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Linking algal growth inhibition to chemical activity: Baseline toxicity required 1% of saturation

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Highlights

- Algal growth inhibition was linked to chemical activity
- The chemical activity range for baseline toxicity (0.01-0.1) was supported
- Baseline toxicity (EC_{50}) required 1% of saturation
- 1‰ of saturation is suggested as PNEC for baseline toxicity of individual compounds
- 0.1‰ of saturation is suggested as PNEC for baseline toxicity of mixture constituents

Abstract

Recently, high-quality data were published on the algal growth inhibition caused by 50 non-polar narcotic compounds, of which 39 were liquid compounds with defined water solubility. In the present study, the toxicity data for these liquids were applied to challenge the chemical activity range for baseline toxicity. First, the reported effective concentrations (EC_{50}) were divided by the respective water solubilities (S_{water}), since the obtained EC_{50}/S_{water} ratio essentially equals the effective chemical activity (Ea_{50}). The majority of EC_{50}/S_{water} ratios were within the expected chemical activity range of 0.01-0.1 for baseline toxicity, and none of the ratios were significantly below 0.01. On a practical level, these findings suggest EC_{50} values for baseline toxicity to be at or above 1% of water solubility, which would have been accurate or conservative for all 39 liquids with defined water solubility in the applied dataset. On an environmental risk assessment level, predicted no-effect concentrations (PNECs) for baseline toxicity could even be set as a percentage of saturation, which can easily be extended to mixtures. However, EC_{50} values well below 1% of saturation can still occur and would be a direct indication of excess toxicity.

Keywords: Algal toxicity; Chronic toxicity; Baseline toxicity; QSAR; Chemical activity

1. Introduction

In a recent study, Aruoja and co-workers (Aruoja et al., 2014) determined the algal growth inhibition caused by 50 non-polar narcotic compounds and generated a quantitative structure-activity relationship (QSAR) between effective concentrations (EC_{50}) and octanol to water partition coefficients (Log K_{ow}, Fig. 1a). The data, and the accompanying QSAR developed for baseline toxicity, are useful within a regulatory risk assessment context, in that they provide additional guidance with respect to estimating EC_{50} values based on molecular descriptors. However, the dataset of Aruoja et al. (2014) also provides an opportunity to challenge the recently proposed chemical activity range for baseline toxicity (Reichenberg and Mayer, 2006; Mayer and Holmstrup, 2008; Mackay et al., 2009; Mackay et al., 2014). The chemical activity (a) quantifies the energetic level of an organic compound relative to the energetic level in its pure liquid (reference state, a=1), and the chemical activity of a liquid is thus defined between 0 and 1 (Reichenberg and Mayer, 2006). Several recent experimental and modelling studies have proposed, and to some degree also confirmed, that baseline toxicity requires a chemical activity of at least 0.01-0.1 (e.g., Reichenberg and Mayer, 2006; Mayer and Holmstrup, 2008; Mackay et al., 2009; Smith et al., 2010; Mackay et al., 2011; Lee et al., 2013; Mackay et al., 2014). While the experimental studies tend to be limited in terms of tested compounds, the modelling studies have involved data selection and estimation of data. Thus, larger datasets of experimental toxicity data, which can easily be converted to chemical activity, can profitably complement the reported experimental and modelling studies.

The interpretation of toxicity data on a chemical activity basis can be achieved using an approach that is both relatively simple and elegant. Specifically, when a liquid compound is dissolved in water, and has limited water solubility (S_{water}), the ratio of EC_{50}/S_{water} provides

a unitless metric that essentially equals the effective chemical activity (Ea_{50}) (Ferguson, 1939; Reichenberg and Mayer, 2006). The dataset reported by Aruoja et al. (2014) is thus well suited for assessing the utility of the chemical activity approach within a risk assessment paradigm for several reasons: First, the algal growth inhibition tests were conducted in closed vessels without headspace and at reduced algal density, which both minimises the loss of test compound. Second, the majority of test compounds were liquids, which simplifies the conversion from aqueous concentration to chemical activity. Finally, a wide range of chemical groups were included (Aruoja et al., 2014). Thus, the aims of the present study are: (1) to convert toxicity data published by Aruoja and co-workers to chemical activity, (2) to challenge the proposed chemical activity range of 0.01-0.1 for baseline toxicity and finally (3) to provide a framework for how the obtained findings can be used in practise.

2. Methods

Algal growth inhibition tests of 50 non-polar narcotic compounds were recently reported by Aruoja and co-workers (Aruoja et al., 2014). The 72-h tests were conducted in closed vessels without headspace and at reduced algal density in order to minimise losses of test compound (Mayer et al., 2000). Inhibition of growth rate was used as the toxicity endpoint and expressed as effective concentrations (EC_{50}). In the present study, we determined the ratio of EC_{50} (mg L^{-1}) and water solubility (S_{water}, mg L^{-1}) for all the liquid compounds with defined water solubility, for which this ratio essentially equals the effective chemical activity (Ea_{50}). Test compounds that were either water miscible (n=9) or solids (n=2) were excluded from this data analysis, because their Ea_{50} not simply can be approximated as ratio of EC_{50} and S_{water}. The EC_{50}/S_{water} ratios of the remaining 39 test compounds were

plotted as a function of their respective Log K_{ow} (Fig. 1b. See Supplementary Table 1 for a list of the 39 liquids). Additional data from closed algal growth inhibition tests were found in the literature for 14 of the 39 test compounds (Hsieh et al., 2006; Lin et al., 2005). The additional data were included in Supplementary Fig. 1 for validation and as an additional reference, in the absence of analytical exposure confirmation in the study by Aruoja et al. (2014).

The water solubilities and Log K_{ow} values given by Aruoja and co-workers were double-checked in the PhysProp Database (SRC Inc.), and the following four corrections were made; the water solubilities of diethylether, 1,2-dichlorobenzene and pentachloroethane were corrected to 60400 mg L^{-1}, 156 mg L^{-1} and 490 mg L^{-1}, respectively, while the Log K_{ow} value of 1,2-dichlorobenzene was corrected to 3.43. In all other cases, data were used as reported by Aruoja et al. (2014).

3. Results and discussion

The EC_{50}/S_{water} ratios for the 39 non-polar narcotic liquids were essentially within the chemical activity range of 0.01-0.1 (Fig. 1b). These ratios represent effective chemical activities (Ea_{50}) for liquids with limited water solubility (e.g. S_{water} < 1-10 g L^{-1} or Log K_{ow} ≥ 2, Supplementary Fig. 2), whereas they still roughly approximate Ea_{50} for the more water soluble compounds (Ferguson, 1939; Reichenberg and Mayer, 2006). In this way, the EC_{50}/S_{water} ratios shown in Fig. 1b clearly support the recently established chemical activity range for baseline toxicity (Reichenberg and Mayer, 2006; Mayer and Holmstrup, 2008; Mackay et al., 2009; Mackay et al., 2014). This finding was confirmed by additional toxicity data for 14 of the 39 test compounds (Supplementary Fig. 1). Again, the EC_{50}/S_{water} ratios

were essentially within the expected range of 0.01-0.1 for baseline toxicity, and none of the ratios (in total n=56) were significantly below 0.01 (Supplementary Fig. 1).

The obtained findings are in good agreement with previously reported observations, but more importantly provide additional insight. First, previous studies establishing and supporting the chemical activity range for baseline toxicity have mainly relied on acute toxicity data, whereas the present study not only supports the chemical activity range for baseline toxicity, but also extends this with chronic toxicity data. Second, the obtained findings complement an earlier study, in which algal growth inhibition caused by hydrophobic organic solids was related to chemical activity with special emphasis on toxicity cut-off phenomena (Mayer and Reichenberg, 2006). In combination, the present study and the study by Mayer and Reichenberg (2006) support the chemical activity range of 0.01-0.1 for baseline toxicity for a wide range of compounds, covering the Log K_{ow} range of 1-7. Finally, the obtained findings are in overall agreement with the target lipid model by Di Toro and co-workers (Di Toro et al., 2000) and also the critical membrane range suggested by van Wezel and Opperhuizen (van Wezel and Opperhuizen, 1995). In this way, we are not establishing an entirely new relationship between exposure and baseline toxicity, but rather offering an alternative perspective and interpretation.

In a practical perspective, the findings of the present study may have a very simple implication. While QSARs are normally applied to estimate EC_{50} values for untested contaminants, this might not be necessary in instances where baseline toxicity is identified as the predominant mode of action, which is typical of non-polar narcotic compounds. EC_{50} values could be set to 1% of the water solubility, which for the dataset of Aruoja and co-workers would have been accurate or conservative for all 39 liquids (Fig. 1b). This

approach might even be extended to the estimation of no-effect concentrations (PNEC): Baseline toxicity is a general type of toxicity and characterized by a rather narrow species sensitivity distribution (van Wezel and Opperhuizen, 1995; McCarty and Mackay, 1993), which has been supported in previous studies linking baseline toxicity to chemical activity in algae (Mayer and Reichenberg, 2006), invertebrates (Mayer and Holmstrup, 2008; Smith et al., 2010; Schmidt et al., 2013a) and fish (Veith et al., 1983; Mackay et al., 2009; Seiler et al., 2014; Mackay et al., 2014). Additionally, chemical activity-response curves for baseline toxicity do generally have rather steep slopes (Mayer and Holmstrup, 2008; Smith et al., 2010; Schmidt et al., 2013a), which leads to a close correspondence between EC_{50} values and lower effect concentrations (e.g., EC_{10} and NOEC) (Chen et al., 2009). Due to the rather narrow species sensitivity distribution and the steep slopes, a general PNEC of 1‰ of saturation (i.e., a=0.001) is expected to be protective with regards to the baseline toxicity of individual contaminants for a wide range of species. Another important perspective of relating toxicity to a percentage of saturation is associated to baseline mixture toxicity, which generally follows “activity addition” (Smith et al., 2013; Schmidt et al., 2013b). The contribution of an individual compound to baseline mixture toxicity could thus be kept below 10%, when setting the PNEC at 0.1‰ of saturation, although additional research would be useful in improving our overall understanding of how to set these values.

The findings of the present study do not indicate that toxicity per se will require at least 1% of saturation, since several modes of action can lead to excess toxicity beyond baseline toxicity. In this respect, EC_{50} values well below 1% of saturation would be a direct indication of excess toxicity and should trigger further testing and assessment of the compound. We believe this to be an area of interest warranting additional research. In

particular, it would be of interest to assess the feasibility of the chemical activity approach for contaminants exhibiting excess toxicity, due to more specific modes of action.

4. Conclusions and perspectives

Effective chemical activities (Ea_{50}) were approximated for 39 non-polar narcotic liquids, and the algal toxicity data supported the established chemical activity range of 0.01-0.1 for baseline toxicity. More practically, these findings suggest that effective concentrations (EC_{50}) for baseline toxicity will generally be at or above 1% of water solubility. This provides a simple yet sound basis for setting predicted no-effect concentrations of untested compounds, identified as baseline toxic contaminants, as a fraction of saturation. 1‰ of saturation appears to be protective for the baseline toxicity of individual compounds, whereas a lower level (e.g. 0.1‰ of saturation) could be set in order to limit the contribution of an individual compound to the baseline toxicity of a mixture. EC_{50} values well below 1% of saturation would be a direct indication of excess toxicity, and should trigger further testing and assessment of the compound. This study provides valuable insight regarding the relationship between chemical activity and chronic toxicity data for baseline toxic contaminants. We recommend that additional work be targeted towards further challenging the chemical activity range for baseline toxicity, with additional work focussing on refining appropriate thresholds of saturation based on addressing the risk assessment of mixture toxicity and addressing the feasibility of the chemical activity approach for compounds that have a more specific mode of action.

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Supplementary material

Supplementary Table 1, Fig. 1 and Fig. 2 associated with this article can be found, in the online version, at http://....

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