for numerous, sometimes successful, QSAR analyses.[11] Whilst the current databases are of considerable use, it is noted that more, and better, information could be derived from ecotoxicity studies which could improve the quality of the data generated,[48] especially if coupled to robust principles of ecotoxicity testing.[49]

To progress the prediction of acute toxicity, clearer domains of narcotics are required, incorporating where possible further non-test evidence, whether it be *in vitro*, *in chemico* or other. This would assist in the definition of the chemical domains of narcosis as well as allowing for the development of robust QSARs. There is also a requirement to define the applicability domains of QSARs for specific toxicity better, as well as consideration of improved parameterisation of the specific aspect of the toxicity.

*(Q)SARs for Chronic Toxicity*

Chronic, or prolonged, toxicity tests are required to determine the effects of long-term, repeated exposure to substances. This is more realistic of “typical” exposures to pollutants, thus the outcomes are very important for environmental risk assessment. These tests rely on exposing organisms to

increasing concentrations and deriving a “No Observed Effect Concentration” (NOEC) or “Lowest Observed Effect Concentration” (LOEC) from the data. For environmental species, the NOEC would be chosen whereby there is no deviation from the viability of control population. As such, the reported NOEC does not represent the actual NOEC but rather is dependent on the concentrations tested. This must be borne in mind when modelling. In comparison to QSARs for acute toxicity, such models for chronic toxicity are restricted by the number and type of data and the subtlety and often unknown nature of the mechanisms of action. In addition, the practice of deriving a NOEC has in itself been seen as controversial, the elaboration of which is important for the development of QSARs for chronic toxicity.[49,50]

There appear to be fewer chronic toxicity data available for modelling than for acute toxicity. In addition, as toxicity is evaluated only on the reduction of viability or other effect as compared to a control group, there is little, or no, information regarding mechanism of action. For some tests, e.g. reproduction, some mechanistic understanding may be implied, but commonly not organ level effects are recorded. Therefore, for chronic toxicity, much more reliance will need to be placed on molecular responses and a more detailed assessment of organ level effects. There is a clear opportunity here for data from high-throughput *in vitro* assays to provide further input for modelling and to support other prediction methods.

Due to the paucity of chronic toxicity data and their inherent variability, there are few (reliable) QSAR models for this endpoint. Predictions are available for a number of species from the US EPA’s ECOSAR software, although these QSARs are poorly described or evaluated. There appear to be possibilities to develop QSARs when the data are well reviewed and it is performed on a mechanistic basis e.g. Austin and Eadsforth found reasonable QSARs for the NOECs of a limited number “unreactive” compounds which may, potentially, be considered to be acting by an unspecific mechanism(s) of action and NOEC may be related to critical body burden.[52] Whilst successful for limited groups of compounds, the whole concept of QSAR development for chronic toxicity based on unspecific mechanisms of toxicity needs further, and more detailed, investigation to make it more broadly applicable.

One area of chronic toxicity that has been significantly addressed through (Q)SAR modelling is the prediction of events that may lead to endocrine disruption. For instance, there are a number of (Q)SAR models that relate to oestrogen,[53-54] androgen[55] and thyroid[56] binding. There are a number of *in silico* techniques that have been applied to these, and other, endpoints ranging from the development of 2-D alerts, QSAR models, to pharmacophores.[54-57] In addition, knowledge of receptors and interactions can be modelled. Of particular interest is the ability to build homology models that may allow for extrapolation of effects from one species to another.[58-59] There are several reasons for the proliferation of models for endocrine disruption, undoubtedly including the importance of this endpoint, the current lack of an *in vivo* test system and the relative abundance of data for receptor binding which are amenable to modelling. As such, it does demonstrate that given mechanistic understanding and suitable data, (Q)SAR modelling is possible for modes of toxicity.

Thus, the development of QSARs for chronic toxicity is poorly developed in many areas, with exceptions such as (Q)SARs for endocrine disruption showing what may be possible. This would seem to be an area where mechanistic interpretation is crucial and where molecular biology, and even pharmacological, information may play a very important role to help understand the more complex and subtle mechanisms of action. There is also a role to develop robust databases of quality assured toxicological information.

*AOPs, 21st Century Toxicology, Big Data, Species Extrapolation and Beyond*

The topic of Adverse Outcome Pathways (AOPs) has swamped toxicology since the first paper from Ankley et al.[60] It is often forgotten that it was devised to formulate a framework for complex, subtle environmental effects with much work in AOPs being taken up by the mammalian toxicology community; for an up to date view of the coverage of AOPs the reader is referred to the AOP wiki.[61] However, the concept can prove to be a unifying metric for environmental toxicology, especially for non-lethal adverse effects. Here we have the possibility of identifying mechanisms and supporting grouping through molecular biology approaches.[62-63] This can support mechanistic comprehension and the creation of (QSAR) models from knowledge of Molecular Initiating Events (MIEs).[46] In addition, with regard to grouping and read-across, information from AOPs will enable better justification of the similarity and read-across hypotheses.[64-66] Overall, information from AOPs will assist in the better development and utilisation of (Q)SARs, especially for chronic toxicity, by providing mechanistic knowledge on which to formulate models. However, whilst there has been progress in developing "conceptual" frameworks of mechanistic information through the AOP paradigm, fundamental progress is still required in defining the AOP, or network of AOPs, that defines narcosis. Several starting points for this AOPs are available[60,67] and they have been reviewed critically as to their progress and potential.[68] The application of AOPs will need to be considered as a network, rather than the traditional linear depiction. The availability of knowledge, and potentially rapid measurement of (high-throughput *in vitro*) data may potentially allow for the rapid development of these "networked"-AOPs for environmental endpoints.

The consideration of the perturbation of biochemical pathways is at the heart of what is often termed 21st Century Toxicology. This approach is, in part at least, closely entwined with the development of AOPs and the analysis of compilations of "big data". There is a growing wealth of resources that fall into the overused term of "big data". For environmental endpoints these include ToxCast[69] which provides information across a broad set of receptors to species specific resources through to compilation of information from high-throughput test systems to non-vertebrate organisms such as *Caenorhabditis elegans*.[70] It is beyond the scope this article to provide insight into these new resources, rather their impact on the development of the QSARs can be considered. The information from such resources will undoubtedly support mechanistic classification to support the use of a particular QSAR to predict the effects of a compound (including the support of grouping to allow for read-across). In addition, they may provide data which can be modelled directly, knowledge of effects to species seldom considered before, e.g. *C. elegans*, will expand the applicability of models. It been recognised, however, that whilst fashionable "big data" are not the panacea for all the problems in this field, with the demonstration of relevance and curation of data being fundamental; to this end a number of recommendations have been made recently regarding the use of such information.[6]

We will never know how many species there are in the world, through the realms of fauna and flora. Additionally it is recognised that whilst environmental risk assessment aims to be protective of all species, it will never achieve that goal. The paradigm has, so far, been to select sentinel species representative of trophic levels with the aim of obtaining information on the most sensitive. QSAR models are typically for single species and, often, limited to a single life stage with measurements made under controlled laboratory conditions. There are limited opportunities to extrapolate to other species either directly, sometimes termed Quantitative Activity-Activity Relationships (QAARs)[71] or through the incorporation of other descriptors to help account for inter-species differences (Quantitative Structure Activity-Activity Relationships (QSAARs)).[72] These are simplistic approaches and based on nothing more than seeking correlations when comparable data are

available. However, understanding of the new technologies, big data resources and homology of receptors and physiology will make these extrapolations much more sophisticated. For instance, approaches are being made to understand species differences from the level of evolutionary biology. Whilst ambitious, with the growth, speed and reducing cost of genomic screening, this may provide valuable information supporting the modelling of MIEs etc.[73] This all-encompassing consideration of evolutionary genomics may allow for the rational expansion of (Q)SAR models across species through the combined understanding of mechanistic effects and how these are impacted by inter-species physiology and biochemical pathways.

Thus, to develop read-across and (Q)SAR approaches better, consideration should be given how to incorporate information from AOPs and when and where this information can be applied. Strategies are required to utilise the "big data" resources to facilitate mechanistic understanding to underpin prediction of toxicity within and between species; issues such as adopting best practices and ensuring transparency and open access to data have already been recognised.[6]

*QSARs for Mixtures*

Regulatory toxicology has focussed on the assessment of single chemicals, hence the data that has been provided for modelling, and the necessity for that modelling, has also been for single chemicals. There are a small number of QSARs for mixtures.[74-76] To increase uptake in this area and make the use of QSARs for mixture more widespread some investigation of the problem is required. The development of QSARs for mixtures will, in part, be driven by the endpoint. For quantitative endpoints e.g. acute lethality, there are well established principles of additivity etc. These may provide the basis for predictions of mixtures, but requires knowledge of mechanism of action. For instance, the toxicity of a mixture of chemicals known to act by non-polar narcosis can be predicted accurately, whereas adding in further mechanisms may mean the additivity is lost.[76] Alternatively, for chronic toxicities, consideration of individual components of a mixture may be required, i.e. screening of single chemicals which an overall call on toxicity. There is a clear, and as yet largely unexplored potential, to link assessment of mixtures and the involvement of QSAR to AOPs whereby the MIEs, and also networked-AOPs, could be utilised to advantage. The overall concept could be linked to some form of ecological Threshold of Toxicological Concern (eco-TTC; the use of thresholds of exposure considered to not to cause harm), where only compounds at a significant concentration need to be considered.[77-78]

To increase uptake of QSARs for mixtures, more fundamental knowledge is required about mixture toxicology, implying more data. Once these cornerstones are in place *in silico* models, including QSARs, can be developed from defined strategies. The understanding of the toxicology of mixtures should not imply *in vivo* testing, indeed the opposite, much could be derived from careful experiments using omics and other high-throughput technologies.

*Conclusions*

This article attempts to summarise briefly over 100 years of research and thinking particularly the past 50 years of concerted effort to understand and predict, from chemical structure, the effects of xenobiotics to environmental species. As such, it can only be cursory in nature, allowing for the identification of main trends and isolation of specific areas of progress and need for further work. It seems that more than ever, to create more robust *in silico* models and (Q)SARs in particular, we

need mechanistic understanding and we need to incorporate it into models wherever possible. To obtain this we need to harness the increasing data resources of test results and, in particular, the opportunities evolving from AOPs and the conversion of mechanistic information from omics into usable AOPs.

At the current time we have accurate QSAR models for the acute aquatic toxicity of non-polar narcotic compounds – the key to utilising these models is the correct and full definition of the (applicability) domain of non-polar narcosis. For specific mechanisms of aquatic toxicity, we have increasing knowledge and it may be that read-across may be more applicable than QSAR alone; identification, definition and clarification of these specific mechanisms is needed, especially with regard to elucidation of the most sensitive species. Much work, starting with the development of robust databases and mechanistic interpretation is required to develop better QSARs for chronic toxicity, a broader range of representative species and for mixtures. The key to making progress in the development of QSARs for environmental effects is to embrace, harness and utilise correctly the new technologies and frameworks providing detailed mechanistic background and inspiration can be gained from the recent report of the National Academy of Sciences.[6] In addition, correct use of QSAR predictions must be ensured either as standalone estimates or as part of more formal testing strategies.

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