

APPENDIX A: THE ACTIVITY CONCEPT AND THE RELATIONSHIP BETWEEN FUGACITY/ACTIVITY WITH TOXICITY AND CRITICAL BODY BURDEN (CBB)

Chemical activity and lipid volume fraction are dose metrics that have direct relationships to disruptive narcotic concentrations in cell membranes are chemical activity and the volume fraction in lipid. Chemical activity and lipid volume fraction provide other insights and applications for toxicity test data; however, these metrics are also constrained by some of the same technical issues discussed for critical body residue data earlier in the report. Chemical activity (a , unitless) is closely related to the lipid based critical body residue as activity in the lipid phase is a product of the chemical's mole fraction in the lipid and the activity coefficient in that phase (Ferguson, 1939). The activity coefficients of nonpolar narcotics in octanol (often an assumed surrogate for organic phase toxicity target sites) are relatively constant (Mackay *et al*, 2009; 2011). Chemical activity is directly related to concentration. For example, the chemical activity in water is the fraction of saturation of the chemical's liquid state water solubility. Activity thus expresses the proximity to saturation directly and can readily identify inadvertent experimental supersaturation in various phases (i.e. water and air). It also can explain the apparent non-toxicity of high melting point solid solutes such as hexachlorobenzene because these chemicals cannot achieve an activity coefficient of 1.0 in aqueous solution (Escher and Fenner, 2011), being constrained to lower activities and lower lipid concentrations by the low fugacity ratio. Chemical activity can be measured or estimated in the physical environment or in organisms (Mayer and Reichenberg, 2006; Mayer *et al*, 2009; Jahnke *et al*, 2011), calculated from toxicity test exposure concentrations or fugacities in water or air (Ferguson, 1939; Mackay *et al*, 2009) or calculated using multimedia mass balance models, thus providing a direct link to environmental fate, exposure, and toxicity evaluations in screening-level risk assessment (Mackay and Arnot, 2011).

When considering the activity and fugacity concepts discussed in Chapter 1, it is important to understand whether the chemical under consideration is a solid, liquid or vapour at the environmental temperature. In particular, for those substances that are solid at the ambient temperature, there is a need to establish what the super-cooled liquid vapour pressure is. This is defined as the vapour pressure that a solid would have at ambient temperature (e.g. 25°C) if it were a liquid at 25°C. The super-cooled liquid vapour pressure cannot be measured directly but can be calculated or measured indirectly using gas chromatographic retention times (Mackay, 2001). Correlations exist between the vapour pressure of substances in the solid state and their super-cooled liquid counterparts. The ratio between the solid vapour pressure and the super-cooled liquid vapour pressure is called the fugacity ratio (F). The fugacity ratio can be approximated with knowledge of the substance melting point (Yalkowsky *et al*, 1979; Cole and Mackay, 2000) as:

$$F = \exp[-6.79(T_M/T-1)] \dots\dots\dots \text{Equation 1}$$

where T_M is the melting point in Kelvin and T is the ambient temperature. The constant value of -6.79 is derived by dividing a typical value of $56 \text{ J mol}^{-1} \text{ K}$ assumed for entropy of fusion (ΔS_{fus}) with the gas constant ($R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$). For example, solid naphthalene has a melting point of 318 K and a water solubility of 0.24 mol m^{-3} at 25°C. Its estimated fugacity ratio (F) is 0.634 and thus its hypothetical (and unmeasurable) liquid solubility is therefore $0.24/0.634 \text{ mol m}^{-3}$ or 0.382 mol m^{-3} . Further information on more accurate determination of fugacity ratios (F) can be found in Yalkowsky *et al* (1979) and Mackay (2001).

In simple terms, fugacity can be viewed as a measure of the tendency of a component of a liquid mixture to escape, or vaporise, from the mixture. The fugacity of a component in a mixture is essentially the pressure that it exerts in the vapour phase when in equilibrium with the liquid mixture. The fugacity of a substance can be deduced for a chemical in solution from its concentration. At concentrations low enough to negate intermolecular interactions between solute molecules, fugacity and concentration are linearly related by $C = Zf$, where C is the concentration (mol/m^3), f is the fugacity (Pa) and Z is termed the fugacity capacity ($\text{mol/m}^3 \text{ Pa}$). The fugacity capacity in (Z_w) water can be deduced from the Henry's law constant (H , $\text{Pa m}^3/\text{mol}$) where $Z_w = 1/H$.

The fugacity capacity in water can be defined as $1/v_w \gamma_i f_R$; where v_w is the molar volume of water, f_R the reference fugacity and γ_i is the activity coefficient of a particular chemical i . The reference fugacity is the fugacity that the solute will tend to in the pure liquid state when the mole fraction is 1.0 and γ_i is also 1.0. It is therefore the fugacity of the pure liquid solute at the temperature and pressure of the system. In a similar way, the fugacity capacity for octanol can be defined as $Z_o = 1/v_o \gamma_i f_R$, where v_o is the molar volume of octanol. This is further elaborated below.

When considering the potential for toxicity of non-polar narcotic substances, the property of interest is the hydrophobicity of the substance. The fundamental determinant of hydrophobicity is the solute's activity coefficient (γ_i) in water. This property can be viewed as the ratio of the activity (or fugacity) of the solute to the activity (or fugacity) that the solute would have if it were in a solution consisting entirely of pure solute. The activity coefficient can also be regarded as the inverse expression of solubility, where a solute that is only sparingly soluble in a solvent (e.g. water), will have a high activity coefficient. When expressed as a mole fraction, the activity coefficient is the reciprocal of the solubility (Mackay, 2001). Substances with an activity coefficient that is less than 20 can be considered highly soluble in water, or even miscible. Mackay (2001) defined a pseudo-solubility as $1/\gamma_i v_s$. For a substance that is miscible in water, which will then behave like an ideal liquid where $\gamma_i = 1.0$, the solubility approaches $1/v_s$ which is the density of the solvent (mol/m^3). Mackay has estimated this as $55,500 \text{ mol/m}^3$ for water ($10^6 \text{ g/m}^3 / 18 \text{ g/mol}$), or 55.5 mol/L . In comparison, a

poorly water soluble substance, like DDT (water solubility of 5.5 µg/L; 10⁻⁷ mol/L) has an activity coefficient (γ_i) of over 500,000. Table A1 from Mackay *et al* (2009) defines the relationships between the above parameters.

Table A1: Dependence and relationships of substance physicochemical properties with its activity coefficient in water (γ_w) as an indicator of hydrophobicity

Property	Relationships to γ_w
Fugacity ratio F , i.e. ratio of solid to liquid solubility (S) or vapour pressure (P) at temperature T (K)	$F = P_S^S/P_L^S = S_S^S/S_L^S \approx \exp^{(6.79(1-T_M/T))} \approx 10^{(0.01(298-T_M))}$ T_M is melting point (K) if $T_M < T$, $F = 1.0$
Solubility of liquid state chemical in water S_L^S (mol m ⁻³)	$S_L^S = 1/(\gamma_w v_w)$ where v_w is molar volume of water (18 x 10 ⁻⁶ m ³ mol ⁻¹)
Solubility of solid state chemical in water S_S^S (mol m ⁻³)	$S_S^S = F S_L^S = F/(\gamma_w v_w)$
Octanol-water partition coefficient K_{ow}	$K_{ow} = \gamma_w v/(\gamma_o v_o)$ where γ_o is the activity coefficient in octanol and v_o is the molar volume of water-saturated octanol (126.6 x 10 ⁻⁶ m ³ mol ⁻¹)

A suite of other useful substance property parameters (K_{OA} , K_{AW} , solubility in air / vapour pressure etc.) which describe the behaviour of substances in the environment, are dependent upon the activity coefficient of that substance in different media (e.g. water, air, soil and sediment). When partition coefficients e.g. octanol-water (K_{ow}), but also air-water (K_{AW}), and octanol-air (K_{OA}) are used, the fugacity ratio (F) does not need to be factored in as is the case for the solubility of solids since F cancels out between the concentration ratios in the two media. These additional partition coefficient parameters are useful in the application of multimedia environmental distribution models and have been discussed extensively in the literature (Mackay *et al*, 2009).

The concept of activity has proven to be extremely useful in providing a framework for directly comparing the toxic potency of chemicals in both air- and water-breathing animals (Reichenberg and Mayer, 2006; Mackay *et al*, 2009) and that are measured or predicted in the environment. Measured concentrations from bioassays were converted to activities in water by comparison to the saturation concentration (i.e. water solubility of the liquid-state chemical). For poorly defined phases such as biota or sediments, the concentrations could be converted into fugacities using the appropriate Z values. Multimedia models in many cases have fugacity as the output, and fugacities or concentrations can be converted to activities and can be compared with the activity levels required to cause acute or chronic toxicity in a variety of water and air-respiring organisms, i.e. risk characterisation using the chemical activity concept, rather than concentrations.

When chemical concentration is expressed as quantity per unit weight or volume (e.g. mg kg^{-1} and mg L^{-1}) it is often difficult, and not scientifically meaningful (Mackay *et al*, 2009), to compare the toxic potency of chemicals to organisms in environmental media (e.g. air, water, soil, sediment). As mentioned above, these units are external metrics which cannot be used to accurately describe the critical body burden found to cause effects in various organisms. Unfortunately, the toxicological literature has rarely incorporated this knowledge and has instead made extensive use of external dose metrics in this manner that cannot be easily compared (Mackay *et al*, 2009). The standardised use of molar concentration ($\text{mol}\cdot\text{L}^{-1}$) normalises potency for chemicals with different molecular weights. However, two different chemical structures with the same mode of action (MoA), such as benzene and naphthalene present at the same molar concentration can appear to have vastly different (eco)toxicities and therefore this approach is considered a major cause of fragmentation in the approach to assess chemicals for environmental hazard (Mackay *et al*, 2009).

In a recent study present study, McCarty *et al* (2013) calculated chemical activity from the evaluated critical body residue data (n=161 observations for 29 chemicals) using 2 methods: from estimated chemical fugacities in the test organisms and chemical solubilities in membranes. McCarty *et al* (2013) assumed that all types of biological lipids are equivalent to octanol in their capacity to dissolve the test chemicals. Details of the fugacity concept and activity calculations have been described elsewhere (Mackay, 2001; Mackay *et al*, 2009). For the first approach, fugacity capacities for lipid were calculated using octanol as a surrogate for lipid (ZL, moles per cubic meter lipid pascals) and fugacities (f, pascals) were calculated from the lipid-normalised critical body residues (moles per cubic meter lipid) as f/Z (Mackay, 2001). Chemical activities were then calculated from the fugacities using liquid-state vapour pressures for the solutes (PL, pascals) as f/PL . For the second approach, liquid-state chemical solubilities in lipid (SL, moles per cubic meter lipid) were estimated assuming a constant activity coefficient in octanol for all chemicals and the water-saturated molar volume of octanol (v_O , $126.6 \times 10^{-6} \text{ m}^3/\text{mol}$). Chemical activities were then calculated as critical body residue-lipid/SL. For chemicals that are solids at 25 °C, the liquid-state, subcooled PL and SL properties can be calculated from the solid-state estimates and the fugacity ratios as described elsewhere (Mackay *et al*, 2009). Henry's law constants, octanol/water partition coefficient (K_{OW}), and melting point data required for these calculations can be obtained from EPI Suite 4.1 (US EPA, 2011) with measured data selected preferentially over quantitative structure–activity relationship predictions. It is recognised that the true liquid-state vapour pressures and lipid and solubilities in octanol will differ to some degree from these approximations. Despite differences in the chemical activity estimation methods, approximately 80% of the activities for the same data point were within a factor of 3.