



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

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

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17 **Researchgate:**

18 [https://www.researchgate.net/publication/264980441 Linking algal growth inhibition to
19 chemical activity Baseline toxicity required 1 of saturation](https://www.researchgate.net/publication/264980441_Linking_algal_growth_inhibition_to_chemical_activity_Baseline_toxicity_required_1_of_saturation)

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25 **Highlights**

- 26 • Algal growth inhibition was linked to chemical activity
- 27 • The chemical activity range for baseline toxicity (0.01-0.1) was supported
- 28 • Baseline toxicity (EC_{50}) required 1% of saturation
- 29 • 1‰ of saturation is suggested as PNEC for baseline toxicity of individual
30 compounds
- 31 • 0.1‰ of saturation is suggested as PNEC for baseline toxicity of mixture
32 constituents

33 **Abstract**

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

48

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

50 **1. Introduction**

51 In a recent study, Aruoja and co-workers (Aruoja et al., 2014) determined the algal growth
52 inhibition caused by 50 non-polar narcotic compounds and generated a quantitative
53 structure-activity relationship (QSAR) between effective concentrations (EC_{50}) and octanol
54 to water partition coefficients ($\text{Log } K_{ow}$, Fig. 1a). The data, and the accompanying QSAR
55 developed for baseline toxicity, are useful within a regulatory risk assessment context, in
56 that they provide additional guidance with respect to estimating EC_{50} values based on
57 molecular descriptors. However, the dataset of Aruoja et al. (2014) also provides an
58 opportunity to challenge the recently proposed chemical activity range for baseline toxicity
59 (Reichenberg and Mayer, 2006; Mayer and Holmstrup, 2008; Mackay et al., 2009; Mackay
60 et al., 2014). The chemical activity (a) quantifies the energetic level of an organic
61 compound relative to the energetic level in its pure liquid (reference state, $a=1$), and the
62 chemical activity of a liquid is thus defined between 0 and 1 (Reichenberg and Mayer,
63 2006). Several recent experimental and modelling studies have proposed, and to some
64 degree also confirmed, that baseline toxicity requires a chemical activity of at least 0.01-
65 0.1 (e.g., Reichenberg and Mayer, 2006; Mayer and Holmstrup, 2008; Mackay et al., 2009;
66 Smith et al., 2010; Mackay et al., 2011; Lee et al., 2013; Mackay et al., 2014). While the
67 experimental studies tend to be limited in terms of tested compounds, the modelling
68 studies have involved data selection and estimation of data. Thus, larger datasets of
69 experimental toxicity data, which can easily be converted to chemical activity, can
70 profitably complement the reported experimental and modelling studies.

71

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

75 a unitless metric that essentially equals the effective chemical activity (E_{a50}) (Ferguson,
76 1939; Reichenberg and Mayer, 2006). The dataset reported by Aruoja et al. (2014) is thus
77 well suited for assessing the utility of the chemical activity approach within a risk
78 assessment paradigm for several reasons: First, the algal growth inhibition tests were
79 conducted in closed vessels without headspace and at reduced algal density, which both
80 minimises the loss of test compound. Second, the majority of test compounds were liquids,
81 which simplifies the conversion from aqueous concentration to chemical activity. Finally, a
82 wide range of chemical groups were included (Aruoja et al., 2014). Thus, the aims of the
83 present study are: (1) to convert toxicity data published by Aruoja and co-workers to
84 chemical activity, (2) to challenge the proposed chemical activity range of 0.01-0.1 for
85 baseline toxicity and finally (3) to provide a framework for how the obtained findings can be
86 used in practise.

87

88

89 **2. Methods**

90 Algal growth inhibition tests of 50 non-polar narcotic compounds were recently reported by
91 Aruoja and co-workers (Aruoja et al., 2014). The 72-h tests were conducted in closed
92 vessels without headspace and at reduced algal density in order to minimise losses of test
93 compound (Mayer et al., 2000). Inhibition of growth rate was used as the toxicity endpoint
94 and expressed as effective concentrations (EC_{50}). In the present study, we determined the
95 ratio of EC_{50} ($mg L^{-1}$) and water solubility (S_{water} , $mg L^{-1}$) for all the liquid compounds with
96 defined water solubility, for which this ratio essentially equals the effective chemical activity
97 (E_{a50}). Test compounds that were either water miscible ($n=9$) or solids ($n=2$) were
98 excluded from this data analysis, because their E_{a50} not simply can be approximated as
99 ratio of EC_{50} and S_{water} . The EC_{50}/S_{water} ratios of the remaining 39 test compounds were

100 plotted as a function of their respective Log K_{ow} (Fig. 1b. See Supplementary Table 1 for a
101 list of the 39 liquids). Additional data from closed algal growth inhibition tests were found in
102 the literature for 14 of the 39 test compounds (Hsieh et al., 2006; Lin et al., 2005). The
103 additional data were included in Supplementary Fig. 1 for validation and as an additional
104 reference, in the absence of analytical exposure confirmation in the study by Aruoja et al.
105 (2014).

106

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

113

114

115 **3. Results and discussion**

116 The EC_{50}/S_{water} ratios for the 39 non-polar narcotic liquids were essentially within the
117 chemical activity range of 0.01-0.1 (Fig. 1b). These ratios represent effective chemical
118 activities (Ea_{50}) for liquids with limited water solubility (e.g. $S_{water} < 1-10 \text{ g L}^{-1}$ or Log $K_{ow} \geq$
119 2, Supplementary Fig. 2), whereas they still roughly approximate Ea_{50} for the more water
120 soluble compounds (Ferguson, 1939; Reichenberg and Mayer, 2006). In this way, the
121 EC_{50}/S_{water} ratios shown in Fig. 1b clearly support the recently established chemical activity
122 range for baseline toxicity (Reichenberg and Mayer, 2006; Mayer and Holmstrup, 2008;
123 Mackay et al., 2009; Mackay et al., 2014). This finding was confirmed by additional toxicity
124 data for 14 of the 39 test compounds (Supplementary Fig. 1). Again, the EC_{50}/S_{water} ratios

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

127

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

143

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

150 approach might even be extended to the estimation of no-effect concentrations (PNEC):
151 Baseline toxicity is a general type of toxicity and characterized by a rather narrow species
152 sensitivity distribution (van Wezel and Opperhuizen, 1995; McCarty and Mackay, 1993),
153 which has been supported in previous studies linking baseline toxicity to chemical activity
154 in algae (Mayer and Reichenberg, 2006), invertebrates (Mayer and Holmstrup, 2008;
155 Smith et al., 2010; Schmidt et al., 2013a) and fish (Veith et al., 1983; Mackay et al., 2009;
156 Seiler et al., 2014; Mackay et al., 2014). Additionally, chemical activity-response curves for
157 baseline toxicity do generally have rather steep slopes (Mayer and Holmstrup, 2008; Smith
158 et al., 2010; Schmidt et al., 2013a), which leads to a close correspondence between EC₅₀
159 values and lower effect concentrations (e.g., EC₁₀ and NOEC) (Chen et al., 2009). Due to
160 the rather narrow species sensitivity distribution and the steep slopes, a general PNEC of
161 1‰ of saturation (i.e., $a=0.001$) is expected to be protective with regards to the baseline
162 toxicity of individual contaminants for a wide range of species. Another important
163 perspective of relating toxicity to a percentage of saturation is associated to baseline
164 mixture toxicity, which generally follows “activity addition” (Smith et al., 2013; Schmidt et
165 al., 2013b). The contribution of an individual compound to baseline mixture toxicity could
166 thus be kept below 10%, when setting the PNEC at 0.1‰ of saturation, although additional
167 research would be useful in improving our overall understanding of how to set these
168 values.

169

170 The findings of the present study do not indicate that toxicity per se will require at least 1%
171 of saturation, since several modes of action can lead to excess toxicity beyond baseline
172 toxicity. In this respect, EC₅₀ values well below 1% of saturation would be a direct
173 indication of excess toxicity and should trigger further testing and assessment of the
174 compound. We believe this to be an area of interest warranting additional research. In

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

177

178

179 **4. Conclusions and perspectives**

180 Effective chemical activities (E_{a50}) were approximated for 39 non-polar narcotic liquids,
181 and the algal toxicity data supported the established chemical activity range of 0.01-0.1 for
182 baseline toxicity. More practically, these findings suggest that effective concentrations
183 (EC_{50}) for baseline toxicity will generally be at or above 1% of water solubility. This
184 provides a simple yet sound basis for setting predicted no-effect concentrations of
185 untested compounds, identified as baseline toxic contaminants, as a fraction of saturation.
186 1‰ of saturation appears to be protective for the baseline toxicity of individual compounds,
187 whereas a lower level (e.g. 0.1‰ of saturation) could be set in order to limit the
188 contribution of an individual compound to the baseline toxicity of a mixture. EC_{50} values
189 well below 1% of saturation would be a direct indication of excess toxicity, and should
190 trigger further testing and assessment of the compound. This study provides valuable
191 insight regarding the relationship between chemical activity and chronic toxicity data for
192 baseline toxic contaminants. We recommend that additional work be targeted towards
193 further challenging the chemical activity range for baseline toxicity, with additional work
194 focussing on refining appropriate thresholds of saturation based on addressing the risk
195 assessment of mixture toxicity and addressing the feasibility of the chemical activity
196 approach for compounds that have a more specific mode of action.

197

198

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206

207 **Supplementary material**

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

210

211

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