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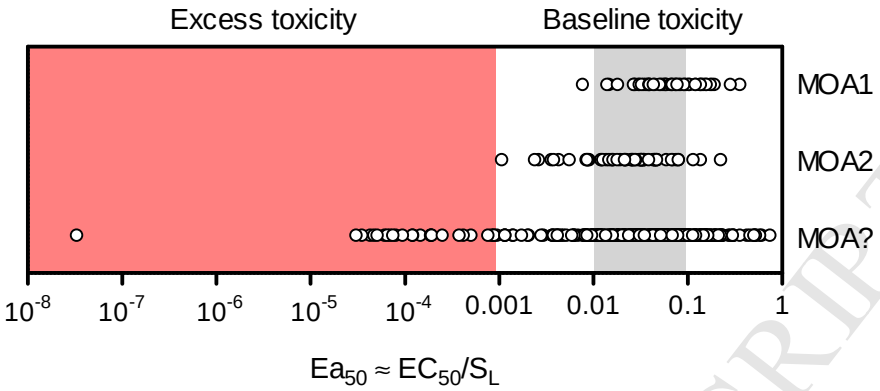
Graphical abstract



TITLE

Linking algal growth inhibition to chemical activity: Excess toxicity below 0.1% of saturation

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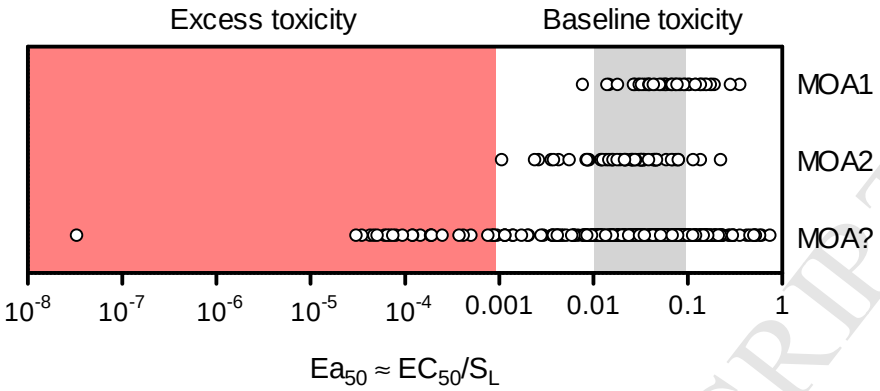
ABSTRACT

Chemical activity quantifies the energetic level of an organic compound relative to its pure liquid [0-1], and several studies have reported that baseline toxicity generally requires chemical activities of 0.01-0.1. The first aim was to challenge this chemical activity range for baseline toxicity. Algal growth inhibition data (median effective concentrations, EC_{50}) were compiled from two recent studies and included 108 compounds categorised as non-polar (mode of action, MOA1) and polar (MOA2) narcotics. These data were linked to chemical activity by (1) plotting them relative to a regression for (subcooled) liquid solubility (S_L), which served as visual reference for chemical activity of unity and (2) determining EC_{50}/S_L ratios that essentially equal median effective chemical activity (Ea_{50}). Growth inhibition required chemical activity >0.01 for MOA1 and >0.001 for MOA2 compounds. The second aim was to identify compounds exerting excess toxicity, i.e., when growth inhibition occurred at chemical activity <0.001. From a recent review with 2323 data entries, 315 EC_{50} values passed our selection criteria. 280 of these EC_{50} values were within or near the baseline toxicity range (Ea_{50}>0.001), and 25 compounds were found to exert excess toxicity (Ea_{50}<0.001). Of these compounds, 16 are pesticides or precursors. Methodologically, this study includes two methods for translating EC_{50} values into the chemical activity framework, each having advantages and limitations. Scientifically, this study confirms that baseline toxicity generally requires chemical activities of 0.01-0.1 and extends the application of the chemical activity approach beyond baseline toxicity, by demonstrating its utility to identify compounds that exert excess toxicity.

Graphical abstract



Keywords: Algal growth inhibition; Chemical activity; Baseline toxicity; Excess toxicity; Environmental risk assessment

Highlights

- Algal growth inhibition data were compiled for a wide range of organic compounds
- Toxicity data were linked to chemical activity using two complementary methods
- Toxicity required chemical activity >0.01 for MOA1 and >0.001 for MOA2 compounds
- Excess toxicity was identified at chemical activity <0.001 (0.1% of saturation)
- The chemical activity approach is suggested for prioritising compounds of concern

1. INTRODUCTION

The environmental risk assessment of hydrophobic organic compounds (HOCs) is based on exposure and effect assessments and is often a time-consuming and costly endeavour. Focusing resources on and attention to the most toxic compounds would thus be highly desirable. However, this prioritisation is often not straightforward because the base set of toxicity data, expressed as median effective concentrations (EC_{50}) or median lethal concentrations (LC_{50}), offers no direct information on the intrinsic toxicity (potency) of the compounds. One crucial question in this respect is whether a given compound exerts only baseline toxicity or additionally excess toxicity. Baseline toxicity (narcosis) is defined here as a non-specific and reversible disturbance of the functioning of cell membranes, whereas excess toxicity is defined as toxicity beyond narcosis, i.e., reactive or specific toxicity (Verhaar et al., 1992; van Wezel and Opperhuizen, 1995). Excess toxicity initiates at a (much) lower level of exposure as compared to baseline toxicity (Verhaar et al., 1992; van Wezel and Opperhuizen, 1995). Thus, a scientifically sound, transparent and practical way to identify compounds with excess toxicity relative to baseline toxicity would be highly valuable to chemical risk assessors in the process of prioritising compounds for further and more comprehensive assessments.

One path forward is more extensive toxicity testing that includes measurements of critical body residues (CBRs) or even critical target concentrations (McCarty et al., 1991; Escher et al., 2011; van der Heijden et al., 2015), since baseline toxicity of HOCs typically requires internal concentrations of 2-8 mmol kg^{-1} wet weight (McCarty and Mackay, 1993) or 40-160 mmol kg^{-1} lipid (van Wezel and Opperhuizen, 1995). CBRs within this range would thus indicate baseline toxicity, whereas markedly lower CBRs would indicate excess toxicity. Unfortunately, for most compounds such CBRs are not (yet) available and their

79 estimation from existing toxicity data (i.e., based on external concentrations) is associated
80 with significant uncertainty and critical assumptions (McCarty, 2015).

81 Another path forward is the use of Quantitative Structure-Activity Relationships
82 (QSARs). QSARs, as applied to toxicity data, are models relating effect concentrations to
83 molecular structures or physicochemical properties, and they are well established within
84 environmental risk assessment (US EPA, 2016; OECD and ECHA, 2017). Numerous
85 QSARs have been developed over the years for different compounds, organisms and
86 toxicological endpoints (Könemann, 1981; Schultz et al., 2003). By plotting toxicity data
87 (e.g., EC_{50} values) for uncharacterised compounds with appropriate QSARs established
88 for baseline toxicity, agreement with the QSAR indicates baseline toxicity whereas EC_{50}
89 values well below the regression indicates excess toxicity. Still, the uncertainty and
90 assumptions inherent in QSARs call for careful evaluation and validation of each model
91 before using it for prioritising compounds based on their excess toxicity.

92 A new approach, receiving increased attention, is to relate toxicity to chemical activity
93 (Gobas et al., 2018). Chemical activity (a) quantifies the energetic level, and not the mass
94 concentration, of a HOC relative to the energetic level in its pure liquid (reference state,
95 a=1; Reichenberg and Mayer, 2006). Several studies have linked toxicity to chemical
96 activity, and baseline toxicity for neutral HOCs has been reported to initiate within the
97 rather narrow range of chemical activity 0.01 to 0.1 (Reichenberg and Mayer, 2006; Mayer
98 and Reichenberg, 2006; Mackay et al., 2009; Mackay et al., 2014). This range is fairly
99 independent of the type of HOC and the target organism. The explanations for these
100 observations are: (1) chemical activity controls equilibrium partitioning (from high to low
101 chemical activity) and thereby the diffusive uptake and internal distribution of HOCs in
102 organisms (Di Toro et al., 1991; Reichenberg and Mayer, 2006; Schmidt et al., 2013), (2)

the site of toxic action for baseline toxicity is lipid membranes, and the target site is thereby relatively alike across organisms (van Wezel and Opperhuizen, 1995) and (3) the activity coefficients in lipid/oil are small and similar across HOCs, as exemplified by measurements for polycyclic aromatic hydrocarbons (Mayer et al., 2009). These findings are in line with the “Ferguson Principle” (Ferguson, 1939), the Target Lipid Model (Di Toro et al., 2000) and the aforementioned CBR concept (van Wezel and Opperhuizen, 1995). The well-defined range for baseline toxicity thus implies that compounds exerting toxicity at a chemical activity (well) below the lower limit for baseline toxicity (i.e., a=0.01) exert excess toxicity (Reichenberg and Mayer, 2006).

In a recent publication by Schmidt and Mayer (2015), the reported chemical activity range for baseline toxicity was supported by new algal growth inhibition data for 39 organic liquids, which were all characterised as non-polar narcotics according to the Verhaar classification scheme (Aruoja et al., 2014; Verhaar et al., 1992). On a practical level, these findings suggested that baseline toxicity requires exposure corresponding to 1% of liquid saturation, i.e., 1% of the water solubility for the liquid compounds (Schmidt and Mayer, 2015).

In the present study, we applied the chemical activity approach to much larger datasets on algal toxicity, which included a wide range of solid and liquid organic compounds, several expected modes of toxic action (MOA) and also several algal species. First, we extended the previously published analysis on the algal growth inhibition caused by non-polar narcotic liquids (MOA1; Schmidt and Mayer, 2015) with additional published effect data on solid compounds and compounds characterised as polar narcotics (MOA2) from the same research group (Aruoja et al., 2014; Aruoja et al., 2011). The aim of this extended analysis was to challenge, and possibly confirm, the chemical activity range of

127 0.01 to 0.1 for baseline toxicity with data on 108 compounds characterised as baseline
128 toxicants. Second, we selected data from a recent review by Fu and co-workers (Fu et al.,
129 2015), which includes 2323 data entries for 1081 compounds across 26 algal species, for
130 further analysis. The strategy of this analysis was to expand and illustrate the utility of the
131 chemical activity approach, with the aims of identifying and quantifying excess toxicity and
132 thereby compounds of greater concern. The working hypothesis was that the conversion of
133 concentration-based toxicity data into the chemical activity framework facilitates the direct
134 identification and quantification of excess toxicity relative to baseline toxicity. This would
135 make the chemical activity approach truly operational for screening-level risk assessment
136 of existing and emerging environmental contaminants and thereby a support tool for
137 regulatory decision-making (Mackay et al., 2011).

138 Two different and complementary methods were used to translate the concentration-
139 based toxicity data from the literature into the chemical activity framework, i.e., to link
140 toxicity to chemical activity: (1) EC_{50} values were plotted against octanol to water partition
141 ratios (K_{ow}), and a regression for (subcooled) liquid solubility (S_L) was then used as visual
142 reference for chemical activity of unity (Mayer and Reichenberg, 2006) and (2) EC_{50} values
143 were divided by estimated S_L values in order to determine median effective chemical
144 activities (Ea_{50}, unitless; Reichenberg and Mayer, 2006; Schmidt and Mayer, 2015).
145 Finally, compounds exerting toxicity at chemical activity below 0.001 (i.e., below 0.1% of
146 saturation) were identified as compounds with excess toxicity relative to baseline toxicity.
147

148 **2. DATA AND METHODS**

149

150 *2.1. Selection of data*

For the extended analysis on baseline toxicity, we selected two datasets published by Aruoja and co-workers (Aruoja et al., 2014; Aruoja et al., 2011). These datasets reported the algal growth inhibition caused by 50 compounds characterised as non-polar narcotics, i.e., MOA1 (Aruoja et al., 2014) and 58 compounds characterised as polar narcotics, i.e., MOA2 (Aruoja et al., 2011), according to the Verhaar classification scheme (Verhaar et al., 1992). The compiled dataset with 108 compounds included 66 liquids and 42 solids, of which nine compounds were water miscible and eight compounds were ionisable. All algal tests were carefully conducted in the same laboratory, using the green algae *Raphidocelis subcapitata* (until recently named *Pseudokirchneriella subcapitata*) and with an exposure duration of 72 h. In this way, these high-quality data form a consistent and reliable dataset for challenging, and possibly confirming, the chemical activity range for baseline toxicity.

For the analysis aiming at identifying and quantifying excess toxicity, we selected data from a recent review by Fu and co-workers (Fu et al., 2015). Fu et al. compiled algal toxicity data from a wide range of published studies and two databases in order to evaluate the data quality and the relationship between toxicity and hydrophobicity, i.e., generation of QSARs (Fu et al., 2015). The compiled dataset includes 2323 data entries for 1081 compounds across 26 algal species. Data for the present analysis were selected to meet the following criteria: (1) the compounds are predominately neutral at pH 6-8, (2) the compounds have log K_{ow}≥2.00, (3) the test organisms are freshwater green algae, (4) the test duration is 48 or 72 h and (5) the toxicity endpoint is inhibition of growth rate rather than reduction in yield or integral (Christensen et al., 2009). The acid dissociation constant of a given compound was used to determine its fraction of ionised molecules at pH 7, and compounds with ≤10% ionised molecules were characterised as neutral at pH 6-8. A total

175 of 315 data entries for 253 compounds fulfilled these five criteria. The compounds were
176 tested with the four algae species *Raphidocelis subcapitata*, *Scenedesmus obliquus*,
177 *Chlorella pyrenoidosa* and *Desmodesmus subspicatus*.

178 In the Aruoja studies, EC_{50} values were expressed in mg L^{-1}, whereas in the Fu
179 review, toxicity was expressed as log $1/EC_{50}$ (mol L^{-1}). Before further data analysis, all
180 data were standardised as log EC_{50} in the unit of mmol L^{-1}. The molar masses needed for
181 the standardisation were retrieved from the US EPA based EPI Suite^{TM} program (US EPA,
182 2017).

183

184 *2.2. Data analyses*

185 We applied two different and complementary methods to translate the concentration-
186 based data into the chemical activity framework, i.e., to link toxicity to chemical activity.

187 Following Method 1, log EC_{50} (mmol L^{-1}) was plotted against log K_{ow}, and a
188 regression for (subcooled) liquid solubility (S_L, mmol L^{-1}) was then used as visual reference
189 for chemical activity of unity (Mayer and Reichenberg, 2006). The rationale behind is, that
190 the chemical activity of an HOC is unity in its pure liquid (i.e., at liquid solubility/saturation).
191 For compounds that are liquid at standard conditions, the liquid solubility is simply the
192 water solubility. For compounds that are solid at standard conditions, the subcooled liquid
193 solubility is the water solubility of the hypothetical liquid state of the compound, i.e., the
194 water solubility had the solid compound been a liquid (Schwarzenbach et al., 2003). Lines
195 representing chemical activity levels of 0.1, 0.01 and 0.001 were also included in the plot.
196 The regression for S_L (mmol L^{-1}) was published by Mackay and co-workers (Mackay et al.,
197 1980):

198

$$S_L \approx \frac{1797}{K_{ow}} \quad \Rightarrow \quad \log S_L \approx 3.25 - \log K_{ow} \qquad (1)$$

The regression is based on solubility data for 45 HOCs with log K_{ow} values ranging from 1.97 to 7.11 (Mackay et al., 1980). The number 1797 (Eq. 1) is essentially an estimate of the (subcooled) liquid solubility or pseudo-solubility in octanol (mmol L^{-1}) and is, for many HOCs, similar in magnitude to the solubility in the lipid phase. Additional regressions were collected and used in parallel analyses (Mackay, 2000; Jain and Yalkowsky, 2001; Di Toro et al., 2007).

Following Method 2, the EC_{50} (mmol L^{-1}) of each compound was divided by a compound-specific S_L (mmol L^{-1}), as this ratio essentially equals the median effective chemical activity (Ea_{50}, unitless) for compounds with log K_{ow}≥2 and defined water solubility (Reichenberg and Mayer, 2006; Ferguson, 1939). The EC_{50}/S_L ratios were then plotted against their respective log K_{ow}, and lines representing chemical activity levels of 1, 0.1, 0.01 and 0.001 were included in the plot to serve as visual references. A given S_L value (mmol L^{-1}) was estimated as the ratio of the water solubility (S_w, mmol L^{-1}) of the compound and its maximum chemical activity (a_{max}, unitless; Reichenberg and Mayer, 2006), which equals its fugacity ratio:

$$S_L \approx \frac{S_w}{a_{max}} \qquad (2)$$

The maximum chemical activity is by definition 1 for liquid compounds. Solid compounds crystallise before reaching a chemical activity of unity, and the thermodynamic stability of the crystal structure of a given compound then defines its a_{max} (Mayer and Reichenberg, 2006). For each solid compound, a_{max} (unitless) was estimated from its melting

223 temperature (T_M, K) and the ambient temperature (T, 298 K) according to Yalkowsky et al.
224 (Yalkowsky et al., 1979), assuming the entropy of melting to be 56 J mol^{-1} K^{-1} (i.e.,
225 Walden's rule):
226
227 $$a_{max} \approx e^{6.8\times(1-\frac{T_M}{T})} \quad (3)$$
228
229 The log K_{ow}, S_w and T_M values used in the analyses were all retrieved from EPI Suite™
230 (US EPA, 2017). The program was operated in the batch mode function (input using
231 SMILES) for the KOWWIN v1.68, WSKOWWIN v1.42 and MPBPWIN v1.43 modules and
232 empirical and predicted values were compiled for those three parameters respectively. If
233 available the experimental values were used, otherwise the estimated values were used.
234
235 *2.3. Identifying and quantifying excess toxicity*
236 Excess toxicity was identified visually relative to the regression for S_L. Toxicity below
237 chemical activity 0.001, which corresponds to 0.1% of liquid saturation, was identified as
238 excess toxicity. For the identified compounds, Ea_{50} values were estimated in order to
239 quantify the excess toxicity. Following Method 1, Ea_{50} was quantified as the ratio of EC_{50}
240 and S_L, estimated from the regression for S_L (Eq. 1). Following Method 2, Ea_{50} was
241 quantified as the ratio of EC_{50} and compounds-specific S_L, estimated from S_w and a_{max}
242 (Eqs. 2 and 3).
243
244 **3. RESULTS AND DISCUSSION**
245
246 *3.1. Challenging the chemical activity range for baseline toxicity*

247 The extended analysis on baseline toxicity included 108 compounds characterised as
248 MOA1 and MOA2 compounds. The first chemical activity based analysis of these toxicity
249 data was done using a regression for S_L (Fig. 1A), that served as visual reference for
250 chemical activity of unity (Method 1; Mayer and Reichenberg, 2006). Also, lines
251 representing chemical activity of 0.1, 0.01 and 0.001 were added, and the chemical activity
252 range for baseline toxicity (a=0.01-0.1) was shaded to visually help interpretation (Fig. 1A).
253 The EC_{50} values for all MOA1 compounds (from Aruoja et al., 2014) were within or very
254 near the reported range for baseline toxicity of 0.01 to 0.1, whereas the EC_{50} values for the
255 MOA2 compounds (from Aruoja et al., 2011) were largely within the chemical activity
256 range of 0.001 to 0.1 (Fig. 1A). The average EC_{50}/S_L ratio was 0.085 for the MOA1
257 compounds (range: 0.008 to 0.356, n=50) and 0.034 for the MOA2 compounds (range:
258 0.001 to 0.221, n=58), with the S_L values being estimated via log K_{ow} (Eq. 1, Fig. 1A).
259 Further, the method proved applicable for both liquid and solid compounds (Fig. 1A) with
260 an average EC_{50}/S_L ratio of 0.070 for the liquid compounds (range: 0.003 to 0.356, n=66)
261 and somewhat lower ratios for the solid compounds (average: 0.038, range: 0.001 to
262 0.173, n=42).

263 The second chemical activity based analysis of the 108 narcotic compounds was
264 done by calculating EC_{50}/S_L ratios (≈Ea_{50}) and then plotting these ratios against log K_{ow}
265 (Method 2, Fig. 1B). In the same plot, lines representing chemical activity of 1, 0.1, 0.01
266 and 0.001 were added, and the chemical activity range for baseline toxicity (a=0.01-0.1)
267 was shaded to visually help interpretation (Fig. 1B; Schmidt and Mayer, 2015). The total
268 range of Ea_{50} values was clearly larger for both the MOA1 and MOA2 compounds when
269 using this method relative to the first method, with the lowest Ea_{50} value being below
270 0.0001. The average EC_{50}/S_L ratio was 0.057 for the MOA1 compounds (range: 0.001 to

271 0.352, n=50) and even lower for the MOA2 compounds (average: 0.010, range: 0.0001 to
272 0.062, n=58), with the S_L values being estimated via S_w and a_{max} (Eqs. 2 and 3, Fig. 1B).
273 Again, the method proved applicable for both liquid and solid compounds (Fig. 1B).
274 Thus, overall the analysis performed using Method 1 (Fig. 1A) confirmed the reported
275 chemical activity range for baseline toxicity of MOA1 compounds (0.01-0.1), whereas
276 MOA2 compounds exerted their toxicity within a somewhat larger range (0.001-0.1). The
277 analysis performed using Method 2 (Fig. 1B) was less clear with respect to confirming the
278 chemical activity range for baseline toxicity, as the Ea_{50} values were generally lower and
279 spanned a larger range. Based on the two data analyses, we selected Method 1 (Fig. 1A)
280 for subsequent analyses due to (1) the clear results with narcotic compounds, (2) its
281 simplicity and practicality and (3) the minimised risk of error propagation (see also section
282 3.3). Chemical activity of 0.001 was chosen as the operational threshold for identifying
283 excess toxicity in the subsequent analysis of a larger set of algal growth inhibition data, as
284 none of the narcotic compounds were below this threshold when following Method 1.
285
286 *3.2. Identifying and quantifying excess toxicity*
287 The chemical activity based analysis aiming at identifying and quantifying excess
288 toxicity included 315 data entries, covering 253 compounds across four algal species,
289 selected from a recent review by Fu and co-workers (Fu et al., 2015). The plot used for
290 analysing this larger set of algal growth inhibition data (Fig. 2) was created as described in
291 section 3.1, and results from the different algal species are highlighted in Fig. S1. The vast
292 majority (88.9%) of the EC_{50} values were within the chemical activity range of 0.001 to 1
293 (Fig. 2, white symbols). Based on the extended analysis on baseline toxicity (see Section
294 3.1), these compounds were characterised as baseline narcotics towards the tested algae.

A total of 27 data entries for 25 compounds (corresponding to 8.6% of the data) had EC_{50}/S_L ratios below 0.001 and were thus identified as compounds exerting excess toxicity (Fig. 2, red symbols). The 25 compounds and associated EC_{50}/S_L ratios are listed in Table 1, and the ratios ranged from 3.3×10^{-8} to 9.4×10^{-4}. Of these compounds, 16 are registered as pesticides, biocides or precursors (Table 1). Excess toxicity (i.e., reactive or specific toxicity) to algae was thus to be expected a priori for these 16 compounds, and the analysis thus validated the chemical activity approach for identifying chemicals exerting excess toxicity. The excess toxicity of the remaining nine compounds on the list is very interesting and could trigger further investigations and assessments. The graphical display of excess toxicity in Fig. 2 has been previously suggested by Maeder and co-workers (Maeder et al., 2004) in their development of the concept of "Toxic Ratio" (TR) as an indicator of intrinsic toxicity for PBT (persistent, bioaccumulative and toxic) evaluations. The scientific basis of the TR concept was the pioneering studies by Veith, Könemann, Lipnick and their colleagues (Veith et al., 1979; Könemann, 1981; Lipnick et al., 1987).

Eight compounds, corresponding to just 2.5% of the data, had EC_{50}/S_L ratios above 1 and thus EC_{50} values above the estimated liquid solubility (Fig. 2, grey symbols). Five of these eight compounds had log K_{ow}>6 and/or air to water partition ratios (K_{aw}, L L^{-1}) approaching unity, which makes these compounds very challenging to test in terms of establishing, maintaining and measuring exposure concentrations in the tests. Compounds with high log K_{ow} are generally difficult to dissolve and they tend also to sorb to the algal biomass or exudates, which can lead to freely dissolved concentrations in the test being markedly lower than the nominal concentrations (Mayer et al., 2000). Compounds with K_{aw} approaching unity are prone to evaporative losses from open tests and still have considerable losses when conducting a closed test with headspace (Mayer et al., 2000;

319 Birch et al., 2017). For these five compounds, the $EC_{50}/S_L>1$ can be used as a valuable
320 trigger for additional quality checking of the original toxicity data. For the other three
321 compounds, it remains less clear whether the $EC_{50}/S_L>1$ is due to errors and uncertainty in
322 the EC_{50} value or errors and uncertainty related to the estimated log K_{ow} and S_L.
323
324 *3.3. Two complementary methods to link toxicity to chemical activity*
325 There are several reasons for using Method 1, applying a regression for S_L. It is very
326 convenient to read EC_{50}/S_L as a distance between an EC_{50} value and the regression for S_L
327 in the logarithmic plot. Additionally, S_L (mmol L^{-1}) is approximately inversely related to the
328 activity coefficient in water (γ_{water}, L $mmol^{-1}$), which largely determines the membrane to
329 water partitioning of those compounds that are well dissolved in the membrane (i.e., low
330 activity coefficient in the membrane, $\gamma_{membrane}$, L $mmol^{-1}$). This method allows a chemical
331 activity based analysis of toxicity data without any data conversion. This is not only very
332 simple and practical, it also minimises the propagation of errors associated to input data
333 and model assumptions. The disadvantages of this method are related to the underlying
334 assumptions for regressions for S_L, and particularly the assumption of the constant entropy
335 of melting (i.e., Walden's rule; Yalkowsky et al., 1979). Further, different regressions for S_L
336 have been published and could lead to different results. However, these differences were
337 found to be limited in a recent study (Mayer and Schmidt, 2017) and also in the present
338 study, at least when plotting and analysing the data in a logarithmic framework (Figs. S2 to
339 S4).
340 Following Method 2, the compound-specific S_L values can be estimated from
341 regressions for S_L or using more sophisticated methods. Alternatively, activity coefficients
342 can be used to translate EC_{50} to Ea_{50} values ($Ea_{50}=EC_{50}\times\gamma_{water}$; Reichenberg and Mayer,

2006). The advantage of this method is thus the flexibility for using different simple and more advanced estimation methods, with the possibility of obtaining very accurate S_L estimates for well-characterised compounds. Conversely, the disadvantage of the method is the possibility for considerable error propagation from various input data and model assumptions. Indeed, the increased Ea_{50} range in Fig. 1B, as compared to Fig. 1A, could be caused by error propagation associated to the conversions, more specifically error and uncertainty in the input variables (S_w and T_M) and the applied equations (Eqs. 2 and 3). Diminishing the risk of error propagation would include empirical determination of accurate S_w values and the use of compound-specific entropy of melting. For many HOCs, such data are not (yet) available.

Following the two methods, S_L was either used as a visual reference or as a conversion factor for the analyses within the chemical activity framework. A special issue is thus related to water miscible compounds, not having a defined solubility value. Aruoja and co-workers included nine water miscible compounds in their study (Aruoja et al., 2014). Following Method 1, these compounds were still within the expected chemical activity range for baseline toxicity (Fig. S5), whereas applying Method 2 to these water miscible compounds involved the assignment of a pseudo-solubility value (1000000 mg L^{-1}, Fig. S6). We decided to exclude water miscible compounds from the larger analysis of the dataset from Fu and co-workers (within selection criterion (2), Section 2.1) and suggest for future studies that conversion from EC_{50} to Ea_{50} preferably should be done using activity coefficients ($Ea_{50}=EC_{50}\times\gamma_{water}$). However, it should be recognised that estimates of γ_{water} for miscible compounds are relatively scarce (e.g., Sherman et al., 1996) and therefore values calculated using property prediction software (e.g., SPARC or CosmoTHERM) will likely have to be used.

Another special issue relates to ionisable compounds. Eight ionisable compounds were included in the original dataset by Aruoja and co-workers (Aruoja et al., 2011). Following Method 1, the EC_{50}/S_L ratios for these compounds were, somewhat surprisingly, within or very near the expected chemical activity range for baseline toxicity (Fig. S5). Following Method 2, the compounds had EC_{50}/S_L values within or near the chemical activity range 0.001 to 0.01 (Fig. S6). Future analyses with ionisable compounds should determine the applicability domain for the chemical activity approach when assessing these challenging compounds, and no ionisable compounds were included in the data selected from the review by Fu and co-workers (selection criterion (1), Section 2.1).

3.4. Application of the chemical activity approach within risk assessment and regulatory decision-making

The present study included two methods of analysing an algal growth inhibition dataset of 108 MOA1 and MOA2 compounds within a chemical activity framework. Using a regression for S_L, the analysis overall confirmed the reported chemical activity range for baseline toxicity of MOA1 compounds (a=0.01-0.1), whereas MOA2 compounds exerted their toxicity within a somewhat larger range (a=0.001-0.1). It is now straightforward to use these ranges for assessing new toxicity data of existing and emerging environmental contaminants, with the purpose of characterising them as being in the baseline toxicity range or with excess toxicity relative to baseline toxicity (as illustrated in the data analysis of the second dataset, Fig. 2). It remains crucial to use strict quality criteria on all input data and also to be critical with regards to the applicability domain of the chemical activity approach. While it is obvious that the approach is best suited for organic compounds with log K_{ow}>2 and that are predominantly neutral at pH 6-8, additional work is now required to

set the limits of the applicability domain. However, we expect a rather wide applicability domain of the chemical activity approach for the type of analysis done here, since some error (e.g., factor 2 ≈ 0.3 log units) is acceptable when interpreting toxicity data on a logarithmic scale with the purpose of distinguishing compounds as being either in the baseline toxicity range or exerting excess toxicity (see also Figs. S2 to S4).

We envision that this simple and fast characterisation of compounds within these two groups can guide risk assessment and decision-making, as well as help focus further (testing) efforts on those compounds with significant excess toxicity relative to baseline toxicity.

4. CONCLUSION

Chemical activity based analyses of algal growth inhibition data were performed to (1) challenge the chemical activity range for baseline toxicity (a=0.01-0.1) and (2) identify and quantify excess toxicity. Plotting the EC_{50} values relative to a regression for S_L confirmed the chemical activity range for baseline toxicity of MOA1 compounds (a=0.01-0.1), whereas the MOA2 compounds exerted toxicity within a somewhat larger range (a=0.001-0.1). The method was then applied for analysing a large dataset with several expected MOA, and the chemical activity of 0.001 was chosen as the operational threshold for identifying excess toxicity. In this analysis, 25 compounds were identified with excess toxicity relative to baseline toxicity, and 16 of them are registered for use as pesticides, biocides or precursors and thus expected to have reactive or specific MOA. The remaining nine compounds could trigger further investigations and assessments. On the scientific level, this study extends the application of the chemical activity approach beyond baseline toxicity and demonstrates its utility for comparing toxicity data across compounds and

415 species and to identify compounds with excess toxicity relative to baseline toxicity. On the
416 practical level, these findings imply that excess toxicity occurs below 0.1% of liquid
417 saturation. On the risk assessment level, it is now straightforward to use these limits for
418 assessing new toxicity data with the purpose of characterising them as being in the
419 baseline toxicity range or exerting excess toxicity. This could also help industries at an
420 early stage to identify compounds with considerable excess toxicity.
421

422 **ACKNOWLEDGEMENTS**

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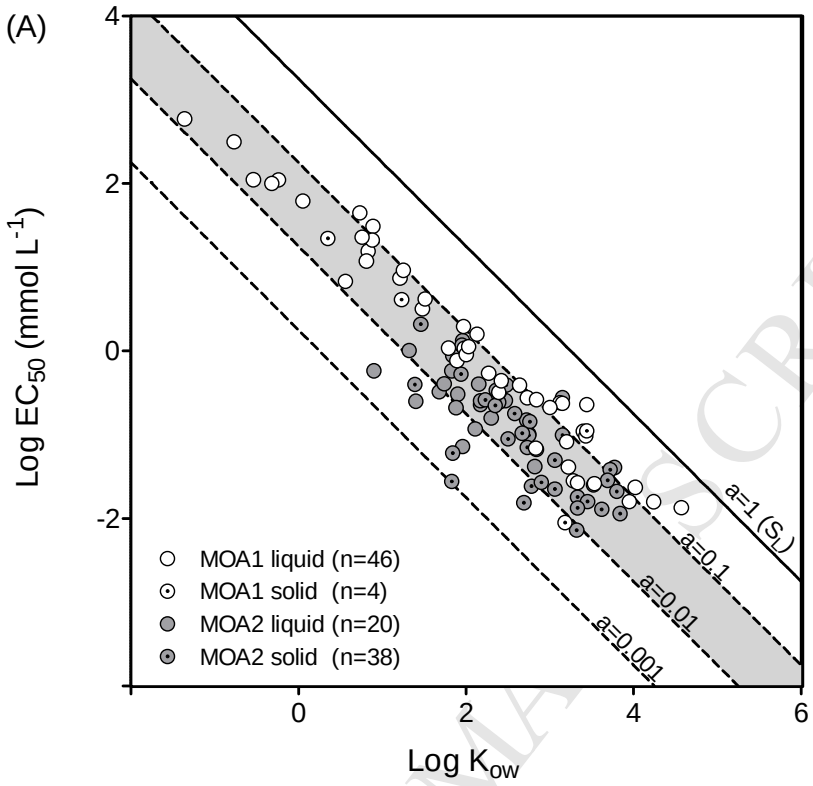
425 the ECOTOC liaison team for good discussions on the work leading to the present paper.
426

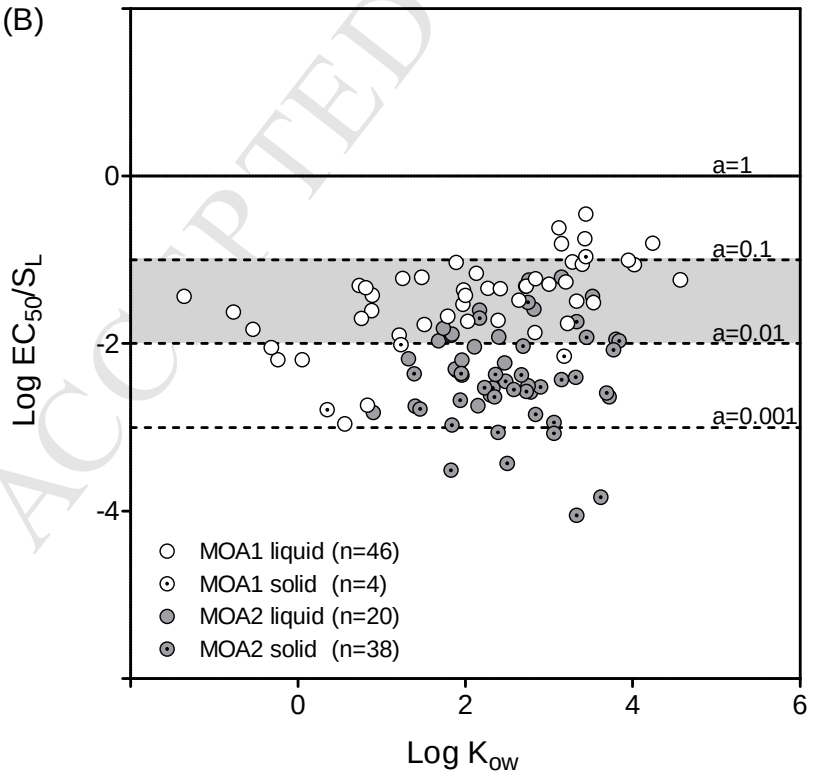
427 **APPENDIX A. SUPPLEMENTARY DATA**

428 Supplementary data related to this article can be found at …

429 **FIG. 1**

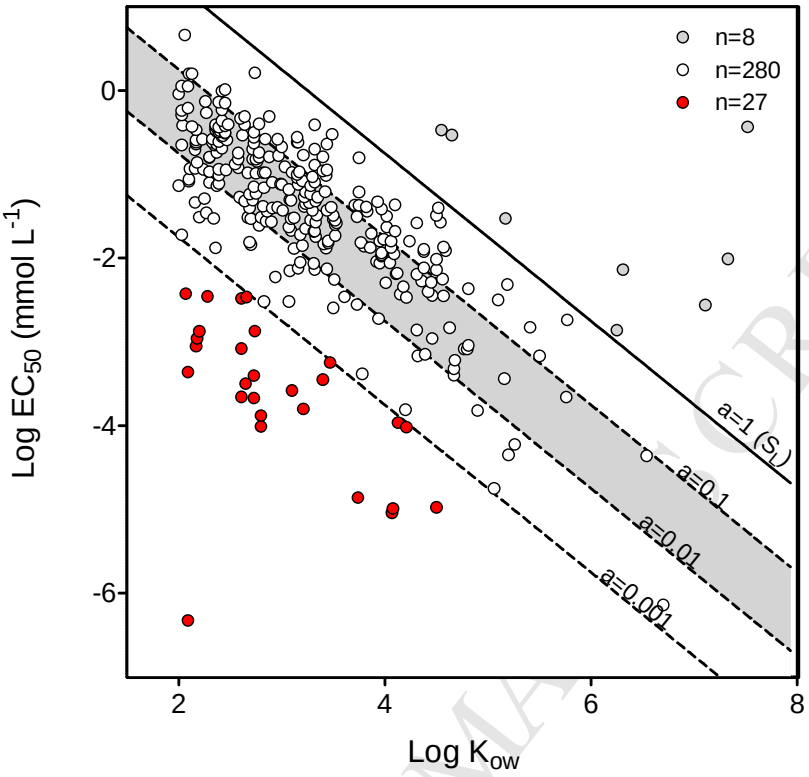


430



431

432 **FIG. 2**



433

Table 1. The 25 compounds with excess toxicity relative to baseline toxicity (Fig. 2). All values are from tests with algae *Raphidocelis subcapitata* (see also Fig. S1).

CAS number	Compound	Common name	Log K_{ow} [a)]	EC_{50}/S_L [b)]	Pesticide ? [c)]
76-06-2	Trichloronitromethane	Chloropicrin	2.09	0.000000033	Insecticide & nematicide
545-06-2	Trichloroacetonitrile		2.09	0.000030	Insecticide (former)[d)]
1014-70-6	2,4-bis(ethylamino)-6-methylthio-1,3,5-triazine	Simetryn	2.80	0.000035	Herbicide
886-50-0	2-tert-butylamino-4-ethylamino-6-methylthio-1,3,5-triazine	Terbutryn	3.74	0.000043	Herbicide
100-14-1	4-nitrobenzyl chloride		2.61	0.000051	
28159-98-0	2-tert-butylamino-4-cyclopropylamino-6-methylthio-1,3,5-triazine	Cybutryne	4.07	0.000060	Algistat, antifoulant, biocide & herbicide
30125-65-6	2-tert-butylamino-4-amino-6-methylthio-1,3,5-triazine		2.73	0.000065	Degradation product of Cybutryne
51218-49-6	n-propoxyethyl-n-chloroacetyl-2,6-diethylaniline	Pretilachlor	4.08	0.000069	Herbicide
97-00-7	1-chloro-2,4-dinitrobenzene		2.17	0.000074	Pesticide (precursor)[d)]
117-80-6	2,3-dichloro-1,4-naphthoquinone	Dichlone	2.65	0.000080	Algicide & fungicide
122-34-9	2,4-bis(ethylamino)-6-chloro-1,3,5-triazine	Simazine	2.18	0.000093	Herbicide
58-27-5	2-methyl-1,4-naphthoquinone	Menadione	2.20	0.00012	Fungicide (former precursor)[d)]
5329-12-4	2,4,6-trichlorophenylhydrazine		2.73	0.00012	
5915-41-3	2-tert-butylamino-4-chloro-6-ethylamino-1,3,5-triazine	Terbuthylazine	3.21	0.00014	Algicide, herbicide & microbiocide
33693-04-8	2-tert-butylamino-4-ethylamino-6-methoxy-1,3,5-triazine	Terbumeton	3.10	0.00019	Herbicide
23184-66-9	2',6'-diethyl-N-butoxymethyl-2-chloroacetanilide	Butachlor	4.50	0.00019	Herbicide
1912-24-9	2-ethylamino-4-isopropylamino-6-chloro-1,3,5-triazine	Atrazine	2.61	0.00019	Herbicide
122-57-6	Benzalacetone		2.07	0.00025	
91-59-8	2-naphthylamine		2.28	0.00037	
110-66-7	1-pentanethiol		2.74	0.00042	
28249-77-6	S-4-chlorobenzyl diethylthiocarbamate	Thiobencarb	3.40	0.00050	Herbicide
1484-13-5	9-vinylcarbazole		4.13	0.00082	
111-88-6	1-octanethiol		4.21	0.00087	
350-30-1	3-chloro-4-fluoronitrobenzene		2.66	0.00088	
95-33-0	N-cyclohexyl-2-benzothiazolelsulfenamide		3.47	0.00094	

[a)]octanol to water partition ratio (US EPA, 2017); [b)]ratio of median effective concentration and (subcooled) liquid solubility (via Eq. 1); [c)]Pesticides registered in the PPDB: Pesticide Properties DataBase (PPDB, 2017);[d)]TOXNET(U.S. National Library of Medicine, 2017).

FIGURE CAPTIONS

Fig. 1. (A) Regression for (subcooled) liquid solubility (S_L, mmol L^{-1}, a≈1) as a function of K_{ow} (Mackay et al., 1980) and lines representing chemical activity levels of 0.1, 0.01 and 0.001. A total of 108 EC_{50} values (mmol L^{-1}) are plotted against their K_{ow}. (B) Ratios of the 108 EC_{50} values (mmol L^{-1}) and respective (subcooled) liquid solubility (S_L, mmol L^{-1}) are plotted against K_{ow}. Shaded areas are the chemical activity range 0.01 to 0.1 for baseline toxicity, which corresponds to 35 and 345 mmol L^{-1} lipid, respectively, at an average activity coefficient of 0.29 L mol^{-1} (Mayer et al., 2009).

Fig. 2. Regression for (subcooled) liquid solubility (S_L, mmol L^{-1}, a≈1) as a function of K_{ow} (Mackay et al., 1980) and lines representing chemical activity levels of 0.1, 0.01 and 0.001. A total of 315 EC_{50} values (mmol L^{-1}) are plotted against their K_{ow}. Grey symbols: compounds with EC_{50}/S_L>1; red symbols: compounds with excess toxicity relative to baseline toxicity, in correspondence with Maeder et al. (2004). The shaded area is the chemical activity range 0.01 to 0.1 for baseline toxicity, which corresponds to 35 and 345 mmol L^{-1} lipid, respectively, at an average activity coefficient of 0.29 L mol^{-1} (Mayer et al., 2009).

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576

Highlights

- Algal growth inhibition data were compiled for a wide range of organic compounds
- Toxicity data were linked to chemical activity using two complementary methods
- Toxicity required chemical activity >0.01 for MOA1 and >0.001 for MOA2 compounds
- Excess toxicity was identified at chemical activity <0.001 (0.1% of saturation)
- The chemical activity approach is suggested for prioritising compounds of concern