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# Ecotoxicity of lanthanides to *Daphnia magna*: insights from elemental behavior and speciation in a standardized test medium

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## ABSTRACT

Lanthanides (LNs) are a group of 15 elements with steadily increasing economical importance due to their multiple uses in technologies essential for sustainable ecological, digital and energetic transitions. Although knowledge on LN ecotoxicology has greatly improved over the last decade, uncertainty persists with regard to their actual hazard and risk in freshwater environments. In particular, only limited information is available on i) the actual relationships between LN speciation vs. ecotoxicological responses in standardized laboratory tests and ii) the existence of regular and predictable patterns in LN ecotoxicity (expressed as *e.g.*, EC<sub>50</sub>) along the LN series. The present paper provides the first report on the ecotoxicity of all lanthanides (except Pm) for the freshwater crustacean *Daphnia magna* along with an unprecedented level of detail on LN speciation in the exposure medium.

Experimental data show that exposure concentrations can decrease by up to 95 % over the test duration, with the percentage decrease being inversely related with LN atomic mass. Thermodynamic speciation calculations confirm the possible formation of insoluble species, mainly LN carbonates. However, the corresponding theoretical solubility limits do not fully agree with measured concentrations at the end of the tests. Experimental verification of exposure concentrations (as a minimum at the beginning and end of laboratory tests) remains therefore mandatory to reach proper conclusions as to the ecotoxicity of each LN. A decreasing trend in ecotoxicity can actually be observed along the LN series when temporal changes in the exposure concentrations are properly accounted for. However, this trend remains dependent on exposure time and selected exposure metrics. This and other caveats must be considered in future research to reach a community-based consensus for the proper hazard and risk assessment of LN towards daphnids and other aquatic organisms.

**Keywords:** aquatic ecotoxicity, technologically critical elements, exposure metrics, hazard assessment, risk assessment

## 1. INTRODUCTION

The transition from a global economy dominated by fossil fuels to one based on renewable energy sources and the increasing digitalization of our society will both lead to a more intensive use of several mineral resources that are collectively termed critical raw materials (Bobba et al., 2020; IEA, 2021, 2023). Some Rare Earth Elements (REY), which include lanthanides (LN), Yttrium and Scandium, are needed in a large number of strategic, interlinked technologies and sectors such as wind energy generation, electric mobility (fuel cells and traction motors), robotics, drones, information and communication technologies at large, and medical applications (Bau & Dulski, 1996; Bobba et al., 2020). The increasing demand for REY has raised a) geopolitical issues in relation to supply chains (Bobba et al., 2020; IEA, 2021, 2023), b) prospections for new resources across the globe (Dushyantha et al., 2020; Pell et al., 2021), c) concerns about the disruption of their natural biogeochemical cycles (Hissler et al., 2016; Kulaksız & Bau, 2013; Lerat-Hardy et al., 2019; Louis et al., 2023; Martin et al., 2021), and d) concerns about the insufficient knowledge as to their potential environmental and human health risks (Blinova et al., 2020; Gonzalez et al., 2014; Malhotra et al., 2020).

Specific challenges arise when assessing REY ecotoxicity. One of the most important lessons already learned by the ecotoxicological community is that the test media and experimental conditions (notably pH values) used in (standardized) ecotoxicity testing cause lanthanides to precipitate as insoluble chemical species such as carbonates, phosphates and hydroxides (Blinova et al., 2018; Gonzalez et al., 2014; Kang et al., 2022; Lachaux et al., 2022). It is also noteworthy that these chemical speciation issues are independent of the chosen biological model because they arise from the chemical properties of LN and the composition of the test media. The formation of such precipitates leads to variable exposure conditions during ecotoxicity tests, which makes the choice of exposure metrics particularly critical when deriving concentration vs. response relationships for hazard and risk assessment. Proposed solutions include modification of standard media to avoid, in principle, the formation of precipitates (Aharchaou et al., 2020; Blinova et al., 2018; El-Akl et al., 2015; Ma et al., 2016; Revel et al., 2023; Vukov et al., 2016) or the choice of exposure metrics based on analytically measured concentrations at the end of the tests or based on geometric means between initial and final filterable concentrations (Lachaux et al., 2022; Vukov et al., 2016). However, modifications in standard procedures should be consensus-based not to sacrifice reliability, *sensu* Roth and Ciffroy (2016), for relevance (note that we mean no criticisms to the scientific validity of the approaches adopted in any of the cited studies). Furthermore, modification of test medium does not necessarily avoid the formation of colloidal-sized precipitates at circumneutral pH values (El-Akl et al., 2015; Revel et al., 2023). Meaningful and informative comparisons among studies on LN ecotoxicity must therefore give the utmost consideration to LN behavior and speciation in the different test media and experimental conditions (Gonzalez et al., 2014).

*Daphnia magna* is a well-studied model filter-feeder used in standardized ecotoxicity testing (ISO, 2012; OCDE, 2004). However, information on its responses following exposure to REY remains quite limited with a few acute ecotoxicity studies on selected LN (Blinova et al., 2018; González et al., 2015; Lachaux et al., 2022; Ma et al., 2016; Revel et al., 2023) and a handful of works on chronic ecotoxicity, transgenerational effects and life-history traits (Blinova et al., 2018; Blinova et al., 2022; Deng et al., 2022; Galdiero et al., 2019; Lürling & Tolman, 2010; Shu et al., 2023). Studies on the effects of selected LN mixtures are also available and suggest that LN mixtures could follow a concentration addition model for mixture ecotoxicity (Lachaux et al., 2022; Romero-Freire et al., 2019). However, to the best of our knowledge, there is no peer-reviewed investigation published to date that compares the effects of the full LN series (i.e., 14 elements when radioactive Pm is excluded) on *D. magna*.

Because lanthanides exhibit regular variation in their physico-chemical properties from Lanthanum (La) to Lutetium (Lu), ecotoxicologists have been looking for similar predictable patterns in ecotoxicity along the LN series or for ecotoxicological predictions based on physico-chemical properties (Le Faucheur et al., 2021). The possible presence of ecotoxicological trends along the LN series is attractive in that it would allow to predict the effects of all LN from a reduced number of studies on selected LN. Apart from its fundamental importance, this scenario would result in considerable time and money savings without compromising reliability and relevance of ecotoxicological results; a highly desirable situation for both fundamental and applied research in this field. The few studies examining at least ten LN have identified both increasing and decreasing trends in their ecotoxicological effects along the LN series (Blaise et al.,

97 2018; Borgmann et al., 2005; Lin et al., 2022; Manusadžianas et al., 2020; Weltje, 2002). Except for Weltje  
98 (2002), these observations require further confirmation because of missing or incomplete information on  
99 LN speciation in the exposure medium, a key factor when looking for trends along the LN series (Gonzalez  
100 et al., 2014).

101 The present study provides the first report on the acute ecotoxic effects of all LN to the model species  
102 *D. magna* according to the norm ISO 6341 (2012). The behavior and speciation of LN in the exposure  
103 medium are examined with an unprecedented level of detail to i) provide reliable and relevant data for  
104 hazard and risk assessment, ii) explore the existence of ecotoxicity trends along the complete LN series for  
105 this model organisms, and iii) summarize evidence-based recommendations to ensure maximum reliability  
106 and relevance in future research on the ecotoxicity of LN.

## 107 2. METHODS

### 108 2.1. Chemicals

109 All lanthanides salts (chlorides or nitrates) were purchased from Sigma-Aldrich and had a minimal purity  
110 of 99% (Table S1). Chemicals for preparing the test medium for acute tests with *Daphnia magna* were also  
111 purchased from Sigma-Aldrich. Concentrated hydrochloric acid (HCl Suprapur 30%) and concentrated nitric  
112 acid (HNO<sub>3</sub> 67%, Prolabo) were from VWR. Ultrapure water for washing/preconditioning of plasticware and  
113 preparing test medium and LN stock/working solutions was obtained from a Veolia Purelab® Chorus  
114 apparatus.

### 115 2.2. Ecotoxicity testing with *Daphnia magna*

116 Parthenogenetic specimen of *D. magna* were reared in Lefebvre-Czarda (LC)-Volvic medium (Table S2)  
117 at 20 °C and under a 16h light : 8 h dark photoperiod (light intensity 35 μmol/s/m<sup>2</sup>). The growth medium  
118 was changed three times a week and daphnids were fed an algal mixture of *Chlorella vulgaris*,  
119 *Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus* (2.5, 5, and 2.5 × 10<sup>6</sup> cells/day/daphnia,  
120 respectively) after each medium renewal.

121 Acute immobilization tests (48 h) were performed according to international guideline NF EN ISO 6341  
122 (2012) at 20 ± 2 °C in the dark as per guideline specifications. Daphnids less than 24 h old were used in all  
123 tests and were obtained from parent organisms aged from two to six weeks. Organisms were not fed during  
124 the tests. The composition of the standardized ISO 6341 medium was: CaCl<sub>2</sub> × 2H<sub>2</sub>O (294 mg/L), MgSO<sub>4</sub> ×  
125 7H<sub>2</sub>O (123 mg/L), NaHCO<sub>3</sub> (64.7 mg/L), and KCl (5.75 mg/L). Calculated medium hardness was approx 250  
126 mg/L as CaCO<sub>3</sub>.

127 In range-finding tests (data not shown), daphnids were exposed to a series of seven dilutions of all LN  
128 (except the radioactive Pm) at concentrations ranging from 0.05 to 45 mg/L of the corresponding salt (see  
129 Table S3 for details and the equivalent elemental concentrations). This range had to be extended up to  
130 1,750 mg/L of LN salts to identify the appropriate test concentrations for the definitive tests with La, Nd,  
131 Sm, Tb, Dy, Ho, Er and Tm (Table S3). Preliminary tests were performed at an initial pH of 7.8 units, as  
132 recommended by the ISO 6341 norm, and of 6.5 units to reduce the visible precipitation of lanthanides  
133 carbonate species without compromising organisms' survival. In the preliminary tests performed at the  
134 initial pH value of 7.8, observed mortality did not span the 0–100% range required for optimal EC<sub>x</sub>  
135 estimations. Definitive tests were therefore carried out only at an initial pH of 6.5 units.

136 In definitive tests, minimum and maximum nominal exposure concentrations (expressed as elemental  
137 values) were 0.7 to 15 mg/L and 20 to 70 mg L<sup>-1</sup>, respectively. A dilution series of nine or ten concentrations  
138 plus one control (test medium without added LN) were used for each element (Table S4). Test tubes were  
139 preconditioned for 24 h with the corresponding test solutions to minimize adsorption losses on test vials.  
140 The preconditioning solutions were discarded before starting the experiments. Four replicate exposures (10  
141 mL total volume) with five organisms each were used for all test concentrations. Two independent definitive  
142 tests were performed for each element. During each test, immobilization of daphnids was evaluated after  
143 24 and 48 h of exposure (Table S4). The pH of exposure solutions was measured in control tubes at t = 0 and  
144 48 h and also in selected exposure concentrations at t = 48 h. Initial pH was therefore assumed to be  
145 homogenous for all exposure concentrations. Although this assumption could have been violated in some  
146 cases, a proper discussion of ecotoxicological results remains possible thanks to the presence of linear  
147 relationships between pH and added LN (see sections 4.1 and 4.4). Potassium dichromate was used as a

148 positive control with EC<sub>50</sub> 24 h values between 0.6 and 2.1 mg/L of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> as recommended by the ISO  
149 6341 norm (2012).

### 150 **2.3. Analytical verification of exposure concentrations in the test medium**

151 Analytical determinations were carried out by Inductively Coupled Plasma – Mass Spectrometry (ICP-  
152 MS, Nexion 350x, Perkin Elmer). The following isotopes were measured: <sup>139</sup>La, <sup>140</sup>Ce, <sup>141</sup>Pr, <sup>146</sup>Nd, <sup>147</sup>Sm,  
153 <sup>153</sup>Eu, <sup>157</sup>Gd, <sup>159</sup>Tb, <sup>163</sup>Dy, <sup>165</sup>Ho, <sup>166</sup>Er, <sup>169</sup>Tm, <sup>173</sup>Yb, <sup>175</sup>Lu. Rhenium (<sup>187</sup>Re) was used as internal standard.  
154 Limits of detection (LOD) and quantification (LOQ) ranged from 0.1 to 0.8 µg/L (Table S5). LOD Analytical  
155 and procedural blanks were prepared and analyzed using the same analytical procedure as for samples. The  
156 accuracy of the whole analytical protocol was validated using international certified material (BCR-668-  
157 mussel tissues) from LGC standards. The recovery of LN ranged from 105 to 116 % (Table S5).

158 Actual exposure concentrations were analytically verified at t = 0 and 48 h for total (unfiltered)  
159 concentrations and at t = 0, 5, 24 and 48 h for filterable concentrations. Filtrations were performed with  
160 0.45 µm syringe filters (Millex 33 mm diameter, mixed cellulose esters – MCE; Millipore, reference  
161 SLHA033SB, lot R7HA27577). The day before use, filters were washed with 10 mL of diluted (10% v/v) HCl,  
162 rinsed three times with 10 mL of ultrapure water and stored at 4 °C in the dark in a zip-bag with a few drops  
163 of MilliQ water to prevent drying of the membranes. Filtered and unfiltered solutions were collected in 15  
164 mL falcon tubes previously preconditioned in two baths of diluted 10% v/v HNO<sub>3</sub> followed by two baths of  
165 milliQ water. The soaking time was 24 h in each bath. Except for t = 0 h, all solutions for analytical  
166 measurements were incubated in the presence of daphnids as described for ecotoxicity tests. The first 2 mL  
167 of filtered solutions were discarded and any living daphnids or visible debris were eliminated from the  
168 unfiltered samples before transfer into the falcon tubes. All samples were acidified at 1% v/v with  
169 concentrated nitric acid and stored at 4 °C in the dark pending analysis. Analyses at t = 0 and 48 h were  
170 performed in duplicate for both total and filterable concentrations. Measurement precision was estimated  
171 using the formula:

$$172 \quad (1) \quad Precision = \sqrt{abs\left(\frac{m_1 - m_2}{2}\right)}$$

173 where  $m_1$  is the first replicate measurement,  $m_2$  is the second replicate measurement and  $abs$  is the  
174 absolute value of the difference.

175 Analytical verifications were carried out on solutions initially amended with a concentration  
176 corresponding to the 24 h EC<sub>50</sub>, hereinafter C<sub>EC50</sub>, determined in the first round of definitive test. Tubes for  
177 the analytical verification of LN concentrations were incubated in parallel with the second definite tests and  
178 pH values were verified at t = 0 and 48 h for selected concentrations (see sections 3.3 and 3.4). For selected  
179 elements Lanthanum (La), Neodymium (Nd), Gadolinium (Gd) and Ytterbium (Yb), total and filterable  
180 concentrations were measured also in solutions amended with the minimum (C<sub>min</sub>) and maximum (C<sub>MAX</sub>)  
181 test concentrations (Table S4). These additional measurements were performed in duplicate at t = 0, 24 and  
182 48 h using solutions where daphnids were initially added (except at t = 0 h). The pH was checked in all the  
183 C<sub>min</sub> and C<sub>MAX</sub> solutions immediately before filtration and/or transfer to falcon tubes for analysis. Procedures  
184 for filter cleaning and removal of debris from unfiltered solutions were as previously described.

### 185 **2.4. Speciation of lanthanides in the test medium**

186 Speciation calculations were performed using VisualMinteq (VM) version 3.1. The precipitation of  
187 carbonate and hydroxide complexes was allowed as ‘possible solid phases’. The default VM database  
188 specifies values for carbonate solubility products only for La, Neodymium (Nd), Samarium (Sm), Europium  
189 (Eu), Gd, Dysprosium (Dy) and Yb. Additional calculations were therefore performed using the carbonate  
190 solubility products determined by Firsching and Mohammadzadei (1986) who provided values for all  
191 lanthanides except Ce (Table S6). The value reported by Li et al. (2004) was used for Ce<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> solubility.  
192 Possible precipitation of sulfate and nitrate complexes was not considered because it requires LN  
193 concentrations well above those used in our experiments; *i.e.* above tens or hundreds of mM for LN<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>  
194 precipitation (Judge et al., 2023; Moldoveanu et al., 2024) and in the molar range for LN(NO<sub>3</sub>)<sub>3</sub> precipitation  
195 (Siekierski & Salomon, 1985).

196 Input temperature was fixed at 20 °C, medium alkalinity was specified as 0.831 meq/L CaCO<sub>3</sub> and the  
197 Debye-Hückel equation was used for activity correction. Calculations were performed in pH range 5 to 9  
198 using increments of 0.2 units and for nominal concentrations from 10 ng/L (5.7–7.2 × 10<sup>-11</sup> M) to 50 mg/L  
199 (2.9–3.6 × 10<sup>-4</sup> M). The upper and lower limit of the molar concentration ranges refer to La and Lu,  
200 respectively. The nominal concentration range used for speciation calculation covered the range of tested  
201 and measured concentrations in the present study. For each element, theoretical solubility limits were  
202 estimated from speciation calculation by subtracting the percentage of precipitated concentrations from  
203 the corresponding nominal values.

204 Speciation was also modelled for solutions amended with concentrations corresponding to the 24 h  
205 EC<sub>50</sub> obtained in the first round of definitive tests for each element. In this case, the elemental concentration  
206 for VM runs was entered as the time-weighted mean (TWM) of the 24 h C<sub>EC50</sub> to account for the  
207 concentrations decreases observed over the test duration (see the Results section for details). The TWM  
208 was calculated from the experimentally measured concentrations at 0 and 24 hours using the formula  
209 (OECD, 2012):

$$210 \quad (2) \quad TWM = \frac{Conc_{t(x)} - Conc_{t(x+1)}}{\ln(Conc_{t(x)}) - \ln(Conc_{t(x+1)})}$$

211 where: Conc<sub>t(x)</sub> is the measured concentration at t = 0; Conc<sub>t(x+1)</sub> is the measured concentration at 24;  
212 Ln is the Napierian logarithm of the measured concentrations at any time 't'.

213 Because TWM concentrations are mathematical estimates, the corresponding pH values to specify for  
214 VM calculations were estimated as follows. Changes in pH were monitored between 0 and 48 h for several  
215 concentrations of all tested elements (Table S7). Except for Praseodymium (Pr), linear relationships existed  
216 between pH at 48 h and LN nominal concentrations. These relationships were used to estimate the  
217 expected pH values for the calculated TWM concentrations and for the concentrations measured at t = 24  
218 and 48 h (Table S8). In the case of Pr, the pH value of the concentration closer to the TWM EC<sub>50</sub> was used.  
219 It will be shown later that pH changes were minor between 24 and 48 h.

## 220 2.5. Data analysis and statistics

221 The presence of statistically significant linear relationships between variables was verified using the  
222 standard Pearson least squares method and a robust MM estimation method that is less sensitive to outliers  
223 and non-normally distributed errors (Susanti et al., 2014; Venables & Ripley, 1999). The letters MM indicate  
224 that the estimation is of the maximum likelihood type, but do not form an acronym. Rather, they refer to  
225 the type of estimation procedure as explained in Susanti et al. (2014). Linear relationships were obtained  
226 with R version 3.6.0 (R Core Team 2019 R Core Team, 2019. R: A Language and Environment for Statistical  
227 Computing. R Foundation for statistical computing, Vienna. <https://www.R-project.org>). Graphs were  
228 prepared using Sigma Plot (version 14).

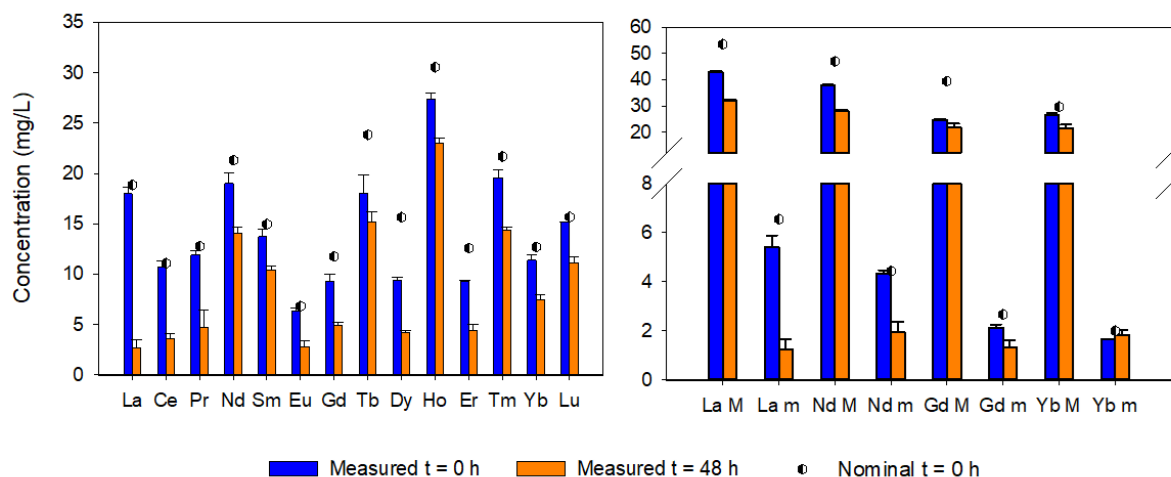
229 Effect concentrations (EC<sub>x</sub>) were calculated with the Excel macro REGTOX (version EV7.0.7) using the  
230 Hill model. The macro and the related documentation are available in French at:  
231 [https://www.normalesup.org/~vindimian/fr\\_index.html](https://www.normalesup.org/~vindimian/fr_index.html).

## 232 3. RESULTS

### 233 3.1. Total concentrations in the test medium

234 Measured levels of LN in control solutions were always below the corresponding analytical detection  
235 limits (Table S5). The relative standard deviation (RSD) for replicate measurements of total LNs  
236 concentrations was less than 10% at t = 0 and usually within 30% at t = 48h. Total concentrations at t = 0  
237 were within 20% of the expected nominal values except for solutions amended with Dy at C<sub>EC50</sub> where  
238 measured values were 60% of the expected ones (Fig. 1 and Table S9). This discrepancy, possibly due to  
239 dilution errors, does not affect the objectives of the present paper nor the data interpretation presented  
240 below. At the end of the tests (48 h), total concentrations in solutions initially amended with C<sub>EC50</sub> were  
241 within 25% of initial values for Nd, Tb, Ho, Tm, Sm and Lu, but had decreased by more than 40% for the  
242 other elements (Fig. 1A and Table S9). Total concentrations also decreased by more than 50% in medium  
243 aliquots spiked with C<sub>min</sub> of La, Nd, and Gd (6.55, 4.44 and 2.67 mg/L, respectively), but remained relatively

244 constant for Yb (2.0 mg/L). In solutions spiked with  $C_{MAX}$ , total concentrations (La = 53.5, Nd = 47.0, Gd =  
 245 39.4 and Yb = 29.6 mg/L) showed little variation over 48 h (Fig. 1B, Table S9).



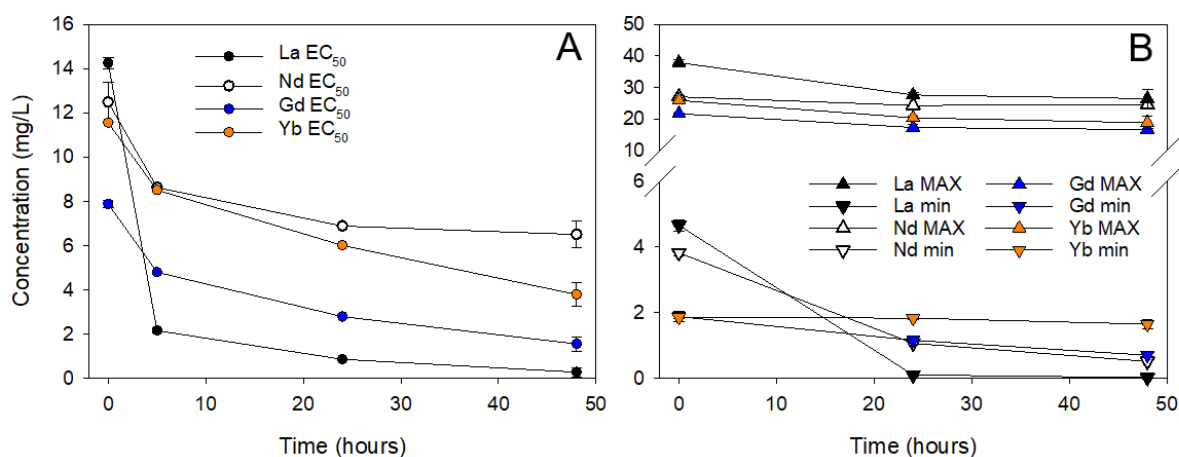
246  
 247 Figure 1. (A) Total concentrations of lanthanides (LN) measured at 0 and 48 hours in solutions initially  
 248 amended with LN concentrations equal to the corresponding  $EC_{50}$  for *D. magna*. (B) Same as panel A  
 249 for solutions initially amended with the maximum (LN M) and the minimum (LN m) concentrations of  
 250 La, Nd, Gd and Yb used in ecotoxicity tests. All measurements were performed in duplicate. Error bars  
 251 were calculated using Formula 1 in the text. Nominal values expected at  $t = 0h$  are indicated for  
 252 comparison purposes in both panels.

### 253 3.2. Filterable concentrations in the test medium

254 In solutions amended with  $C_{EC50}$ , the ratio between total and filterable concentration was between 80  
 255 and 120% for all elements except Nd (65%), Pr (77 %) and Sm (77%) (see Table S9 for raw results and section  
 256 4.2 for discussion). In the same solutions, filterable concentrations decreased by 50–98% over the test  
 257 duration according to three temporal patterns as described in figure 2A for selected elements and in Table  
 258 S9 and Figure S2 for the entire LN series. In pattern one (La and Ce), filterable concentrations decreased by  
 259 more than 80% within 5 h from the start of the incubation and fell below 5% of the initial values after 48  
 260 hours. In pattern two (Pr, Sm, Eu, Gd, Er, Dy, Yb), the concentration decrease was less rapid and reached  
 261 70–95% after 48 hours. In pattern three (Nd, Tb, Tm, Ho, Lu), the overall concentration decrease was less  
 262 than 50% with little changes between 24 and 48h. After 48 h, the differences between total and filterable  
 263 concentrations were less than 2-fold for Nd, Tb, Dy, Ho, Er, Tm, Yb, and Lu, but between 3- and 34-fold for  
 264 the other elements of the series. Small differences between total and filterable concentrations suggest  
 265 either a higher solubility or the formation of precipitates that were not retained by the 0.45  $\mu m$  filters.

266 In solutions amended with  $C_{min}$  and  $C_{MAX}$ , the ratio between total and filterable concentrations was  
 267 between 85 and 110% except for Nd at  $C_{MAX}$  (see section 4.2 for discussion). At  $C_{min}$ , the behavior of La, Nd,  
 268 Gd and Yb essentially agreed with the observations at  $C_{EC50}$ , while filterable concentrations remained within  
 269 30% of the initial values for  $C_{MAX}$  scenarios (Fig. 2B, Table S9).

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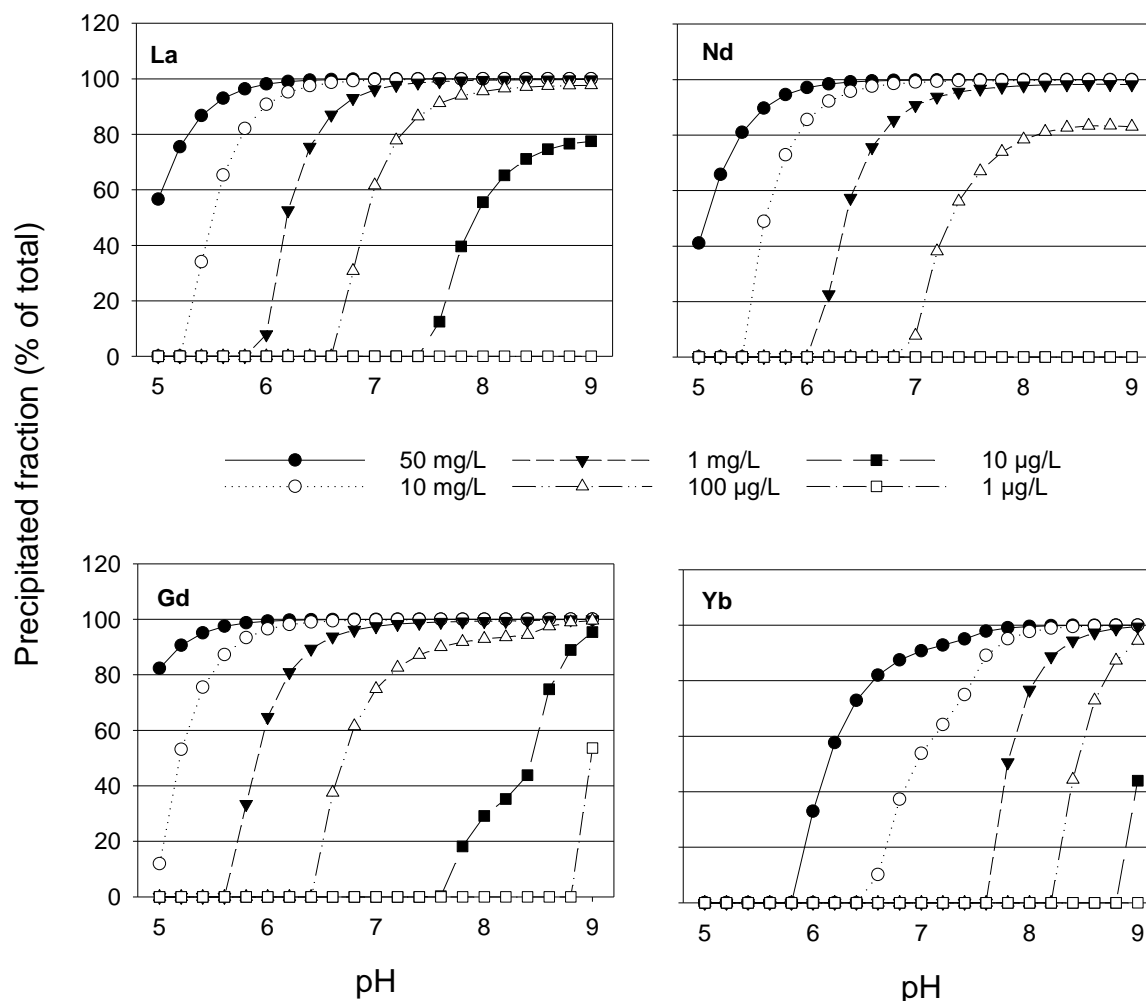
Figure 2. (A) Temporal changes in the filterable concentrations (< 0.45  $\mu\text{m}$ ) of La, Gd, Nd, Yb for solutions initially amended with lanthanides concentrations equal to the corresponding EC<sub>50</sub> at 24 h. The selected elements are representative of the three different behaviors across the whole lanthanides series (see text for details and Table S9 and Fig. S2 for full results). Measurements at t=0 and t=48h were performed in duplicate. (B) Same as panel A, but for solutions initially amended with maximum (MAX) and minimum (min) tested concentrations. All measurements performed in duplicate. Error bars may be smaller than the corresponding symbols.

### 290 3.3. Theoretical solubility of lanthanides in the test medium

291 The present section will focus on speciation results obtained using the carbonate solubility product ( $K_{\text{SP}}$ )  
292 established by Firsching and Mohammadzadei (1986), except for La and Ce. In the case of La, using the  $K_{\text{SP}}$   
293 of Firsching and Mohammadzadei (1986) clearly overestimated La solubility compared with experimental  
294 results. The default  $K_{\text{SP}}$  in the VisualMinteq (VM) database was therefore preferred (Table S6). An indicative  
295  $K_{\text{SP}}$  for  $\text{Ce}_2(\text{CO}_3)_3$  was taken from Li et al. (2003), because no value for Ce was available in Firsching and  
296 Mohammadzadei (1986).

297 In the pH range from 6.0 to 7.8 (covering the values measured in ecotoxicity tests, Table S7), formation  
298 of insoluble carbonate species was predicted for all LNs and for concentrations as low as 10  $\mu\text{g/L}$  (Fig. 3).  
299 Formation and precipitation of hydroxides,  $\text{LN}(\text{OH})_3$ , was also predicted for Er, Tm, Yb and Lu.





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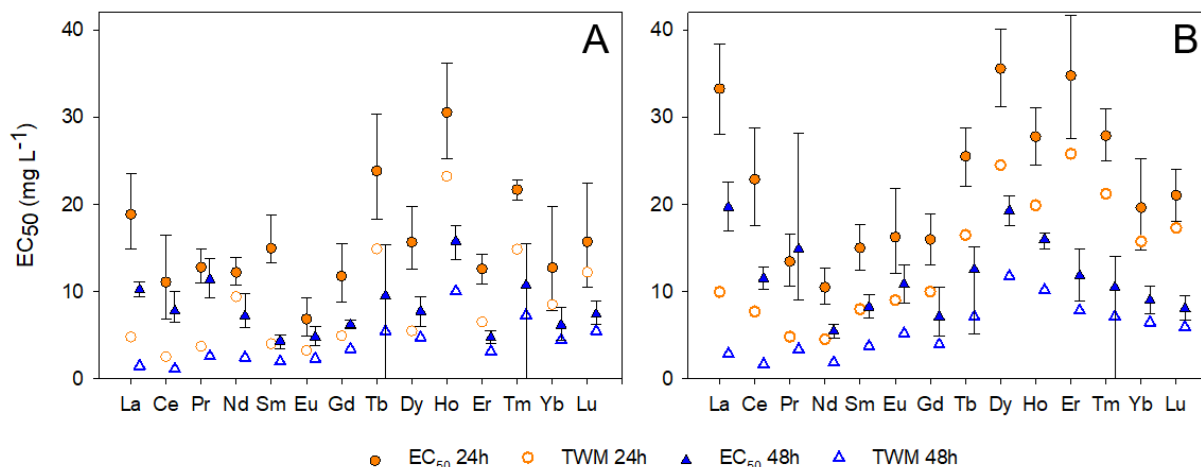
Figure 3. Theoretical solubility of La, Nd, Gd and Yb as a function of pH in ISO 6341 medium for acute toxicity testing with *Daphnia magna*. Model calculations were performed for theoretical concentration from 1 µg/L to 50 mg/L. No precipitation was predicted in theoretical solutions amended with 10 and 100 ng/L (plots not shown).

### 305 3.4. Lanthanides ecotoxicity

306 In the first round of definitive test, nominal EC<sub>50</sub> ranged from 6.84 to 30.5 mg/L and from 4.70 to 15.7 mg/L  
307 after 24 and 48 h of exposure, respectively (Fig. 4a; Table S10). To account for the decrease in LN  
308 concentrations over time (section 3.1), the TWM EC<sub>50</sub> were calculated using Formula 2. For the 24h TWM  
309 EC<sub>50</sub>, the experimental data presented in section 3.1 (*i.e.*, the filterable concentrations at t = 0 and t = 24 h)  
310 were directly entered into Formula 2. For the 48h TWM EC<sub>50</sub>, the nominal 48h EC<sub>50</sub> values were used as  
311 input for the concentrations at t = 0 h. The corresponding LN concentrations at 48h were estimated using  
312 Formula 5 (see section 4.2) and then used as input for the concentrations at 48h in Formula 2. Note that  
313 the experimental measurements at 48h (section 3.1) could not be used for this purpose, because they refer  
314 to medium aliquots initially spiked with concentrations equal to the 24h EC<sub>50</sub>. Results from the calculation  
315 procedure are summarized in Table S11. A detailed explanation of the calculation procedure is provided in  
316 the caption of Table S11 in the readme file accompanying the supporting information. For comparison  
317 purposes, the same procedure was also performed for the 24h TWM EC<sub>50</sub> estimated using Formula 4 (Table  
318 S11).

319 The TWM 24h EC<sub>50</sub> calculated using experimental values differed from the nominal EC<sub>50</sub> by more than  
320 2-fold for La, Ce, Pr, Sm, Eu, Gd and Dy (Fig. 4A). The 24h TWM EC<sub>50</sub> estimated using Formula 4 were within  
321 20 % of those obtained using experimental data for La, Pr, Eu, Tb, Ho, Tm, Yb, Lu and within a factor of two  
322 for the remaining elements except Nd (Table S11). Differences between nominal and TWM 48h EC<sub>50</sub> were

323 more than 2-fold for La, Ce, Pr, Nd, Sm and Eu (Table S11). The observations confirm that TWM correction  
 324 would avoid underestimating the ecotoxicity of La, Ce, Pr, Sm, Dy and, to a lesser extent, Eu and Gd (Fig.  
 325 4A). For the second round of definitive tests, 24h and 48h EC<sub>50</sub> ranged from 10.5 to 35.6 mg/L and 5.4 to  
 326 19.7 mg/L, respectively (Table S10, Fig. 4B). The TWM corrections were performed using Formulas 6 and 7  
 327 (Table S11), leading to results and conclusions equivalent to those discussed for the first round of tests.



328

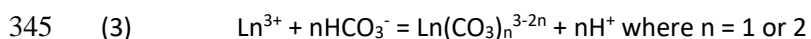
329 Figure 4. Nominal and Time-Weighted Mean (TWM) EC<sub>50</sub> values for specimen of *Daphnia magna*  
 330 exposed to lanthanides for 24 or 48 h in the first (panel A) and second (panel B) series of definitive  
 331 tests. Bars represent 95% confidence intervals for nominal EC<sub>50</sub> (estimation not possible for TWM).  
 332 Values for 24 and 48 h have been slightly offset for graphical representation purposes.

333

## 4. DISCUSSION

### 334 4.1. Temporal changes in total LN concentrations

335 In the present study, total LN concentrations at t = 0 h were in good agreement with expected values,  
 336 but showed an unexpected decrease at the end of the test for several LN (Fig. 1 and Table S9). Although LN  
 337 tend to form insoluble precipitates in the test medium, we cannot exclude that the decrease in total LN  
 338 concentrations could partly originate from adsorption losses onto the tubes walls despite preconditioning  
 339 of the test tubes. However, daphnids can actively ingest LN precipitates forming into the test medium and  
 340 contribute to the observed losses, although the extent of such ingestion is element-dependent (Revel et al.,  
 341 2023). The effect of LN addition on the pH of the test medium (Tables S7, S8, S9) can also contribute to the  
 342 observed temporal changes in their concentrations. The inverse relationship between added LN  
 343 concentrations and pH (Table S8) reflects the formation of Ln<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> complexes in test solutions along with  
 344 the release of protons according to the following equilibrium (Luo & Byrne, 2004):



346 As for alkaline waters in equilibrium with atmospheric CO<sub>2</sub> (Luo & Byrne, 2004), carbonate complexes  
 347 are expected to dominate LN speciation regardless of the added concentrations. The formation and  
 348 subsequent precipitation of such complexes consume the alkalinity of the test medium (provided by the  
 349 NaHCO<sub>3</sub> component), reduce its buffering capacity, shift its pH toward more acidic values via the release of  
 350 protons (Formula 3) and eventually increase LN solubility at high concentrations (see section 3.3, Tables S7  
 351 and S9). The formation of protons following LN addition to the test medium explains why pH remained  
 352 acidic in medium aliquots amended with C<sub>MAX</sub> (Table S9) and why pH and LN concentration were inversely  
 353 correlated (Tables S7 and S8). However, medium pH increased from the beginning to the end of the tests  
 354 in control exposures and in medium aliquots amended with C<sub>EC50</sub> and C<sub>min</sub> (Tables S7 and S9). These  
 355 observations can be explained by considering that test tubes were opened to the atmosphere; *i.e.*, the  
 356 carbonate system (H<sub>2</sub>CO<sub>3</sub> – HCO<sub>3</sub><sup>-</sup> – CO<sub>3</sub><sup>2-</sup>) of the medium could equilibrate with atmospheric CO<sub>2</sub> over time.  
 357 In control exposure, the protons added to lower the initial pH to 6.5 (section 2.2) shifted the equilibrium of

358 the carbonate system toward to formation of carbonic acid (H<sub>2</sub>CO<sub>3</sub>). The newly formed H<sub>2</sub>CO<sub>3</sub> will dissociate  
 359 into H<sub>2</sub>O and CO<sub>2</sub>, with CO<sub>2</sub> evading from the solution into the atmosphere. The overall outcome of this  
 360 process is a removal of protons from the medium resulting in an increase in pH. In solutions amended with  
 361 C<sub>EC50</sub> and C<sub>min</sub>, both processes (Formula 3 and the equilibrium of the carbonate system) contributed to  
 362 determine the pH of the solutions at the end of the test.

363 Considering that pH differences could exceed one unit across the exposure range (Table S7), LN  
 364 speciation was not necessarily homogenous for all exposure concentrations. The ecotoxicological  
 365 significance of such variability remains to be ascertained and will be further discussed in section 4.4.

#### 366 4.2. Temporal changes in filterable LN concentrations

367 Filtrable concentrations at t = 0 agreed well with the corresponding total ones except for Pr, Nd, and  
 368 Sm (section 3.2 and Table S9). Although filters were acid washed and preconditioned before use (section  
 369 2.3), we cannot completely exclude possible adsorption losses onto filter membranes. Possible (element  
 370 specific) filtration artifacts may require specific investigations, especially when comparing results from  
 371 different studies or combining them for regulatory purposes (see section 5).

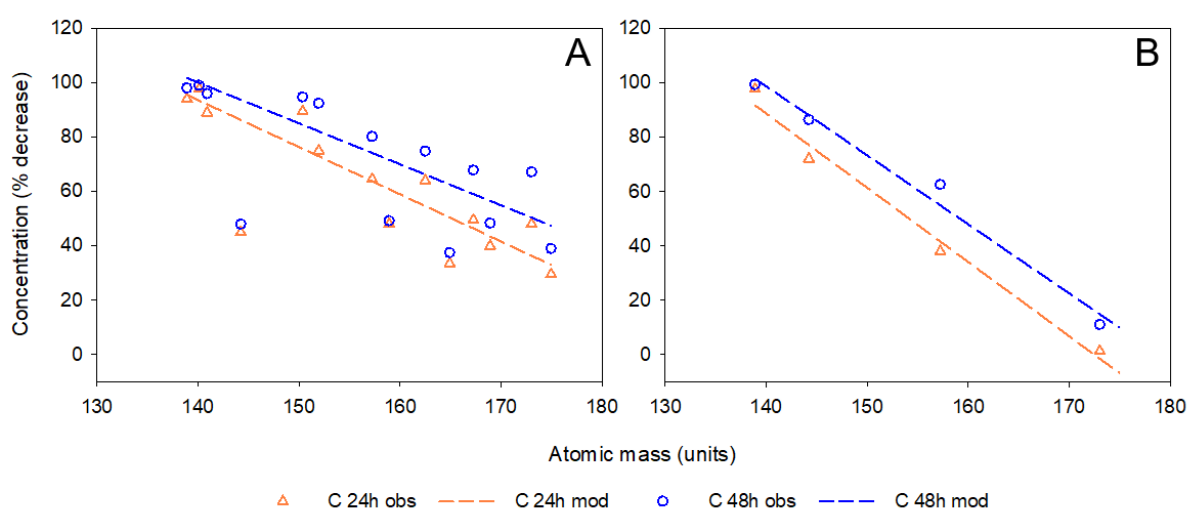
372 Assuming that our results are internally coherent (same filter type and batch, same procedures for all  
 373 elements), the decrease in filterable concentrations was always more marked than the corresponding total  
 374 ones, suggesting the presence of insoluble LN particles large enough to be retained by the pores of 0.45 μm  
 375 filters (see also section 3.3). Inverse linear correlations (Formulas 4 to 7) existed between the decrease in  
 376 filterable concentrations at 24 and 48 h (expressed as % of initial concentrations) and LN atomic masses in  
 377 solutions amended at C<sub>EC50</sub> and C<sub>min</sub> (Fig. 5). Robust MM regressions showed a better fit compared with  
 378 conventional partial least-squares regressions for the C<sub>EC50</sub> scatterplots, while the performance of the two  
 379 regressions was equivalent for C<sub>min</sub> (data not shown). For the sake of clarity, only robust MM regressions  
 380 will be presented and discussed.

381 (4)  $\%Loss_{C_{EC50\_24hMM}} = (-1.7353 \pm 0.2517) \textit{ atomic mass} + (337 \pm 40) \quad R^2 = 0.70$

382  
 383 (5)  $\%Loss_{C_{EC50\_48hMM}} = (-1.5065 \pm 0.3772) \textit{ atomic mass} + (311 \pm 59) \quad R^2 = 0.54$

384  
 385 (6)  $\%Loss_{C_{min\_24hMM}} = (-2.7296 \pm 0.2438) \textit{ atomic mass} + (471 \pm 38) \quad R^2 = 0.98$

386  
 387 (7)  $\%Loss_{C_{min\_48hMM}} = (-2.5407 \pm 0.2461) \textit{ atomic mass} + (454 \pm 38) \quad R^2 = 0.98$



388

389 Figure 5. (A) Scatterplots of percentage decrease in lanthanides concentrations vs. atomic mass at 24  
 390 and 48 h in solutions initially amended with LN concentrations equivalent to the corresponding C<sub>EC50</sub>  
 391 (panel A) and to the minimum tested concentration C<sub>min</sub> (panel B). Triangles and circles correspond  
 392 the observed (obs) percentage concentration decreases calculated from experimental data. Dotted

393 lines are the corresponding modeled (mod) linear fits to the observed values using robust MM  
394 regressions (Formulas 4 to 7). Note that a 100% decrease means a filterable concentration equal to  
395 zero after 24 or 48h, while a 0% decrease means that concentrations did not change over time. See  
396 text and Figure S1 for the actual meaning of modelled values above 100% or below 0.

397 The better goodness-of-fit at 24 h reflects the presence of elements showing little concentration  
398 decrease between 24 and 48 h (*i.e.*, pattern three in Figs. 2 and S2). Regression slopes were higher for  
399 solutions amended with  $C_{\min}$  (Formulas 6 and 7) than with  $C_{EC50}$  (Formulas 4 and 5). These results indicate  
400 that i) the concentrations of lanthanides decreased faster in solutions amended with  $C_{EC50}$  than with  $C_{\min}$   
401 and ii) the differences in concentration decrease between  $C_{\min}$  and  $C_{EC50}$  progressively became more  
402 important from La to Lu (see Fig. S1 for details and examples). In solutions amended with  $C_{EC50}$ , the observed  
403 percentage decrease in Nd concentrations (atomic mass = 144.24) was markedly lower than expected at  
404 both 24 and 48h (Fig. 5). We do not have a definite explanation for Nd behavior, but we notice that El-Akl  
405 et al. (2015) reported the formation of Nd-colloidal particles with an approximate size of 60 nm in a non-  
406 complexing medium test medium for freshwater algae. Particles of such sizes can probably pass through  
407 the pores of standard 0.45  $\mu\text{m}$  filters. However, it remains unclear while only Nd would form such small-  
408 sized particles compared with other LN. Some modelled % losses fall outside the 0–100 % range; *i.e.* the  
409 actual boundaries for experimental observations. These model artifacts do not invalidate the clear trend in  
410 increasing LN solubility from La to Lu in the ISO 6341 test medium for acute ecotoxicity testing with *D.*  
411 *magna*. The proposed linear models can be used to optimize analytical efforts to check for exposure  
412 concentrations in ISO test medium for *D. magna* over the test duration (at least for concentration up to  
413  $C_{EC50}$  and with the possible exception of Nd).

414 Although decreasing total or filterable LN concentrations have been previously reported in standardized  
415 test media for *D. magna* or *Daphnia* spp. (Barry & Meehan, 2000; Blinova et al., 2018; González et al., 2015;  
416 Lachaux et al., 2022; Ma et al., 2016; Vukov et al., 2016), the present study provides the first data of the  
417 different temporal trends in concentration decrease across the whole LN series. Overall, LN solubility  
418 appears to be time and concentration dependent. Correct interpretation of LN ecotoxicity data therefore  
419 needs a detailed follow up of changes in exposure concentrations over test duration.

420 We acknowledge that TWM (Formula 2) is just one of the available approaches to account for decreasing  
421 exposure concentrations during ecotoxicity tests. For example, using geometric means instead of TWM  
422 concentrations would result in lower, average exposure concentrations over the test duration in the case  
423 of 24h  $EC_{50}$  for the first round of definitive tests (Table S11). More refined calculations of the TWM are also  
424 possible when exposure concentrations are verified at multiple time points during ecotoxicity tests. The  
425 data available for  $t = 0, 5$  and 24 h (Figure 2 and Table S9) provide a useful example in this sense. First, the  
426 average exposure concentrations between 0–5 h (0.21 days) and 5–24 h (0.79 days) can be calculated using  
427 a slightly modified version of Formula 2 that was presented earlier:  
428

429 (8) 
$$\text{Average concentration}_{\text{period}} = \frac{\text{Conc}_{t(x)} - \text{Conc}_{t(x+1)}}{\ln(\text{Conc}_{t(x)}) - \ln(\text{Conc}_{t(x+1)})} * \text{Days}$$

430 As already seen for Formula 2,  $t(x)$  and  $t(x+1)$  refer to concentration at the beginning and at the end of each  
431 exposure period. The calculated average concentration during each period is then multiplied by the  
432 duration of the exposure period itself (in days). Finally, the overall TWM is obtained by summing the average  
433 concentration for each period and dividing by the total duration in days; *i.e.*, one day in the present case.  
434 We notice that Formula 8 simplifies to Formula 2 when measurements are available only at 0 and 24 h.

435 The TWM obtained with Formula 8 are lower than those obtained using Formula 2, except for Nd, and  
436 in good agreement with the corresponding geometric means (Table S11). Considering the variety of possible  
437 approaches, the research community should reach a consensus about the preferred calculation method(s)  
438 to estimate average exposure concentrations during ecotoxicity tests with LN when their concentrations  
439 are not stable over the entire test duration. This consensus is especially necessary to facilitate comparisons  
440 across studies used for regulatory decisions.

441 Caution is also required to understand the impact of adding large quantities of LN to the test medium.  
442 Indeed, LN concentrations remained within  $\pm 30\%$  of the initial values in solutions amended with  $C_{\text{MAX}}$  for  
443 La, Nd, Gd and Yb; contrary to what observed for  $C_{EC50}$  and  $C_{\min}$  (Figure 2A, B). Furthermore, the pH in

444 solutions amended with  $C_{MAX}$  remained around the initial value of 6.5 units or slightly decreased over time,  
445 again in contrast with observations of  $C_{EC50}$  and  $C_{min}$  where pH increased to about 7.5 units over the test  
446 duration (Table S7 and S9). To the best of our knowledge, these are the first results considering the changes  
447 in pH during standardized ecotoxicity test with *D. magna* following protocol ISO 6341 (2012). These findings  
448 could also bear relevance for correct interpretation of chronic ecotoxicity tests such as the *Daphnia magna*  
449 reproduction test (OCDE, 2012). Depending on the tested LN, exposure concentration can decrease by up  
450 to 80% of initial values in five hours; a span much shorter than the 48 h among medium renewal usually  
451 adopted in chronic tests with *D. magna*.

#### 452 **4.3. Theoretical solubility of LN**

453 At the initial pH for the definitive ecotoxicity tests (range from 6.4 to 6.6, Table S7), all LN concentrations  
454 were above the corresponding theoretical solubility except for the  $C_{min}$  of Er, Tm, Yb and Lu (Table S12). The  
455 decrease in total and filterable LN concentrations over time (Fig. 1, Table S9) therefore agreed with the  
456 modelling results. However, the agreement between modelled and observed LN solubility was far from  
457 perfect, reflecting the limitations of thermodynamic speciation models for our experimental matrix and  
458 conditions.

459 In solutions spiked with  $C_{EC50}$ , measured filterable concentrations at  $t = 24$  and 48 h were consistently  
460 higher than the corresponding theoretical solubility values in the pH range from 6.6 to 7.4 (Table S12). The  
461 pH range 6.6–7.4 covers the corresponding values estimated from concentration–pH relationships in  
462 solutions initially spiked with  $C_{EC50}$  (Table S8). The discrepancies between theoretical and measured  
463 concentrations could simply indicate that the system had not yet reached equilibrium at the end of the  
464 experiments. This explanation holds for La, Ce, Pr, Sm, Eu, Gd, Dy, Er, Tm and Yb. The filterable  
465 concentrations of these elements continuously decreased between 0 and 48 h (Fig. 2) and the trend could  
466 have continued beyond 48 h. On the other hand, the non equilibrium explanation does not apply to Nd, Tb,  
467 Ho, Tm and Lu whose filterable concentrations plateaued after 24 h while remaining above the  
468 corresponding theoretical solubility limits (Fig. 2). One possibility is that the measured filterable  
469 concentrations of these elements included colloidal-sized precipitates small enough to pass through the  
470 filter pores. The presence of colloids could have increased the apparent solubility of these elements  
471 compared with VM calculations that do not consider the particle size of the precipitates. The formation of  
472 such colloidal-sized particles (60 nm in the specific case) has indeed been reported in a simple  
473 ecotoxicological medium spiked with Ce (El-Akl et al., 2015). Furthermore, the filterable elemental fraction  
474 may vary depending on the filter material as well as the characteristics and quantity of filtered waters (Hall  
475 et al., 2002; Horowitz, 2013). While we expect our filtration results to be internally coherent (same medium  
476 and same filter type for all samples), the true percent filterable metal contained in medium ISO6341 may  
477 well vary depending on methodological choices for filtration. Finally, the conditional nature of VM  
478 modelling parameters could also contribute to the observed differences for all LNs (Rodríguez de San Miguel  
479 et al., 2023).

480 The reasoning developed for solutions amended with  $C_{EC50}$  also applies to experiments using  $C_{min}$  for La,  
481 Nd, Gd and Yb. In solutions amended with  $C_{MAX}$  for the same elements, all daphnids died within 24 h and  
482 pH values at  $t = 48$  h had dropped below the initial value of 6.5 (Table S9). Low pH values are expected to  
483 increase LN solubility and likely contributed to the stability of filterable concentrations in these solutions  
484 (Fig. 2) even if the added LN levels initially exceeded the corresponding theoretical solubilities and  
485 precipitation could be expected (Fig. 3 and Table S12). Furthermore, the death of daphnids likely released  
486 different organic molecules into test solutions. These molecules may also increase LN solubility as observed  
487 for humic acids (Pourret et al., 2007), soil organic matter (Lachaux et al., 2022) and organic ligands such as  
488 oxalate and citrate (Wen et al., 2024), but this hypothesis remains to be verified.

#### 489 **4.4. Comparison of different exposure metrics for lanthanides ecotoxicity**

490 The two rounds of tests gave equivalent results (*i.e.*, overlapping 95% confidence intervals of nominal  
491  $EC_{50}$ ) for Pr, Nd, Gd, Tb, Ho, Tm, Yb, Lu and, at 24 h only, Sm (Table S10). In all other cases (La, Ce, Eu, Dy,  
492 Er, and, at 48 h only, Sm),  $EC_{50}$  were higher in the second round of definitive tests than in the first one (Table  
493 S10, Fig. 4). Except for Eu (see below), we surmise that these differences were caused by the higher pH  
494 values of the exposure solutions during the second round of tests (Table S7).

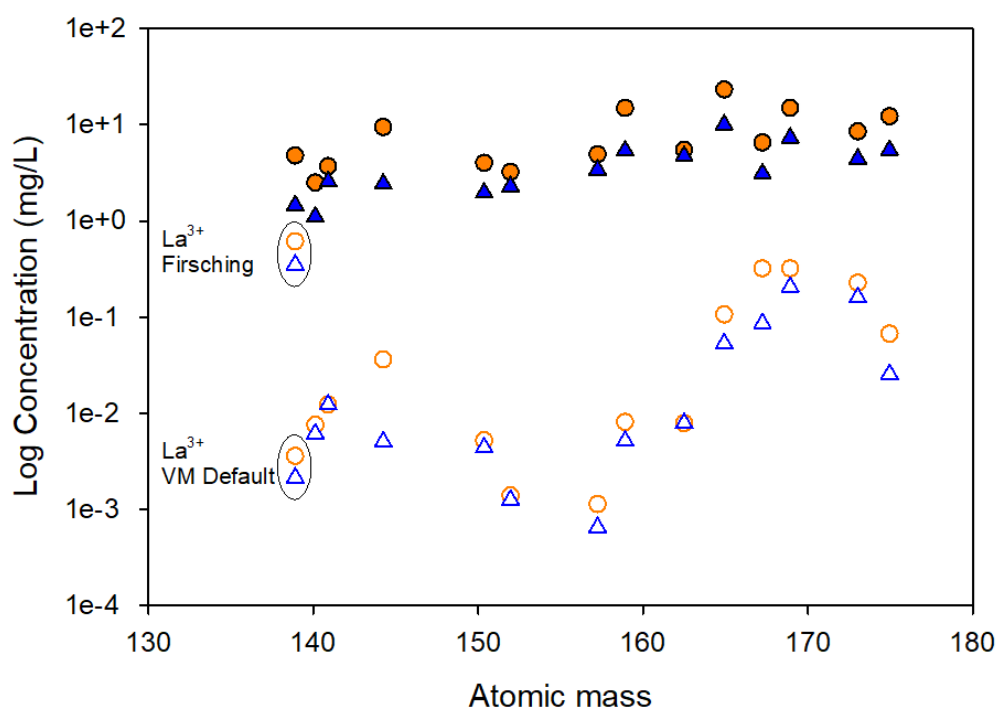
495 In both experiences, pH values were measured at  $t = 0$  h in control solutions (assumed to be  
496 representative of all exposure solutions at the beginning of the tests) and at 48 h for selected  
497 concentrations (Table S7). Measurements of pH after 24 h of exposure were not systematically performed  
498 during definitive tests to avoid additional stress to daphnids. However, available data showed limited pH  
499 variability between 24 and 48 h (Table S9).

500 Statistically significant inverse linear relationships were found between the measured pH values at 48 h  
501 and the initial LN concentration in the test medium (Table S8). These linear relationships were used to  
502 estimate the pH value in hypothetical aliquots of test media spiked with the corresponding nominal  $EC_{50}$   
503 (Table S8). Because pH controls the solubility of  $Ln_2(CO_3)_3$ , a higher pH value at the end of the test (data in  
504 Table S7) implies i) a larger and probably faster decrease in the actual exposure concentrations over the  
505 test duration (see sections 3.2 and 3.3) and hence ii) higher  $EC_{50}$  values in the second round of definitive  
506 tests. The pH explanation does not hold for Eu whose exposure solutions showed lower pH values for the  
507 higher  $EC_{50}$  in the second test series (Table S7 and S8). We acknowledge that the proposed simple  
508 explanation does not account for kinetics processes and for confounding processes linked to the presence  
509 of daphnids in the exposure solutions.

510 Besides the formation of insoluble precipitates, expressing the  $EC_{50}$  in terms of free  $Ln^{3+}$  concentrations  
511 in the dissolved fraction can provide further insight into the ecotoxicity of LNs and increase the reliability  
512 and relevance of concentration–response relationships. Estimation of  $Ln^{3+}$  concentrations was done for  
513 TWM  $EC_{50}$  values using the same assumptions described in section 3.3 for the choice of stability constants  
514 for  $Ln_2(CO_3)_3$  precipitation. These estimations will be presented only for the first series of definitive tests.

515 In absolute terms,  $Ln^{3+}$  based  $EC_{50}$  were between 1 to 10  $\mu\text{g/L}$  for La, Ce, Sm, Eu, Gd, Tb and Dy and in  
516 the tens to hundreds of  $\mu\text{g/L}$  for the remaining elements. Differences between  $Ln^{3+}$  and TWM-based  $EC_{50}$   
517 were between 1 and 3 orders of magnitude at both 24 and 48h of exposure (Fig. 6). The actual  
518 ecotoxicological significance of such estimations depends on how well the constants used in the  
519 thermodynamic models are representative of the true LN speciation in the test medium. For example,  
520 estimates of free  $La^{3+}$  concentrations were three orders of magnitude higher when using the  $La_2(CO_3)_3$   
521 solubility product reported by Firshing et al. (1986) compared with the default value in the VisualMinteq  
522 database (Fig. 6). Further work is needed to experimentally measure the free  $Ln^{3+}$  concentration in exposure  
523 medium and to determine confidence intervals to  $EC_{50}$  values based on TWM or free  $Ln^{3+}$ .

524 Considering the important quantities of LN salts added to the test medium (Table S3) and the tendency  
525 of test medium to get more acidic at high levels of added LN salts, it is important to verify the possible  
526 contribution of these two factors (pH and counterions added along with LNs) onto the survival of *D. magna*.  
527 Available reports (Chen et al., 2012) and online article abstracts (El-Deeb et al., 2011; Zhuang, 1994) suggest  
528 that effects of low pH become visible at values below 5.5 units, lower than those observed in the present  
529 study. In the case of the  $NO_3^-$  and  $Cl^-$  ions, the maximum concentration added along with LN were 76 mg/L  
530 and 46 mg/L, respectively (information on the specific counterion for each LN salt can be found in section  
531 2.1). In the case of nitrates,  $LC_{50}$  reported in the literature for *D. magna* are in the range 323–611 mg/L  $N-$   
532  $NO_3^-$ , *i.e.*, 1,430–2,705 mg/L  $NO_3^-$  (Scott & Crunkilton, 2000). In the case of chlorides,  $LC_{50}$  depends on the  
533 specific salt added to the test medium. Potassium chloride (KCl) has the lowest reported 48h  $LC_{50}$  of 660  
534 mg/L, which corresponds to 314 mg/L of  $Cl^-$  (Mount et al., 1997). Based on this information, we estimated  
535 the toxic units ( $TU = \text{exposure concentration} / LC_{50}$ ) for each LN and the appropriate counteranion at the  
536 maximum concentrations added to the test medium. A worst-case scenario was used for the counteranion  
537 contribution; *i.e.*, the maximum added  $Cl^-$  or  $NO_3^-$  concentration used in the present study was divided the  
538 lowest  $LC_{50}$  reported in the literature. The TU approach allows to compare the potential contribution of LN  
539 and counteranions to the overall ecotoxic effect for the maximum added LN levels. The TU values for  
540 counteranions were below 5% of those for LN in all cases except for Eu in one of the test series (Table S13).  
541 Overall, the contribution of counteranions to the observed ecotoxicity should therefore be secondary  
542 compared with that of the added LN. Depending on the added LN concentrations, future studies may  
543 consider including experimental verification of counterion ecotoxicity.



● TWM 24h (mg/L)  
 ▲ TWM 48h (mg/L)  
 ○ Ln<sup>3+</sup> 24h (mg/L)  
 △ Ln<sup>3+</sup> 48h (mg/L)

544

545 Figure 6. Comparison between 24h and 48h Time Weighed Mean (TWM) EC<sub>50</sub> expressed as total filterable concentrations and free Ln<sup>3+</sup> concentrations. Free Ln<sup>3+</sup> concentrations were estimated using  
 546 VisualMinteq (see section 3.3 for details). Calculations for free lanthanum (La<sup>3+</sup>) are presented using  
 547 two different La<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> solubility products to invite caution when making ecotoxicological inference  
 548 using modelled La<sup>3+</sup> values (see text for details).  
 549

550 The EC<sub>50</sub> values based on nominal values (Fig. 4 and Table S10) agree with those observed in previous  
 551 studies with daphnids and other crustaceans (see the recent data compilation reported in Table S5 of  
 552 Lachaux et al. (2022). Comparison based on measured LN concentrations or on modelled free ion  
 553 concentrations are less straightforward because of the methodological differences among the studies  
 554 containing such information. In the case of Nd, Gd, and Yb, our TWM EC<sub>50</sub> at 48 h (Table S11) are within the  
 555 95% confidence intervals of the values reported by Lachaux et al. (2022) who used the same test medium  
 556 and analogous methodologies. However, our estimations for EC<sub>50</sub> based on modelled free-ion  
 557 concentrations (Fig. 6) are three orders of magnitude lower than those of Lachaux et al. (2022) because of  
 558 different assumptions in model calculations. In particular, we allowed the precipitation of Ln<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> solids  
 559 in the test medium and subtracted the precipitated concentrations from the total ones before calculating  
 560 the free-ion concentrations. Both approaches can be acceptable depending on the experimenter's choice  
 561 as to the complexation and solubility constants in the speciation model (see sections 3.3, 4.3 and 5 for  
 562 further discussion). Blinova et al. (2018) did observe a marked decrease in La, Ce, Pr, No, and Gd  
 563 concentrations in standard artificial freshwater (OCDE, 2004), but did not attempt to calculate 48h EC<sub>50</sub>  
 564 based on measured concentrations. Vukov et al. (2016) reported a 48 h EC<sub>50</sub> of 0.49 mg/L Dy for *D. pulex*  
 565 based on the concentration measured at the end of the text. This value is 10- to 20-fold lower than the 48h  
 566 TWM EC<sub>50</sub> of the present study (Table S11). However, it compares favorably with the filterable Dy  
 567 concentration of 2 mg/L that remained in our exposure medium after 48 hours (Table S9). Shu et al. (2023)  
 568 reported 24 h and 48 h EC<sub>50</sub> of 16.2 and 2.3 mg/L, respectively, for *D. magna* exposed to La according to  
 569 OCDE guideline 202 (OCDE, 2004). These values referred to analytically measured filterable La  
 570 concentrations at t = 0 (after 2h of equilibration following the initial La spike). They are higher than our EC<sub>50</sub>  
 571 based on TWM (Table S11), but difficult to compare with nominal values considering the tendency of La  
 572 concentration to rapidly decrease upon addition to the test medium (Fig. 1). Finally, Ma et al. (2016)

573 substituted NaHCO<sub>3</sub> with NaOH in the test medium for *D. magna* and successfully avoided carbonate  
 574 precipitation when testing the ecotoxicity of Ce. As a result, the filterable (0.22 μm) Ce concentration  
 575 showed little variation between the beginning and the end of the test. Their 48h EC<sub>50</sub> of 1.5 mg/L (based on  
 576 measured concentration) is in excellent agreement with our 48h TWM EC<sub>50</sub> of 1.11 mg/L (1<sup>st</sup> series) and  
 577 1.64 mg/L (2<sup>nd</sup> series) (Table S11), showing that correction for concentration decrease can improve data  
 578 comparability across studies. We are not aware of other studies providing EC<sub>50</sub> based on free ion  
 579 concentrations for *D. magna* or other daphnids, apart from the mentioned comparison with Lachaux et al.  
 580 (2022).

#### 581 4.5. Trends in EC<sub>50</sub> along the lanthanides series

582 Ecotoxicologists are constantly seeking for the presence of regular, predictable variations in EC<sub>50</sub> along  
 583 the LN series, in analogy with the predictable changes in physico-chemical properties such as ionic radius  
 584 and ionization potential. Regular ecotoxicological patterns would allow to focus research efforts on a few  
 585 representative lanthanides from which relevant information could then be derived for the whole series.

586 In the present study, nominal EC<sub>50</sub> values did not show any clear trend along the LN series after 24 or  
 587 48h of exposure (Fig. 4). On the other hand, linear relationships could be derived between the atomic mass  
 588 of lanthanides and the EC<sub>50</sub> values expressed as TWM (Formulas 9 to 13, see Table S14 for the statistical  
 589 significance of regression coefficients and calculation details). For 24 h exposure in the first series of  
 590 experiments, two relationships were derived; one using TWM values calculated from Formula 2 with  
 591 analytically measured concentrations at 0 and 24 h as inputs (Formula 9) and one using TWM estimated  
 592 from Formula 4 (Formula 10). On average, the predicted values differed by 30–40 % from the input TWM  
 593 values, with differences larger than 50% for many elements (Table S15).

$$594 \quad (9) \quad TWM_{24h(1stSeries\_Exp)} = (0.2582 \pm 0.115) \text{ atomic mass} + (-32.0 \pm 18.1) \quad R^2 = 0.29$$

$$595 \quad (10) \quad TWM_{24h(1stSeries\_Mod)} = (0.3010 \pm 0.0898) \text{ atomic mass} + (-37.5 \pm 14.1) \quad R^2 = 0.48$$

$$596 \quad (11) \quad TWM_{48h(1stSeries)} = (0.136 \pm 0.0413) \text{ atomic mass} + (-17.4 \pm 6.5) \quad R^2 = 0.48$$

$$597 \quad (12) \quad TWM_{24h(2ndSeries)} = (0.4516 \pm 0.1013) \text{ atomic mass} + (-56.9 \pm 15.9) \quad R^2 = 0.62$$

$$600 \quad (13) \quad TWM_{48h(2ndSeries)} = (0.1720 \pm 0.0489) \text{ atomic mass} + (-21.3 \pm 7.7) \quad R^2 = 0.51$$

603 Expressing TWM EC<sub>50</sub> in terms of free ion concentrations resulted a clear M-shaped pattern at both 24  
 604 and 48 h (Fig. 6). This pattern resembles the *tetrad effect* linked to the progressive filling of *f* orbitals along  
 605 the lanthanides series (McLennan, 1994) and is also similar to the changes in the energy of electronic  
 606 configuration from La to Lu (Schinzel et al., 2006). In particular, La, Gd and Lu have the largest differences  
 607 between the energy of the highest occupied and lowest occupied molecular orbital and form the most  
 608 stable complexes, which agrees with their higher ecotoxic potential (lower Ln<sup>3+</sup> EC<sub>50</sub>) compared with  
 609 adjacent elements (Fig. 6). However, the solubility products used in the present study (Fig. S3) also showed  
 610 a M-shaped trend when plotted against the atomic mass of the corresponding elements. Otherwise stated,  
 611 the pattern observed in Ln<sup>3+</sup> EC<sub>50</sub> may simply be a model artifact linked to speciation calculation.  
 612 Methodological developments are clearly necessary to obtain experimental measurements of LN speciation  
 613 in ecotoxicological test media and verify the true meaning of this M-shaped pattern in Ln<sup>3+</sup> EC<sub>50</sub>.

614 Few studies examined the ecotoxic effects of the whole LN series and they reached contrasting results  
 615 (Table 1). Data from Blinova et al. (2018) provide the most relevant comparison for the present study and  
 616 suggest a linear decreasing trend for 48 h LC<sub>50</sub> to *D. magna* based on nominal values (La > Ce > Pr > Nd ≈  
 617 Gd). However, a strong decrease in LN concentration was reported over the test duration and the trend  
 618 based on measured concentration could not be validated based on the information provided in the paper.  
 619 Similarly, 21 d chronic LC<sub>50</sub> did not show any trend (La ≈ Gd > Ce ≈ Pr ≈ Nd). Decreasing ecotoxicity along  
 620 the LN series has been reported for *Hyalella azteca*, *Hydra attenuata* and *Danio rerio* based on nominal  
 621 concentrations (Table 1). However, the heaviest lanthanides did not conform to the observed trends for *H.*  
 622 *azteca* and *H. attenuata* (see footnotes in Table 1). These findings (*i.e.*, a decrease in ecotoxicity along the  
 623 LN series) agree with the observations of the present study, but the comparisons cannot be considered fully



624 conclusive due to the missing analytical verification of exposure concentrations in these previous works  
625 (Table 1).

626 At the opposite, increasing ecotoxicity along the LN series was reported for the charophyte alga  
627 *Nitellopsis obtusa* (Manusadžianas et al., 2020) and the bacterium *Aliivibrio fischeri* (Weltje, 2002) (Table  
628 1). In the very simple medium (0.355 M NaNO<sub>3</sub>) used for testing with *A. fischeri*, free Ln<sup>3+</sup> were predicted  
629 to be the dominant species and showed the same trend as total dissolved concentrations (nominal values).  
630 However, total dissolved and free Ln<sup>3+</sup> concentration will likely have different patterns in more complexing  
631 media, especially those containing phosphates or carbonates. Gonzalez et al. (2014) warned about  
632 establishing trends in the absence of detailed and complete information on LN speciation in the various test  
633 media used for different organisms. Until minimum analytical requirements are adopted by the whole  
634 ecotoxicological community, it will remain elusive to establish meaningful and generally valid ecotoxicity  
635 trends along the LN series.

636 In the same vein, it is instructive to examine if differences exist in the ecotoxicity of LN after grouping  
637 them into light rare earth elements (LREE – from La to Gd) and heavy rare earth elements (HREE – from Tb  
638 to Lu); an approach that is widely used by the scientific community (Rétif et al., 2024). We are specifically  
639 interested in differences between LREE and HREE at any given time (24 or 48 h) and for each exposure  
640 metric (nominal EC<sub>50</sub>, TWM EC<sub>50</sub> and EC<sub>50</sub> based on free ion concentrations; Figs. 4 and 6). On average, LREE  
641 appear more toxic than HREE ( $p < 0.05$ ) except when EC<sub>50</sub> are expressed as nominal concentrations after 48  
642 h of exposure (Fig. S4). The use of TWM increases the probability of LREE being more toxic than HREE in all  
643 cases; in agreement with the larger decrease in the exposure concentrations for many LREE (see sections  
644 4.1 and 4.2; Fig. S4). Although similar results have been reported (Lin et al., 2022), these observations  
645 confirm the need to reach a consensus on which metric to use for LN exposure in order to correctly interpret  
646 their ecotoxicological effects.

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Table 1. Summary of studies examining trends in ecotoxicity along the lanthanides series to aquatic organisms. ↓ or ↑ indicate a decreasing or increasing trend in ecotoxicity along the lanthanides series, respectively. [LN] measured refers to analytical verification of exposure concentration; Y, yes; N, no.

Elements	Model organism	Trend (Y/N)	[LN] measured	Medium composition	Reference
La, Ce, Pr, Nd, Gd	<i>Daphnia magna</i>	Y <sup>§</sup> (acute, nominal), ↓ N (chronic)	Y	Acute test: OECD (2004) Chronic test: Lake Water (pH 7.5 ± 0.2, DOC 10.6 mg/L C)	Blinova et al. (2018)
All LN	<i>Danio rerio</i>	Y, ↓	N	According to OECD 203 (2019)	Lin et al. 2022
La, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Er, Lu (and Y)	<i>Hydra attenuata</i>	Y <sup>#</sup> , ↓	N	Hydra medium CaCl <sub>2</sub> , TES, pH 7	Blaise et al. (2018)
All LN	<i>Hyalella azteca</i>	Y, ↓ <sup>§</sup>	Y	Soft water	Borgmann et al. (2005)
All LN except Pr, Ho, Yb	<i>Vibrio fischeri</i>	Y, ↑	N	0.355 M NaNO <sub>3</sub> , pH 5.5	Weltje (2002)
All LN except Ho, Tm, Yb	<i>Nitellopsis obtusa</i>	Y, ↑ <sup>  </sup>	N	Artificial Pond Water KCl, NaCl, CaCl <sub>2</sub> and HEPES (pH 7.5)	Manusadžianas et al. (2020)
All LN except Ho, Tm, Yb	<i>Thamnocephalus platyurus</i>	N	N	Standard freshwater NaHCO <sub>3</sub> , CaSO <sub>4</sub> , MgSO <sub>4</sub> and KCl	Manusadžianas et al. (2020)
All LN	Cell lines of <i>Danio rerio</i> and <i>Onchorynchus mykiss</i>	N	Y	Mixed medium	Fleurbaix et al. (2022)
La, Ce, Gd, Ho	<i>Danio rerio</i>	N <sup>§</sup>	Y	Standard dilution water CaCl <sub>2</sub> , MgSO <sub>4</sub> , NaHCO <sub>3</sub> , KCl	Kang et al. 2022
All LN	<i>Hyalella azteca</i>	N	N	Tap water	Borgmann et al. (2005)
All LN	<i>Chlorella vulgaris</i>	N	N	K <sub>2</sub> HPO <sub>4</sub> , KH <sub>2</sub> PO <sub>4</sub> , (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , KNO <sub>3</sub> (pH just below 7)	Den Dooren de Jong (1965)
All LN	<i>Skeletonema costatum</i>	N	N	f/2 medium without EDTA: NaNO <sub>3</sub> , NaH <sub>2</sub> PO <sub>4</sub> , trace elements and vitamins (pH 8.0)	Tai et al. (2010)

§, The existence of trends has been examined by the authors of the present study, but was not mentioned in the original publication.

||, only for 24 d LC<sub>50</sub> (not for shorter exposure time); #, if Er and Lu excluded from the regression; §, if Tm, Yb and Lu excluded.

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## 5. RECOMMENDATIONS FOR FUTURE STUDIES ON LN ECOTOXICITY

667 The results of the present study highlight a number of caveats that should be addressed to improve the  
668 reliability and relevance of laboratory studies on LN ecotoxicity. The proposed list of caveats (Table 2 and  
669 text below) considers only issues directly linked to the results and approaches used in the present study  
670 and does not claim to be exhaustive.

### 671 *Caveat # 1. Formation of insoluble LN species during ecotoxicity testing*

672 The ecotoxicological community is already well aware of the tendency of LN to form insoluble  
673 precipitates in ecotoxicological test media, especially in the presence of carbonates or phosphates (Blinova  
674 et al., 2018; Lachaux et al., 2022; Ma et al., 2016). Formation of Ce-bearing colloidal particles has been  
675 reported even in a non-complexing medium (0.01 M NaHEPES +  $10^{-5}$  M  $\text{Ca}(\text{NO}_3)_2$ ) spiked with approx. 5  $\mu\text{g}/\text{L}$   
676 of Ce at pH between 6 and 8, with 40 to 70 % of the total concentration being retained by a 3kDa centrifugal  
677 ultrafiltration device (El-Akl et al., 2015). It is therefore advisable to consider that insoluble LN species will  
678 form in any medium and take the necessary analytical steps to verify this hypothesis (Table 2, point #1).

679 The present study suggests that the use analytical verifications based on total concentrations (*i.e.*,  
680 unfiltered samples) should be discouraged because they do not distinguish between soluble and  
681 (nano)particulate LN species (Vukov et al., 2016). Indeed, soluble species and precipitates do not necessarily  
682 contribute to ecotoxicity in the same way (Table 2, point # 3). The norm ISO14442 (ISO, 2006) provides  
683 useful recommendations that are applicable to the hazard assessment of elements tending to form  
684 insoluble precipitates such as LN. Further research should also better characterize the particle size  
685 distribution of LN precipitates, because filtration at 0.45 or 0.22  $\mu\text{m}$  will not detect the presence of colloidal  
686 precipitates smaller than the filter cut-off. Based on Single-Particle ICP-MS, El-Akl et al. (2015) estimated an  
687 average size of 60 nm for Ce particles formed in an exposure medium for short-term uptake experiments  
688 with *Chlamydomonas reinhardtii*. It is highly likely that part of the filterable concentrations reported in the  
689 present and previous studies actually include a certain proportion of particles having passed through the  
690 filter pores.

691 The formation of LN precipitates seems to strongly depend on the pH of the medium. Besides the  
692 theoretical solubility calculations supporting this hypothesis (sections 3.3 and 4.3), the decrease in filterable  
693 LN concentrations was almost suppressed when the quantity of added LN salt was high enough to keep the  
694 pH at values below 6.5 units over the duration of the test (Figures 1 and 2). Measurements of pH values at  
695 the beginning and at the end of ecotoxicity tests therefore provide valuable additional information to i)  
696 verify that LN speciation is similar across the chosen range of exposure concentrations in individual studies  
697 and ii) facilitate comparisons across studies.

698 Finally, we note that modifications of exposure media are a possible alternative to avoid the formation  
699 of LN precipitates and even increasing the environmental relevance of the results (Blinova et al., 2018; Ma  
700 et al., 2016). These options would not eliminate the need for a thorough preliminary verification of LN  
701 precipitation in the chosen matrix across the range of exposure concentrations. Indeed, substituting NaCl  
702 for  $\text{NaHCO}_3$  in ISO medium 6341 (*i.e.*, the medium used in the present study without modification) did not  
703 prevent La and Gd filterable concentrations from decreasing by 50–98 % (La) and 40–70% (Gd) over the  
704 exposure period of 48 h (Revel et al., 2023). On the other hand, Ma et al. (2016) apparently avoided the  
705 formation of Ce precipitates larger than 0.22  $\mu\text{m}$  by substituting NaOH for  $\text{NaHCO}_3$  in the same medium.  
706 Medium modifications should therefore be standardized among research groups to ensure that data across  
707 studies are comparable. A similar reasoning should ideally apply also to the procedures to determine  
708 filterable LN fractions (Horowitz, 2013; Vignati et al., 2006; Weyman et al., 2012).

### 709 *Caveat # 2. Variable exposure conditions for test organisms over the test duration*

710 Although marked changes in LN exposure concentrations have been previously reported (see caveat  
711 #1), the present study shows that concentration decreases can be especially fast at the beginning of  
712 ecotoxicity tests (Figure 2). It is therefore necessary to reach a consensus as to the appropriate exposure  
713 metric to calculate ecotoxicological endpoints for LN. In principle, evaluation of LN ecotoxicity should not  
714 be based on i) nominal or measured concentrations at the beginning of the test, or ii) total concentrations  
715 that may contain a mixture of soluble and precipitated LN species (Vukov et al., 2016); present study). Both

716 choices can lead to a marked underestimation of ecotoxic effects (Fig. 4). Measured concentrations at the  
717 end of the test would be a conservative alternative when exposure concentrations decrease over time.  
718 However, we recommend either TWM or geometric mean values as the best options to estimate the  
719 average exposure concentration during the whole test duration.

720 In the specific case of the test medium ISO 6341 (2012) for *D. magna*, the temporal decrease in filterable  
721 LN concentrations at 24 and 48 h shows a clearly linear trend along the LN series (with the possible  
722 exception of Nd) (Figure 5). Future studies using this medium may therefore rely on Formulas 4 to 7 to  
723 estimate LN concentrations at the end of the tests and provide EC<sub>50</sub> estimates as TWM (present study) or  
724 geometric mean concentrations (Lachaux et al., 2022). It would be beneficial to verify if analogous  
725 relationships exist between LN atomic mass vs. concentration decrease in other standardized  
726 ecotoxicological test media. The existence of such relationship would indeed reduce the analytical  
727 requirements necessary to establish a consensus about the best exposure metrics to evaluate LN  
728 ecotoxicity. Care should also be taken as to the possible effects of adding large quantities of LN on medium  
729 pH. In the present study, we assumed homogenous pH at the beginning of the tests for all exposure  
730 concentrations, but experimental results suggest that this assumption could have been violated in some  
731 cases. Future studies may consider either experimental verification of pH at multiple time points for all  
732 exposure concentrations or pH adjustments at the beginning of the tests. The second option does not  
733 necessarily eliminate the possibility of pH changes during the tests and hence the need for pH monitoring.  
734 Linear relationships between pH and LN concentrations may exist, as for the present study, and be used to  
735 estimate pH values across the exposure range.

### 736 *Caveat # 3. Unknown ecotoxicological role of precipitates*

737 The results of the present study do not allow to establish if the observed biological responses in *D.*  
738 *magna* were caused solely by the free ionic LN species, whose concentrations may have varied during the  
739 tests and were only a tiny fraction of filterable ones (Fig. 6), or by combined effects of soluble and  
740 particulate LN species. Considering that LN precipitation is probably ubiquitous in standard ecotoxicological  
741 media, we provide a few suggestions on how to tackle this important issue based on current knowledge.

742 Revel et al. (2023) recently observed that particle formation was accompanied by different effects and  
743 tissue distributions in daphnids exposed for 48 h in medium aliquots initially amended with soluble La and  
744 Gd in the same concentration range. As discussed in *caveat #1*, both elements formed insoluble precipitates  
745 albeit La did so to a greater extent. Micro- and nano-XRF analysis showed that La concentrated almost  
746 totally in the digestive tract, while Gd was apparently taken up and distributed across different tissues. Only  
747 Gd caused a clear ecotoxicological response in the exposed organisms with 48h EC<sub>50</sub> of 13.9 mg/L (nominal  
748 value) or 8.22 mg/L (filterable concentration at the end of the test); comparable with the present study.  
749 The absence of La ecotoxicity suggests that precipitates were not available for uptake by internal organs,  
750 while the observed Gd effects cannot be univocally ascribed to soluble or precipitated forms. These results  
751 were confirmed by Revel et al. (2024) who suggested using total or nominal concentrations as exposure  
752 metrics for LN ecotoxicity, in contrast with our recommendations at *caveat #1*.

753 Identifying the possible role of precipitates in LN ecotoxicity is related, but not equivalent, to  
754 understanding the ecotoxicity of nanoparticulate LN forms. LN-bearing nanoparticles are engineered  
755 materials of known composition and primary particle-size distribution. This contrasts with the formation of  
756 precipitates following addition of soluble LN salts into the test medium. LN precipitates have variable  
757 composition, *i.e.*, different types of precipitates can be present at the same time, and unknown particle size  
758 distribution. Their concentration may also change over the test duration depending on the stability of  
759 exposure conditions and on the solubility of the precipitates. Specific experimental approaches are required  
760 to identify the presence of precipitates and their possible contribution to LN ecotoxicity in standard  
761 laboratory tests (Table 2).

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Table 2. Caveats, recommendations and open issues for properly testing LN ecotoxicity to *Daphnia magna* and aquatic organisms in general. The recommendations are applicable to other ecotoxicological media where LN precipitation may occur. Open issues indicate possible priorities for future research and consensus-building (also see Table S16 for a more detailed presentation of each caveat). TWM, Time-Weighted Mean; LN, lanthanides.

#	Caveats	Recommendations / Possible approaches	Open issues
1	Formation of insoluble chemical species in the test medium	Provide appropriate analytical verification of filterable exposure concentrations. Verification of pH values very useful.	Presence of colloidal particles undetected by filtration. Speciation differences across the range of exposure concentration. Definition of standard non-complexing media.
2	Changeable exposure conditions for test organisms	Derive EC <sub>x</sub> using experimentally measured filterable concentrations and TWM or geometric means.	Relative contribution of soluble and particulate LN species to observed ecotoxic responses. Technical developments to measure LN bioavailable concentrations in test medium. Differences in filter type/performance can affect comparisons across studies.
3	Unknown and contrasting ecotoxicological roles of LN precipitates	Modify test medium to avoid formation of precipitates. Verify the absence of precipitates by <i>e.g.</i> , ultrafiltration or single-particle ICP-MS.	Contribution of precipitated species to ecotoxicity. Accumulation of precipitates in the gut of filter-feeding organisms during tests.
4	Use of free ionic LN concentrations as an improved exposure metric	Remember assumptions and limitations of thermodynamic speciation models.	Untested assumption that only Ln <sup>3+</sup> contribute to toxicity. Dynamic aspects of LN speciation.
5	Conditional character of stability and solubility constants in thermodynamic speciation models	Strive to combine speciation calculation and experimental verification of model predictions.	Developing techniques for direct determination of LN speciation in test media.
6	Ecotoxicity trends along the LN series may be artefacts related to methodological limitations and experimental choices	Do not establish trends in the absence of minimum verification of exposure concentrations (see # 1 and 2) and Table 1.	True trends require determination of the actual LN bioavailable fraction.
7	Inappropriate comparison between exposure metrics from ecotoxicity testing and environmentally measured levels	Clearly report what is being compared. Consider the implications of the different exposure metrics from ecotoxicity testing in terms of possible underestimation or overestimation of the risk.	Matrix effects on LN speciation remain to be fully understood. Field-based studies using DGTs may provide hints on LN bioavailability in natural conditions. Reporting conventions to be defined.

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776 Alternatively, exposure media could be aged after spiking with soluble LN forms and the precipitates  
777 removed by filtration, ultrafiltration or centrifugation before ecotoxicity testing in analogy to approaches  
778 proposed in the ecotoxicity of engineered nanoparticles (Sørensen & Baun, 2015). Results from the present  
779 study suggest that formation of particles larger than 0.45 µm (nominal cut-off) becomes progressively less  
780 important along the LN series (Figs. 1 and 2). However, the time to reach stable filterable concentrations  
781 varies among LN (Fig. 2). The choice of the appropriate filter cut-off also requires further information on  
782 the particle size distribution of the precipitates. Such considerations may equally apply to chronic  
783 ecotoxicity testing because theoretical calculations suggest that the solubility limits could be as low as a  
784 few tens of micrograms per liter depending on specific elements and medium pH (Fig. 3 and Table S12).  
785 These solubility limits are comparable to, or lower than, the lowest chronic ecotoxicity values reported by  
786 Shu et al. (2023) and Lüring and Tolman (2010) for La (59 µg/L and 100 µg/L, respectively in artificial media),  
787 but higher than Ce and Er concentrations of 1 µg/L used by Galdiero et al. (2019) to study multigenerational  
788 effects. Comparison with the chronic values determined by Blinova et al. (2018) for La, Ce, Pr, Nd and Gd  
789 (range 300 – 490 µg/L) is hindered by the use of a natural lake water at pH 7.5 and containing 10.6 mg/L of  
790 dissolved organic carbon.

#### 791 *Caveats # 4 and 5. Using modelled free ion concentrations as an improved exposure metric*

792 According to the scientific underpinnings of the biotic ligand model (BLM), ecotoxicological responses  
793 are controlled by the bioavailable elemental fraction that binds to specific biological ligands. The  
794 bioavailable fraction is mostly controlled by the free ion concentration although contributions from other  
795 elemental species are possible (Campbell & Fortin, 2013). While suitable analytical techniques are being  
796 explored (Janot et al., 2021; Leguay et al., 2016; Nduwayezu et al., 2016), free ion concentrations for LN are  
797 usually estimated using thermodynamic speciation models. This approach facilitates comparisons across  
798 studies using different exposure media and provides ecotoxicity estimation more relevant for risk  
799 assessment. However, it has some conceptual limitations that researchers should understand to avoid  
800 overstretching the environmental significance and implications of experimental results (Table 2, points # 4  
801 and 5).

802 The choice of  $\text{Ln}^{3+}$  as a metric of exposure assumes that only free ions contribute to the observed  
803 ecotoxic effects. In support of this assumption, MacMillan et al. (2018) found good overall relationships  
804 between the modelled  $\Sigma\text{REY}$  free ion concentrations and REY accumulation by indigenous zooplankton in  
805 Canadian lakes. On the other hand, studies with a variety of model organisms provide contrasting evidence  
806 as to the bioavailability of Ln-containing particles formed in ecotoxicological media following the addition  
807 of soluble LN forms (Aharchaou et al., 2020; El-Akl et al., 2015; Revel et al., 2023; Vukov et al., 2016). The  
808 problem is exacerbated when comparing  $\text{Ln}^{3+}$  concentrations in ecotoxicological media and in natural  
809 waters.

810 Although insoluble precipitates can dominate LN speciation in the laboratory, LN in natural waters can  
811 be strongly associated with colloids and occurring as complexes with dissolved natural organic matter (Janot  
812 et al., 2021; Marsac et al., 2021; Martin et al., 2021; Pinheiro & Rotureau, 2023). Thermodynamic speciation  
813 calculations in the presence of natural organic matter still suffer from conceptual shortcomings that could  
814 lead to erroneous estimations of  $\text{Ln}^{3+}$  in natural waters (Tesfa et al., 2022; Town et al., 2019). Similarly,  
815 speciation calculation in a given standard ecotoxicological medium (or across ecotoxicological media) will  
816 yield different results depending on the stability constants chosen for modelling by the experimenters as in  
817 the case of Lachaux et al. (2022) vs. the present study. The choice to allow or not for the precipitation of  
818 insoluble species during speciation modelling (provided that the necessary solubility products are available)  
819 can also affect the comparability of free ion based metrics across different studies. Theoretical solubility  
820 limits can also vary markedly depending on the choice of solubility values for the possible precipitate  
821 species. In the present study, solubility limits could differ by over 10-fold when comparing calculations using  
822 the 'Firsching' and the 'Default VM' databases for carbonate solubility (Table S12), notably in the case of  
823 La. Finally, estimation of free ion concentrations based on nominal or total concentrations and filterable  
824 concentrations at the beginning of the test should be avoided for the same reasons discussed in caveat #1.

#### 825 *Caveat # 6. Identifying meaningful trends along the LN series*

826 The previous caveats show that defining a generally valid ecotoxicological trend along the LN series  
827 remains elusive at the current state of knowledge, but also provide some hints to ensure that future

828 research provides valid results for verifying the actual existence of such trends. In particular, results from  
829 studies not providing minimum analytical information on exposure concentrations (Table 1) should be  
830 considered of limited reliability for hazard and risk assessment purposes until further verification. Trends  
831 based on average, measured exposure concentrations (or free ion concentrations derived from such  
832 measurements) may provide better information, but are also subject to further screening as more  
833 knowledge on LN speciation and bioavailability becomes available in both standardized test media and  
834 natural waters. The results of the present study do not end the quest for the presence or absence of regular,  
835 generally valid ecotoxicological patterns along the LN series in relation to their acute ecotoxicity toward  
836 *Daphnia magna*. They do however show a clear trend in the concentration decrease (Fig. 5), suggesting the  
837 best approaches to avoid underestimating LN ecotoxicity.

#### 838 *Caveat # 7. Comparing laboratory-derived exposure metrics with natural concentrations*

839 Risk assessment is based on the comparison between the EC<sub>50</sub> derived from laboratory studies (hazard  
840 assessment) with the measured ambient concentrations in the case of site-specific investigations or  
841 probable environmental concentrations to establish environmental quality standard on a wider scale  
842 (European Commission, 2019). At the current state of knowledge, the utmost care is required to avoid both  
843 overestimation and underestimation of the potential risks arising from (increasing) concentrations of LN  
844 following anthropogenic impacts (Table 2). According to the present study, laboratory exposure metrics  
845 derived from nominal concentrations (or concentrations measured at the beginning of the tests) would  
846 clearly underestimate risk. At the opposite extreme, comparing properly derived EC<sub>50</sub> with total elemental  
847 concentrations in natural water would likely overestimate the actual risk because LN bound to  
848 environmental particles will be considered equivalent to the dissolved (bioavailable) fraction. Studies  
849 attempting risk assessment should therefore be germane as to the analytical details of the concentrations  
850 being compared. An intriguing possibility would be to base risk assessment on comparisons of time-  
851 averaged, DGT-labile concentrations measured in both laboratory media and natural freshwater by  
852 extending approaches proposed for in situ ecological risk in sediments (Gu et al., 2020; Gu et al., 2022;  
853 Paller et al., 2019). Extrapolation of laboratory-based results to real-field situations also needs more  
854 information on how the variable composition of natural aqueous matrices in terms of major ions (e.g., Ca,  
855 Mg) or organic matter content affects LN uptake by and ecotoxicity to biota. Protective effects of Ca and  
856 organic matter against LN ecotoxicity have already been reported (Aharchaou et al., 2020; El-Akl et al.,  
857 2015; Lachaux et al., 2022; Vukov et al., 2016), but generally accepted models to predict such effects still  
858 have to be developed.

## 859 **6. Concluding remarks and way forward**

860 Reliable exposure vs. response relationships are the foundation of hazard and risk assessment.  
861 Understanding the dynamic and complex speciation of LN is therefore a missing cornerstone in standardized  
862 ecotoxicity tests for these elements. In the absence of such information, it is not possible to choose the  
863 appropriate metric to link exposure to LN with the measured biological responses even in controlled  
864 laboratory conditions.

865 The experimental results from the present study show that a statistically significant linear relationship  
866 exists between the atomic mass and the solubility of LN in the standard ISO 6341 test medium for *D. magna*,  
867 implying that reliable ecotoxicological endpoints must explicitly account for changes in LN concentration  
868 over the test duration. On the other hand, ecotoxicological trends along the LN series appear dependent  
869 on exposure time and the selected exposure metrics, highlighting the need for the definition of shared  
870 experimental and reporting procedures across the research community on this aspect. Given the difficulties  
871 in obtaining stable exposure concentrations of LN in many test media, one may even question the ability of  
872 standardized methods to provide reliable results on the ecotoxicity of LN to aquatic organisms. However,  
873 similar issues would equally apply to New Approach Methodologies (NAM), such as in vitro screening, and  
874 to any test method unless they adopt test media allowing a thorough control of LN speciation.

875 In our opinion, addressing the caveats discussed in section 5 will provide stronger basis for mutual  
876 acceptance of data across studies and help to reconcile standardized tests with the much more complex  
877 environmental reality (Vignati, 2021). Similarly, ecotoxicity trends along the LN series will remain conditional  
878 (*i.e.*, dependent on the chosen exposure metric and exposure time, among the other variables) until precise

879 information becomes available as to the LN form(s) capable of interacting with test organisms.  
880 Alternatively, approaches based on LN bioaccumulation may be pursued to determine the critical body  
881 residues (CBR) capable of inducing adverse effects in *D. magna*. Such approaches are becoming increasingly  
882 attractive thanks to new techniques allowing a detailed mapping of element distribution across *D. magna*  
883 tissues. In this case, measurements of LN accumulation on a mass base (*i.e.*, µg LN per gram tissue) should  
884 be completed by more complex approaches allowing to map and, ideally, quantifying the LN distribution  
885 across *D. magna* tissues.

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## 893 **Data, scripts, code, and supplementary information availability**

894 Data and supplementary information are available online: <https://doi.org/10.57745/NW0GF9>  
895 (*Vignati et al., 2023*)

## 896 **Conflict of interest disclosure**

897 The authors declare that they comply with the PCI rule of having no financial conflicts of interest in  
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