Defining the role of chemical activity in environmental risk assessment within the context of mode of action: Practical guidance and advice

29-30 October 2015
Snowbird, Utah, USA

Workshop Report No. 29

Jointly organised by

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and

RIFM (Research Institute for Fragrance Materials)

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1. SUMMARY

Chemical activity has recently been promoted as a useful concept for interpreting and classifying ecotoxicological data and for performing environmental risk assessment of chemicals. The most common approach to estimate chemical activity in the aqueous phase is as the fraction of the water solubility (liquid or sub-cooled liquid, if the substance is a solid at room temperature). Accordingly, LC50s from acute and chronic aquatic toxicity tests can readily be converted to lethal chemical activities (La50s) using the appropriate water solubility. For example, La50s for baseline toxicants have been shown to cluster around a value of 0.01 for chemicals spanning a large range of hydrophobicity. While the chemical activity approach is attractive due to its apparent simplicity, it is important to recognise that there can be substantial challenges regarding the implementation of the concept as a practical environmental risk assessment tool. The goal of this Experts Workshop entitled, "Defining the role of chemical activity in environmental risk assessment: Practical guidance and advice" was thus to assess the feasibility of the chemical activity concept as a risk assessment tool, highlighting where the concept is and is not useful. The workshop was a recommendation of ECETOC Technical Report no. 120, and follows the work of Cefic LRI project ECO16.

Workshop participants concluded that there were both opportunities and challenges with respect to the chemical activity concept. The opportunities identified include:

- Chemical activity is a useful metric that can directly relate chemical exposure and toxicity more effectively than concentration, because concentrations are media-dependent while activity applies to all media, allowing exposure and toxicity to be expressed on a common basis.
- Activity provides a good metric for characterising baseline toxicity for single non-polar organic chemicals and mixtures of non-polar organic chemicals.
- Activity data is a useful metric for discriminating between baseline toxicity (MOA 1 and MOA2), which occurs at activities between 0.01 and 0.1, and excess toxicity, which occurs at activities less than 0.01.
- Activity can also be used to identify poor quality data, such as toxicity data from experiments where dosing concentrations were above the solubility of the chemical in the exposure medium, and exposure data from experiments subject to background contamination.
- The application of activity to describe the toxicity of mixtures of non-polar organic chemicals represents a novel tool in chemical risk assessment that can be particularly useful in addressing chemical risks in real world environments.

The challenges associated with advancing the chemical activity concept within environmental risk assessment include:

- Application of the chemical activity concept to chronic toxicity endpoints and reactive and specifically acting chemicals needs to be better understood. In order for relationships to advance there is a need to refine existing chemical toxicity classification schemes using a variety of tools, including the use of Adverse Outcome Pathways (AOPs) and Omics data.
- Translation from concentration to activity is crucial in studies where existing data are converted into the chemical activity space. However, this translation can be challenging and can add error to measurement error.

- Quantification of the uncertainties/error in measured or estimated water solubilities needs to be addressed, as well as clarification/guidance regarding the conversion of concentration data to chemical activity using partition coefficients.
- Improved communication of the activity concept is a major issue and will be central to future application and impact. Communication of the activity approach to a non-scientific audience may not be easy. Whether a broader acceptance of the chemical activity framework can be achieved might also be a matter of semantics.

Suggestions for future research were separated into three primary themes, (i) the chemical activity concept, (ii) application of the chemical activity approach and (iii) classification of chemicals. Given that a major challenge associated with advancing the chemical activity concept relates to its application towards chemicals with specific toxicological modes of action, a common theme between each of the primary themes was the recommendation for a need to refine existing chemical toxicity classification schemes. Suggestions for refining chemical classification include the adoption of weight-of-evidence approaches that include AOPs and omics data, in which conversion of concentration data to chemical activities may prove useful, particularly with respect to possibly helping to better understand observations of the cytotoxic-burst phenomenon reported for *in vitro* data. To help better communicate the utility of the chemical activity concept within environmental risk assessment, there is a need to further advance the effectiveness of the concept as applied to chemicals known to act as baseline toxicants. Specifically, continued efforts are needed to further demonstrate the application of the concept towards :

- Data rich chemicals, in which the conversion of concentration data to chemical activities enable information to be presented as a single ‘currency’. Such actions thus provide a basis for comparisons of data both temporally and spatially, enabling better utilisation of all existing data, and helps to facilitate the process towards assessing and managing risks.
- Activity-based species sensitivity distributions, in which toxicity tests could be conducted at controlled chemical activity in order to provide an improved estimate of actual sensitivities between species.
- Application of the chemical activity concept to interpret and estimate mixture toxicity.
- Development of an online freely available chemical activity calculator.

2. WORKSHOP OVERVIEW

2.1 Introduction

Society is facing a variety of challenges in environmental risk assessment (ERA): growing concerns about the effects of multiple stressors (both chemical and non-chemical); risks associated with exposure to complex mixtures; and demands to quantify local site-specific risks. At the same time, risk assessors are seeking to provide a more efficient framework on which to address these emerging problems and questions in a manner that reduces cost and the use of laboratory animals. This workshop assessed the applicability of using the thermodynamic chemical activity concept for organic chemicals in the interpretation of effects data within the context of environmental risk assessment.

Chemical activity and effect concentrations

The concept of thermodynamic chemical activity has been shown to be a useful approach for relating exposure to acute toxicity endpoints (Mackay *et al*., 2011; Ecetoc, 2013; Mackay *et al*., 2014; Ferguson, 1939; Mayer and Holmstrup, 2008; Smith *et al*., 2010) but can also be used to help understand the environmental fate and distribution of chemicals, analogous to the use of fugacity (Mackay 1979; Di Toro *et al*., 1991; Franco *et al*., 2011; Trapp *et al*., 2010; Reichenberg and Mayer, 2006; Mackay and Arnot, 2011).

The most common approach to estimate chemical activity in the aqueous phase is as the fraction of the water solubility (liquid or sub-cooled liquid, if the substance is a solid at room temperature), i.e.

$$a = \frac{C_W}{S_W^L} \qquad (1)$$

where C_W is the concentration of the chemical in the aqueous phase (e.g., mg/L) and S_W^L is the water solubility (liquid or sub-cooled liquid). Equation 1 thus results in a dimensionless metric of between 0 and 1, which provides a quantitative measure of the fraction of saturation in the aqueous phase observed in the environment/test system. If Raoult's law holds, activity will equal the mole fraction. Alternatively, toxicity data (e.g., LC_{50}s, EC_{50}s) can also be expressed in terms of chemical activity by replacing C_W with the selected endpoint concentration, i.e.

$$La_{50} = \frac{LC50}{S_W^L} \qquad (2)$$

Considerable effort has recently been invested towards defining the chemical activity domain of non-polar neutral organic chemicals that act as acute lethal baseline toxicants, where La_{50} values are >0.01 (Reichenberg and Mayer, 2006; Schmidt and Mayer, 2015; Mackay *et al*., 2014). Fewer studies, however, have addressed chemicals with excess toxicity, La_{50} are <0.01 (Reichenberg and Mayer, 2006; Schmidt and Mayer, 2015; Mackay *et al*., 2014). Consequently, current understanding and application of the chemical activity concept imply that the tool could be readily used to assess the environmental risk of non-polar organic chemicals that act as baseline toxicants. Additionally, by estimating chemical activity using equation 2, based on data obtained from toxicity studies, it might be possible to differentiate between baseline and excess toxicity. Excess toxicity (Te) is commonly defined as the ratio of the effect concentration that represent base line toxicity and the observed effect concentration (Lipnick et al., 1987a, b).

The chemical activity approach for toxicity illustrated in equation 2 assumes external concentrations (i.e. in water, sediment, soil, air) approximate concentrations in organisms and at the target site. For baseline toxicity this has proved beneficial in reducing variability in external effect concentrations as a result of normalising against the sub-cooled liquid water solubility of the chemical, for organic chemicals that are solids at the temperature of the test, or simply the water solubility of liquid organic chemicals. The approach thus provides a method for comparing effect concentrations for non-polar neutral organics exhibiting baseline toxicity across (1) compounds; (2) species; and (3) environmental media (Smith *et al*., 2010; Reichenberg and Mayer, 2006) Furthermore, given the additive nature associated with baseline toxicity, it is also possible to sum the chemical activities associated with mixtures of non-polar neutral organic chemicals to assess the potential risks associated with mixture exposure (Smith *et al*., 2013; Schmidt *et al*., 2013a).

Whereas there are numerous examples that relate chemical activity to acute baseline toxicity, there are limited studies that have attempted to assess the relationship between chemical activity and excess toxicity and chronic effects. Additionally, the approach has also seen limited application to miscible and ionisable organic chemicals. These limitations were identified as important data gaps within the ECETOC task force report (Ecetoc, 2013) and represent an important driver for initiating discussions aimed at addressing approaches for possibly expanding the applicability domain of chemical activity.

1. Modes of action in ecotoxicology and classification schemes

Numerous classes of compounds have specific modes of action (Lipnick *et al*., 1987a, b; Escher and Hermens, 2002; McCarty and Mackay. 1993). Several reporter gene assays are available to study specific mechanisms and modes of action, including for example genotoxicity, oxidative stress and hormonal effects (Scholz *et al*., 2013; van der Linden *et al.,* 2014). Examples of experimental research in the area of ecotoxicology include: uncouplers of the oxidative phosphorylation (Escher and Schwarzenbach, 2002), acetylcholine esterase inhibitors (de Bruijn and Hermens, 1993) and alkylating agents (reactive compounds) (Hermens, 1990) Also polar narcosis is sometimes regarded as a mode of action different from “non-polar narcosis” (Roberts and Costello, 2003; McCarty *et al*., 1992). Systematic studies into mode of action (MOA) in *in vivo* fish toxicity studies are scarce. The early work of McKim and co-workers from the US EPA based in Duluth, Minnesota, is a good example of detailed and pioneering studies into modes of action (McKim *et al*., 1987).

Classifying compounds according to their mode of action is not a trivial exercise. An example of an attempt to develop a clear classification system is the approach proposed by the US EPA Duluth laboratory (Russom et al., 1997). In their classification system a number of requirements for the assignment of a MOA to a specific compound are defined, including: (a) results from fish acute toxicity syndrome studies, (b) literature data on mechanistic studies, (c) joint toxicity data, (d) behaviour syndromes, (e) excess toxicity (Te)[1] and (f) similarity in chemical structure or chemical properties.

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[1] Excess toxicity is defined as the ratio of the predicted effect concentration for base line toxicity and the observed effect concentration.

Most other classification systems adopt simpler approaches, which are typically based on items e) and f) from the list of requirements above. Structural alerts or rules are then applied to assign a MOA to a chemical (Verhaar *et al*., 1992; Enoch *et al*., 2008). Recently there has been considerable effort in developing an adverse outcome pathway (AOP) framework, related to developing greater mechanistic insight regarding specific modes of action by studying the chain of events that occur following a molecular initiating event (MIE) with a target up to the whole organism effect level (Ankley *et al*., 2010; Russom *et al.,* 2014). It is believed that the chemical activity approach could provide a complementary approach towards an improved understanding of an AOP, as it has the potential to link exposure with the MIE in a single metric. However, defining where it is and where it isn't useful would be very helpful in effectively illustrating the added value of chemical activity. Conversely, the concepts of AOP and MIE could be very useful in classification of chemicals, which could lead to improved ability to study relationships with chemical activity.

2. Modes of action, interactions, target sites

The target site for narcosis is the cell membrane. For specifically acting compounds also, the target can be located in the cell membrane (for example a specific protein). However, the location of the target site can also be in a more aqueous environment such as the cytosol (aqueous phase inside the cell) or blood. Differences in internal distribution may also lead to a shift in the mode of action for chemicals within a certain class, for example a shift from a specific mode of action to narcosis (Freidig *et al*., 1999). Interaction of compounds with a target may vary from reversible van der Waals interactions, hydrogen bonding or irreversible covalent binding (Escher and Hermens, 2002).

3. Dose metrics, dynamic aspects and modelling

As described above, chemical activity represents a measure of effective exposure, which can also be a useful metric in toxicokinetic studies. It is notable that, for specifically acting chemicals, the dynamic aspects (interaction with a target) are additional factors that can influence the final effect concentration. Consequently the influence of exposure time on effect concentrations is often related to a kinetic parameter, and represents a non-equilibrium scenario. For specifically acting chemicals, the effect of time may also be related to toxicodynamics, for instance, in the case of an irreversible interaction of a compound with its target (Legierse et al., 1999). A simple dose related parameter may thus represent an inappropriate metric for quantifying the response in a dose-response relationship, and exposure time may be needed as an additional parameter. A nice example is a study from Gülden et al. (2010) where "area under the curve" was successfully applied in an analysis of cytotoxic potency of H2O2 in cell cultures. An additional challenge is with respect to assessing relationships between an incipient effect concentration and the concept of chemical activity. It is generally acknowledged that modelling of both the kinetic as well as the dynamic aspects will lead to a better understanding of the effects of compounds with more specific modes of action and also of differences in species sensitivity (Ashauer and Brown, 2008; Jager and Kooijman, 2005; Kretschmann et al., 2012; Nyman et al., 2014). It is anticipated that these modelling approaches may provide insight on appropriate dose parameters in ecotoxicology of compounds with modes of action other than baseline toxicity.

4. *Uncertainty in key physicochemical property data*

Although calculating chemical activity in the aqueous phase appears to be a relatively straight-forward exercise, it is important to recognise that uncertainty exists in both the toxicity data (e.g., LC_{50}s) and the water solubility data. For chemicals which are solids at the system temperature, an additional consideration is that the sub-cooled liquid water solubility is estimated from the water solubility of the solid using the Fugacity Ratio (F):

$$S_W^L = \frac{S_W^S}{F} \qquad (3)$$

A simplified approach to estimate the Fugacity Ratio (F) at 25 °C is as shown below,

$$\log F = -0.01(T_M - T) \qquad (4)$$

where T_M is the melting point of the chemical and T is the system temperature. Implicit to this simplified approach is the applicability of Walden's Rule, which states that the entropy of melting (ΔS_M) is 56.5 J/K•mol. The equation for estimating the Fugacity Ratio in a more expanded form is as follows.

$$\log F = \frac{-\Delta S_M}{2.303RT}(T_M - T) \qquad (5)$$

Consequently, an improved understanding related to the uncertainty surrounding the assumption of ΔS_M, as well as the propagation of error associated with uncertainty and variability in both the water solubility value used and the effect concentrations that have either been measured or are based on nominal concentrations, and the uncertainty associated with the melting point temperature of solids are required (Muller and Klein, 1992, Yalkowsky *et al*., 1994; Ran *et al*., 2002; Lian and Yalkowsky, 2014; Jain *et al*., 2004a; Jain and Yalkowsky, 2006, 2007; Tetko *et al*., 2014). A greater appreciation of the influence of uncertainty is believed to be useful in helping to align chemical activity values to specific modes of action.

5. *Estimating chemical activity in non-aqueous media (i.e. biota)*

Because acute toxicity data may be reported in terms of internal concentration (i.e., critical body burden) and non-equilibrium conditions may necessitate the estimation of internal concentrations and chemical activity in biota, the reliability of methods to calculate chemical activity in non-aqueous phases also requires careful consideration. Mackay *et al*. (2011) suggest that chemical activity in non-aqueous phases can be calculated in an analogous fashion to aqueous phases, i.e.,

$$La_{50} = \frac{CBR}{S_{\mathbf{B}}^L} \qquad (6)$$

where CBR is the Critical Body Residue (i.e., internal LC_{50}) and S_B^L is the solubility of the chemical in the organism, which is estimated as:

$$S_B^L = K_{BW} S_W^L$$

where K_{BW} is the biota-water partition coefficient. Adding to the uncertainty discussed above, are thus the challenges associated with assessing the uncertainties aligned with equation 6. Alternatively, the internal lethal (or effect) concentration can also be estimated from external LC_{50} using toxicokinetic (TK) models. Given the various methods that can be used in obtaining chemical activity, guidance is thus needed if the concept is to be routinely and transparently applied within a risk assessment framework.

6. *Feasibility of applying the chemical activity concept to miscible organic chemicals (MOCs)*

Whereas the concept of chemical activity has been widely applied to non-polar neutral organics, a key challenge is assessing how to apply the concept to very hydrophilic chemicals (i.e., organic chemicals that are miscible at the temperature of the system of interest). In the case of MOCs there is no quantifiable limit to the solubility of the chemical in water. 'Empirical' water solubilities of miscible chemicals (e.g., methanol, ethanol, acetone), however, may still be reported, for instance, as 10^6 mg/L in the EPISUITE database.

For neutral organic chemicals, chemical activity can be calculated using the following alternative expression:

$$a = \chi_i \gamma_W \tag{7}$$

where χ_i is the concentration of the chemical in the aqueous phase expressed as a mole fraction and γ_W is the (dimensionless) activity coefficient of the chemical in water (at the given mole fraction). γ_W for neutral organic chemicals span multiple orders of magnitude, which follows from the inverse relationship between the activity coefficient and water solubility. For example, the activity coefficients at infinite dilution for 1-butanol and benzo(a)pyrene are 50 and 10^8 respectively (Schwarzenbach *et al.*, 2003). For more hydrophobic chemicals, γ_W in dilute solution and at saturation are typically within 30% and any concentration-dependence of the activity coefficient can be ignored (Schwarzenbach *et al.*, 2003). For more hydrophilic (miscible) compounds, γ_W will exhibit a stronger concentration-dependence. However, as per the definition of γ_W, γ_W tends towards a value of 1 as the mole fraction of the chemical in aqueous solution increases.

There are numerous experimental techniques available for measuring γ_W at infinite dilution and empirical data are available for some miscible chemicals e.g., γ_W = 1.6, 3.7 and 7.0 for methanol, ethanol and acetone respectively (Schwarzenbach *et al.*, 2003). As both the upper bound (γ_W at infinite dilution) and lower bound ($\gamma_W = 1$) is known for these miscible chemicals, LC_{50}s can be expressed using chemical activity (i.e., converted to Ea_{50}s), at least as a bounded first approximation, i.e.,

$$Ea_{50} = \chi_i (LC50) \gamma_{W,\inf} \tag{8}$$

where $\chi_i(LC_{50})$ is the LC_{50} expressed as a mole fraction. Equation 8 thus represents a possible approach to enable an estimate of chemical activity in relation to an effect concentration. Further assessment, however, is required to better understand the feasibility of the approach and to quantify uncertainties and define potential limitation (Yalkowsky *et al.*, 1994; Lian and Yalkowsky, 2014; Schwarzenbach *et al.*, 2003; Sherman *et al.*, 1996; Brockbank *et al.*, 2013; Ingram *et al.*, 2011; Hilal *et al.*, 2004).

7. Feasibility of applying the chemical activity concept to ionisable organic chemicals (IOCs)

The applicability of the chemical activity concept to ionisable organic chemicals (IOCs) represents an additional challenge. Firstly, the water solubility of IOCs is a function of the solubility of the compound and the degree of ionisation (He and Yalkowsky, 2004; Jain *et al*., 2006) where the degree of ionisation is a function of pH and pK_a. The type and concentration of counterion(s) present in solution are also considerations for determining the apparent solubility limit (Chowan, 1978; Serajuddin, 2007). Even if the apparent water solubility can be estimated, the relevance of using this estimate in chemical activity calculations (such as Equation 1) has not yet been fully addressed. While methods to estimate activity coefficients for IOCs have been proposed (Franco and Trapp, 2010), it is unclear if these approaches are congruent with the methods for neutral organic chemicals and hence can be used in the same manner (i.e. Equation 7). IOCs also exhibit different partitioning behaviour compared to neutral organic chemicals (Avdeef *et al*., 1998; Escher *et al*., 2000a,b; Armitage *et al*., 2013) complicating the estimation of the biota-water partition coefficient (or more appropriately, the biota-water distribution ratio, D_{BW}). Furthermore, in addition to challenges associated with estimating a chemical activity for IOCs, there is the complexity associated with aligning a derived chemical activity to an effect concentration. The key challenge here is that IOCs, examples of which include active pharmaceutical ingredients and pesticides, are unlikely to act strictly as baseline toxicants, but will have one or more specific modes of action.

2.2 Workshop structure

The 2-day workshop began with in an introductory session designed to stimulate participant understanding of chemical activity by presentation of material covering the fundamental thermodynamic principles that form the basis of the concept, as well as a brief introduction of the outline of each work group's topic. Initial refinement and expansion of work group questions resulted from this introductory session.

The remainder of the workshop was structured around the three work groups (WGs) using a series of key questions to focus discussion. Between 10-14 experts were assigned to each specific work group. Activities in the WGs included presentations, substantial group discussion and writing periods and regular plenaries to update participants on progress and changes that occurred in topic development.

Workshop participants were invited based on their expertise in areas such as environmental toxicology, environmental chemistry, and environmental risk assessment. Thirty-eight individuals participated, representing academia, government, and industry. The workshop was held prior to the SETAC North America meeting in Salt Lake City, 29-30 October, 2015, at the Snowbird Resort in Utah.

2.3 Workshop aims and objectives

The aim of the workshop was to assess the applicability of using chemical activity in the interpretation of effects data within the context of environmental risk assessment, with an emphasis on the following key themes:

- To demonstrate the feasibility of using the chemical activity concept as an environmental risk assessment tool for neutral organic chemicals classified as baseline toxicants, including non polar and polar compounds.
- To determine the extent of chemical and toxicological domain for the use of the chemical activity concept as it is applied to polar and non-polar narcotics and to compounds with modes of action beyond narcosis and baseline toxicity for both acute and chronic ecotoxicological effects.
- To explore alternative methods for classifying the toxicological mode of action for chemicals, including the role of adverse outcome pathways in classification, to explore alternative dose metrics, and to assess the role of chemical activity as a potential complementary approach.
- To address issues of uncertainty and applicability domain with respect to physicochemical properties of organic chemicals, including miscible and ionisable organic chemicals, in applying the chemical activity concept to environmental risk assessment.

This workshop was a recommendation of ECETOC Technical Report no. 120 and follows the work of Cefic LRI project ECO16.

3. PRESENTATION SUMMARIES

3.1 Foundational aspects of the concept of chemical activity

Philipp Mayer
DTU-Environment, Technical University of Denmark, Denmark

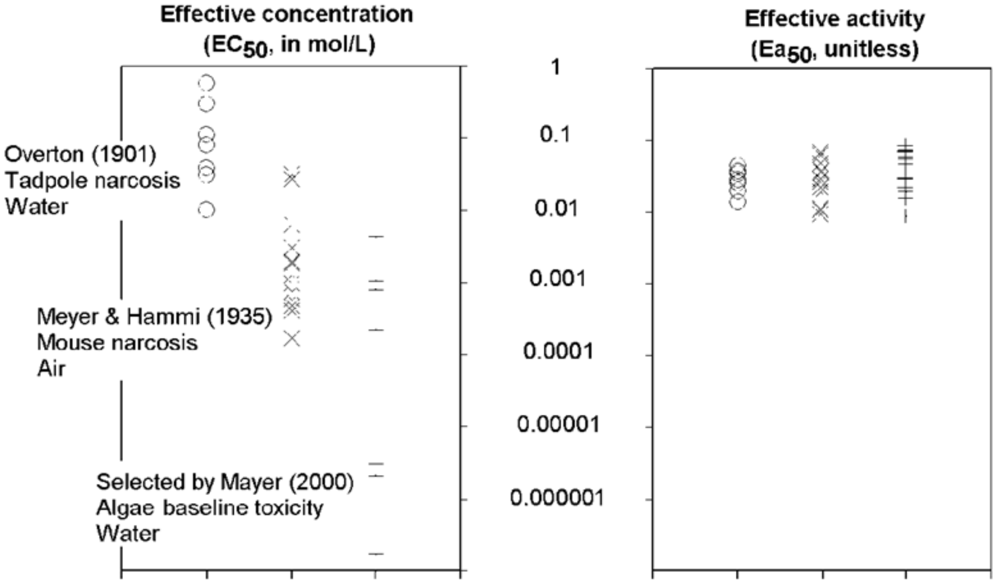
The chemical activity of an organic chemical quantifies its potential for spontaneous physicochemical processes, such as diffusion, sorption, and partitioning. For instance, the chemical activity of a sediment contaminant determines its equilibrium partitioning concentration in sediment-dwelling organisms and differences in chemical activity determine the direction and extent of diffusion between environmental compartments (Di Toro *et al*., 1991; Reichenberg and Mayer, 2006). This makes chemical activity a meaningful and well-defined exposure parameter that is closely linked to fugacity and freely dissolved concentration (Reichenberg and Mayer, 2006). The conversion of published toxicity data (i.e. effective concentrations, EC50) to chemical activity space (i.e. effective chemical activities, Ea50) provided the first indication that narcosis for different chemicals, organisms and exposure media occurs within a relatively narrow range of chemical activity (Reichenberg and Mayer, 2006; Meyer, 1899; Overton, 1901; Ferguson, 1939; Mayer and Holmstrup, 2008) (Figure 3.1.1). During the last 10 years several studies have confirmed this for the "baseline toxicity" of non-polar organic chemicals and their mixtures (Mayer and Holmstrup, 2008; Mackay *et al*., 2011; Smith *et al*., 2013; Thomas *et al*., 2015).

The first part of the presentation emphasised the physical meaning of chemical activity, by clarifying that "mass concentration" and "chemical activity" are two complementary dimensions for chemicals in the environment, exactly like we know from other areas (heat content versus temperature; water content versus water activity). The second part focused on how chemical activity can be measured and controlled in environmental research and testing, which has the very important implication that chemical activity goes beyond modelling and re-calculations of mass based data. The third part summarised recent experimental studies that link toxicity to chemical activity.

This presentation initiated the discussions at the workshop of why and how chemical activity can help to:

1. predict baseline toxicity,
2. link exposure between media,
3. compare sensitivities between species,
4. assess excess toxicity of chemicals and
5. add up the exposure for mixtures?

Figure 3.1.1. Three series of toxicity data with different organisms, compounds, and means of administration plotted as the median effective concentration (EC50) and the median effective activity (Ea50). The EC50 values span several orders of magnitude, whereas the Ea50 values are relatively constant for a wide range of agents, targets, and routes of exposure (Figure taken from Reichenberg and Mayer, 2006).



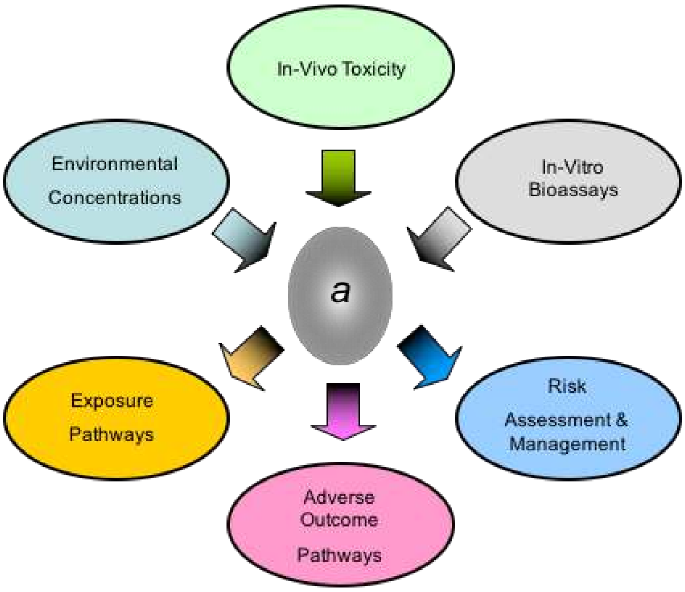
3.2 Application of the Activity Approach

Frank A.P.C. Gobas[1], **S. Victoria Otton**[2], **Laura F. Tupper-Ring**[3] **and Meara A. Crawford**[4]
[1]School of Resource and Environmental Management, Simon Fraser University, Canada, [2] Simon Fraser University, Canada, [3] Simon Fraser University, Canada, [4] Independent consultant, Canada

Thermodynamic activity (also frequently referred to as chemical activity) was first proposed by Lewis (1901, 1907) and has been successfully applied in many areas of science, engineering and medicine to better understand and describe the behaviour of chemical substances and pharmaceutical drugs. In the field of environmental chemistry and toxicology. Thermodynamic activity has also been put forward as a theoretically well founded and practical metric for studying and managing environmental contaminants (Mackay and Arnot, 2011). Thermodynamic activity is a multimedia exposure parameter that provides a metric for comparing, relating and combining exposure and toxicity data from various sources, which is useful for environmental risk assessment and management.

The thermodynamic activity approach can be defined as the expression of chemical exposure and toxicity data in terms of thermodynamic activity with the purpose to (i) study and assess the distribution or fate of chemicals in the environment; (ii) to study and assess the toxicity and modes of toxic action of chemicals; and (iii) to conduct risk assessment and management of chemicals at specific locations and in the general environment (Figure 3.2.1).

Figure 3.2.1: Conceptual diagram illustrating the activity approach as a method for expressing chemical exposure and toxicity data in terms of thermodynamic activity with the purpose to (i) establish exposure pathways of chemicals in the environment; (ii) to study and assess the toxicity and modes of toxic action of chemicals; and (iii) to conduct risk assessment and management of chemicals at specific locations and in the general environment.



While the activity approach has been used for many years in environmental chemistry and toxicology to study the environmental distribution and toxicity of chemicals, it has rarely been used to make management and regulatory decisions. The only documented example to date where the application of thermodynamic activity played a role in environmental decision making was a relatively recent decision by Environment

Canada to reverse its original assessment of the chemical Decamethylcyclopentasiloxane (D5) from toxic to non-toxic within the context of the Canadian Environmental Protection Act (Gobas *et al*., 2015).

Figure 3.2.2 activities (unitless) in waste water treatment plant (WWTP) effluents, ambient water, ambient sediment, plankton, invertebrates, fish, birds, terrestrial mammals and marine mammals from different locations in the Northern hemisphere (black filled circles) in relation to the maximum activity (a=1, red line) and various NOECs (Gobas et al., 2015) in sediment and soil dwelling invertebrate and plant species at 25oC (dashed grey lines).

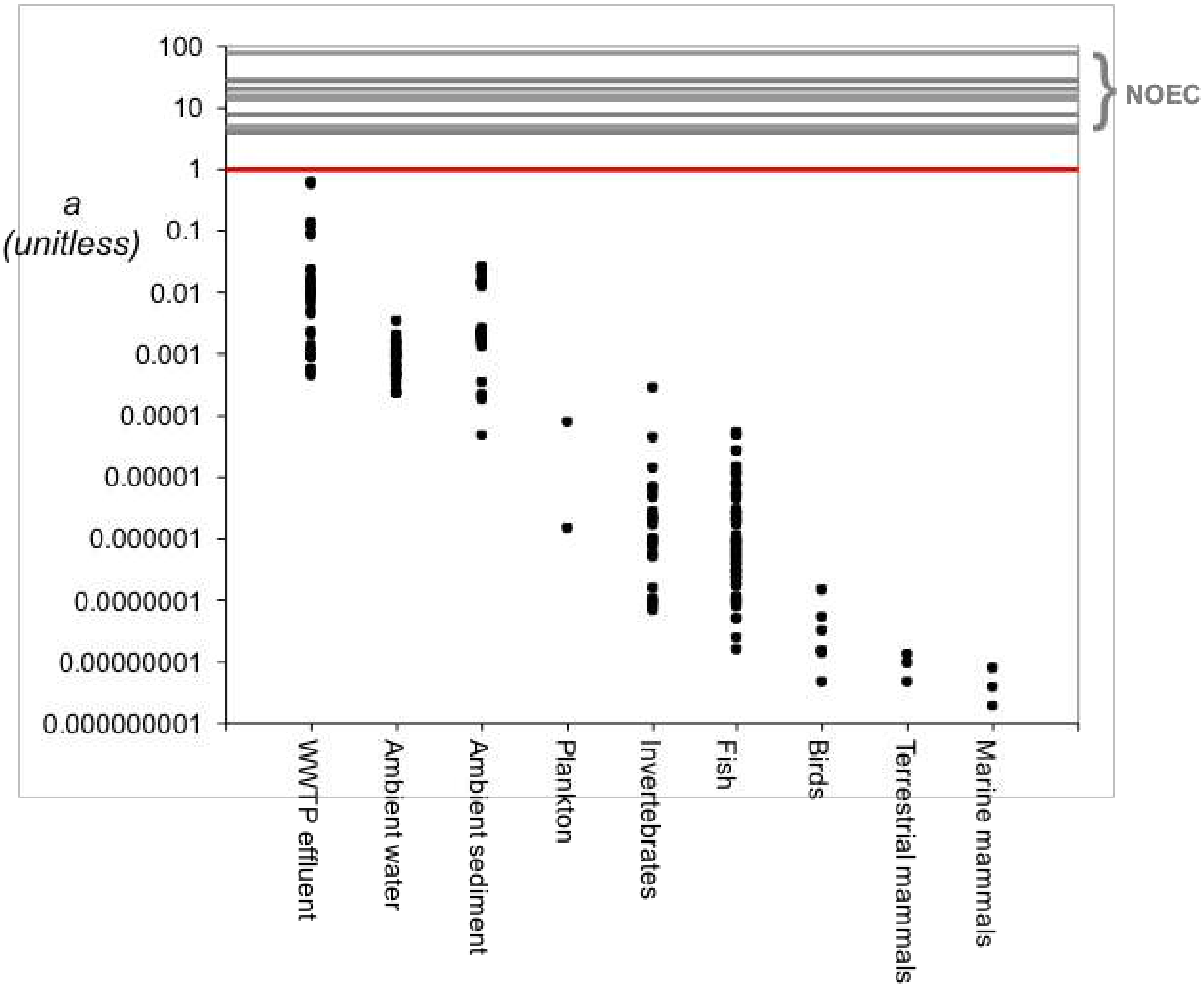


Figure 3.2.2, which summarises the activity based analysis of D5, shows all environmental concentration and toxicity data, expressed in terms of the dimensionless thermodynamic chemical activity for D5 in a single plot. Figure 3.2.2 illustrates that activities of D5 in the environment range by orders of magnitude and are always less than 1, which is the maximum activity that a liquid chemical like D5 can achieve in the environment. In contrast, activities of D5 associated with reported no-effect concentrations in toxicity tests are well above 1. Activities above 1 cannot occur in the environment and are most likely the result of

experimental artefacts in the toxicity experiments due to dosing at concentrations above D5's solubility in water and/or sorptive capacity in sediments and soils.

The activity approach has also been applied to the environmental risk assessment of the plasticiser di-ethyl-hexyl-phthalate ester or DEHP and mixtures of phthalate esters. This risk assessment illustrates the application of activity to include large numbers of field based concentrations of phthalate esters in various environmental media and data from *in-vivo* and *in-vitro* toxicity studies in a comprehensive risk assessment (Tupper-Ring, 2015).

There are many more possible applications of the thermodynamic activity approach. Thermodynamic activity can be used to develop environmental quality guidelines or criteria because of its ability to express medium specific environmental criteria in terms of generally applicable thermodynamic activities. Thermodynamic activity is also a useful method in green chemical design for establishing a maximum ceiling above which a chemical's thermodynamic activity and corresponding concentrations in the environment cannot go. If the maximum activity of a chemical falls below the apparent toxicity threshold, the chemical (on its own, i.e. in absence of other chemicals with a similar mode of toxic action) cannot exert known toxicity. Activity is also useful for monitoring of risks in the environment (Booij and Smedes 2010; Jahnke *et al*., 2012, 2014). For example, monitoring data from passive samplers can be expressed in terms of thermodynamic activity and then compared to toxicological data also expressed in terms of thermodynamic activity. Thermodynamic activity can also play an important role in characterising the toxicity of mixtures of chemicals (Reichenberg and Mayer. 2006).

In conclusion, the application of the thermodynamic activity approach provides many opportunities to advance the study and management of chemicals in the environment. The approach complements the adverse outcome pathway analysis by providing a dose metric to link response levels from the initiation event to the whole organism level.

3.3 Modes of action in ecotoxicology: classification, chemical activity and dose metrics

Joop Hermens
Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

The chemical activity concept is a promising approach in the understanding and prediction of ecotoxicological effect concentrations of organic contaminants. This concept has successfully been applied to compounds that are known to act via non-polar narcosis (Reichenberg and Mayer, 2006). Application of this concept to compounds beyond non-polar narcosis to other modes of action (MOA) is not yet explored. It is believed that the chemical activity approach could provide a complementary approach towards an improved understanding of an Adverse Outcome Pathway (AOP), as it has the potential to link exposure with the Molecular Initiating Event (MIE) in a single metric.

Different systems have been developed to classify chemicals according to their mode of action (MOA) (Russom *et al*., 1997; Verhaar *et al*., 1992). In this presentation, the focus was on the Verhaar classification system that distinguishes four MOA classes:

MOA 1, non-polar narcosis,
MOA 2, polar narcosis,
MOA 3, modes of actions related to reactive chemicals and
MOA 4, specific modes of action.

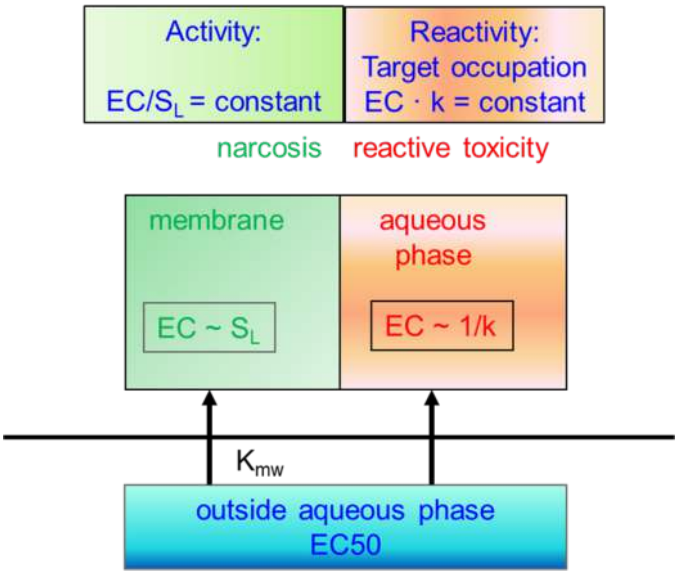
Chemical activities for effect data of compounds with other modes of action than narcosis are scarce. There are a few publications with initial analyses of fathead minnow LC50 data (Mackay *et al*., 2011, 2014). In this presentation, the Utrecht guppy LC50 data set was used to illustrate how chemical activity can be applied in the analysis of acute fish toxicity data. Chemical activity was calculated from the LC50 data and the estimated aqueous solubility. The following observations were presented and discussed:

- Chemical activities for MOA 1 compounds are in the range known for narcosis and the variability in activities is strongly reduced in comparison with aqueous effect concentrations.
- Activities for MOA 2 are slightly lower.
- Chemical activities of MOA 3 and 4 compounds cover a broad range and vary between 10-1 and 10-6.

While chemical activities of MOA 3 and 4 are often substantially lower than of MOA 1 chemicals, the more hydrophobic compounds tend to have activities close to values for MOA 1 compounds. An example with acrylates was discussed in more detail at the workshop based on data analysed by Freidig *et al*. (1999) This example was also used to show that information about the target site and target site environment is important in the analysis of effect data. The same example also shows that chemicals from one particular class may have multiple MOA’s. MOA 3 chemicals will also act via narcosis and which MOA dominates depends on the internal distribution within an organism or within a cell.

The examples presented show that plotting data as chemical activity represents a useful tool for potentially interpreting toxicity data. It easily shows baseline- and excess toxicity for MOA 3 and 4. For modelling effects of MOA 3 and 4 chemicals, more complex toxicokinetic and dynamic (TKTD) approaches (Ashauer *et al*., 2015; Jager *et al*., 2011) are essential to obtain a better understanding of effect data.

Figure 3.3.1. Two modes of action of reactive chemicals: narcosis and "reactive toxicity. These two modes of action can be active at the same time. EC is effect concentration, SL is the subcooled liquid solubility, Kmw is the membrane-water partition coefficients and k is a rate constant of chemical with a nucleophile



3.4 Challenges and potential limitations: physicochemical properties

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Chemical activity (*a*), as it has been developed for neutral organic chemicals, is commonly estimated as the fraction of water solubility (liquid or sub-cooled liquid, if the chemical is a solid at room temperature): i.e.

$$a = \frac{C_W}{S_W^L} \qquad (9)$$

where C_W is the concentration of the chemical in the aqueous phase (e.g., mg/L) and S_W is the water solubility (liquid or sub-cooled liquid for organic chemicals that are solids at the temperature of the test system). Equation 1 thus results in a dimensionless metric of between 0 and 1, which effectively provides a ratio of the energetic level in the aqueous phase observed in the environment/test system relative to the energetic level at the limit of solubility in water. Although estimating chemical activity using equation 1 is a relatively straight-forward exercise, it is important to acknowledge that uncertainty exists within both the numerator and the denominator. For instance, depending on how C_W is derived it can include analytical error, associated with empirical methods, propagation of an error if estimated using an environmental fate model, or uncertainty related to the accuracy of a nominal concentration used in a toxicological test. Similarly S_W will also have inherent uncertainty associated with values derived either empirically or estimated using a quantitative structure property relationship (QSPR).

The emphasis of this presentation was to highlight the influence that uncertainty in S_W can have on estimates of chemical activity, which is meant to help stimulate discussion within the work group and focus activities towards approaches that could be used to help quantify the relative magnitude of error in S_W.

In an attempt to provide preliminary insight regarding the variance that might exist in water solubility measurements, 233 chemicals reported by Mackay *et al*. (2006) were assessed with respect to their availability of solubility data. A graphical plot of the results is shown in Figure 3.4.1, which summarises 2440 solubility measurements for the 233 chemicals. The dataset reported by Mackay *et al*. (2006) are believed to provide a relatively good indication of the variance that might exist in empirically derived solubility data, with the majority of chemicals having >10 separate solubility measurements. A general observation from Figure 3.4.1 is that as solubility decreases the relative magnitude of the uncertainty increases, thus implying caution when relying on a limited number of solubility measurements for relatively insoluble organic chemicals (i.e. <0.01 mg/L).

Figure 3.4.1. Variability in reported water solubility measurements for 233 neutral organic chemicals represented as box and whisker plots for each individual chemical (primary axis)compiled from Mackay et al. (2006).

Green boxes represent the upper 25th percentile of data that are above the median value, whereas the orange boxes represent the lower 25th percentile of data that are below the median value. The maximum and minimum values are represented as the end of the whiskers above and below the median value, respectively. The number of data points included for each chemical plotted in the box and whisker plots are plotted as bars in relation to the secondary axis.

QSPRs used to estimate water solubility can also introduce different levels of uncertainty depending on the relative performance of the estimation method in relation to the chemical under investigation. Specifically, where test chemicals have properties that differ from those used in deriving the QSPR, it can be demonstrated that a higher level of uncertainty will undoubtedly be associated with the estimated value. Consequently, the use of estimated values of S_W will require greater insight with respect to the performance of an individual QSPR in relation to the chemical being assessed. For organic chemicals that are solids at environmentally relevant temperatures, additional caution is warranted, particularly with respect to the manner by which melting point data are obtained and assumptions associated with Walden's Rule in relation to the assumption that ΔS_M = 56.5 J/mol K, when estimating fugacity ratios and deriving the sub-cooled liquid solubility of the chemical.

Lastly, the chemical activity concept has been demonstrated to work well for neutral organic chemicals, however, challenges currently exist with respect to applying equation 1 to miscible organic chemicals which do not have a quantifiable S_W, and to ionisable organic chemicals, for which deriving S_W may be problematic depending on the extent of ionisation that may exist, and which is a function of pH, ionic strength and on the influence of counter ions that may be present.

4. SYNDICATE SESSIONS

4.1 Syndicate Session 1: Full utilisation of the chemical activity concept for non-polar organic chemicals (Log $K_{OW} \geq 2$)

Participants

Moderator:	P. Mayer
Moderator:	F. Gobas
Rapporteur:	A. Jahnke
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	J. Appel
	T. Henry
	D. Knapen
	L. McCarty
	T. Parkerton
	S. Nørgaard Schmidt
	F. Smedes
	J. Tunkel
	D. van den Meent

Introduction

Thermodynamic activity (also frequently referred to as chemical activity) was first proposed by Lewis (1901) and has been successfully applied in many areas of science, engineering and medicine to better understand the behaviour of chemical substances and pharmaceutical drugs. In the field of environmental chemistry and toxicology, the activity approach has also been put forward as a theoretically well founded and practical approach to study and manage environmental contaminants (Di Toro *et al.*, 1991; Reichenberg and Mayer, 2006; Mackay and Arnot, 2011). Chemical activity is a multimedia exposure parameter, which provides a metric for comparing, relating and combining exposure and toxicity data from various sources, which represents a potentially useful tool for environmental risk assessment and management.

The overall objective of WG1 was to explore the practical utilisation of the chemical activity concept for non-polar (i.e., neutral) organic chemicals in environmental risk assessment. Non-polar organic chemicals with a log $K_{OW} \geq 2$, were the focus of the discussions, because the concept of chemical activity has been largely developed for organic chemicals with these properties. Hence, any potential application of the activity approach in environmental assessment and management is most feasible and likely for non-polar organic chemicals with a log $K_{OW} \geq 2$, as this represents the current understanding of the domain of applicability. The chemical activity of non-polar organic chemicals can both be measured and calculated. Non-polar organic chemicals often only exert baseline toxicity, which has been demonstrated to initiate in a well-defined and narrow range of chemical activities, of between 0.01-0.1, thus providing the potential for a novel approach for conducting risk assessments. This rather narrow activity range related to the initiation of baseline toxicity for non-polar organic chemical also provides a basis for the assessment of mixtures of non-polar organic chemicals, whereby the chemical activity of each of the individual components of a mixture can be summed.

The assessment of the effects of chemical mixtures has been a long-term goal in the field of environmental toxicology, and therefore the relationship between mixture effects for non-polar baseline toxicants and chemical activity provides a potentially novel method for interpreting the toxicity of mixtures.

In an effort to help shape the discussion within the workgroup, participants of WG1 presented their own individual research commenting on:

1. Current or potential applications of thermodynamic activity in their area of research and/or management of the environmental risks of commercial chemicals.
2. Actual or potential merits of the application of thermodynamic activity in their area of research and/or management.
3. Possible limitations of the application of thermodynamic activity in their area of research and/or management.
4. Areas for further research to advance the application of thermodynamic activity in their area of research and/or management.

The purpose of the presentations, questions and subsequent discussions was to address the potential application of the activity approach in environmental risk assessment and management.

Summary of presentations and the resulting discussions:

Each of the presentations given during the WG1 session, and summarised here, led to stimulating discussions, which were useful in challenging the limits of applying the chemical activity approach in risk assessment and management of chemicals, as well as emphasising where the approach is to be most useful.

The role of passive dosing in ecotoxicological test studies for non-polar organic chemicals with log $K_{OW} \geq 2$ was presented, in which it was demonstrated how baseline toxicity is well related to chemical activity, and that the absence or presence of baseline toxicity for solid chemicals depends on their maximum chemical activity. The data presented demonstrated how baseline toxicity of individual PAHs was initiated at chemical activities between 0.01-0.1 (Schmidt *et al*., 2013b) and further showed that effects caused by mixtures of two to three PAHs could be plotted on a single “sum activity” (∑a) response curve, indicating the same activity range (Schmidt *et al*., 2013a). Two additional studies were presented that:

1. demonstrated how mixtures of individually non-toxic PAHs could cause toxic effects (Smith *et al*., 2013)
2. assessed the influence of combining stressors, i.e., chemical and drought stress (Schmidt *et al*., 2014).

Lastly, recent research involving pyrethroids, which were shown to exhibit toxicity at much lower activities, gave strong evidence of the excess toxicity related to the exposure of this group of chemicals. Data mining activities presented at SETAC Salt Lake City were also discussed that partly covered an algal growth inhibition study (Schmidt and Mayer, 2015) and partly included additional work by Christensen *et al*., 2009.

Additional experimental evidence of baseline toxicity occurring between 0.01 and 0.1 for PAHs and chlorinated benzenes was also presented and discussed (Tcaciuc, 2015). In an effort to explore the use of the chemical activity concept for complex real-world mixtures, seven contaminated sediments were equilibrated with polyethylene for use in further toxicity experiments. For these seven sediments, the extracts from the equilibrated polyethylene were run on a GCxGC-FID to separate the compounds based on boiling point and polarity. Using the unidentified chemicals' positions in GCxGC space, estimates of partitioning into lipids (and therefore the potential for baseline toxicity) were calculated and this information was then used for predictions of baseline toxicity of mixtures. Passive dosing experiments using *Daphnia magna* confirmed that this method predicted baseline toxicity for six of the sediments with the seventh sediment showing signs of excess toxicity (40% immobilisation of *D. magna* instead of the <5% predicted) (Tcaciuc, 2015).

As a further illustration of the application of the chemical activity concept, its use in interpreting data from various environmental media was presented and discussed. Environmental media that could be characterised as being rich in "transporter agents", and which effectively facilitate equilibration, i.e., sediment rich in organic carbon (Mäenpää *et al*., 2011; Jahnke *et al*., 2012) and lipid-rich biota (Jahnke *et al*., 2009), enabled the measurement of equilibrium partitioning data and comparison of measured activity ratios using polymer "chemometers" (Jahnke *et al*., 2014). Conversely, passive sampling in environmental media such as water, typically requires an extrapolation to equilibrium, which can be done based on the loss of performance reference compounds spiked before sampling is initiated (Booij and Smedes, 2010; Rusina *et al*., 2010).

The relationship between chemical activity and the adverse outcome pathway for baseline toxicity was also discussed. Due to non-specific interactions in relation to the molecular initiating event, a complete adverse outcome pathway (AOP) for baseline toxicity with causal relationships of high scientific confidence has been difficult to delineate. At least two different putative pathways, mainly focusing on apical endpoints, have been described in the literature. An important topic for debate focused on which cellular membranes are being targeted by baseline toxicants. Given the fact that membranes are dynamic structures incorporating many different regulatory processes, it is reasonable to assume that different membrane types are capable of reacting differently to the presence of baseline toxicants, leading to potential specific effects on membrane-bound processes (e.g., electron transport chain). While the chemical activity range of 0.1 to 0.01 for acute baseline toxicity (i.e., mortality) has been established with a relatively high level of confidence, a high level of uncertainty remains with respect to sub lethal effects. Since organisms are capable of activating compensatory mechanisms at lower exposure levels – before the onset of system breakdown and failure – organelle membrane-specific processes could possibly cause sub lethal effects to be significantly different among organelles, tissues and biological species, at different chemical activities. The relationship between baseline toxicants and chemical activity for chronic effects thus represents a key data gap that needs to be addressed in order to better assess the utility of the chemical activity in risk assessing chemicals where exposure is characterised by a steady-state emission at low concentrations.

The group briefly discussed reactive chemicals and noted that this could be an area where the chemical activity concept could help in the development of better predictive tools. Discussion was supported by reference to a study reporting on the toxicity of a series of alkyllactates to daphnia, algae and fish (Bowmer *et al*., 1998), which discussed potential modes of action for this group of chemicals across different species. It was emphasised that the ecotoxicity profiles of many chemical groups with a specific mode of action may differ greatly, e.g. triazine herbicides which inhibit photosynthesis have severe effects on plants including green algae - while unsurprisingly for fish and crustaceans their effects are in line with baseline toxicity (see Solomon and Cooper, 2008) but there are more complex examples. Esters such as the alkyllactates hydrolyse readily to a polar alcohol, and a reactive lactic acid molecule in the cells of an organism. However, different toxicity responses across algae, crustaceans and plants are known to occur as a result of exposure to this class of chemicals. Mode of action is thus not so much a property of the chemical but of its (complex) interaction with a species. In the regulatory context, there is a role for novel tools in predicting aquatic toxicity, and it was suggested that chemical activity may have a complementary role to play. However, it was pointed out that such tools needed to be robust, validated, and well packaged prior to being implemented into existing hazard and risk assessment frameworks, as further emphasised below.

A compilation of available acute toxicity data for zebrafish for hydrocarbons, alcohols, ethers, ketones and chlorinated solvents was also presented and discussed. LC_{50} data were expressed in terms of activity and found to occur at a value of > 0.01, further supporting the relationship between chemical activity and non-polar organic chemicals that act as acute baseline toxicants. Recent toxicity test results obtained using passive dosing yielded Ea_{50} data that were less variable than earlier literature data. Available chronic toxicity data across a range of fish species focusing largely on embryo-larval survival and larval growth effect endpoints when expressed in terms of activity fell above 0.001. A positive trend was also noted between the activity corresponding to chronic effects in fish and the log K_{OW} of the test substance. An increasing number of substances with log K_{OW} > 6 were observed not to be chronically toxic at activities > 0.01. Furthermore, experiments were shown to assess the potential extension of the chemical activity concept to complex petroleum substances. The results reported chemical activities for acute effects data for 11 organisms including fish, invertebrate and algal test species ranging from 0.06 to 0.39 and three chronic studies with daphnia, trout and algae approximately an order of magnitude lower falling between an estimated activity of 0.01 and 0.05. These results suggested that no. 2 fuel oil exhibits chronic effects at chemical activities that are generally consistent with that observed for acute baseline toxicity.

The group discussed whether chemical activities should preferably be measured in various media and toxicity tests conducted at controlled activities, or whether it would be sufficient to translate existing monitoring and toxicity data into the chemical activity space. There was consensus that while directly measured data generally are preferable, a translation of existing data can also lead to enhanced understanding on the thermodynamic controls of the environmental fate and toxicity of environmental pollutants.

Discussions based on the following questions which had been distributed in advance to the workshop participants are outlined below.

1. *Can 0.01 (i.e., 1 % of liquid solubility) be used as a chemical activity benchmark to distinguish baseline toxicity and excess toxicity?*
2. *Could the observation of non-toxicity at chemical activity of 1 (100 % of liquid solubility) be used for categorising a chemical as being non-toxic?*
3. *Is it possible and meaningful to set a general predicted no-effect activity (PNEA) for baseline toxicity?*
4. *Is it possible and meaningful to set a general predicted no-effect activity (PNEA) for mixtures with regards to baseline toxicity?*
5. *Is it scientifically correct to assess the sorptive capacity or “solubility” of neutral hydrophobic organic chemicals in non-aqueous phases as the product of the non-aqueous-water partition coefficient and the aqueous solubility?*
6. *What are the inherent assumptions in a comparison of activities of neutral organic chemicals among various environmental media?*
7. *Is it possible and meaningful to include an activity framework in AOP analysis?*

8. *What are the low-hanging fruits for the application of the activity approach?*

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1. *Can 0.01 (i.e., 1 % of liquid solubility) be used as a chemical activity benchmark to distinguish baseline toxicity and excess toxicity?*

The group agreed that the available evidence in the scientific literature with respect to this question provides compelling evidence that this is indeed the case. While the approach in itself is valid, the exact number may have to be adjusted, e.g., for instance better characterisation is needed between acute versus chronic baseline toxicity. For chronic toxicity, the threshold is expected to be lower than for acute toxicity. Establishing and using a chemical activity threshold, such as at 0.01, is seen as a good starting point. For instance, in relation to assessing the potential risks to the exposure of complex mixtures in the environment, baseline toxicity may be the most critical issue due to the additivity of the activity of hundreds of compounds present at low concentrations. Setting an activity of 0.01 is a useful way to distinguish between baseline and excess toxicity, i.e., if effects occur at activities below the threshold, other modes of toxic action may be implied. The use of a chemical activity threshold value does not, however, allow for conclusions about which mode of action other than baseline toxicity occurs.

From a regulatory perspective, the suggested threshold was considered useful for screening and prioritisation of chemicals that are subject to risk assessment. Another advantage mentioned was that the activity concept offered a way to avoid or reduce animal testing. However, the group emphasised the importance of defining careful rules regarding the applicability domain of the chemical activity concept, since the approach is currently limited to chemical effects associated with baseline toxicity of parent compounds and their mixtures, while it does not include various types of excess toxicity nor the effect of metabolites and other degradation products.

2. *Could the observation of non-toxicity at chemical activity of 1 (100 % of liquid solubility) be used for categorising a chemical as being non-toxic?*

The group agreed that this kind of concept already was contained in regulatory documents. However, it needs to be more carefully framed, e.g., rather than ”non-toxic” discussing ”apparently non-toxic” chemicals. While the concept in itself might be useful, the question of how to apply it was discussed.

WG1 participants agreed that the statement was generally acceptable, but that it needs to be qualified. Issues that were identified in this context that need particular awareness were the following:

- kinetics, in particular in acute toxicity testing
- diversity in physicochemical properties
- that the concept is limited to the parent compound toxicity
- levels above solubility that might occur in the environment

Another aspect that was addressed in the context of this question was how the chemical activity concept provides a stronger scientific approach for interpreting chemicals that may apparently be non-toxic, which represents an improvement relative to the use of an arbitrary toxicity cut-off at log K_{OW} of 5.5, as used in current practice.

3. *Is it possible and meaningful to set a general predicted no-effect activity (PNEA) for baseline toxicity?*

The group agreed that a related PNEA value is meaningful, as a fraction of saturation and in case of lethal effects. A particular advantage is that no additional toxicity data are needed to set this limit, potentially (i) reducing the need for additional testing and use of laboratory animals, (ii) providing less uncertainty in the risk assessment process, and (iii) reducing the need to use assessment factors currently used in the derivation of PNEC values. However, the same restrictions and uncertainties discussed above apply regarding the applicability domain. Specifically, the use of PNEA values in risk assessment would only be useful for non-polar organics with log $K_{OW} > 2$ and for which no excess toxicity is possible, i.e. that the only mode of action for the chemical is baseline toxicity. It was briefly discussed by which factor below the range where baseline toxicity initiates (i.e., 0.01-0.1) this threshold should be defined, but the value itself needs to be set by the regulatory community, which most likely would benefit from the input of additional data.

4. *Is it possible and meaningful to set a general predicted no-effect activity (PNEA) for mixtures with regards to baseline toxicity?*

This question triggered much discussion. The group agreed that the conclusions of Q3 could be extended to the baseline toxicity of mixtures, meaning that a PNEA on a $\sum a$ basis is sound and feasible, where the mode of action is limited to baseline toxicity. In these instances, the concept of chemical activity can help to identify the main drivers of toxicity while at the same time determining the baseline toxic potential of the mixture. The concept was considered particularly useful for site-specific risk assessment and for monitoring the reduction of toxicity during remediation of contaminated sites. However, as above, the limitations to the applicability domain need to be taken into account.

The group, however, could not form a consensus on whether it is meaningful and reasonable to set a chemical activity limit for individual compounds to constrain their contribution to the baseline toxic potential of environmental mixtures (e.g., 1 or 10 %) (see, e.g., Schmidt and Mayer, 2015). Some group members found this difficult to apply without knowing the composition of the environmental mixtures, whereas other members argued that such an approach was needed in order to account for the multitude of chemical emissions and the large number of chemicals present in the environment. The group did not reach any consensus on this question.

5. *Is it scientifically correct to assess the sorptive capacity or “solubility” of neutral hydrophobic organic chemicals in non-aqueous phases as the product of the non-aqueous-water partition coefficient and the aqueous solubility?*

The work group stressed the importance of taking into account the uncertainties. As an initial step, good quality non-aqueous/water distribution coefficients are needed, and a discussion was initiated whether the quality of the calculated values was sufficient, without coming to a conclusion. Hence, a substantial knowledge gap was identified for which more research is needed.

6. *What are the assumptions in a comparison of activities of neutral organic chemicals among various environmental media?*

A: For measured activities

Activity measurements require equilibrium partitioning between the sampler and the medium and also that the depletion of the medium is kept at a negligible level (i.e., below 5 %) during the sampling. For the issue of measuring activities in sediment either in the field (*in situ*) or with samples brought to the laboratory (*ex situ*), data were reported that showed higher activity measurements for *ex situ* compared to *in situ* sampling. Hence, the extent to which the activity can be conserved when bringing samples into the lab was discussed.

B: For calculated activities

When no measured activities are available, total concentration data, e.g., from monitoring programs, can be converted to activities. This approach is followed under the assumption of equilibrium partitioning and a certain capacity for one compartment, and the quality of the translation is a function of scaling to log K_{OW}. In general, the work group recommends using good modelling practice, i.e., clearly describing the methods and assumptions. In particular, uncertainty analysis needs to be taken into account.

7. *Is it possible and meaningful to include an activity framework in AOP analysis?*

Using activity has clearly been identified as an alternative exposure basis and is expected to be a useful tool for exploring AOPs.

In addition to the first 7 questions addressed above, the group defined an additional question (below) to trigger brainstorming.

8. *What are the low-hanging fruits for the application of the activity approach?*

See Chapter 5 for details of opportunities to apply the activity approach.

4.2 Syndicate Session 2: Classification of chemicals according to MOA and chemical activity or other dose metrics for chemicals with specific mode of action

Participants

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	V. Otton
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	P. Thomas
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Introduction

While the chemical activity concept has been applied in the analysis of toxicity data for baseline toxicity, this concept could also be valuable for compounds with other modes of action (MOA). A recent ECETOC report on "Activity based relationships for aquatic ecotoxicology data" listed examples of estimated activities for baseline toxicants as well compounds with other MOA (ECETOC, 2013). Classification into MOA is an essential element in analysing toxicity data. In addition to classification based on chemical structure, other novel techniques such as "omics" and high throughput screening (HTS) can become powerful tools in the analyses of MOA and within the adverse outcome pathways approach (AOPs). In addition to chemical activities, other dose metrics can be appropriate to analyse and understand differences in toxicity of compounds with a MOA beyond baseline toxicity.

Objectives

The objectives of this WG 2 were:

- To determine the extent of chemical and toxicological domain for the use of the chemical activity concept as it is applied to neutral non-polar organic chemicals and to compounds with modes of action beyond baseline toxicity for both acute and chronic ecotoxicological effects.
- To explore alternative methods for classifying the toxicological mode of action for chemicals, including the role of adverse outcome pathways in classification and to explore alternative dose metrics, and to assess the role of chemical activity as a potential complementary approach.

The participants discussed the following three themes during three breakout sessions:

Theme 1: Data for chemical activity (beyond baseline toxicity)
Theme 2: Modes of action (MOA) and classification
Theme 3: (Quantitative) adverse outcome pathways (AOP) – chemical activity and other dose metrics.

A brief introductory text has been prepared in advance for each of the three themes and finalised during the workshop (see Background information below). Each participant has agreed with this text.

Background information

Theme 1: Data for chemical activity (beyond baseline toxicity)

The theme was introduced with a presentation of the data from the ECETOC report "Activity based relationships for aquatic ecotoxicology data". The presentation included acute and chronic algae, daphnids and fish effect data for MOA 1 and 2[2].

Acute fish tox data for fathead minnow and guppy

The chemical activity concept is already applied in the analysis and prediction of effect data of chemicals that act via non-polar baseline toxicity (see working group 1). Compounds with other MOAs are often "more toxic" (potent) than these base-line toxicants, at least if the toxicity data are interpreted on a K_{ow} scale (Russom *et al.*, 1997; Verhaar *et al.*, 1992) or as a plot of effect concentrations versus the sub-cooled liquid solubility (ECETOC, 2013). Comparing effect data of compounds with chemical activities is more direct because comparisons can be made simply based on one parameter instead of a K_{ow} regression. The EPA fathead minnow LC50 data represent a high quality dataset from which various toxicological modes of action can be assessed (Russom *et al.*, 1997). Mackay (Mackay *et al.*, 2014), for instance, have plotted the data from the EPA fathead minnow database demonstrating the applicability domain of the chemical activity approach for baseline toxicants (see Figure 4.2.1). From this study, as well a number of other publications, it is generally accepted that acute baseline toxicity occurs within an activity range of between 0.01 and 0.1. In a recently published ECETOC report, the chemical activity approach was applied to acute toxicity data for a whole range of chemicals covering different MOAs (ECETOC, 2013).

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[2] In the report, we often refer to MOA 1, 2, 3 and 4. This terminology is based on the Verhaar classification system: MOA 1: non-polar narcosis, MOA 2: polar narcosis, MOA 3: modes of actions related to reactive chemicals, MOA 4: specific modes of action.

Figure 4.2.1. Fathead minnow acute toxicity data - chemical activity against water solubility. LC50 data from EPA (Russom et al., 1997). Figure reproduced from (Mackay et al., 2014)

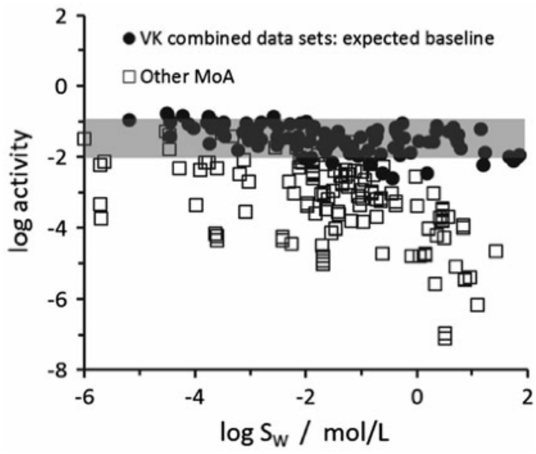


Figure 4.2.1 reveals some interesting trends. In particular, the chemical activity of the more hydrophobic chemicals (low S_w) with “other modes of action” is closer to the range representing baseline toxicity of 0.01-0.1 (grey band) than the less hydrophobic compounds. Similar trends have been observed for the 96-hour LC50 to guppy of reactive chemicals, for example for reactive acrylates (Freidig and Hermens, 2000; Freidig *et al.*, 1999). The most plausible explanation for these trends is related to internal distribution of these compounds inside the organisms (Figure 3.3.1). The target site for reactive compounds is often in an aqueous environment inside the organism. An example is the interaction of reactive compounds with intracellular glutathione. More hydrophobic compounds will accumulate mainly in the cell membrane and the concentration in a more aqueous phase (intracellular or blood) will be relatively low. For those chemicals, narcosis overrules the more specific MOA and this explains the shift towards the base line activity range.

Figure 4.2.1 also includes data for polar narcosis compounds (MOA 2). Based on internal membrane concentration, these two classes (MOA 1 and 2) merge (Escher and Schwarzenbach, 2002; Vaes *et al*., 1998). Additional analyses are needed to get more understanding of chemical activity of MOA 1 and MOA 2 compounds.

Theme 2: Chemically-based methods for determining Modes of action (MOA) and classification

The theme was introduced with a presentation on the topic of MOA and classification, which was followed by a presentation on the application of omics in classifying chemicals according to their MOA.

Chemistry-based Modes of action (MOA) and classification

Classifying compounds according to their mode of action is important in the interpretation of ecotox data and in developing predictive models. A good example of a clear classification system is the one that was developed by the EPA Duluth lab (Russom *et al.*, 1997). In this system a number of requirements for the assignment of a MOA to a specific compound are defined, including (a) results from fish acute toxicity syndromes and behaviour studies, (b) joint toxicity data, (c) excess toxicity (Te) and (d) similarity in chemical structure or chemical properties (e.g. reactivity towards nucleophiles) with compounds with a known MOA. This classification system has been applied to the fathead minnow database (Russom *et al.*, 1997).

Most other classification systems are simpler and in fact are based on chemical structure. Structural alerts or rules are then applied to assign a MOA to a chemical. The Verhaar classification scheme (Verhaar *et al.*, 1992) is another system based on four classes of chemicals representing very broad MOAs. Chemicals within these MOA's include: (i) inert chemicals, (ii) less inert chemicals, (iii) reactive chemicals and (iv) specifically acting chemicals). An updated and improved version for the Verhaar classification scheme was recently published by Enoch *et al*. (Enoch *et al.*, 2008). Automated versions to classify compounds are developed as part of an OECD toolbox. The OECD toolbox includes several other classification systems for MOA assignment (see http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm). More recently, omic approaches also have been applied in this field. According to Dom, "transcriptomics tools hold considerable promise to be used in biological response based mechanistic profiling of potential (eco)toxicants" (Dom *et al.*, 2012).

<u>Biologically-based mode of action classification</u>

As it pertains to the application of chemical activity as a predictor of toxicity, at present our current understanding and data strongly supports the utility of chemical activity for predicting non-polar narcosis toxicity (Verhaar category 1). Data evaluating the applicability of chemical activity to other modes of action, such as reactive toxicity or specifically-acting toxicities, including those that cause chronic and/or sub lethal adverse effects are currently lacking. Therefore, discrimination of chemicals as predominantly baseline/non-polar narcosis toxicants versus other modes of action can have significant value for determining whether a chemical activity-based toxicity prediction is a sound basis for a risk assessment/risk management decision. The classification strategies described above are largely chemical-structure based. However, emerging biological pathway-based tools have potential to provide complementary or orthogonal approaches for binning chemicals into broad mode of action categories.

As an example, evaluation of US EPA's ToxCast data set has identified a phenomenon of a "burst" of pathway-based activity at or near the concentrations that elicit overt cytotoxicity (Judson *et al.*, 2015). This "burst" of activity across a wide range of assays largely associated with generalised toxic stress may serve as the high throughput *in vitro* analog to "baseline toxicity". Judson *et al*. have proposed the use of Z-scores to identify assay responses that occur in the region of the non-specific cytotoxic burst and to distinguish these responses from those that may reflect specific biological activities against particular pathways or biological targets (Judson *et al.*, 2015). Examining the overall chemical space encompassed by the ToxCast chemicals, it was broadly identified that pharmaceuticals and pesticides (compounds designed to interact with specific targets) were the most likely to have biological activities at concentrations well below the cytotoxic burst region. In contrast, while industrial chemicals often showed a diversity of biological activities, those were more likely to be reflective of generalised toxic stress and were activated at or very near the cytotoxic burst region. These results all allude to the potential of using such biologically-based high throughput data to differentiate narcosis/baseline type toxicants for which chemical activity approaches can be applied with good predictive confidence, versus those for which more pathway/target specific approaches may be needed.

While the current assumption is that the “cytotoxic burst” phenomenon is an *in vitro* analog to baseline toxicity, this assumption has not been explored experimentally. This represents an interesting topic for research moving forward. In the near term, one could envision two efforts which might provide insights into the validity of this assumption. First, based on the data of Judson (Judson *et al.*, 2015), concentrations associated with the “cytotoxic burst” could be expressed as chemical activity to test the hypothesis that these concentrations would be equivalent to activity in the 0.1-0.01 range. Second, it would be useful to apply structure-based MOA classification schemes to the ToxCast chemical library and examine the agreement (or lack thereof) between chemical structure-based identification of putative baseline (MOA 1,2) toxicant and biologically-based identification of baseline toxicants as based on the cytotoxic burst analysis. Kramer (Kramer *et al.*, 2009) compared *in vitro* and fathead minnow *in vivo* toxicity and found that not all specifically acting chemicals were poorly predicted with *in vitro* cytotoxicity, indicating baseline toxicity and excess toxicity are both leading to the observed toxicity. Active metabolites have been little considered *in vitro* and lack of consideration may lead to misclassifying chemicals in MOA classes.

Beyond the utility for discriminating predominantly baseline or non-specific toxicants (which may include certain reactive MOAs) from those with potential to interact with specific biological targets/pathways at much lower concentrations, high throughput toxicology datasets can also provide a finer resolution classification of chemicals that fall into broad Verhaar category IV or excess toxicity categories. It would be expected that solubility-based chemical activity would not necessarily be a robust predictor of potency for many of these specific modes of toxicity. An illustrative example is the case of the stereoselectivity of many enzymes and receptors. While stereoisomers would have similar solubility they can have dramatically different biological potency. In these cases structural features and/or physical/chemical properties other than those closely linked to solubility would be needed to describe the chemical space likely to interact potently with these targets. Nonetheless, there may prove to be certain targets for which chemical activity may be good predictors. These would likely be targets that are found in membranes or other lipid-rich regions of the cell and are fairly promiscuous in terms of the chemical structures with which they bind or react. A potential research exercise would be to examine correlations between chemical activity and potency of ToxCast chemicals in specific assays and identify those for which a strong relationship exists. One could then examine the localisation and function of those targets in more detail and begin to investigate whether there is a scientifically-plausible theoretical basis on which to expect that activity-based predictions would have value for predicting chemical potency against those targets. This finer resolution of MOA categorisation based on activity in various pathway-based high-throughput toxicology assays can be mapped to the concept of molecular initiating events (MIE). Whereas baseline toxicants can be expected to act similarly on a broad range of organisms, life stages, sexes, to cause overt mortality through non-specific membrane interactions, more specifically-acting toxicants may show considerable selectivity in terms of the taxa, life stages, sexes, etc. that are sensitive/susceptible to their effects. The AOP framework is intended to establish and describe the scientifically-credible links between perturbation of a particular MIE, as may be captured/assessed via a high throughput assay, and the downstream biological consequences that may be expected within specific biological domains. Divergent effects and sensitivities among these different biological domains can be represented in an AOP network, allowing this finer resolution definition of chemical mode(s) of action to be linked to relevant hazards. While chemical activity can be viewed as a useful predictive framework for linking non-selective baseline toxicity to a fairly universal outcome of acute lethality, AOPs provide the framework to link these more specific toxicities to their more specific and selective outcomes. Thus, the approaches are complementary rather than redundant in the context of an overall predictive framework for chemical safety evaluation.

The use of toxicogenomics for understanding the mechanisms underlying chemical toxicity in (eco)toxicology has become common practice. Generally, the application of transcriptomics is more routine and advanced than the application of proteomics and metabolomics in toxicological studies. In particular, use of QPCR for targeted measurement of transcript levels of genes known to be implied in the toxic mechanism of interest is now routine in many toxicological studies. In addition, measurements of the whole transcriptome, or at least larger subsets, are often performed using microarray or next generation sequencing techniques. There are two main ways in which such toxicogenomics datasets can help to identify the MOA of previously uncharacterised chemicals. 1) The MOA is inferred from direct biological interpretation of the data based on toxicological knowledge, for example through pathway analysis identifying enrichment of a receptor activated pathway. 2) A more literal application of the classification concept is to use clustering algorithms to group chemicals according to their expression profiles or signatures without necessarily knowing the functions of the genes contributing to the classification. Although the use of toxicogenomics data for regulatory applications can be envisaged in the long term, for instance aiding in the selection of appropriate QSAR models, early attempts to apply omics-based classification strategies have been limited due to several factors. Firstly, the resolution for distinguishing among MOAs depends on intrinsic limitations of the techniques applied. More importantly, the vast number of MOAs that exist, the different levels of definition of MOAs that are being used (e.g. endocrine disruption in general versus oestrogen agonism, oestrogen antagonism, androgen agonism etc.), and the – often limited – numbers of MOAs and chemicals per MOA that are included in profiling analyses contribute to a generally low resolving power. Furthermore, the use of outgroups containing chemicals with MOAs strongly differing from those of interest (a standard practice in more traditional classification approaches such as phylogenetic analyses) has often been neglected, although using outgroups as a reference improves the interpretation of differences and similarities among chemicals. Additionally, both technical (e.g. inter-lab variability, poor standardisation of protocols) and biological (e.g. age, time, handling stress, exposure concentration) variability have complicated these efforts. Due to these complexities, there has been an ongoing debate about the potential of toxicogenomics for classification of chemicals according to their MOA, with only rare success stories in ecotoxicology.

From these limitations and experiences, it has become clear that large-scale efforts with an important bio-informatics component are needed. Useful advances have been made in other scientific fields and recently also in ecotoxicology that could aid in using toxicogenomics for classification of chemicals, with regulatory applications following in the longer term. One example is the MNI (MOA by Network Identification) approach used by Ergün *et al*. (2007) to study prostate cancer in a clinical setting. The authors used a large multi-cancer transcriptional expression dataset containing different sources of variation (drug treatments, cell lines, patient samples) in order to construct a co-expression network reflecting the average behaviour of genes in cancer. Subsequently, they used this network as a background to filter out genes that are specific (i.e., respond differently from what is expected based on the network) for different stages of prostate cancer. They were thus able to build a molecular classifier to distinguish between non-recurrent and recurrent primary prostate cancer, which is of high diagnostic value. Using a large database and relatively simple mathematical models, they achieved a resolving power that would not have been possible based on standard transcript expression analysis workflows. A recent example in ecotoxicology is the study of Antczak (Antczak *et al.*, 2015) in which *Daphnia magna* were exposed to 26 organic chemicals to study the mechanism for basal toxicity. The mechanisms involved in narcosis, especially related to sub lethal effects, are still poorly understood, although the use of QSARs for prediction of baseline acute toxicity (i.e., mortality) has become common practice. The authors built a network integrating physicochemical features of the chemicals with affected pathways, and pathways with organismal toxicity. The results indicated a link between transcriptional changes involved in intracellular calcium mobilisation and narcosis. They validated these findings by showing that exposure to a calcium ATPase pump inhibitor was able to reproduce a large part of the differential expression signature of narcotics.

For an appropriate use of QSARs for predicting narcosis toxicity (acute and chronic, lethal and sub lethal), it is essential to determine whether chemicals act through narcosis or have (additional) specific MOA(s). In this respect, regulatory agencies are increasingly demanding biological mechanistic information to support MOA designation to justify the use of a QSAR model. Since aquatic toxicity tests using algae, invertebrates and fish embryos are considered alternative testing methods, they could be used to collect acute toxicity data on new unknown compounds without the need to use animals. Subsequently, analysis of the concordance between predicted toxicity based on acute QSAR models for narcosis and the acute experimental data may suggest that the chemical acts through narcosis. However, this MOA designation would be solely based on mortality data and would not take sub lethal effects into account. Subsequent application of acute-to-chronic ratios for prediction of chronic toxicity may not always be appropriate, since other mechanisms as well as inter-species differences in sensitivity may become more important after long-term exposure to low chemical concentrations. For these reasons, toxicogenomics data could play a role in biologically supporting the proper MOA designation needed to justify the use of QSARs. Combined with *in vitro* assays and *in vivo* measurements of sub lethal endpoints at different levels of biological organisation (biochemistry, physiology, behaviour, etc.), toxicogenomics are considered as an important source of biological support for increasing confidence in risk assessment. Given the maturing methodologies, the increasing scale of studies, and the growing bioinformatics component, a revival of the use of toxicogenomics for classifying chemicals according to MOA may be underway.

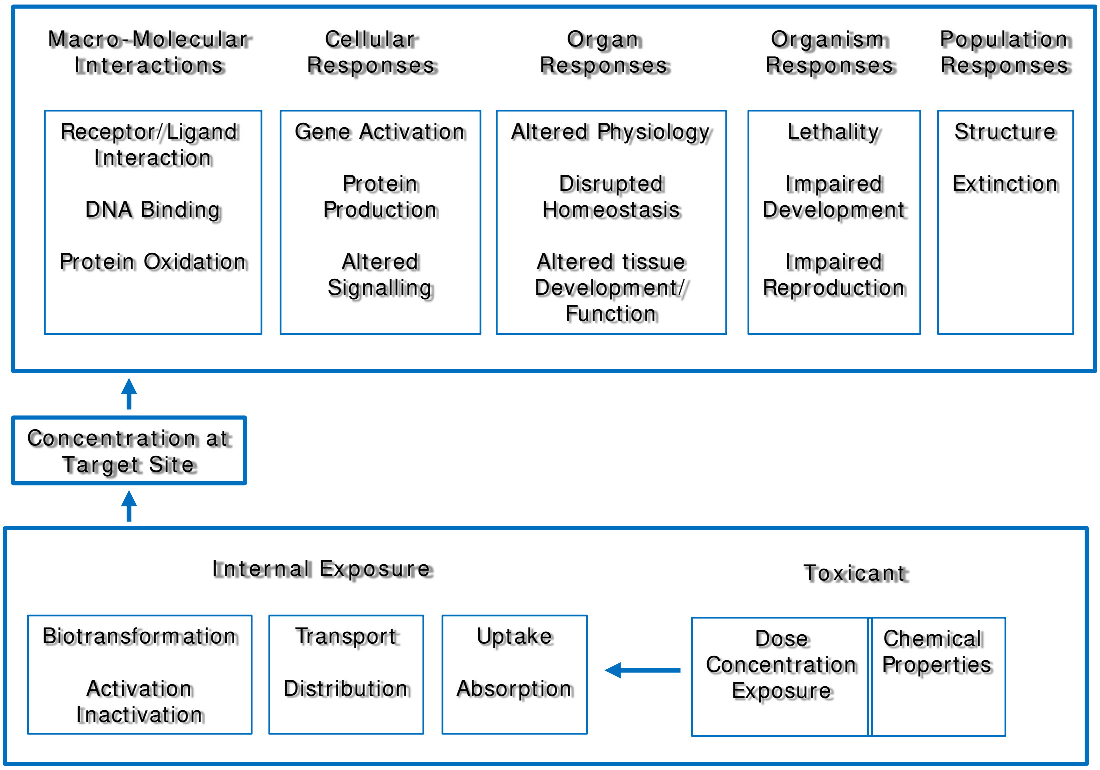
Theme 3: Quantitative adverse outcome pathways (AOP) – chemical activity and other dose metrics

The theme was introduced with a presentation on the topic of Quantitative Adverse Outcome Pathways and a presentation on Dose metrics.

Mode of action is a very broad term and does not directly refer to the underlying processes. If we take reactive chemicals or alkylating agents, for example: there are numerous more specific processes or targets that such compounds may interfere with (DNA, a specific enzyme etc.). The terminology applied within the context of AOP (Ankley *et al.*, 2010; Russom *et al.*, 2014; Villeneuve *et al.*, 2014; Villeneuve and Garcia-Reyero, 2011) is more precise. According to Ankley (Ankley *et al.*, 2010) and the OECD (OECD, 2013): “an Adverse Outcome Pathway (AOP) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect (see Figure 4.2.3). AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning”.

The AOP concept is useful to interpret and study the effects of chemicals in a hazard assessment. In hazard characterisation and risk assessment we need also quantitative information about dose-response relationships as well as toxicokinetic data, including uptake, internal distribution and biotransformation. For a more complete analysis of the whole process from the dose to the overall effect, information about rate or equilibrium constants of the underlying intermediate steps is needed as well. We could refer here to quantitative AOP analysis. An example of a quantitative AOP was recently published by Villeneuve (Villeneuve *et al.*, 2015). Such complete analyses are also presented in so-called Toxicokinetic/Toxicodynamic models (TKTD). A nice example of such a more complete quantitative AOP or TK-TD model is a study from Kretschmann (Kretschmann *et al.*, 2012) for the effects of organophosphates. The TK-TD model used includes parameters for all underlying processes such as uptake, elimination, biotransformation and interaction with the target.

Figure 4.2.3. Different steps in a quantitative adverse outcome pathway (AOP). Modified from (Ankley et al., 2010; OECD, 2013).



Because they were intended to link molecular-level biological perturbations elicited by chemicals to hazards considered relevant to risk assessment and regulatory decision-making (e.g., impacts on human health or survival, growth, or reproduction of wildlife populations) AOPs are bioactivity-based, not chemical specific. They are employed to generalise and predict the pattern(s) of effects *any* chemical that perturbs a particular target/MIE (with sufficient potency and duration) could be expected to produce. The challenge to applying AOPs in predictive toxicology then is understanding what constitutes sufficient potency and duration of interaction for different chemicals. Thus, while the AOP itself does not explicitly consider chemical-specific properties, application of AOPs must consider those properties in order to assess how much of a delivered chemical (in a laboratory/bioassay context) or ambient concentration can actually reach the target site of action and for how long.

In the prediction of the overall organism responses from the applied dose, a number of modelling approaches are relevant and these include:

- Toxicokinetic and Toxicodynamic (TK/TD)
- Physiologically based Toxicokinetic (PBPK)
- Reverse Dosimetry
- Dose-Response and Biologically Based Dose-Response (DR)

These same models are also important in the prediction of *in vivo* effects from *in vitro* data and there is much similarity in quantitative AOP and quantitative *in vitro-in vivo* extrapolation (QIVIVE). There are several examples, also in high throughput screening in the ToxCast program, where these models are applied in the extrapolation of *in vitro* to *in vivo* effects. In these examples, concentrations in blood are set equal to a concentration in an *in vitro* assay. The *in vivo* toxicity is then predicted from *in vitro* data using reverse dosimetry. Similar studies have been published by Louisse *et al*. (Louisse *et al.*, 2010). Bioavailability issues and kinetic analyses of partitioning processes are essential in these approaches and here chemical activity may play an important role as well (Wetmore *et al.*, 2012).

The dose of a compound is an essential element in quantitative AOP, TKTD modelling and in quantitative *in vitro-in vivo* extrapolations (see figure 4.2.3). A number of dose metrics can be applied, including nominal and total concentrations, as well as freely dissolved and internal concentrations (Escher and Hermens, 2004; Groothuis *et al.*, 2015). Also chemical activity is a powerful metric to express the dose. In particular in multi-compartment systems, chemical activity has its strengths. As indicated by Reichenberg (Reichenberg and Mayer, 2006), "the chemical activity of a substance - as well as its fugacity - is by definition the same throughout a system that has reached thermodynamic equilibrium. In that case, the measured chemical activity in one phase applies to the other phases as well. This is true regardless of the degree of heterogeneity, the number and diversity of sorptive sites, and the organic matter quality". Because of this, chemical activity is very useful in the interpretation of toxicity data from *in vitro* assays, where total concentrations leading to an effect may be affected by protein binding, while chemical activity and freely dissolved concentration should be independent of that (Armitage *et al.*, 2014; Kramer *et al.*, 2010).

For some specific compounds or MOA's (or AOP's), effects are related to a time integrated dose and this will particularly be the case when the MIE is based on an irreversible mechanism. In those cases, a time integrated exposure, such as an area under the curve (AUC) or target occupation is a more suitable dose metric (Gülden *et al.*, 2010; Legierse *et al.*, 1999). Also in TKTD modelling, the factor time is inherently included in the analyses and modelling of effect data (Ashauer and Brown, 2008; Ashauer *et al.*, 2015).

To the extent that chemical activity or other exposure metrics could improve the accuracy/precision with which the dose and duration of chemical exposure at the target site of the MIE are described, their application could be expected to improve and enhance the utility of quantitative AOPs for predictive toxicology. These approaches could provide more precise definition of point of departure, as a generalisable description of an equivalent dose and duration of chemical exposure, needed to produce the effects observed for a reference compound. In the context of weight of evidence evaluation for AOPs, the use of dose-metrics that can more readily account for significant differences in study design, would aid the evaluation of whether apparent deviations from dose-response concordance among different key events is simply an explainable result of disparate study designs or whether it actually represents grounds for reducing confidence in the causal relationships depicted in the AOP and/or outright rejection of that AOP. The bottom-line relative to quantitative application of AOPs in predictive risk assessment is that higher precision dosimetry estimates, whether they be derived from chemical activity, free concentration estimates, IVIVE, AUC or other approaches, should aid and strengthen the quantitative application of AOP knowledge for predictive risk assessment applications.

At present, these concepts remain largely unexplored. However, the first case studies in the quantitative application of AOPs are underway (Villeneuve *et al.*, 2014). Initial sets of toxicodynamic model predictions which capture key feedback and compensatory mechanisms known to operate along the reproductive endocrine axis have been generated using the simple steady state assumption that chemical concentration in water is equivalent to the free concentration in plasma and that relative potency at the target site can be defined simply based on nominal concentration in an *in vitro* assay. Should those initial case studies fail to produce reasonable estimates of *in vivo* biological response, a logical next step would be to redo the model simulations using more sophisticated approaches to predict the internal dose in the organism from the external concentration in the exposure media and/or apply more refined *in vitro* dose-metrics in an attempt to provide a more accurate characterisation of potency that more accurately considers how much chemical actually reached the target site within the bioassay. These are near-term case studies that could provide insights into the extent to which toxicokinetic considerations and alternative dose-metrics could improve the accuracy of quantitative AOP-based predictions.

Summary of resulting discussions

The participants discussed the following three themes during three breakout sessions:

Theme 1: Data for chemical activity (beyond baseline toxicity)
Theme 2: Modes of action (MOA) and classification
Theme 3: (Quantitative) adverse outcome pathways (AOP) – chemical activity and other dose metrics.

Theme 1: Data for chemical activity (beyond baseline toxicity)

The following questions were discussed during the workshop:

1. Can we identify suitable data sets with effect concentrations from which chemical activities can be derived, or are there publications that report chemical activities (for chemicals beyond non-polar baseline toxicity)?
2. Can the chemical activity approach be helpful in the identification of compounds with modes of action other than non-polar baseline toxicity?
3. Can the chemical activity approach be useful to interpret differences in effect concentrations of MOA1 and MOA2 chemicals?
4. Are there enough data to estimate the range in chemical activity of compounds with a certain mode of action? What are the advantages of applying a chemical activity concept to compounds with specific modes of action?
5. Can we link the chemical activity approach to the TTC concept (threshold of toxicological concern)?

6. Can we apply chemical activities in the interpretation and understanding of effects of complex mixtures with baseline toxicity only and multiple MOAs; as well as of individual compounds with "multiple" modes of action?

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1. *Can we identify suitable data sets with effect concentrations from which chemical activities can be derived, or are there publications that report chemical activities (for chemicals beyond non-polar baseline toxicity)?*

Only a few studies interpret acute toxicological effect data using the chemical activity concept (Mackay *et al*., 2014; McCarty *et al*., 2013; Mayer and Reichenberg, 2006). However, several data sets in the open literature report effect concentrations that can be applied to derive chemical activity using experimental or estimated data for subcooled liquid solubility. Many of these data sets include non-polar organic compounds (MOA 1) (McGrath and Di Toro, 2009), while other sets report data for MOA 1, 2, 3 and 4 chemicals (Barron *et al*., 2015; Russom *et al*., 1997; Verhaar *et al*., 1992). Also the European Chemicals Agency (ECHA) has developed toxicity databases[3]. Other data sets are known to exist but need to be made available.

The group recommended that for calculating reliable chemical activity data, high quality toxicity data as well as reliable experimental or estimated values for solubility (or subcooled liquid solubility) are needed. More detailed information about estimation of solubility is presented in WG3.

2. *Can the chemical activity approach be helpful in the identification of compounds with modes of action other than non-polar baseline toxicity?*

As discussed in WG1, chemical activities related to acute effects on survival for compounds that act via MOA 1 are in a rather narrow range (between 0.01 and 0.1). There were indications from a recently published ECETOC report (ECETOC, 2013) that chronic activities could also be interpreted using the chemical activity concept, based on data obtained from regulatory studies, and that, as expected, the chemical activities derived were lower than those for chemical activities calculated based on acute toxicity data. However, it is notable that the accuracy of the relationship was hampered by data quality and transparency related to methods used to allocate substances as MoA 1 or MoA 2. It was concluded that if for a given chemical the calculated chemical activity is lower than 0.001, especially at lower log K_{ow} (<4), that there is a high likelihood that the chemical has a more specific MOA, for instance MoA 4 in the Verhaar system or is a more reactive compound, i.e. MoA 3. Consequently, based on the analysis reported in the ECETOC report, it can be argued that the chemical activity concept is useful in differentiating chemicals as being either baseline toxicants or as having the potential to illicit excess toxicity. As log K_{ow} increases above 4, the difference in chemical activities between baseline and excess toxicity is less obvious.

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[3] See the database Information on Chemicals from the European Chemicals Agency (http://echa.europa.eu/information-on-chemicals)

3. *Can the chemical activity approach be useful to interpret differences in effect concentrations of MOA1 and MOA2 chemicals?*

Initial analyses that are reported in the ECETOC report (ECETOC, 2013) have shown that chemical activities of compounds that act via so-called polar narcosis (MOA 2) are lower than the range of 0.01-01. The group agreed that a more detailed analysis of the data is needed to improve the overall understanding of the difference between MOA 1 and MOA 2. It is not clear why chemical activity for MOA 2 compounds is lower than the range 0.01-0.1 and how this relates to the observation that internal membrane concentrations for MOA 1 and MOA 2 chemicals are very similar.

The group agreed that this topic will be explored in more detail in a follow-up manuscript planned to be prepared following publication of this ECETOC workshop report.

4. *Are there enough data to estimate the range in chemical activity of compounds with a certain mode of action? What are the advantages of applying a chemical activity concept to compounds with specific modes of action?*

Data directly assessing the chemical activity of chemicals with modes of action beyond MoA 1 and 2 are currently not readily available. Nonetheless, WG2 participants acknowledged that there could be advantages in applying the chemical activity concept for chemicals with specific modes of action associated with them. Expressing toxicity data using chemical activities will very easily show an “enhanced” toxicity as compared to the 0.01-0.1 range that is valid for MOA 1. Applying the chemical activity beyond MoA 1 and 2, however remains a challenging area, and is revisited in the responses to other questions posed to the group. A more detailed analysis of MOA 3 and 4 chemicals is needed to arrive at clear conclusions about the applicability of the activity concept to compounds from MOA 3 and 4 classes. Such an analysis will be performed and results will be presented in a manuscript planned to be prepared following publication of this ECETOC workshop report. It was recognised that chemicals may have multiple MOA’s, especially with respect to chronic exposure, and that for acute exposures baseline toxicity may present itself prior to a specific MOA, particularly in instances where the exposure is relatively short, and the concentration in the cell membrane is much higher than the concentration at the target site. This will occur in particular for the more hydrophobic chemicals.

5. *Can we link the chemical activity approach to the TTC concept (threshold of toxicological concern)?*

During the meeting, a short presentation was given regarding an application of the chemical activity concept in relation to the concept of threshold of toxicological concern (TTC), in particular for chemicals whose only mode of action is baseline toxicity. Using aqueous concentrations, QSAR models and sensitivity distribution analyses have been developed in the literature to predict no-effect concentrations at the ecosystem level. In this approach, hazardous concentration for 5 % of the species (HC5) has been proposed as a no-effect level for MOA 1 compounds (van Leeuwen *et al*., 1992). An initial analyses of HC5 values shows that recalculating HC5 for baseline toxicants (i.e. MOA 1) into chemical activities leads to a constant chemical activity for the NOEC(ecosystem). This approach is useful within a TTC concept. At present, the TTC for the aquatic environment for MOA I is based on aqueous concentrations and is determined by the most toxic (most hydrophobic) compound (De Wolf *et al*., 2005).). A TTC based on chemical activity adds value in that the approach acknowledges the effect of hydrophobicity. The group regarded this as an interesting approach and suggested to work this out in more detail.

The approach will be presented in more detail in an upcoming manuscript planned to be prepared following publication of this ECETOC workshop report.

It must be emphasised that such an approach is useful for MOA 1, and the applicability to other MOA's is not feasible.

6. *Can we apply chemical activities in the interpretation and understanding of effects of complex mixtures with baseline toxicity only and multiple MOAs; as well as of individual compounds with "multiple" modes of action?*

There was a clear consensus that the chemical activity concept is very useful in interpreting and estimating the effects of complex mixtures that are solely comprised of MOA 1 and 2 chemicals, since chemical activities can simply be added (see also discussion from WG1). WG2 noted that the applicability of the chemical activity concept to mixtures consisting of chemicals that act as MOA 3 and 4 is currently outside the applicability domain and not feasible at the moment. An exception is that in mixtures that contain compounds from MOA 1 and other modes of action, the chemical activity approach is useful to predict the contribution of all compounds (including the contributions from MOA 3 and 4) to the baseline toxicity.

Theme 2: Modes of action (MoA) and classification

The following questions were discussed during the workshop:

1. Which major modes of action (MOA) are relevant in analysing ecotox data?
2. How can we classify compounds according to their mode of action? What kinds of classification systems are available and how reliable are they? Do we need more precise and specific classifications?
3. New approaches in classification are using "omics data". Are there many examples and how do they relate to more traditional classification schemes?
4. How can chemistry based approaches impact on definition and identification of MOA?
5. Are the current classifications schemes for acute MOA appropriate for chronic endpoints?

1. *Which major modes of action (MOA) are relevant in analysing ecotox data?*

Examples of major modes of action that are well documented in ecotoxicological research are: baseline toxicity (non-polar, polar, ester, amine); uncouplers; electrophilic (can be sub-categorised); specific: Central Nervous System (CNS), Acetylcholine-esterase inhibitors, other neurotoxicity, oestrogenicity, photosynthesis inhibition.

2. *How can we classify compounds according to their mode of action? What kinds of classification systems are available and how reliable are they? 4. How can chemistry based approaches impact on definition and identification of MOA?*

Verhaar and Russom are coded classification schemes and readily available but should be used with appropriate caveats, in particular attention should be paid to check the chemical applicability domain for untested compounds. It was recognised that Verhaar classes offer a simple approach to group MoAs and due to its simplicity, that this method could be used as a basis for a more complete scheme with some modifications:

- Subcategorisation of the major classes could be valuable (for example different classes for reactive compounds etc.).
- Updating of chemical information is needed.
- For specifically acting and reactive chemicals, a secondary consideration would be what is the target of action and can chemical activity be applied in a useful and relevant manner.

It was noted that chemistry based approaches can be a combination of alerts and physico-chemical properties. It was also recognised by the group that chemical reactivity data (glutathione (GSH) depletion, Direct Peptide Reactivity Assay (DPRA), although optimised for skin sensitisation) as well as information from other endpoints (skin sensitisation) could be helpful but need to be supported experimentally. The group concluded that biologically based HTS assays (high throughput screening) can be useful in identifying MOAs. It was noted that while the current assumption is that the “cytotoxic burst” phenomenon is an *in vitro* analog to baseline toxicity, this assumption has not been explored experimentally. This represents an interesting topic for research moving forward.

The group recommended to create a separate activity to amend existing classification schemes for non-baseline toxicity chemicals and to consider the inclusion of esters (perhaps, as in Russom scheme, to include as a sub-class of non-polar narcosis). Such an activity should also include a discussion on improvement of classification systems of MOA, including chemistry based approaches (see above), high throughput screening and omics approaches (see question 3).

3. *New approaches in classification are using “omics data”. Are there many examples and how do they relate to more traditional classification schemes?*

The group discussed new approaches in classification based on “omics data”. There are two main ways in which omics such as toxicogenomics datasets can help to identify the MoA of previously uncharacterised chemicals. 1) The MoA is inferred from direct biological interpretation of the data, based on toxicological knowledge. 2) A more literal application of the classification concept is to use clustering algorithms to group chemicals according to their expression profiles. Although regulatory agencies are increasingly demanding biological mechanistic information to support MoA designation to justify the use of QSAR models, regulatory application of toxicogenomics data is at this point limited, largely due to high technical and biological variability. Large scale efforts based on bio-informatics are needed to overcome the current limitations, and it is further recommended that efforts should be focused on increasing the applicability of omics for identifying MoAs of untested chemicals (including assigning chemicals as either MoA 1 or 2) and specifically on sub-lethal effects and species differences.

5. *Are the current classifications schemes for acute MOA appropriate for chronic endpoints?*

The group also discussed the applicability of the chemical activity concept to chronic toxicity data. The following questions were raised:

- For acute baseline toxicants does an ACR of 10:1 hold, and if so can chemical activity be applied to determine chronic endpoints?
- Do specifically acting chemicals have a higher, or more variable ACR?
- How can we accommodate species differences in ACR and effects?

Time was too short to discuss these questions in detail, although some positive evidence was provided supporting the last point. It was noted that more chemical activity research should be performed on chronic effects. This aspect will be elaborated upon in the manuscript which is planned as a product of the workshop.

Theme 3: (Quantitative) adverse outcome pathways (AOP) – chemical activity and other dose metrics

The following questions were discussed during the workshop:

1. General
    - Are the concepts of adverse outcome pathway (AOP) and molecular initiating event (MIE) useful in classification of chemicals (which could lead to improved ability to study relationships with chemical activity)?
    - Can the chemical activity concept provide a complementary approach towards an improved understanding of an adverse outcome pathway (AOP)?

Has chemical activity the potential to link exposure with the molecular initiating event (MIE) in a single metric?

2. Specific
    - Is the chemical activity concept useful for all kinds of target sites and target site environments?
    - Can chemical activity be applied as valuable input parameter in a quantitative AOP, a TKTD model and in *in vitro-in vivo* extrapolations?
    - Are there alternative promising dose metrics or parameters for the evaluation of the effects of reactive compounds or of compounds with specific modes of action?

1. General

- *Are the concepts of adverse outcome pathway (AOP) and molecular initiating event (MIE) useful in classification of chemicals (which could lead to improved ability to study relationships with chemical activity)?*
- *Can the chemical activity concept provide a complementary approach towards an improved understanding of an adverse outcome pathway (AOP)?*
- *Has chemical activity the potential to link exposure with the molecular initiating event (MIE) in a single metric?*

A brief discussion was held on the linkage between MOA classification and the AOP (adverse outcome pathway) construct. It was agreed that AOP can support MOAs. AOP may help identify MIEs which could form the basis of classification schemes, AOP may help understand interspecies differences and chronic toxicity. It was also noted that few reliable AOPs exist at this time and that more research on AOPs is required.

The group agreed that chemical activity may be applied as a valuable input parameter in a quantitative AOP as it generally improves quantifying exposure and may also give information about the bioavailability of a compound. Chemical activities thereby (i) offer the potential to better define “points of departure” from where the organism can handle the perturbation to where the perturbation is fatal to the organism, (ii) offer an improved ability to use diverse studies to evaluate overall concordance of the relationships depicted in the AOP and (iii) represents a critical link of environmental concentrations to potency.

The group does not support the suggestion that chemical activity can be linked to the molecular initiating event (MIE) in one single metric. Only for baseline toxicity, chemical activity represent a single metric because there is no specific target molecule and the potency for all baseline toxicity compounds is similar. For other modes of action, effects are related to a number of processes (uptake, interaction with a target or receptor) and this cannot be related to one single metric.

2. Specific

- *Is the chemical activity concept useful for all kinds of target sites and target site environments?*
- *Can chemical activity be applied as valuable input parameter in a quantitative AOP, a TKTD model and in in vitro-in vivo extrapolations?*
- *Are there alternative promising dose metrics or parameters for the evaluation of the effects of reactive compounds or of compounds with specific modes of action?*

Chemical activities, free concentration (measured or modelled approaches) and internal concentration in an organ or cell are promising dose metrics to describe the bioavailable fraction. For *in vitro-in vivo* extrapolations, chemical activity forces consideration of the bioavailable fraction.

Dose metrics such as concentrations or chemical activities are useful in the analysis of effects of chemicals with a key event that represents a reversible interaction. For irreversible interactions with the target site, as is often the case for MOA 3 compounds, time is an important variable as well because “damage” is cumulative for irreversible interactions. In those cases, time integrated exposure measures such as area under the curve (AUC) could be an interesting dose metric (Gülden *et al*., 2010).

It was suggested that TKTD modelling is an interesting approach to analyse or predict effects of MOA 3 and MOA 4 chemicals as it includes parameters for all underlying processes including, uptake, elimination, biotransformation, interaction with the target, etc. It may also prove useful to classify compounds into distinguishable MOA subgroups.

Conclusions

- AOPs and biologically based assays will be useful to differentiate further MOA classes 3 and 4 which in turn open these roads for chemical activity to improve bioavailability metrics;
- Verhaar classes offer a simple approach to differentiate between expected specific, reactive and MOA 1 and 2 for acute exposure with some recommendations (see below) if the chemical domain is covered;
- In specifically acting and reactive classes a secondary consideration would be what is the target of action and can chemical activity be applied in a useful relevant manner;
- Chemical activity seems best applied to MOA 1 and 2 with significant research effort necessary to see to what extent the application can be broadened to MOA 3 and 4;
- Initial analyses of HC5 values have shown that recalculating HC5 into chemical activities leads to constant “threshold of toxicological concern”. This concept is interesting and could be useful in risk assessment;
- New approaches in classification based on “omics data” are promising. The variability in omics data is presently high and the regulatory applicability is still limited. Large scale efforts with an important bio-informatics component are needed to overcome the current limitations and it is further recommended that efforts should be focused on increasing the applicability of omics for identifying MOAs of untested chemicals (including assigning MOA 1 chemicals) and specifically on sub-lethal effects and species differences.

4.3 Syndicate Session 3: Challenges and potential limitations to the application of the chemical activity concept for ecological risk assessment – Physicochemical properties and partitioning

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Uncertainty in key physicochemical property data

The main objective of this work group (WG3) was to address issues of uncertainty and applicability domain with respect to physicochemical properties of organic chemicals, including miscible and ionisable organic chemicals, in applying the chemical activity concept to environmental risk assessment. The following sections are formatted around a set of questions (listed below) addressing critical challenges and potential limitations in using chemical activity in environmental and biological systems:

1. Uncertainty in key physicochemical property data

1. *How reliable are available water solubility data? How reliable are current approaches for estimating water solubility in the absence of empirical data?*
2. *How reliable are available melting point data? How reliable are current approaches for estimating melting points in the absence of empirical data?*
3. *How reliable is Walden's Rule given the wide range of chemical structures for which the chemical activity concept may be applied to?*
4. *How reliable are available methods to estimate the entropy of melting (ΔS_M) from chemical structure?*
5. *Taken together, what is the expected uncertainty in chemical activity calculations for 'data poor' chemicals?*

2. Calculation of chemical activity in non-aqueous phases (biota)

1. *Is the octanol-water (K_{OW}) paradigm sufficiently accurate for estimating K_{BW} (i.e. biota-water partition coefficient)? When is it necessary to consider more sophisticated approaches (e.g., ppLFERs) for estimating K_{BW}?*

3. Application of the chemical activity concept to miscible organic chemicals (MOCs)

1. *To what extent are empirically-based chemical activity coefficients available for miscible chemicals and how reliable are these data?*
2. *How reliable are computational approaches (e.g., UNIFAC, COSMOTherm, SPARC) for estimating chemical activity coefficients for miscible chemicals?*
3. *Case Study: Are the chemical activities corresponding to LC50s for 'narcotic miscibles' calculated using Equation 7 consistent with expectations (i.e., Ea50s ~ 0.01)?*
4. *Given the (relatively) low affinity for lipids and other non-lipid organic matter, what modifications to the approach for estimating K_{BW} (see above) are necessary?*

4. Application of the chemical activity concept to ionisable organic chemicals (IOCs)

1. *To what extent can approaches to calculate chemical activity for neutral organic chemicals be expanded/modified to IOCs? Are methods for estimating the activity coefficients of electrolytes (e.g., Debye–Hückel approach) (Trapp et al., 2010) compatible with methods for neutral organic chemicals?*
2. *Case Study: Can the intrinsic water solubility (i.e., water solubility of the neutral form) and fraction of chemical in neutral form in solution be used to calculate chemical activity from LC50s?*

In addressing each of the questions listed above, the major topics addressed in this section thus include a review of the uncertainties in critical physicochemical properties, the data needed to calculate chemical activity, the calculation of chemical activity in water, the calculation of chemical activity in biota, and the application of the chemical activity to miscible organic chemicals (MOCs) and ionisable organic chemicals (IOCs), and which summarises the nature of the discussions that occurred during the workshop within this workgroup. It should be noted that the topics covered in this section reflect the expertise within the workgroup, which may be stronger in some areas than others. Nonetheless, the participants within the group have made every effort to best articulate key data gaps, and where possible make recommendations regarding how data gaps might be best addressed.

1. Uncertainty in key physicochemical property data for neutral organic chemicals

It can be argued that one of the key physicochemical properties influencing the overall behaviour of hydrophobic organic chemicals in the environment and biological systems is the chemical activity coefficient (γ) in aqueous solution. This is because water in the environment and biological systems provides an important phase through which chemicals are transported. A measure of the hydrophobicity of a chemical can be attained by quantification of γ, which can provide an understanding of how a chemical partitions between water and other environmental phases (Sandler, 1996).

The γ of an organic chemical describes the relative degree of deviation from ideality, as described by Raoult's law, in which under ideal conditions the activity of a chemical (a) is equal to the mole fraction (χ). In environmental and biological systems, where organic chemicals are dissolved in water, a is proportional to χ, and γ is the proportionality constant that describes this relationship. Thermodynamically, γ describes the excess Gibbs free energy (G) associated with non-ideal solutions.

$$G = RT \ln\gamma \qquad (10)$$

where R and T represent the universal gas law constant and temperature, respectively, thus quantifying the deviation from ideal behaviour.

At equilibrium, the chemical activities of chemicals in the water and organic or lipid-like phases in the environmental and biological systems are equal, or alternatively:

$$\chi_w\gamma_w = \chi_o\gamma_o \qquad (11)$$

where the subscripts ‘w’ and ‘o’ refer to water and an organic or lipid-like phase in the environment or biological system.

For many organic chemicals, γ_o does not show considerable variation. For example, γ_o in octanol for a range of neutral organic chemicals is relatively constant at about 2.5 (Mackay *et al*., 2014; Sandler, 1996). Consequently, the partitioning process between water and various organic phases will be largely influenced by γ_w, which varies several orders of magnitude between organic chemicals (Schwarzenbach *et al*., 2003). The octanol-water partition coefficient (K_{OW}), for instance, is the ratio of solubility in octanol and water, and is typically used as a metric of hydrophobicity. It can be shown, however, that the activity coefficient in water is the key parameter that influences the magnitude of K_{OW} (Andren *et al*., 1987; Chiou, 1981; Llinàs *et al*., 2008; Sandler, 1996; Schwarzenbach *et al*., 2003), as well as solubility in water (S_w), whereby:

$$S_w = 1 / \gamma_w v_w \qquad (12)$$

where v_w is the molar volume of water, both K_{ow} and S_w are important input parameters for a wide range of models aimed at assessing fate and transport of organic chemicals.

Whereas K_{OW} and S_w strongly influence the behaviour of chemicals in environmental and biological systems, there are considerable challenges associated with quantifying each of these properties. A key challenge is related to limited availability of high-quality empirical data sets of reliable and reproducible measurements for many organic chemicals (Llinàs *et al*., 2008). For instance, in their review of solubility and K_{OW} data for the relatively well-studied organochlorine pesticide DDT, Pontollio and Eganhouse (2001) observed that the data reported in the literature tend to be populated by multi-level references, citation errors, and data errors, with reported property values spanning several orders of magnitude. Consequently, given the large degree of variance in the reported data, combined with the lack of information to fully evaluate the quality of the original data, the ability to define a true solubility value for DDT, for instance, represents a substantial challenge (Pontolillo and Eganhouse, 2001). Given the challenges associated with a well-studied chemical such as DDT, for less-well studied chemicals where there may only be a single empirically derived S_w value, it is potentially not practically possible to assign an estimate of uncertainty against that single available value.

The current situation is thus problematic, particularly given the relative importance of K_{OW} and S_W in estimating chemical behaviour, whereby the use of erroneous data as input to environmental fate and physiologically based pharmacokinetic (PBPK) models can result in high uncertainty in assessing chemical risk and efficacy (Mackay *et al*., 2009; Pontolillo & Eganhouse, 2001; Tesconi & Landis, 2013). While various efforts have been initiated to improve the reliability of empirical measurements towards the development of more robust *in silico* tools (Hewitt *et al*., 2009; Llinàs *et al*., 2008), for the vast number of chemicals used in commerce, establishing reproducibility of physicochemical property data between laboratories and analytical methods is rarely assessed. Thus, current practice continues to rely heavily on K_{OW} and S_W measurements obtained from a single laboratory study and/or output obtained from a single estimation method. Where the use of a single value is the only option, the ability to quantify the uncertainty represents a substantial challenge. Empirical solubility data are typically measured for one chemical at a time. Toxicity tests can use co-solvents which may enhance the solubility of individual chemicals, particularly for higher molecular weight/low solubility chemicals. Limitations of this co-solvent effect may be relevant in both a laboratory settings (e.g., when measuring solubility of large, low-soluble chemicals), and also in environmental monitoring scenarios (e.g., organic materials or other chemicals in environmental samples acting as co-solvents for the target chemical). Key objectives for participants within this workgroup were thus to consider the influence of uncertainty in physicochemical properties in relation to estimating a thermodynamic chemical activity which might be used within environmental risk assessments, and to propose approaches that might be adapted for applying the chemical activity concept to nonpolar, miscible, and ionisable organic compounds.

1) How reliable are available water solubility data? How reliable are current approaches for estimating water solubility in the absence of empirical data?

In an attempt to provide preliminary insight regarding the variance that might exist in water solubility measurements, data in relation to 233 neutral organic chemicals reported by Mackay *et al*. (2006) were assessed with respect to their availability of solubility data. An illustration of the results is shown in Figure 3.4.1, which summarises 2440 solubility measurements for the 233 chemicals included in the assessment. The dataset reported by Mackay *et al*. (2006) are believed to provide a relatively good indication of the variance that might exist in empirically derived solubility data for neutral organics, with the majority of chemicals having more than ten separate solubility measurements. A general observation from Figure 3.4.1, is that as solubility decreases the relative magnitude of the uncertainty increases, thus implying caution when relying on a limited number of solubility measurements for relatively insoluble organic chemicals (i.e. <0.01 mg/L).

In addition to the challenges of assessing the variance and uncertainty associated with measured physicochemical properties are the challenges in assessing the applicability domain and uncertainties in property data obtained from estimation methods. In the absence of empirical water solubility data, various estimation methods, such as the WATERNT v1.01 and WSKOWWIN models within U.S. EPA's EPISUITE, are heavily relied upon, particularly in estimating exposure concentrations. The U.S. EPA's EPISUITE empirical database underlying the WATERNT v1.01 submodule contains water solubility data for 5764 chemicals (1128 in training set, 4636 in validation set), and represents one of the most widely used estimation methods. The reported water solubilities in the training set range from $4 \cdot 10^{-7}$ to $1 \cdot 10^{6}$ mg/L ($9 \cdot 10^{-13}$ to 22 mol/L) while the reported water solubilities in the validation set range from $4 \cdot 10^{-8}$ to $6 \cdot 10^{6}$ mg/L ($7 \cdot 10^{-14}$ to 50 mol/L). Chemicals with reported water solubilities equal to $1 \cdot 10^{6}$ mg/L are likely to be miscible organic chemicals (MOCs) (e.g., some organic solvents) and values greater than this should be considered suspect. A subset of these data were used to train and validate the EPISUITE WSKOWWIN submodule (1450 in training set, 902 in validation set).

The average deviations of the WATERNT v1.01 predictions for the training and validation set are 0.355 and 0.796 log units, respectively, which corresponds to factors of approximately 2.5 and 6.0, respectively. Similar performance was found for the WSKOWWIN v1.42 submodule. However, substantially larger errors can be obtained for some chemicals (i.e., greater than two orders of magnitude).

It is notable that while the various estimation packages within EPISUITE tend to be widely used, largely due to being easily and freely accessible, there do exist a myriad of methods for estimating water solubility. For example, water solubility can be estimated based on correlation with a variety of descriptors. Dearden (2006) summarise a large number of quantitative structure property relationships that have been derived since 1990, and categorised the descriptors used in estimating water solubility as the following: log K_{OW} with or without melting point; atom/group contributions; physicochemical and quantum chemical descriptors; and topological indices. Statistical techniques prior to 1990 were based on linear regression, but artificial neural networks began to be used after 1990, with partial least squares statistics and descriptor selection by genetic algorithm also being used.

Dearden (2006) also notes that the development of estimation methods currently relies on the use of diverse compound libraries, which is particularly important in the development of new active pharmaceutical ingredients (APIs), where good understanding of aqueous solubility is a critical component in estimating oral absorption. While the performance of the various estimation methods reviewed by Dearden (2006) is highly variable, a general observation is that the degree of uncertainty with using an estimation method depends largely on whether or not the test chemicals being assessed have structural similarities to the chemicals used in the training set.

It is notable that the relationship between K_{OW} and S_W has been widely used in the development of methods for estimating S_W. For instance, Hansch *et al* (1968) demonstrated that for a heterogeneous data set of organic liquids that:

$$\text{Log } S_W = -1.339\log K_{OW} + 0.978 \text{ } (n=156, r^2 = 0.874, s = 0.472) \quad (13)$$

For organic chemicals that are solids at ambient temperature, it is necessary to consider the energies associated with the dissolution process. When a solid dissolves in water, the first step is to envision a melting step to a sub-cooled liquid, where the enthalpic and entropic changes cause the solubility of the solid to be less than that of its sub-cooled liquid. The difference between the two is proportional to the melting point T_m of the solid. In an effort to better estimate the solubility of solids, Yalkowsky and Valvani (1980) proposed the following:

$$\text{Log } S_W = 0.8 - \log K_{OW} - 0.01(T_m - 25) \quad (14)$$

Additional challenges associated with organic chemicals that are solids at ambient temperatures is further explored below, but here we attempt to capture how various estimation methods have evolved from these early observations. For instance, efforts to improve the performance of estimating S_W for nonpolar organic chemicals include the use of an additional descriptor of molecular size to account for the influence of energy required for cavity formation between water molecules. For polar solutes, in addition to molecular size descriptors to account for hydrogen bonding, atomic charge, polarisability, and polar surface area have all been utilised. Nonetheless, a general trend can be observed, whereby estimation methods tend to perform better for soluble chemicals than for insoluble chemicals. This may be related to the quality of the measured data for poorly soluble chemicals, as discussed above.

Another key challenge in estimating the S_W for organic chemicals relates to the availability and performance of methods for ionisable organic chemicals (IOCs). Active pharmaceutical ingredients (APIs) for instance consist of a large number of IOCs, encouraging Hewitt *et al*. (2009) to address a solubility challenge directly aimed at estimating the solubility of 32 API using a high quality training set of 97 chemicals. Based on a rigorous process assessing the quality of measured data and ensuring an appropriate applicability domain for a large number of estimation methods, Hewitt *et al*. (2009) report on the performance for the following models: ChemSilico (CSLogWS), Optibrium (StarDrop), Pharma Algorithms, SPARC, and seven different modules used within Simulation Plus (YINAN; UIQBB; LGGAV; A69EM; NSLIC; AM108; OLASM)). Included in their evaluation is also the results of a new *in silico* consensus tool, which gave relatively good performance (R^2 = 0.60; s = 0.68; RMSE = 0.90) (Hewitt *et al*., 2009). Consequently, methods that combine the output of a large number of estimation methods that have an applicability domain relevant to the chemicals being assessed appear to lead to lower uncertainty regarding the S_W of a chemical under investigation. Indeed, more recently Cappelli *et al* (2013) also assessed the performance of five different estimation methods (ACD, T.E.S.T. 4.0.1, ADMET Predictor 6.0, and the two EPI Suite 4.1 solubility estimation modules) using 400 chemicals with experimental values, and found that the consensus method reported by the T.E.S.T. 4.0.1 estimation method performed better than other methods (R^2 = 0.658).

A key message from this section is that the uncertainty/error in measured or estimated water solubilities translates directly into uncertainty/error in the calculated chemical activity. In the absence of empirical data for chemicals of interest, it is strongly recommended that multiple estimation software and/or other techniques (e.g., polyparameter Linear Free Energy relationships; ppLFERs) be utilised to assess the uncertainty in the solubility estimates. Note that while a high level of agreement between various estimates increases the level of confidence, and confers precision in the predicted values, it does not necessarily guarantee accuracy.

2) How reliable are available melting point data? How reliable are current approaches for estimating melting points in the absence of empirical data?

For chemicals that are a solid at the ambient temperature in the system of interest, it is important to use the chemical's sub-cooled liquid properties, and not properties of the solid-state chemical, when calculating activity. If sub-cooled liquid property data are not available for a given chemical, then melting point data can be used to convert solid-state properties to sub-cooled liquid properties (e.g., sub-cooled liquid solubility) via the Fugacity Ratio (F). Assuming Walden's Rule (1908) applies (see following section), the Fugacity Ratio can be calculated using the following expression:

$$\log F = -0.01(T_M - T) \qquad (15)$$

where T_M (^oC or K) is a given chemical's melting point and T is the ambient temperature in the system of interest.

The EPISUITE empirical melting point test database (MPBPVP v1.43 submodule) contains reported values for 10051 chemicals, with the reported melting points ranging from –205 to +492 °C. The largest publicly available database was recently compiled and described by Tetko *et al*. (2014). The database is divided into four non-overlapping datasets: i) OCHEM (n = 21883), ii) Enamine (n = 22404), iii) Bradley (n = 2866), and Bergström (n = 277). Note that the OCHEM dataset includes the EPISUITE database mentioned above. In general, the smaller the dataset, the more highly curated (and hence reliable) are the data.

The average prediction error of the MPBPVP v1.43 submodule for the test set (n = 10051) is approximately ± 50 °C and the root mean square error (RMSE) is approximately 64 °C. As with water solubility estimates, larger errors in estimated melting point (e.g., > 200 °C) can occur for some chemicals. Physical state misclassification (i.e., solids predicted to be liquids at 25 °C and vice versa) can also occur but is relatively uncommon; the MPBPVP v1.43 submodule correctly predicts the physical state for 86% of the data points used to evaluate the model.

The performance of the melting point estimation models developed and described in Tetko *et al*. (2014) is modestly improved in comparison to the MPBPVP v1.43 submodule. As discussed in Tetko *et al*. (2014), the RMSEs of estimated melting points are smallest for chemicals with reported melting points between 100 and 200 °C (RMSE ~ 30–40 °C) and largest for chemicals with melting points greater than 250 °C (RMSE ~ 50–90 °C). Because there is generally a positive relationship between melting point and molecular weight, these results suggest that uncertainty/error in predicted melting points will be greater for larger molecules.

It is worth reiterating that uncertainty/error in melting points for liquids are not relevant for chemical activity calculations and that the main concerns are uncertainty/error in predictions for solids and misclassification errors. For example, the average deviation of 50 °C and RMSE of 64 °C corresponds to a potential error in the Fugacity Ratio (F) of a factor of about 3.0 and 4.5 respectively. This factor translates directly into the uncertainty/error of the sub-cooled liquid water solubility estimates. Much larger errors (i.e., two orders of magnitude) are also possible for some chemicals. However, as documented above, the amount of publicly-available melting point data is relatively large (n = 47430 datapoints). While it may seem disappointing that all prediction methods still exhibit relatively large error, it is likely that empirical melting point data will be available for many chemicals of interest.

3) How reliable is Walden's Rule given the wide range of chemical structures for which the chemical activity concept may be applied?

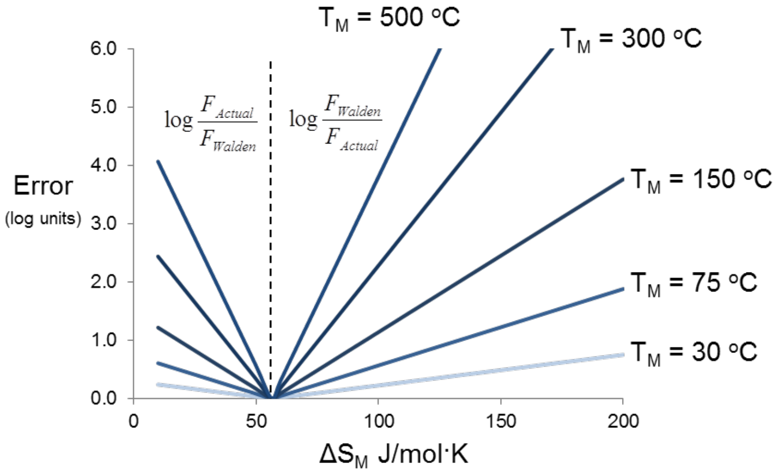
Implicit to the simplified equation for calculating the Fugacity Ratio (F) is the applicability of Walden's Rule (1908), which states that the entropy of melting (ΔS_M) is 56.5 J/K·mol. This entropy value is based on coal tar derivatives and is most applicable to rigid aromatics (e.g., polycyclic aromatic hydrocarbons). For small spherical compounds (e.g., methane, neon), ΔS_M is on the order of 10 J/K·mol (Richard's Rule) (Jain *et al*., 2004b). The more rigorous expression for calculating F is shown below.

$$\log F = \frac{-\Delta S_M}{2.303RT}(T_M - T) \tag{16}$$

The largest empirical database of ΔS_M values we are aware of was compiled by Jain *et al*. (2004b). This database contains 1799 reported ΔS_M values, which range from 0.6 (2,2-paracyclophane) to 232.6 J/K·mol (2-heneicosanone). The average reported ΔS_M values and standard deviation (σ) are 67 and 32 J/K·mol, respectively; the median ΔS_M value is 60 J/K·mol. The empirical ΔS_M database compiled by Dannenfelser and Yalkowsky (1996) (n = 1311) exhibits a larger range (ΔS_M = 0.7–588 J/K·mol) but similar central tendencies (average ΔS_M = 70 J/K·mol, σ = 57 J/K·mol, median ΔS_M = 54 J/K·mol). The largest discrepancies between the reported ΔS_M and Walden's Rule are for long chain alkyl substances (e.g., tristearin, $C_{57}H_{110}O_6$).

The uncertainty/error in the Fugacity Ratio (F) associated with uncertainty/error in ΔS_M depends on the melting point of the chemical of interest. The dependence of the error on melting point is illustrated in Figure 4.3.2 as a function of assumed ΔS_M for hypothetical chemicals/property value combinations.

Figure 4.3.2. Absolute error in estimated Fugacity Ratio (log units) as a function of ΔS_M for hypothetical chemicals with melting points of 30, 75, 150, 300 and 500 °C.



As illustrated in Figure 4.3.2, the uncertainty/error in F (and hence the subcooled liquid state property value) when Walden's Rule does not apply can be much greater for chemicals with larger melting points. For example, the uncertainty/error in F is greater than 6 orders of magnitude for a chemical with ΔS_M = 125 J/K·mol and TM = 500 °C, but less than 1 order of magnitude for any chemical with T_M = 30 °C. While the assessment based on hypothetical chemicals/property value combinations exaggerates the potential for error, in general, it is clear that errors in calculated F values greater than 3 to 5 orders of magnitude could occur, but only for chemicals with high melting points. The central tendency of the ΔS_Mvalues in the databases cited above suggest that large deviations/errors may be uncommon.

4) How reliable are available methods to estimate the entropy of melting (ΔSM) from chemical structure?

Dennenfalser and Yalkowsky (1996) introduced a semi-empirical approach for estimating the entropy of fusion (ΔS_M) using structural features. The equation they proposed is shown below:

$$\Delta S_M = C - R\ln\lambda + R\ln\phi \qquad (17)$$

where C is an entropy of melting constant, λ is a molecular rotational symmetry number, and φ is a molecular flexibility number. The rotational symmetry number is defined as, "the number of positions into which a molecule can be rotated that are identical to a reference position" whereas the flexibility number is a function of chain length and a "flexibility count" or number of torsional angles.

When applied to a subset of chemicals in the Dennenfalser and Yalkowsky ΔS_M database (n = 933), the average absolute error was 12.5 J/K·mol. However, error greater than or equal to 20 J/K·mol occurred for approximately 20% of the chemicals in the database. Similar performance was reported when the same equation was applied to the larger Jain *et al*. (2004b) database (n = 1799). While these results are promising, we are not aware of any automated methods to generate the required input parameters for the ΔS_M equation above.

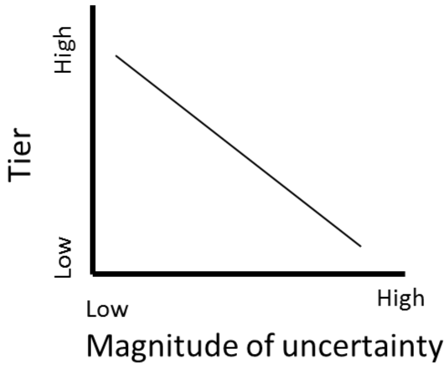
Brown *et al*. (2015) explored the possibility of applying an Iterative Fragment Selection (IFS) approach (Brown *et al*., 2012) to estimate ΔS_M from chemical structure (SMILES code). The IFS-QSAR was trained using 1056 chemicals from the Jain *et al*. (2004b) database and validated using 529 chemicals. Despite neglecting molecular symmetry effects, the performance of the IFS-QSAR was comparable to the original method. As the IFS-QSAR approach can readily be automated, it is hoped that the capability to generate estimates of ΔS_Mcan be soon disseminated to expert and non-expert users.

Brown *et al*. (2015) also generated an IFS-based QSAR for melting point. The two IFS-QSARs were used to generate estimates of T_M and ΔS_M for the 199 solid chemicals in the ΔS_M validation set with empirical melting point data. Fugacity Ratios calculated using the empirical T_M and ΔS_M were then compared to Fugacity Ratios calculated using the IFS-QSAR estimates. In this case study, 77% of the IFS-based Fugacity Ratios were within a factor of three of the empirically-based values; the maximum error was a factor of 34.

5) Taken together, what is the expected uncertainty in chemical activity calculations for 'data poor' chemicals?

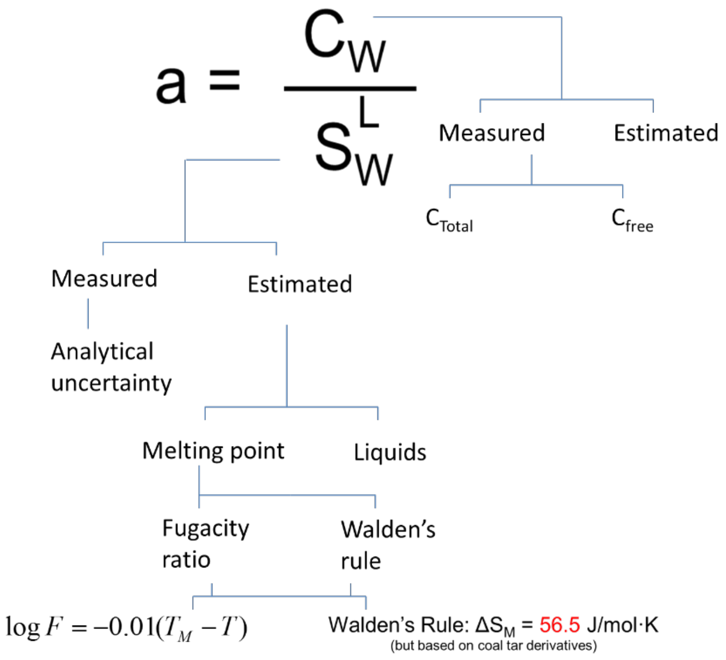
The relative influence of uncertainty in the physicochemical properties of an organic chemical on a chemical activity calculation will be directly related to the quality of input data. It is thus anticipated that calculations based on a limited assessment of S_W, either based on measured, estimated or a combination of both measured and estimated data, will inherently propagate a larger magnitude of uncertainty associated with calculations of chemical activity. Nonetheless, limited or poor quality S_W may still prove useful for screening and prioritisation purposes, or where the chemical activity concept is used at low tiers of assessment. If information based on chemical activity calculations are to be used within higher tiers of assessment it is suggested that efforts be targeted towards reducing the relative magnitude of uncertainty in S_W by ensuring the use of high quality measured data where consistency in S_W can be shown to be relatively consistent between different labs and analytical methods. Figure 4.3.3 qualitatively illustrates our perception of the expected uncertainty in chemical activity calculations in relation to how the calculation might be used in risk assessment. Figure 4.3.3 implies that a higher level of uncertainty can be accepted at lower tiers of assessment, where it is anticipated that input data would be subject to relatively little scrutiny. At higher tiers of assessment it is expected that input data would be of higher quality and receive greater scrutiny, which we believe would help to reduce the relative magnitude of uncertainty in calculations of chemical activity. Figure 4.3.4 illustrates how uncertainties in the various input properties discussed above might influence on the relative magnitude of uncertainty in a calculation of chemical activity.

Figure 4.3.3: Qualitative illustration relating the relative magnitude of uncertainty to different tiers of risk assessment.



It is anticipated that at lower tiers of assessment input data to calculations of chemical activity will be of lower quality and receive less scrutiny than input data used at higher tiers of assessment. This will directly influence the relative magnitude of uncertainty, whereby higher uncertainty may be acceptable in screening and prioritisation, but that higher quality data are necessary at higher tiers of evaluation to effectively reduce the inherent uncertainty that may propagate through the chemical activity calculation.

Figure 4.3.4: Illustration of how uncertainties in various physicochemical properties discussed in this section can propagate to influence the relative magnitude of uncertainty in a chemical activity calculation. Use of high quality measured data can be seen to reduce the propagation of error that might be associated with data based on estimates and various assumptions.



2. Calculation of chemical activity in non-aqueous phases (biota)

1) Is the octanol-water (K_{OW}) paradigm sufficiently accurate for estimating K_{BW}? When is it necessary to consider more sophisticated approaches (e.g., ppLFERs) for estimating K_{BW}?

The two main reasons for estimating chemical activity in non-aqueous phases are to i) convert biomonitoring data in non-aqueous phases (e.g., lipids) to chemical activities and ii) calculate chemical activities in situations where non-equilibrium conditions (i.e., chemical activity in biota ≠ chemical activity in water) are suspected or known to exist. Non-equilibrium conditions between water and biota may exist because of various processes, including i) rapid biotransformation *in vivo* or other kinetic limitations on chemical uptake (e.g., for superhydrophobic chemicals) and ii) biomagnification in food webs (i.e., step-wise increase in chemical activity from prey to predator). Note that rapid biotransformation or other kinetic limitations on chemical uptake can also lead to biodilution in food webs (i.e., trophic dilution).

Chemical activity in biota (a_B) can be calculated analogously to water:

$$a_B = \frac{C_B}{S_B} \tag{18}$$

where C_B is the concentration of the chemical in biota and S_B is the solubility of the chemical (liquid or sub-cooled liquid) in the organism. Although the concept of “solubility in biota” is not an intuitive one, it is believed that it can be approximated for neutral non-polar organics as the product of the water solubility and a biota-water partition coefficient (K_{BW}) (Mackay *et al*., 2011) as shown below:

$$S_B = K_{BW} S_W \tag{19}$$

See Section 4.1 for additional discussion.

As a first approximation, the biota-water partition coefficient for neutral organic chemicals can be estimated as the product of the total lipid content of the organism (f_L) and the octanol-water partition coefficient (K_{OW}), that is:

$$K_{BW} = f_L K_{OW} \tag{20}$$

The main assumptions underlying this expression are that i) lipids represent the dominant storage reservoir in the organisms and ii) octanol is a sufficiently accurate surrogate for lipids.

Broadly speaking, lipids can be divided into two classes, i) storage lipids (e.g., adipose) and ii) membrane lipids (i.e., phospholipids). Polyparameter linear free energy relationships (ppLFERs) are available for both types of lipids (Endo *et al*., 2011; Geisler *et al*., 2012; Geisler *et al*., 2015). In combination with solute descriptors for chemicals of interest (e.g., the UFZ LSER Database) (Endo *et al*., 2015), it is now possible to estimate partition coefficients for storage lipids and membrane lipids for any neutral organic chemical of interest. ppLFERs for structural proteins and plasma proteins are also available (Endo *et al*., 2011, 2012), meaning that sorption to some non-lipid organic matter (NLOM) can also be captured. In other words, the biota-water partition coefficient can be expanded to address partitioning in greater detail, as deemed necessary:

$$K_{BW} = f_{SL} K_{SLW} + f_{ML} K_{MLW} + f_{NLOM} K_{NLOMW} \tag{21}$$

where f_{SL}, f_{ML} and f_{NLOM} are the fractions of storage lipids, membrane lipids and non-lipid organic matter, respectively, and K_{SLW}, K_{MLW} and K_{NLOMW} are the corresponding partition coefficients, respectively.

Based on current knowledge of the performance of the available ppLFERs, it is recommended that more sophisticated approaches for estimating partitioning to biological macromolecules be considered for polar neutral organic compounds (i.e., compounds capable of engaging in hydrogen bonding), whereas the simplified approach is likely sufficient for apolar neutral organic compounds. Chemicals can be screened as 'polar' or 'apolar' by examining the values of the solute descriptors for the H-bond donor (α) and H-bond acceptor (β) parameters. The recommended source of these solute descriptors is the UFZ LSER Database (Endo *et al*., 2015). In the absence of reported data, estimates can be obtained using the ABSOLV estimation software from ACD Labs (www.acdlabs.com/products/percepta /predictors/absolv/).

More sophisticated approaches for estimating biota-water partitioning should also be considered for organisms (or tissues) with very low lipid content (e.g., $f_L < 0.01$), as partitioning to proteins is likely to be more important (deBruyn and Gobas, 2007; Endo *et al*., 2012). Finally, neutral chemicals with large water solubilities and/or small log K_{OW} values (i.e., log $K_{OW} < 2$) should also be treated differently (see section on Miscible Organic Chemicals, MOCs).

3. Application of the chemical activity concept to miscible organic chemicals (MOCs)

As introduced previously, chemical activity in water can be calculated from the concentration of the chemical in the aqueous phase (C_W) and the water solubility (S_W) (liquid or subcooled liquid):

$$a_W = \frac{C_W}{S_W} \tag{22}$$

This expression is problematic for miscible organic chemicals because truly miscible chemicals can be mixed into water up to any mole fraction (χ) i.e., from 0 (not present) to 1 (pure chemical). In other words, a constant water solubility does not exist. However, as discussed earlier, chemical activity can also be estimated using the following expression,

$$a_W = \chi_W \gamma_W \tag{23}$$

Sherman *et al*. (1996) compiled a database of 336 empirically-based activity coefficients derived from various experimental techniques (e.g., gas chromatography, differential static cell equilibrium). These activity coefficients are intended to be representative of the chemical in water at infinite dilution (i.e., γ_W^{∞}).

However, many of the data points were estimated from inverse solubility using the expression below and therefore are activity coefficients at saturation (i.e., γ_w^{sat})

$$\gamma_w^{sat} = \frac{1}{V_W^{MV} S_w^{sat}} \tag{24}$$

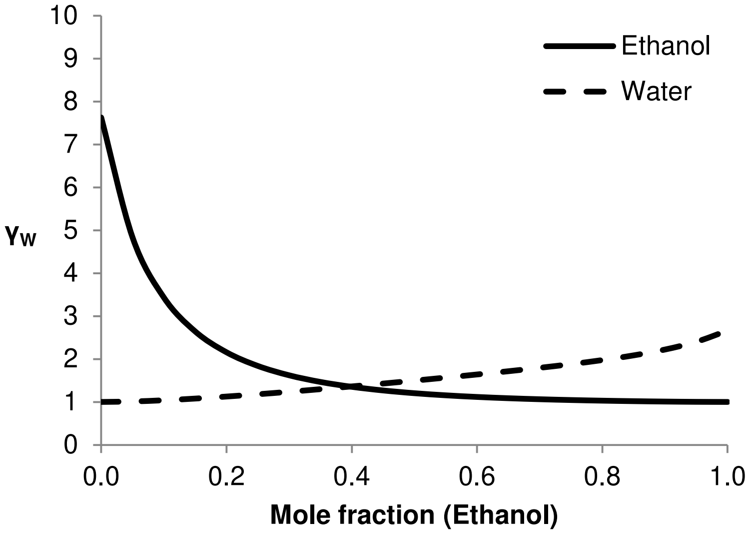
where V_W^{MV} is the molar volume of water (0.018 L/mol) and S_w^{sat} is the solubility of the chemical in water (liquid or subcooled liquid) at saturation. As shown in Table 4.3.1, activity coefficients at saturation tend to be similar to activity coefficients at infinite dilution for more sparingly soluble chemicals (i.e., limited concentration dependence is exhibited).

Table 4.3.1. Reported activity coefficients at saturation and infinite dilution for a set of neutral organic chemicals (Schwarzenbach et al., 2003).

Compound	γ_w^{sat}	γ_w^{∞}
Methanol	Miscible	1.6
Ethanol	Miscible	3.7
Acetone	Miscible	7.0
1-butanol	70	50
Phenol	63	57
Aniline	140	130
3-methylphenol	250	23
1-Hexanol	900	800
Trichloromethane	790	820
Benzene	2500	2500
Chlorobenzene	$1.4 \cdot 10^4$	$1.3 \cdot 10^4$
Tetrachloroethene	$7.5 \cdot 10^4$	$5.0 \cdot 10^4$
Naphthalene	$6.7 \cdot 10^4$	$6.9 \cdot 10^4$
1,2-dichlorobenzene	$6.2 \cdot 10^4$	$6.8 \cdot 10^4$
1,3,5-trimethylbenzene	$1.3 \cdot 10^5$	$1.2 \cdot 10^5$
Phenanthrene	$2.0 \cdot 10^6$	$1.7 \cdot 10^6$
Anthracene	$2.5 \cdot 10^6$	$2.7 \cdot 10^6$
Hexachlorobenzene	$4.3 \cdot 10^7$	$3.5 \cdot 10^7$
2,4,4'-trichlorobiphenyl	$5.6 \cdot 10^7$	$4.7 \cdot 10^7$
2,2',5,5'-tetrachlorobiphenyl	$7.0 \cdot 10^8$	$7.5 \cdot 10^7$
Benzo(a)pyrene	$3.2 \cdot 10^8$	$2.7 \cdot 10^8$

The assumption of limited concentration dependence is not valid for more water soluble chemicals (i.e., $\gamma_W < 100$),as can be seen in Figure 4.3.5. Moreover, the inverse solubility approach cannot be applied to miscible chemicals.

Figure 4.3.5. Estimated activity coefficient of ethanol and water at 25 °C as a function of the mole fraction of ethanol in the solution (generated using the DDBST-UNIFAC online calculator; http://ddbonline.ddbst.com/UNIFACCalculation/UNIFACCalculationCGI.exe).



1) To what extent are empirically-based chemical activity coefficients available for miscible organic chemicals and how reliable are these data?

For the purposes of this assessment, miscible chemicals are assumed to be those listed in the Sherman *et al*. (1996) database that exhibit γ_w^∞ values less than 20 (Mackay, 2001). Based on this criterion, approximately 15% (n = 49) of the empirically-based activity coefficients are for MOCs. The reliability of these data is unclear as very few chemicals have activity coefficients estimated using different techniques. Literature values of activity coefficients for methanol, ethanol, and 1-propanol compiled by Sherman *et al*. (1996) are within a factor of two whereas the literature values for acetone are within a factor of 10.

As we are unaware of any other publicly-available databases, no further evaluation is possible.

2) How reliable are computational approaches (e.g., UNIFAC, COSMOTherm, SPARC) for estimating chemical activity coefficients for miscible organic chemicals?

Activity coefficients can be estimated using ppLFERs (Sherman *et al*., 1996, Schwarzenbach *et al*., 2003) and various software estimation programs (e.g., UNIFAC, SPARC, COSMOtherm). An example of a ppLFER is shown below (Schwarzenbach *et al*., 2003):

$$\ln\gamma_w^{sat} = -\ln p_L^* - 5.72\left[(V_x)^{2/3}\left(\frac{n_{Di}^2 - 1}{n_{Di}^2 + 2}\right)\right] - 5.78\pi - 8.77\alpha - 11.1\beta + 0.0472V_x + 9.49 \qquad (25)$$

where p_L^* is the vapour pressure of the chemical (liquid or subcooled liquid) (Pa), V_X is the molar volume of the chemical (cm3/mol), n_{Di}^2 is the refractive index of the chemical, and π, α and β are solute descriptors for dipolarity, H-bond donor and H-bond acceptor properties.

ppLFERs

Activity coefficients estimated using the ppLFER described above (n = 266) were found to be within a factor of two to three of empirical values and it is suggested that the general performance of this method can be expected to be similar (Schwarzenbach *et al*., 2003).

Sherman *et al*. (1996) evaluated the performance of two ppLFERs using a subset of the values in their database. Note that one of the ppLFERs evaluated in Sherman *et al*. (1996) was trained using the remaining data points. Estimated activity coefficients were within a factor of two or less on average of the empirically-based values (average absolute deviation = 0.3–0.5 ln units). Furthermore, there was no large distinction in ppLFER performance for empirically-based values from inverse solubility data versus other techniques.

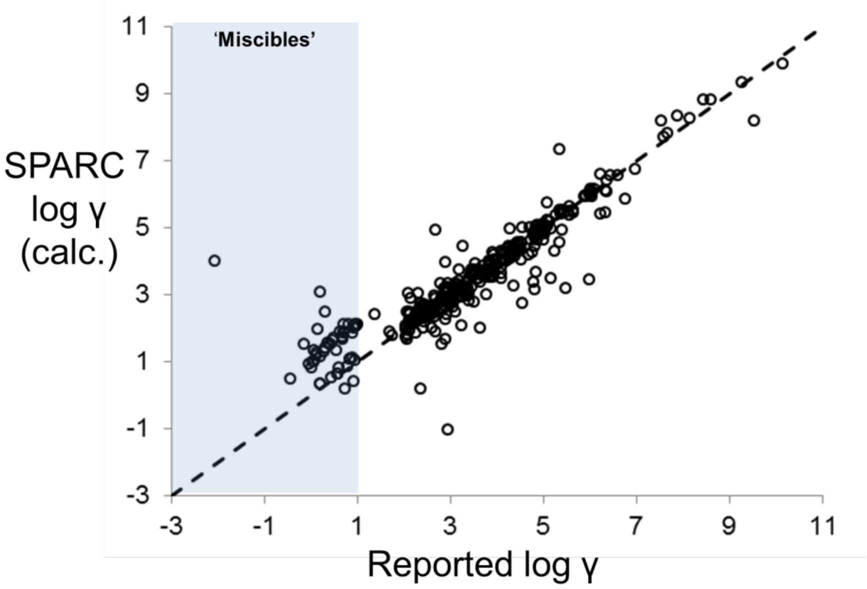
UNIFAC

Sherman *et al*. (1996) also evaluated the performance of UNIFAC using the same subset of data from their compilation. The average absolute deviation was 0.6 ln units (i.e., estimated values were again within a factor of two on average).

SPARC (http://archemcalc.com/sparc-web/calc)

A comparison between reported activity coefficients (n = 326) and those estimated by SPARC are shown in Figure 4.3.6. On average, the estimated values are within a factor of 2.5 of the reported values. However, some large discrepancies can be seen for chemicals with relatively large water solubilities (i.e., small activity coefficients). Accordingly, estimates for other miscible organic chemicals may be biased more than indicated by the average model performance.

Figure 4.3.6. Comparison of reported activity coefficients at infinite dilution and activity coefficients calculated by the SPARC estimation software.



COSMOtherm

COSMO-RS theory, as developed and distributed in the COSMOtherm program suite by COSMOlogic GmbH in Germany[4], is a relatively new and powerful theoretical method for estimation of infinite-dilution activity coefficients in aqueous and non-aqueous solvation environments. Invented by Andreas Klamt in the early 1990s, COSMO-RS is based on a "first-principles" approach, which uses the results of quantum-mechanical density-functional theory (DFT) calculations to estimate the relative Gibbs energy of a molecule in a solvation environment. The technique is "universal" in that it relies on a relatively small set of global parameters, which are optimised in the initial development by simultaneously fitting a wide range of chemical properties over a large and varied chemical set. Once optimised, these are not changed, irrespective of the solute molecule in question or the physico-chemical property sought. This gives a distinct advantage over UNIFAC, which is parameterised for all relevant types of functional group-group interactions.

In a recent evaluation by COSMOlogic in which they tested COSMOtherm's (v 15) ability to estimate activity coefficients compared with published data of two UNIFAC variants with aqueous and organic solvent sets, UNIFAC outperformed COSMOtherm for simple (single functional group) organic molecules (0.39 and 0.17 vs 0.53 RMS error in ln(γ) (Gerber and Soares, 2010). However, for the aqueous chemical set, for which UNIFAC is relatively poorly parameterised, COSMO-therm significantly outperformed UNIFAC, with an RMS error in ln(γ) of 0.79 vs 1.84 and 2.31. Overall, with both aqueous and non-aqueous solvation environments, and with a noted bias toward the more favourable organic solvent data for UNIFAC, COSMOtherm yielded an RMS error of 0.67 in ln(γ) compared with UNIFAC, which gave nearly double this value at 1.26 and 1.11. We

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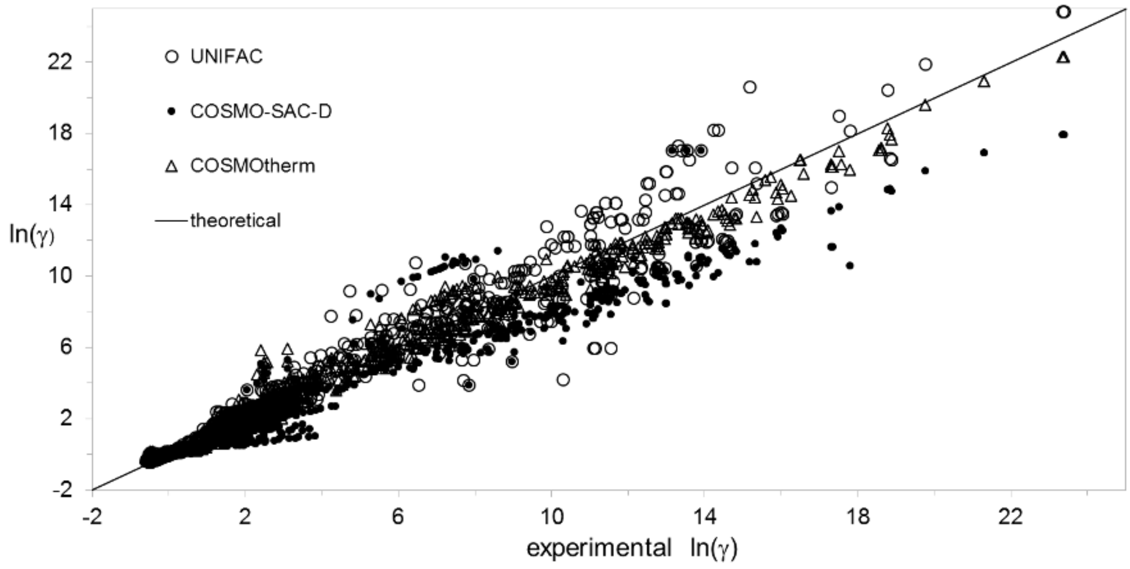
[4] COSMOlogic website: http://www.cosmologic.de/products/cosmotherm.html
See also the foundational publication referenced on the website: Klamt and Schüürmann, 1993.

can therefore anticipate an RMS error of about 0.8 in ln(γ) for aqueous infinite dilution activity coefficients with COSMO-RS. Note that the non-water set contains only 50 different chemical substances of which 16 are alkanes. The chemical diversity is extremely low and as a result this set has little relevance for substances like pharmaceutical, pesticides, fertilisers and fragrances. The chemical diversity of the water-set is somewhat larger, but still all compounds are basically mono-functional, making its relevance to complex molecules again low. As COSMO-RS handles more complex molecules in the same manner as simple molecules, its performance will not change significantly with such environmentally relevant compounds, whereas UNIFAC is expected to perform even less well.

Figure 4.3.7. Comparison of reported activity coefficients at infinite dilution and activity coefficients calculated by the COSMOtherm estimation software.



Summary

Of the 5764 reported water solubilities in the WATERNT database, only ~5% are ≥ $1·10^6$ mg/L (which indicates that they are likely to be MOCs). The general expectation is that activity coefficients for miscible organic chemicals can be estimated within a factor of three or less. Estimated activity coefficients greater than 20 for MOCs imply that a water solubility limit exists and should be considered unreliable. When possible (i.e., if input data are available and users have access to proprietary software), different estimation approaches should be applied in order to assess the level of agreement between model outputs. As discussed above in relation to estimates of S_W, a high level of agreement between various estimation methods increases the confidence in the predictions, however this does not necessarily guarantee accuracy.

Finally, the relatively good performance of the estimation methods for activity coefficients compared to water solubility may be somewhat misleading. As discussed above, activity coefficients and water solubilities are inversely related and therefore the accuracy of estimation methods for the two properties is expected to be similar. Evaluations of activity coefficient estimation methods using the much larger water solubility databases would likely give a better indication of true model performance.

It is thus suggested that chemical activities for MOCs can be calculated using activity coefficients, as opposed to S_W. However, unlike non-polar neutral organic chemicals, where it might be assumed that activity coefficients in lipids do not show much variability between different chemicals, and that at equilibrium, the chemical activities of chemicals in the water and organic or lipid-like phases are equal, it is less well understood if these assumptions are valid for MOCs. In the next section we attempt to address this challenge by assessing relationships between chemical activities and LC50 data for MOCs exerting baseline toxicity.

3) Case Study: Are the chemical activities corresponding to LC50s for 'narcotic miscibles' calculated using Equation 7 [$a_W = \chi_W \gamma_W$] consistent with expectations (i.e., La50s ~ 0.01)?

Acute 96-h toxicity data (LC50s) for three miscible baseline toxicants (Verhaar Class 1) and one miscible non-baseline toxicant (Verhaar Class 3) along with the corresponding lethal chemical activities (La50s) are presented in Table 4.3.2. As shown, the La50s for the baseline toxicants fall within the expected range (0.01–0.1) whereas the La50 for the Verhaar Class 3 chemical is orders of magnitude lower.

Table 4.3.2. Preliminary assessment of the chemical activity hypothesis for miscible organic chemicals.

Compound	**Verhaar Class**	**LC50 (96 h) Fish mg/L**	**Mole Fraction (χ_W)**	**Activity Coefficient (γ_W)**	**La50**
Ethanol	1	13000	0.005	3.7	0.02
Methanol	1	29400	0.016	1.6	0.03
Acetone	1	8300	0.003	7.0	0.02
Formaldehyde	3	50	$3 \cdot 10^{-5}$	2.8	$8 \cdot 10^{-5}$

Verhaar Class taken from ToxTree (http://toxtree.sourceforge.net/predict/); Activity coefficients are from Sherman *et al*. (1996). Toxicity data obtained from the Duluth database of acute toxicities to fathead minnow.

Although further case studies would be useful, it appears that the chemical activity approach can be applied to MOCs categorised as baseline toxicants. However, given the frequency with which MOCs occur in the WATERNT database and the limited environmental relevance of exposure to these compounds, research priorities should focus on reducing uncertainties for sparingly soluble compounds (i.e., apolar and polar neutral organic chemicals), particularly those which are solids at ambient temperatures. Nevertheless, with respect to demonstrating the utility and viability of the chemical activity approach, it is deemed worthwhile to include MOCs in case studies aiming to demonstrate 'proof of concept'.

4) Given the (relatively) low affinity by MOCs for lipids and other non-lipid organic matter, what modifications to the approach for estimating K_{BW} (see above) are necessary?

To more accurately estimate biota-water partitioning for miscible organic chemicals, the freely dissolved concentration in the water (f_W) present in the organism can simply be added to the expression for K_{BW}:

$$K_{BW} = f_L K_{OW} + f_W \text{ or } K_{BW} = f_{SL} K_{SLW} + f_{ML} K_{MLW} + f_{NLOM} K_{NLOMW} + f_W \quad (26)$$

4. Application of the chemical activity concept to ionisable organic chemicals (IOCs)

Of the various questions discussed within this workgroup, it was widely acknowledged that the application of the chemical activity concept to IOCs represents one of the most difficult areas to address, largely due to limited availability of data and models applied to this group of chemicals. The first major issue with applying the chemical activity concept to ionisable organic chemicals (IOCs) is that the total water solubility of such compounds is a function of pH in addition to the properties of the chemical. The presence and identity of counterions in solution can also be an important consideration. These dependencies are illustrated in Figures 4.3.8 and 4.3.9.

Figure 4.3.8 is a generic illustration of the pH-solubility profile of an organic acid; Figure 4.3.9 is the reported pH-solubility profile of naproxen and its various salts (Chowhan 1978, Serajuddin 2007), which clearly demonstrates the sensitivity of water solubility to the type of counterion present.

The pH_{max} is a function of the solubility product (K_{SP}) and hence the counterion(s) present in solution. Below the pH_{max}, the total solubility of an acidic IOC is simply a function of pH and pKa (Figure 4.3.8) and can be estimated from the intrinsic solubility (i.e., solubility of the neutral form of the chemical) and the extent of dissociation (He and Yalkowsky, 2004; Serajuddin 2007), i.e.,

$$S_T = S_N + S_C = S_N (1 + 10^{(pH - pKa)}) \quad (27)$$

Above the pH_{max}, the total solubility is determined by the solubility of the salt complex (i.e., A^-S^+) and is independent of pH (Figure 4.3.8, 4.3.9). As seen in the naproxen example (Figure 4.3.9), the maximum solubility can vary by roughly two orders of magnitude.

Figure 4.3.8. Generic representation of the pH-solubility profile of an organic acid. The pH-solubility profile for an organic base is essentially the mirror image.

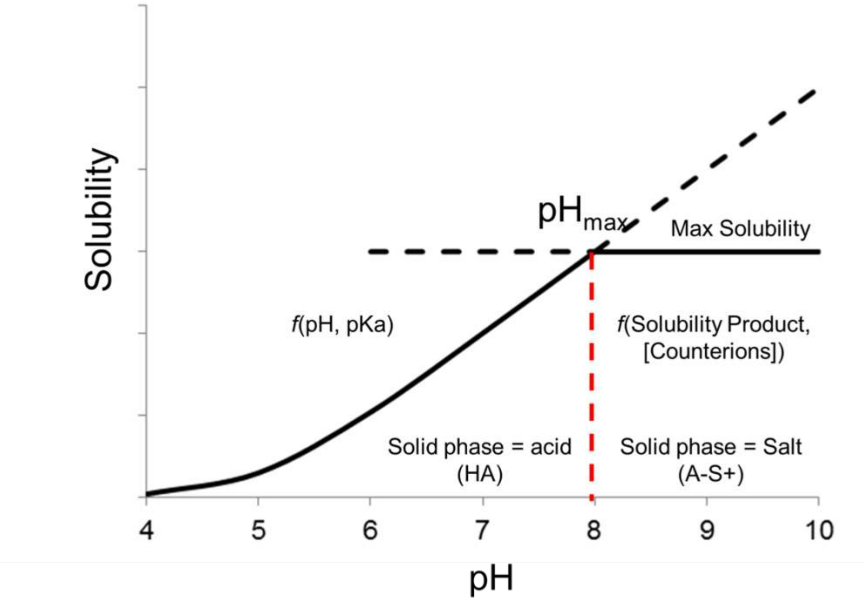
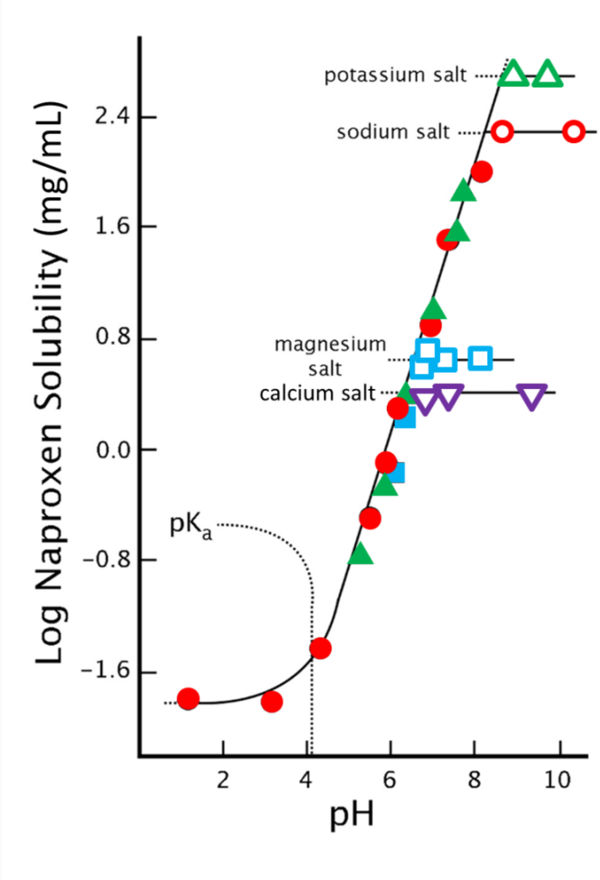


Figure 4.3.9. pH-solubility profile of naproxen (an organic acid with a pKa ~ 4) and its salts (calcium, magnesium, sodium, potassium) in aqueous solution at 25 oC. (Based on Chowhan (1978), Figure above reproduced with permission from Flynn & Roberts 2015)



As aqueous systems in the laboratory and environment can vary greatly in terms of type and concentration of counterions, it is clear that implementing the chemical activity concept is far more challenging for IOCs compared to neutral organics chemicals.

1. To what extent can approaches to calculate chemical activity for neutral organic chemicals be expanded/modified to IOCs? Are methods for estimating the activity coefficients of electrolytes (e.g., Debye–Hückel approach) (Trapp et al., 2010) compatible with methods for neutral organic chemicals?

In an effort to initiate discussion and debate, it is proposed that, as a first approximation, the total chemical activity (a_T) could potentially be calculated using the modified version of the equation for neutral organics, formulated here for an organic acid, i.e.,:

Below pH_{max}

$$a_T = a_N + a_C = \frac{C_N}{S_N} + \frac{C_C}{S_C} = \frac{C_N}{S_N} + \frac{C_N \cdot 10^{(pH-pKa)}}{S_N \cdot 10^{(pH-pKa)}} = 2\frac{C_N}{S_N} \qquad (28)$$

Above pH_{max}

$$a_T = a_N + a_C = \frac{C_N}{S_N} + \frac{C_C}{S_S} = \frac{C_N}{S_N} + \frac{C_N \cdot 10^{(pH-pKa)}}{S_S} \qquad (29)$$

where a_N, C_N, and S_N are the chemical activity, concentration and solubility (sub-cooled liquid) of the neutral form of the chemical, respectively, and a_C, C_C, S_C are the chemical activity, concentration and solubility of the charged form (below pH_{max}), respectively, and S_S is the solubility of the dominant salt complex (i.e., A^-S^+) in the system. Melting points for the neutral chemical (i.e., HA) and the dominant salt complex must be known. It is assumed that i) the IOC is a solid at system temperature, ii) 'solubility addition' applies (Banarjee 1984, Smith *et al*., 2013), and iii) solute-solute interactions are negligible. Assuming that S_S will be greater than S_N, chemical activities below pH_{max} are always greater than chemical activities above pH_{max} (but within a factor of two). However, since the F will be lower, the maximum activity that can be attained, by correcting for F, will be lower (see WG1).

2. Case Study: Can the intrinsic water solubility (i.e., water solubility of the neutral form) and fraction of chemical in neutral form in solution be used to calculate chemical activity from LC50s?

More simplistically, an initial estimate of chemical activity in water for IOCs could be obtained by ignoring the contribution of the charged form and considering only the neutral species, i.e.,

$$a_N = \frac{C_N}{S_N} \tag{30}$$

Note that the concentration of the neutral form of the chemical (C_N) can be calculated from the reported total concentration (or LC_{50}) at any pH using the Henderson-Hasselbalch equation, as shown for an organic acid below:

$$C_N = \frac{C_T}{1 + 10^{(pH - pKa)}} \tag{31}$$

It is important to recognise that even if the approximations presented above are reliable, pH differences between the bulk water phase and biological fluids (e.g., blood, cytoplasm) further complicate the analysis of IOCs due to the 'ion trapping' effect (e.g., Neuwoehner and Escher, 2011). The 'ion trapping' effect refers to the differential accumulation of the charged species in external water vs. internal fluids, as determined by the concentration of the neutral form (assumed equal) and pH-dependent speciation (i.e., ratio of neutral to charged form) in both phases.

Aquatic toxicity data for six pharmaceuticals at three different bulk water pHs are summarised in the following Table 4.3.3 (Boström and Berglund 2015). These data illustrate the commonly reported pH-dependence of aquatic toxicity data based on external water concentration. Irrespective of the equation used, it is clear that chemical activities estimated following Equation 3 will not collapse towards a consistent value but rather will be a function of bulk pH. Such results are in contrast to analyses based on measured or estimated Critical Body Residue (CBR) or membrane concentrations, which tend to cluster around a common value (e.g., Nakamura *et al*., 2008; Neuwoehner and Escher, 2011). To demonstrate this, a CBR-based analysis of aquatic toxicity data for fluoxetine is presented in Table 4.3.4.

Table 4.3.3. Aquatic toxicity data for six pharmaceuticals at three different bulk water pHs. EC50s are reported in terms of total water concentration and concentration of neutral species only (estimated using the Henderson-Hasselbalch equation)

Compound	pKa	pH	EC50 (total) mg/L	EC50 (neutral) µg/L
Fluoxetine				
	10.05 (base)	6.0	27	2.4
		7.5	4.6	13
Sertraline		9.0	0.75	61
	9.47 (base)	6.0	8.5	2.9
		7.5	1.2	12
Naproxen		9.0	0.18	45
	4.84 (acid)	6.0	10	670
		7.5	25	54
Diclofenac		9.0	96	6.6
	4.18 (acid)	6.0	5.2	77
		7.5	11	5.1
Ibuprofen		9.0	41	0.62
	4.41 (acid)	6.0	4.2	110
		7.5	50	40
Ketoprofen		9.0	110	2.8
	4.23 (acid)	6.0	45	740
		7.5	180	99
		9.0	230	122

Table 4.3.4. Aquatic toxicity data for fluoxetine in fish analysed using the CBR approach based on experimental LC50s and bioconcentration factors (BCFs) (Nakamura et al., 2008)

pH	LC50 (mM)	Reported BCF (L/kg)	CBR (mM) BCF * LC50
7	0.018	13	0.23
8	0.0042	37	0.16
9	0.00065	330	0.21

The fact that the CBRs for fluoxetine shown in Table 4.3.4 collapse to a common value requires a common chemical activity in the organism, which could be calculated either from the CBR or an estimate of the internal water concentration. Regardless, while it may be possible to address the pH-dependence of toxicity data of IOCs (e.g., by accounting for ion trapping or using a BCF model to convert external concentrations to internal burdens) (Neuwoehner and Escher, 2011; Armitage *et al.*, 2013), it is obvious that the application of the chemical activity approach to such chemicals is hindered by the additional assumptions and calculations that are likely to be required.

In summary, the simplified approaches for calculating chemical activity for IOCs in water described above suggest it is possible to develop estimation methods for further evaluating the chemical activity hypothesis for IOCs, however, additional research is needed to further support and validate observations reported here.

5. CONCLUSIONS AND RECOMMENDATIONS

The major conclusions and research suggestions identified during the workshop are described in this chapter. No attempt was made to prioritise the research topics during the workshop but the participants were asked to do this prior to this report being finalised.

Conclusions

A key message is that the uncertainty/error in measured or estimated water solubilities translates directly into uncertainty/error in the calculated chemical activity. In the absence of empirical data for chemicals of interest, it is strongly recommended that multiple estimation software and/or other techniques (e.g., polyparameter Linear Free Energy relationships; ppLFERs) be utilised to assess the uncertainty in the model output. Note that while a high level of agreement between various estimates increases the level of confidence, and confers precision in the predicted values, it does not necessarily guarantee accuracy.

Chemical Activity Concept.

The workshop participants concluded that there were a number of opportunities and challenges facing the chemical activity concept.

The opportunities include:

- Chemical activity is a more insightful and relevant metric of chemical exposure than concentration because concentrations are media-dependent while activity applies to all media, allowing exposure and toxicity to be expressed on a common basis.
- Activity provides a good metric for characterising baseline toxicity for single non-polar organic chemicals and mixtures of non-polar organic chemicals.
- Activity data are useful for discriminating between nn-polar narcosis, which occurs at activities between 0.01 and 0.1, and excess toxicity, which occur at activities less than 0.01.
- Activity can also be used to identify poor quality data (McCarty *et al*., 2013) such as toxicity data from experiments where dosing concentrations were above the solubility of the chemical in the exposure medium (e.g., toxicity reported at an activity, $a > 1$), and exposure data from experiments subject to background contamination.
- The application of activity to describe the toxicity of mixtures of non-polar organic chemicals represents a novel tool in chemical risk assessment that can be particularly useful in addressing chemical risks in real world environments.

The challenges are:

- Translation from concentration to activity is crucial in studies where existing data are converted into the chemical activity space. However, this translation can be challenging and can add error to measurement error.
- Improved communication of the activity concept is a major issue and will be central to future application and impact. Communication of the activity approach to a non-scientific audience may not be easy. Whether a broader acceptance of the chemical activity framework can be achieved

might also be a matter of semantics. How can chemical activity be communicated in a comprehensible way and become widely accepted?

Suggestions for communicating the chemical activity concept to a wider audience may require adopting alternative terms that similarly convey the concept of chemical activity, such as “percent of saturation” or “fractional solubility” both of which would also reflect the output from Equation 1. The following suggestions were also proposed:

- An online tool, such as an “activity calculator” provided as an Excel file or interactive website. Such a calculator was used in a SETAC short course on the application of chemical activity, and can be made available for application to existing and new chemicals. A copy of the calculator will be made available on the following website: http://www.rem.sfu.ca/toxicology/models/.
- The characterisation of the error and/or uncertainty in chemical activity needs to be better understood and quantified.
- It is of critical importance to emphasise that at all times the domain of applicability for the activity approach be carefully defined. Current knowledge would limit the applicability domain to non-polar organics log KOW > 2 and predictions of toxicity to MOA 1 and possibly MOA 2 (Baseline toxicity).

It was also concluded that:

- Current ‘chemometer-based’ methods are preferable for hydrophobic chemicals relative to conventional methods. However, credibility needs to be enhanced by improved communication.
- The domain of applicability needs to be carefully defined, and the limitations stated:
    - It works for those chemicals for which training sets exist.
- A number of methods for measuring activity have been identified:
    - passive samplers for more hydrophobic pollutants,
    - head-space approaches for volatile chemicals,
    - utilising concentration data and dividing them by liquid solubility.
- The conversion from concentration to chemical activity by the use of partition coefficients and calculated activity coefficients can be inaccurate.
- There is a need to identify and clearly communicate the domain of applicability for various conversion methods.

Classification of chemicals

- AOPs and biologically based assays will be useful to differentiate further MOA classes 3 and 4, which may then benefit from interpretation using a chemical activity approach to provide improved bioavailability metrics;
- Verhaar classes offer a simple approach to differentiate between expected specific, reactive and narcotic MOAs for acute exposure with some recommendations (see below) if the chemical domain is covered;
- In specifically acting and reactive classes a secondary consideration is to assess the target of action and consider if chemical activity can be applied in a useful relevant manner;
- Chemical activity seems best applied to MOA 1 and 2 with significant research effort necessary to see to what extent the application can be broadened to MOA 3 and 4;

- Initial analyses of HC5 values have shown that recalculating HC5 into chemical activities leads to constant “threshold of toxicological concern”. This concept is interesting and could be useful in risk assessment;
- New approaches in classification based on “omics” data are promising. The variability in omics data is presently high and the regulatory applicability is still limited. Large scale efforts with an important bio-informatics component are needed to overcome the current limitations and it is further recommended that efforts should be focused on increasing the applicability of omics for identifying MOAs of untested chemicals (including assigning chemicals to the narcosis MOA) and specifically on sub-lethal effects and species differences.

Future Research

Suggestions for future research were separated into three themes, (i) the chemical activity concept, (ii) application of the chemical activity approach and (iii) classification of chemicals. The topics listed in the following text were identified as areas where further research could prove important.

Chemical Activity Concept

- QSARs should be re-evaluated in terms of chemical activity.
- Insights into the validity of the assumption that the “cytotoxic burst” phenomenon is an *in vitro* analogue to baseline toxicity. Concentrations associated with the “cytotoxic burst” could be expressed as chemical activity to test the hypothesis that these concentrations would be equivalent to activity in the 0.1-0.01 range. Second, it would be useful to apply structure-based mode of action classification schemes to the Toxcast chemical library and examine the agreement (or lack thereof) between chemical structure-based identification of putative baseline (MOA 1,2) toxicant and biologically-based identification of baseline toxicants as based on the cytotoxic burst analysis.
- Examination of the correlations between chemical activity and potency of ToxCast chemicals in specific assays and identify those for which a strong relationship exists. One could then examine the localisation and function of those targets in more detail and begin to investigate whether there is a scientifically-plausible theoretical basis on which to expect that activity based predictions would have value for predicting chemical potency against those targets.
- Study of the applicability of the concept of chemical activity to chronic toxicity data, exercises to analyse MoA specificities of acute to chronic relationships for consistent data sets, e.g. zebrafish early life stage assay (FELS, OECD guideline 210, 2013) would be helpful.

Classification of chemicals

- Refinement of the Verhaar classification scheme by:
    - Subcategorisation of the major classes;
    - Updating of chemical information;
    - Including information about the target site and target environment;
    - Extending the chemical domain.
- Create a separate activity to improve existing classification schemes for non-narcotic chemicals.

- Develop evidence to support the application of chemical activity to chronic toxicity for non-polar and polar narcotics.
- Test the applicability of chemical activity in deriving threshold of toxicological concern (TTC).
- Expand the application of chemical activity concept for mixtures.
- Omics efforts should be focused on increasing the applicability for identifying MOAs of untested chemicals (including assigning chemicals to the MOA 1) and specifically on sub-lethal effects and species differences.

Application of the activity approach

- Convert and compare critical body burdens to activity. The critical body burden concept has many similarities with the chemical activity approach, and it seems thus useful to relate and contrast data and results from both approaches. As an example, an activity-based conversion of narcosis critical body residue (CBR) data showed that there appeared to be some questionable CBRs in the selected set of baseline toxicant data (McCarty *et al*., 2013).
- Apply the activity approach to data-rich chemicals. The chemical activity approach can be used to convert many different data sets into one “currency”, which then can (i) provide a basis for comparisons of data from different areas, (ii) help in utilisation of more existing data and (iii) facilitate the process towards an overview of the entire data basis for potential assessments and management actions.
- Apply activity to monitoring data sets, which (i) is expected to give better data for heterogeneous and biological media and (ii) will help to connect measurements between environmental compartments.
- Apply the activity concept and activity ratios in order to prioritise and guide monitoring chemicals.
- Activity-based species sensitivity distributions (SSDs). Toxicity tests that were conducted at controlled chemical activity (i.e., via passive dosing) have been published recently. Determining the SSDs from such studies is expected to give an improved estimate of actual sensitivity distributions, since differences in exposure conditions between test methods, which normally confound the distributions, are largely accounted for.
- Develop an activity calculator and make it publicly available.
- Evaluate the agreement between computational and measured data. There are fundamental differences between calculating and measuring chemical activities, and it was found important to distinguish and to compare these two different ways to obtain activity data.
- Use chemical activity to characterise and predict mixture toxicity, which was identified as one of the most important applications of the chemical activity framework during the initial presentations and the discussions.

ABBREVIATIONS

ACR	Acute / chronic ratio
AOP	Adverse outcome pathway
APIS	Active pharmaceutical ingredients
AUC	Area under the curve
CNS	Central Nervous System
D5	Decamethylcyclopentasiloxane
DPRA	Direct Peptide Reactivity Assay
ΔS_M	Entropy of melting
EQS	Environmental Quality Standards
ERA	Environmental risk assessment
F	Fugacity Ratio
G	Gibbs free energy
GSH	Glutathione
IFS	Iterative Fragment Selection
IOCs	Ionisable organic chemicals
KOW	The octanol-water partition coefficient
MIE	Molecular initiating event
MOA	Modes of action
MOCS	Miscible organic chemicals
PNEA	Predicted no-effect activity
QSPR	Quantitative structure property relationship
RMSE	Root mean square error

SSDS	Species sensitivity distributions
Sw	Solubility in water
σ	Standard deviation
TK	Toxicokinetic
TKTD	Toxicokinetic and dynamic
TTC	Threshold of toxicological concern
WWTP	Waste water treatment plant

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APPENDIX B: WORKSHOP PROGRAMME

Thursday 29 October 2015

08:00 –08:30	*Registration and coffee*	
08:30 - 08:50	**Welcome and introductory remarks**	**Malyka Galay Burgos** ECETOC, Belgium
08:50 - 09:20	**Foundational aspects of the concept of chemical activity**	**Philipp Mayer** Technical University of Denmark
09:20 - 9:40	**Application of the “chemical activity” concept**	**Frank Gobas** Simon Fraser University, Canada
9:40 - 10:00	**General information about mode of actions in ecotoxicology**	**Joop Hermens** University of Utrecht, Netherlands
10:00 - 10:20	**Challenges and potential limitations – physicochemical properties**	**Todd Gouin** Unilever, UK
10:20 - 11:00	*Coffee break*	
11:00–12:30	**Breakout into workgroups** ➢ Workgroup 1: “Full utilisation of the chemical activity concept for non-polar organic chemicals (Log $K_{ow} \geq 2$)” ➢ Workgroup 2: “Classification of chemicals according to MOA and chemical activity or other dose metrics for chemicals with specific mode of action” ➢ Workgroup 3: “Challenges and potential limitations to the application of the chemical activity concept for ecological risk assessment – Physicochemical properties & partitioning”	
12:30 - 13:30	*Lunch*	
13:30 –15:30	**Workgroups (continued)** ➢ Workgroup 1: “Full utilisation of the chemical activity concept for non-polar organic chemicals (Log Kow ≥ 2)” ➢ Workgroup 2: “Classification of chemicals according to MOA and chemical activity or other dose metrics for chemicals with specific mode of action” ➢ Workgroup 3: “Challenges and potential limitations to the application of the chemical activity concept for ecological risk assessment – Physicochemical properties & partitioning”	
15:30 - 16:00	*Coffee break*	
16:00 - 17:00	**Plenary: feedback & discussion with panel** *Breakouts report back (5-10 minutes each)*	

Identify key points, consensus and research needs

19:30 *Dinner*

Close of first day

Friday 30 October 2015

08:30–10:30 **Workgroups (continued)**

- Workgroup 1: “Full utilisation of the chemical activity concept for non-polar organic chemicals (Log Kow ≥ 2)”
- Workgroup 2: “Classification of chemicals according to MOA and chemical activity or other dose metrics for chemicals with specific mode of action”
- Workgroup 3: “Challenges and potential limitations to the application of the chemical activity concept for ecological risk assessment. Physicochemical properties & partitioning”

10:30 - 11.00 *Coffee break*

11:00 - 12:00 **Plenary feedback & discussion with panel**

Breakouts report back (5-10 minutes each)

Identify key points, consensus and research needs

12:00 - 13:00 *Lunch*

13:00–15:30 **Breakout into workgroups**

- Workgroup 1: “Full utilisation of the chemical activity concept for non-polar organic chemicals (Log Kow ≥ 2)”
- Workgroup 2: “Classification of chemicals according to MOA and chemical activity or other dose metrics for chemicals with specific mode of action”
- Workgroup 3: “Challenges and potential limitations to the application of the chemical activity concept for ecological risk assessment – Physicochemical properties & partitioning”

15:30 - 16:00 *Coffee break*

16:00 - 17:00 **Breakout into workgroups**

- Workgroup 1: “Full utilisation of the chemical activity concept for non-polar organic chemicals (Log Kow ≥ 2)”
- Workgroup 2: “Classification of chemicals according to MOA and chemical activity or other dose metrics for chemicals with specific mode of action”.
- Workgroup 3: “Challenges and potential limitations to the application of the chemical activity concept for ecological risk assessment – Physicochemical properties & partitioning”

17:00 - 18:00 **Plenary: feedback & discussion with panel**
Breakouts report back (5-10 minutes each)
Identify key points, consensus and research needs

19:30 *Dinner*

Close of Workshop

APPENDIX C: ORGANISING COMMITTEE

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