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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 this 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; Lürding & 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.,

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

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

Experimental results show that a statistically significant linear relationship exists between the atomic mass and the solubility of LN in the standard ISO 6341 test medium, implying that reliable ecotoxicological endpoints must explicitly account for changes in LN concentration over the test duration. On the other hand, ecotoxicological trends along the LN series appear dependent on exposure time and the selected exposure metrics, highlighting the need for the definition of shared experimental and reporting procedures across the research community on this aspect. The most critical and pressing issues include further research on LN speciation in ecotoxicological test media and, for risk assessment purposes, in natural waters along with understanding the bioavailability and ecotoxicological potential of different LN chemical species.

## 2. METHODS

### 2.1. Chemicals

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

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

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

Acute immobilization tests (48 h) were performed according to international guideline NF EN ISO 6341 (2012) at 20 ± 2 °C in the dark as per guideline specifications. Daphnids less than 24 h old were used in all tests and were obtained from parent organisms aged from two to six weeks. Organisms were not fed during 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> × 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 mg/L as CaCO<sub>3</sub>.

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

In definitive tests, minimum and maximum nominal exposure concentrations (expressed as elemental 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 plus one control (test medium without added LN) were used for each element (Table S4). Test tubes were preconditioned for 24 h with the corresponding test solutions to minimize adsorption losses on test vials.

148 The preconditioning solutions were discarded before starting the experiments. Four replicate exposures (10  
149 mL total volume) with five organisms each were used for all test concentrations. Two independent definitive  
150 tests were performed for each element. During each test, immobilization of daphnids was evaluated after  
151 24 and 48 h of exposure (Table S4). The pH of exposure solutions was measured in control tubes at t = 0 and  
152 48 h and also in selected exposure concentrations at t = 48 h. Initial pH was therefore assumed to be  
153 homogenous for all exposure concentrations. Although this assumption could have been violated in some  
154 cases, a proper discussion of ecotoxicological results remains possible thanks to the presence of linear  
155 relationships between pH and added LN (see sections 4.1 and 4.4). Potassium dichromate was used as a  
156 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  
157 6341 norm (2012).

### 158 **2.3. Analytical verification of exposure concentrations in the test medium**

159 Analytical determinations were carried out by Inductively Coupled Plasma – Mass Spectrometry (ICP-  
160 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,  
161 <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.  
162 Limits of detection (LOD) and quantification (LOQ) ranged from 0.1 to 0.8 µg/L (Table S5). LOD Analytical  
163 and procedural blanks were prepared and analyzed using the same analytical procedure as for samples. The  
164 accuracy of the whole analytical protocol was validated using international certified material (BCR-668-  
165 mussel tissues) from LGC standards. The recovery of LN ranged from 105 to 116 % (Table S5).

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

$$180 \quad (1) \quad Precision = \sqrt{abs\left(\frac{m_1 - m_2}{2}\right)}$$

181 where  $m_1$  is the first replicate measurement,  $m_2$  is the second replicate measurement and *abs* is the  
182 absolute value of the difference.

183 Analytical verifications were carried out on solutions initially amended with a concentration  
184 corresponding to the 24 h EC<sub>50</sub>, hereinafter C<sub>EC50</sub>, determined in the first round of definitive test. Tubes for  
185 the analytical verification of LN concentrations were incubated in parallel with the second definite tests and  
186 pH values were verified at t = 0 and 48 h for selected concentrations (see sections 3.3 and 3.4). For selected  
187 elements Lanthanum (La), Cerium (Ce), Gadolinium (Gd) and Ytterbium (Yb), total and filterable  
188 concentrations were measured also in solutions amended with the minimum (C<sub>min</sub>) and maximum (C<sub>MAX</sub>)  
189 test concentrations (Table S4). These additional measurements were performed in duplicate at t = 0, 24 and  
190 48 h using solutions where daphnids were initially added (except at t = 0 h). The pH was checked in all the  
191 C<sub>min</sub> and C<sub>MAX</sub> solutions immediately before filtration and/or transfer to falcon tubes for analysis. Procedures  
192 for filter cleaning and removal of debris from unfiltered solutions were as previously described.

### 193 **2.4. Speciation of lanthanides in the test medium**

194 Speciation calculations were performed using VisualMinteq (VM) version 3.1. The precipitation of  
195 carbonate and hydroxide complexes was allowed as ‘possible solid phases’. The default VM database  
196 specifies values for carbonate solubility products only for La, Neodymium (Nd), Samarium (Sm), Europium

197 (Eu), Gd, Dysprosium (Dy) and Yb. Additional calculations were therefore performed using the carbonate  
198 solubility products determined by Firsching and Mohammadzadei (1986) who provided values for all  
199 lanthanides except Ce (Table S6). The value reported by Li et al. (2004) was used for  $Ce_2(CO_3)_3$  solubility.  
200 Possible precipitation of sulfate and nitrate complexes was not considered because it requires LN  
201 concentrations well above those used in our experiments; *i.e.* above tens or hundreds of mM for  $LN_2(SO_4)_3$   
202 precipitation (Judge et al., 2023; Moldoveanu et al., 2024) and in the molar range for  $LN(NO_3)_3$  precipitation  
203 (Siekierski & Salomon, 1985).

204 Input temperature was fixed at 20 °C, medium alkalinity was specified as 0.831 meq/L  $CaCO_3$  and the  
205 Debye-Hückel equation was used for activity correction. Calculations were performed in pH range 5 to 9  
206 using increments of 0.2 units and for nominal concentrations from 10 ng/L ( $5.7-7.2 \times 10^{-11}$  M) to 50 mg/L  
207 ( $2.9-3.6 \times 10^{-4}$  M). The upper and lower limit of the molar concentration ranges refer to La and Lu,  
208 respectively. The nominal concentration range used for speciation calculation covered the range of tested  
209 and measured concentrations in the present study. For each element, theoretical solubility limits were  
210 estimated from speciation calculation by subtracting the percentage of precipitated concentrations from  
211 the corresponding nominal values.

212 Speciation was also modelled for solutions amended with concentrations corresponding to the 24 h  
213  $EC_{50}$  obtained in the first round of definitive tests for each element. In this case, the elemental concentration  
214 for VM runs was entered as the time-weighted mean (TWM) of the 24 h  $C_{EC50}$  to account for the  
215 concentrations decreases observed over the test duration (see the Results section for details). The TWM  
216 was calculated from the experimentally measured concentrations at 0 and 24 hours using the formula  
217 (OECD, 2012):

$$218 \quad (2) \quad TWM = \frac{Conc_{t(x)} - Conc_{t(x+1)}}{\ln(Conc_{t(x)}) - \ln(Conc_{t(x+1)})}$$

219 where:  $Conc_{t(x)}$  is the measured concentration at  $t = 0$ ;  $Conc_{t(x+1)}$  is the measured concentration at 24;  
220  $\ln$  is the Napierian logarithm of the measured concentrations at any time 't'.

221 Because TWM concentrations are mathematical estimates, the corresponding pH values to specify for  
222 VM calculations were estimated as follows. Changes in pH were monitored between 0 and 48 h for several  
223 concentrations of all tested elements (Table S7). Except for Praseodymium (Pr), linear relationships existed  
224 between pH at 48 h and LN nominal concentrations. These relationships were used to estimate the  
225 expected pH values for the calculated TWM concentrations and for the concentrations measured at  $t = 24$   
226 and 48 h (Table S8). In the case of Pr, the pH value of the concentration closer to the TWM  $EC_{50}$  was used.  
227 It will be shown later that pH changes were minor between 24 and 48 h.

## 228 2.5. Data analysis and statistics

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

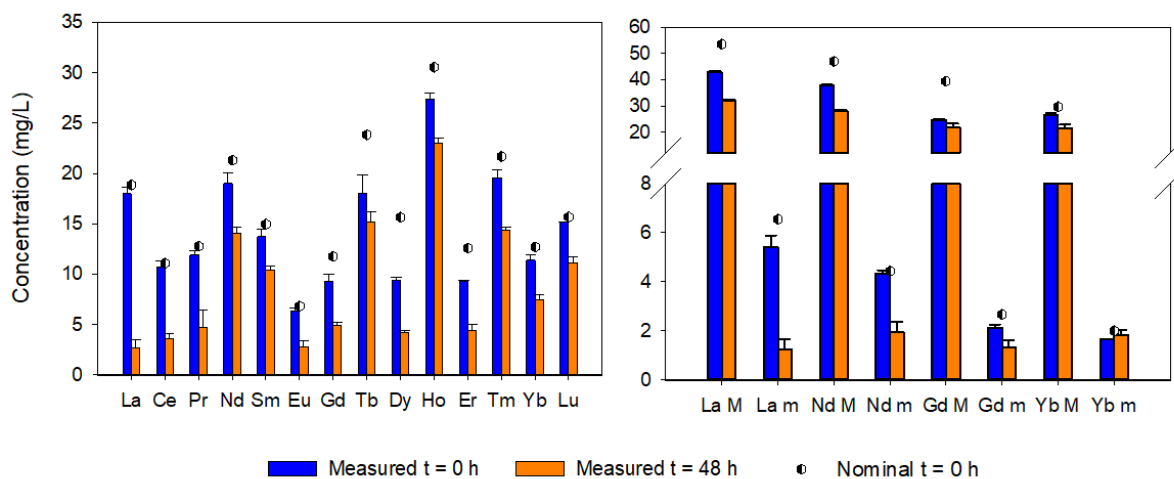
237 Effect concentrations ( $EC_x$ ) were calculated with the Excel macro REGTOX (version EV7.0.7) using the  
238 Hill model. The macro and the related documentation are available in French at:  
239 [https://www.normalesup.org/~vindimian/fr\\_index.html](https://www.normalesup.org/~vindimian/fr_index.html).

## 240 3. RESULTS

### 241 3.1. Total concentrations in the test medium

242 Measured levels of LN in control solutions were always below the corresponding analytical detection  
243 limits (Table S5). The relative standard deviation (RSD) for replicate measurements of total LNs  
244 concentrations was less than 10% at  $t = 0$  and usually within 30% at  $t = 48$ h. Total concentrations at  $t = 0$

245 were within 20% of the expected nominal values except for solutions amended with Dy at  $C_{EC50}$  where  
 246 measured values were 60% of the expected ones (Fig. 1 and Table S9). This discrepancy, possibly due to  
 247 dilution errors, does not affect the objectives of the present paper nor the data interpretation presented  
 248 below. At the end of the tests (48 h), total concentrations in solutions initially amended with  $C_{EC50}$  were  
 249 within 25% of initial values for Nd, Tb, Ho, Tm, Sm and Lu, but had decreased by more than 40% for the  
 250 other elements (Fig. 1A and Table S9). Total concentrations also decreased by more than 50% in medium  
 251 aliquots spiked with  $C_{min}$  of La, Nd, and Gd (6.55, 4.44 and 2.67 mg/L, respectively), but remained relatively  
 252 constant for Yb (2.0 mg/L). In solutions spiked with  $C_{MAX}$ , total concentrations (La = 53.5, Nd = 47.0, Gd =  
 253 39.4 and Yb = 29.6 mg/L) showed little variation over 48 h (Fig. 1B, Table S9).

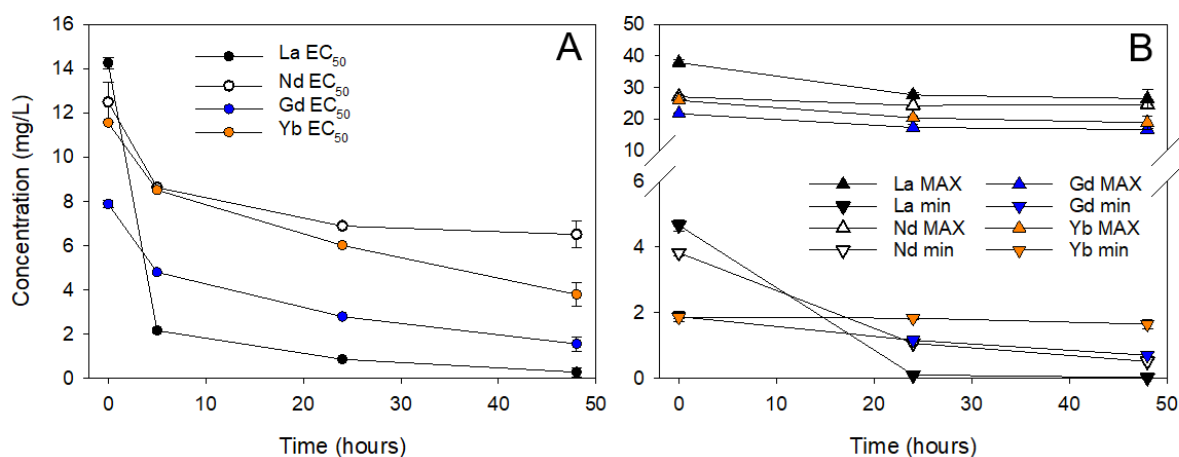


270 Figure 1. (A) Total concentrations of lanthanides (LN) measured at 0 and 48 hours in solutions initially  
 271 amended with LN concentrations equal to the corresponding  $EC_{50}$  for *D. magna*. (B) Same as panel A  
 272 for solutions initially amended with the maximum (LN M) and the minimum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

### 276 3.2. Filterable concentrations in the test medium

277 In solutions amended with  $C_{EC50}$ , the ratio between total and filterable concentration was between 80  
 278 and 120% for all elements except Nd (65%), Pr (77 %) and Sm (77%) (see table S9 for raw results and section  
 279 4.2 for discussion). In the same solutions, filterable concentrations decreased by 50–98% over the test  
 280 duration according to three temporal patterns as described in figure 2A for selected elements and in table  
 281 S9 and Figure S2 for the entire LN series. In pattern one (La and Ce), filterable concentrations decreased by  
 282 more than 80% within 5 h from the start of the incubation and fell below 5% of the initial values after 48  
 283 hours. In pattern two (Pr, Sm, Eu, Gd, Er, Dy, Yb), the concentration decrease was less rapid and reached  
 284 70–95% after 48 hours. In pattern three (Nd, Tb, Tm, Ho, Lu), the overall concentration decrease was less  
 285 than 50% with little changes between 24 and 48h. After 48 h, the differences between total and filterable  
 286 concentrations were less than 2-fold for Nd, Tb, Dy, Ho, Er, Tm, Yb, and Lu, but between 3- and 34-fold for  
 287 the other elements of the series. Small differences between total and filterable concentrations suggest  
 288 either a higher solubility or the formation of precipitates that were not retained by the 0.45  $\mu$ m filters.

289 In solutions amended with  $C_{min}$  and  $C_{MAX}$ , the ratio between total and filterable concentrations was  
 290 between 85 and 110% except for Nd at  $C_{MAX}$  (see section 4.2 for discussion). At  $C_{min}$ , the behavior of La, Nd,  
 291 Gd and Yb essentially agreed with the observations at  $C_{EC50}$ , while filterable concentrations remained within  
 292 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 (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.

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

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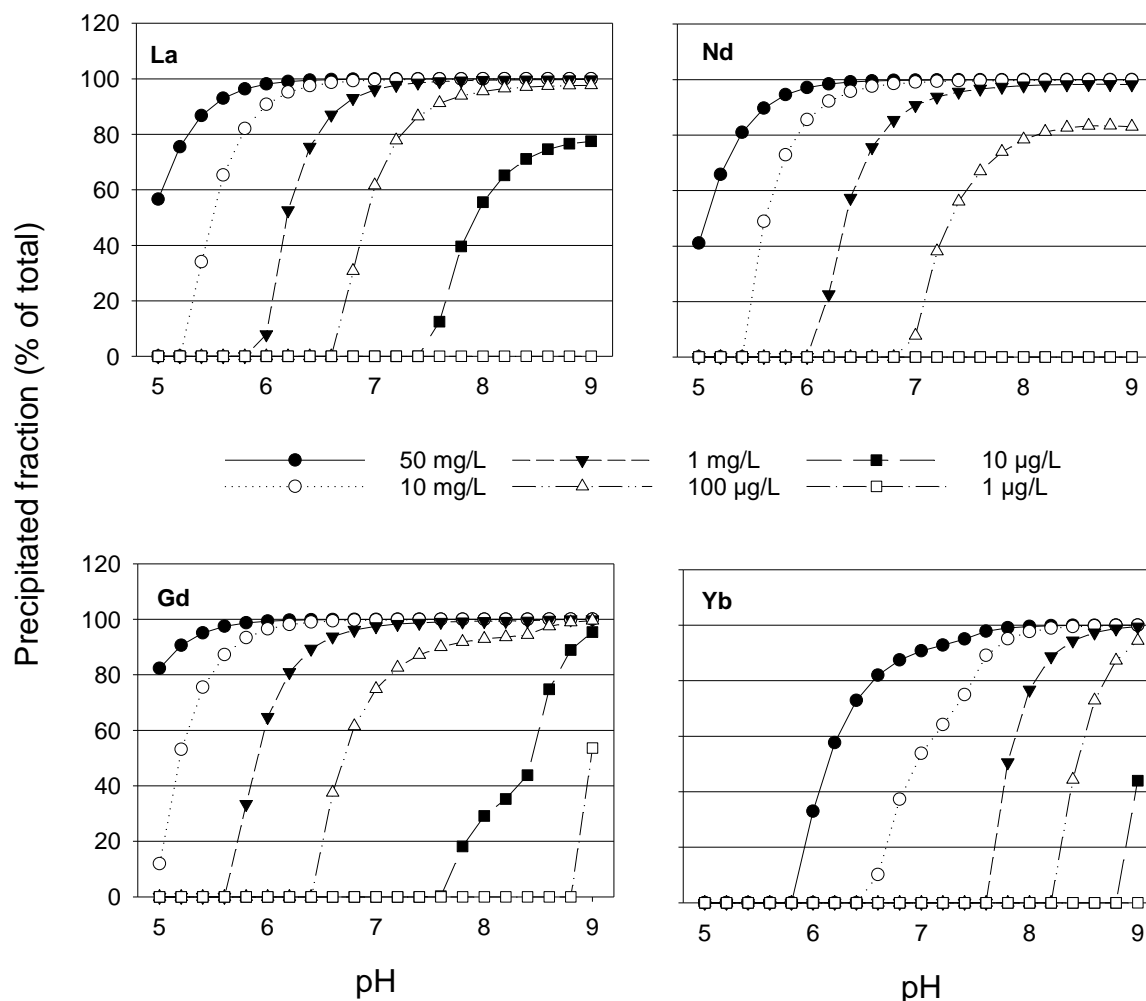
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The present section will focus on speciation results obtained using the carbonate solubility product ( $K_{\text{SP}}$ ) established by Firsching and Mohammadzadei (1986), except for La and Ce. In the case of La, using the  $K_{\text{SP}}$  of Firsching and Mohammadzadei (1986) clearly overestimated La solubility compared with experimental results. The default  $K_{\text{SP}}$  in the VisualMinteq (VM) database was therefore preferred (Table S6). An indicative  $K_{\text{SP}}$  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 Mohammadzadei (1986).

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





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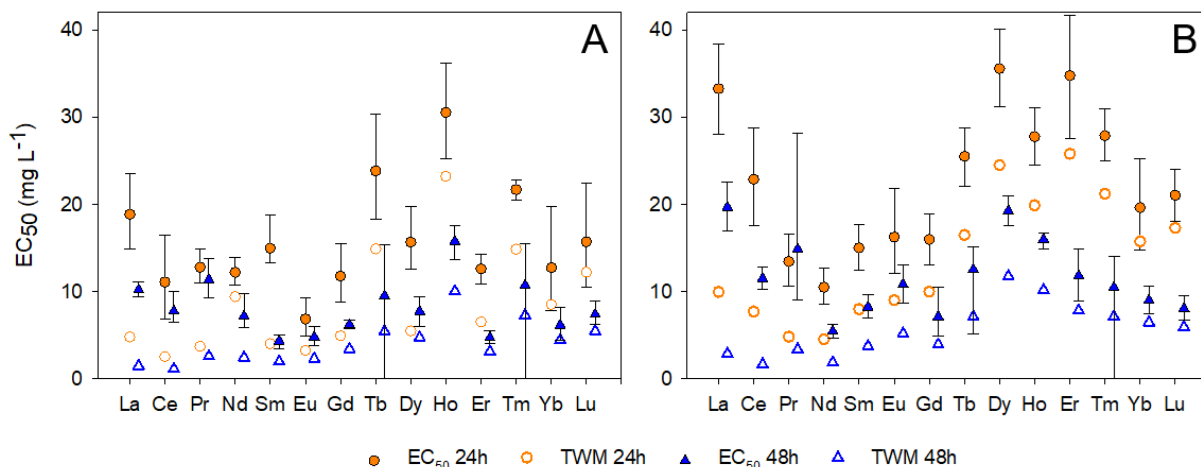
Figure 3. Theoretical solubility of La, Nd, Gd and Yb as a function of pH in ISO 6341 medium for acute toxicity testing with *Daphnia magna*. Model calculations were performed for theoretical concentration from 1 µg/L to 50 mg/L. No precipitation was predicted in theoretical solutions amended with 10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

### 316 3.4. Lanthanides ecotoxicity

317 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  
318 mg/L after 24 and 48 h of exposure, respectively (Fig. 4a; Table S10). To account for the decrease in LN  
319 concentrations over time (section 3.1), the TWM EC<sub>50</sub> were calculated using equation 2. For the 24h TWM  
320 EC<sub>50</sub>, the experimental data presented in section 3.1 (*i.e.*, the filterable concentrations at t = 0 and t = 24 h)  
321 were directly entered into equation 2. For the 48h TWM EC<sub>50</sub>, the nominal 48h EC<sub>50</sub> values were used as  
322 input for the concentrations at t = 0 h. The corresponding LN concentrations at 48h were estimated using  
323 equation 5 (see section 4.2) and then used as input for the concentrations at 48h in equation 2. Note that  
324 the experimental measurements at 48h (section 3.1) could not be used for this purpose, because they refer  
325 to medium aliquots initially spiked with concentrations equal to the 24h EC<sub>50</sub>. Results from the calculation  
326 procedure are summarized in Table S11. A detailed explanation of the calculation procedure is provided in  
327 the caption of Table S11 in the readme file accompanying the supporting information. For comparison  
328 purposes, the same procedure was also performed for the 24h TWM EC<sub>50</sub> estimated using equation 4 (Table  
329 S11).

330 The TWM 24h EC<sub>50</sub> calculated using experimental values differed from the nominal EC<sub>50</sub> by more than  
331 2-fold for La, Ce, Pr, Sm, Eu, Gd and Dy (Fig. 4A). The 24h TWM EC<sub>50</sub> estimated using equation 4 were within  
332 20 % of those obtained using experimental data for La, Pr, Eu, Tb, Ho, Tm, Yb, Lu and within a factor of two  
333 for the reaming elements except Nd (Table S11). Differences between nominal and TWM 48h EC<sub>50</sub> were

334 more than 2-fold for La, Ce, Pr, Nd, Sm and Eu (Table S11). The observations confirm that TWM correction  
 335 would avoid underestimating the ecotoxicity of La, Ce, Pr, Sm, Dy and, to a lesser extent, Eu and Gd (Fig  
 336 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  
 337 19.7 mg/L, respectively (Table S10, Fig. 4B). The TWM corrections were performed using equations 6 and 7  
 338 (Table S11), leading to results and conclusions equivalent to those discussed for the first round of tests.



339

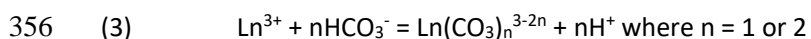
340 Figure 4. Nominal and Time-Weighted Mean (TWM) EC<sub>50</sub> values for specimen of *Daphnia magna*  
 341 exposed to lanthanides for 24 or 48 h in the first (panel A) and second (panel B) series of definitive  
 342 tests. Bars represent 95% confidence intervals for nominal EC<sub>50</sub> (estimation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

344

## 4. DISCUSSION

### 345 4.1. Temporal changes in total LN concentrations

346 In the present study, total LN concentration at t = 0 h were in good agreement with expected values,  
 347 but showed an unexpected decrease at the end of the test for several LN (Fig. 1 and Table S9). Although LN  
 348 tend to form insoluble precipitates in the test medium, we cannot exclude that the decrease in total LN  
 349 concentrations could partly originate from adsorption losses onto the tubes walls despite preconditioning  
 350 of the test tubes. However, daphnids can actively ingest LN precipitates forming into the test medium and  
 351 contribute to the observed losses, although the extent of such ingestion is element-dependent (Revel et al.,  
 352 2023). The effect of LN addition on the pH of the test medium (Table S7, S8, S9) can also contribute to the  
 353 observed temporal changes in their concentrations. The inverse relationship between added LN  
 354 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  
 355 the release of protons according to the following equilibrium (Luo & Byrne, 2004):



357 As for alkaline waters in equilibrium with atmospheric CO<sub>2</sub> (Luo & Byrne, 2004), carbonate complexes  
 358 are expected to dominate LN speciation regardless of the added concentrations. The formation and  
 359 subsequent precipitation of such complexes consume the alkalinity of the test medium (provided by the  
 360 NaHCO<sub>3</sub> component), reduce its buffering capacity, shift its pH toward more acidic values via the release of  
 361 protons (equation 3) and eventually increase LN solubility at high concentrations (see section 3.3, Tables S7  
 362 and S9). The formation of protons following LN addition to the test medium explains why pH remained  
 363 acidic in medium aliquots amended with C<sub>MAX</sub> (Table S9) and why pH and LN concentration were inversely  
 364 correlated (Tables S7 and S8). However, medium pH increased from the beginning to the end of the tests  
 365 in control exposures and in medium aliquots amended with C<sub>EC50</sub> and C<sub>min</sub> (Tables S7 and S9). These  
 366 observations can be explained by considering that test tubes were opened to the atmosphere; *i.e.*, the  
 367 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.  
 368 In control exposure, the protons added to lower the initial pH to 6.5 (section 2.2) shifted the equilibrium of

369 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  
 370 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  
 371 process is a removal of protons from the medium resulting in an increase in pH. In solutions amended C<sub>EC50</sub>  
 372 and C<sub>min</sub>, both processes (equation 3 and the equilibrium of the carbonate system) contributed to  
 373 determine the pH of the solutions at the end of the test.

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

#### 377 4.2. Temporal changes in filterable LN concentrations

378 Filtrable concentrations at t = 0 agreed well with the corresponding total ones except for Pr, Nd, and  
 379 Sm (section 3.2 and Table S9). Although filters were acid washed and preconditioned before use (section  
 380 2.3), we cannot completely exclude possible adsorption losses onto filter membranes. Possible (element  
 381 specific) filtration artifacts may require specific investigations, especially when comparing results from  
 382 different studies or combining them for regulatory purposes (see section 5).

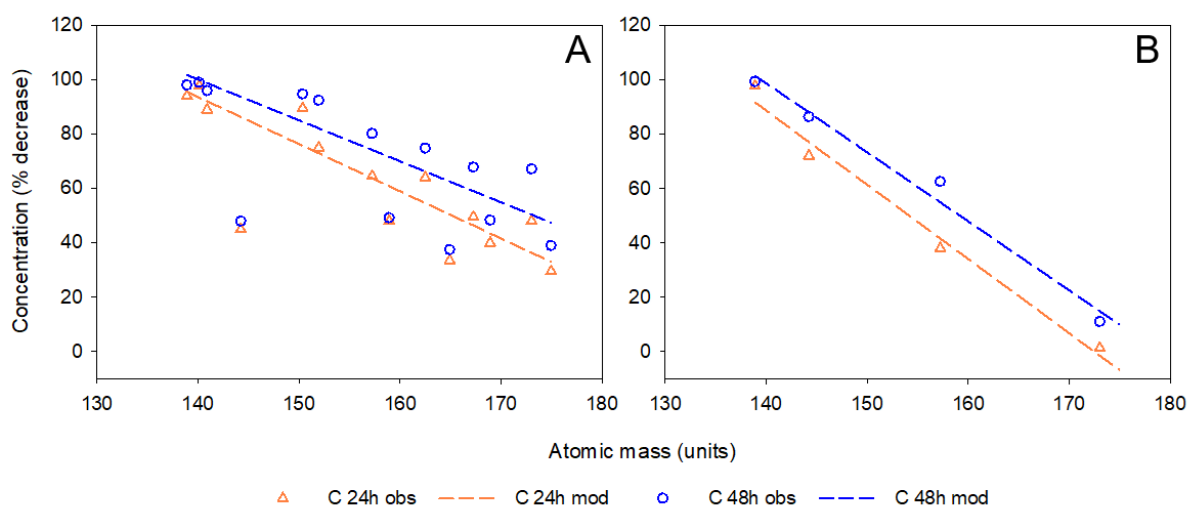
383 Assuming that our results are internally coherent (same filter type and batch, same procedures for all  
 384 elements), the decrease in filterable concentrations was always more marked than the corresponding total  
 385 ones, suggesting the presence of insoluble LN particles large enough to be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

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

393  
 394 (5)  $\%Loss_{C_{EC50\_48hMM}} = (-1.5065 \pm 0.3772) \text{ atomic mass} + (311 \pm 59) R^2 = 0.54$

395  
 396 (6)  $\%Loss_{C_{min\_24hMM}} = (-2.7296 \pm 0.2438) \text{ atomic mass} + (471 \pm 38) R^2 = 0.98$

397  
 398 (7)  $\%Loss_{C_{min\_48hMM}} = (-2.5407 \pm 0.2461) \text{ atomic mass} + (454 \pm 38) R^2 = 0.98$



399

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

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

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

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

431 We acknowledge that TWM (equation 2) is just one of the available approaches to account for  
432 decreasing exposure concentrations during ecotoxicity tests. For example, using geometric means instead  
433 of TWM concentrations would result in lower, average exposure concentrations over the test duration in  
434 the case of 24h  $EC_{50}$  for the first round of definitive tests (Table S11). More refined calculations of the TWM  
435 are also possible when exposure concentrations are verified at multiple time points during ecotoxicity tests.  
436 The data available for  $t = 0, 5$  and 24 h (Figure 2 and Table S9) provide a useful example in this sense. First,  
437 the average exposure concentrations between 0–5 h (0.21 days) and 5–24 h (0.79 days) can be calculated  
438 using a slightly modified version of formula 2 that was presented earlier:  
439

440 (8) 
$$\text{Average concentration}_{\text{period}} = \frac{\text{Conc}_{t(x)} - \text{Conc}_{t(x+1)}}{\ln(\text{Conc}_{t(x)}) - \ln(\text{Conc}_{t(x+1)})} * \text{Days}$$

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

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

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

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

#### 463 **4.3. Theoretical solubility of LN**

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

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

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

#### 500 **4.4. Comparison of different exposure metrics for lanthanides ecotoxicity**

501 The two rounds of tests gave equivalent results (i.e., overlapping 95% confidence intervals of nominal  
502  $EC_{50}$ ) 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,  
503 Er, and, at 48 h only, Sm),  $EC_{50}$  were higher in the second round of definitive tests than in the first one (Table  
504 S10, Fig. 4). Except for Eu (see below), we surmise that these differences were caused by the higher pH  
505 values of the exposure solutions during the second round of tests (Table S7).

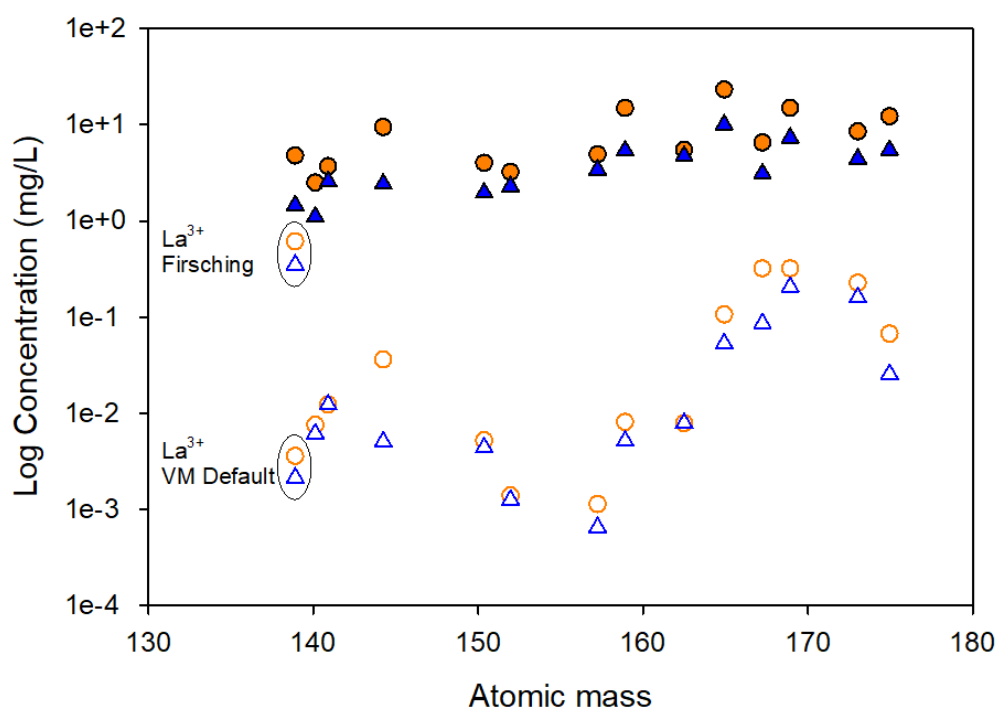
506 In both experiences, pH values were measured at  $t = 0$  h in control solutions (assumed to be  
507 representative of all exposure solutions at the beginning of the tests) and at 48 h for selected  
508 concentrations (Table S7). Measurements of pH after 24 h of exposure were not systematically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

511 Statistically significant inverse linear relationships were found between the measured pH values at 48 h  
512 and the initial LN concentration in the test medium (Table S8). These linear relationships were used to  
513 estimate the pH value in hypothetical aliquots of test media spiked with the corresponding nominal  $EC_{50}$   
514 (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  
515 Table S7) implies i) a larger and probably faster decrease in the actual exposure concentrations over the  
516 test duration (see sections 3.2 and 3.3) and hence ii) higher  $EC_{50}$  values in the second round of definitive  
517 tests. The pH explanation does not hold for Eu whose exposure solutions showed lower pH values for the  
518 higher  $EC_{50}$  in the second test series (Table S7 and S8). We acknowledge that the proposed simple  
519 explanation does not account for kinetics processes and for confounding processes linked to the presence  
520 of daphnids in the exposure solutions.

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

526 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  
527 the tens to hundreds of  $\mu\text{g/L}$  for the remaining elements. Differences between  $Ln^{3+}$  and TWM-based  $EC_{50}$   
528 were between 1 and 3 orders of magnitude at both 24 and 48h of exposure (Fig. 6). The actual  
529 ecotoxicological significance of such estimations depends on how well the constants used in the  
530 thermodynamic models are representative of the true LN speciation in the test medium. For example,  
531 estimates of free  $La^{3+}$  concentrations were three orders of magnitude higher when using the  $La_2(CO_3)_3$   
532 solubility product reported by Firshing et al. (1986) compared with the default value in the VisualMinteq  
533 database (Fig. 6). Further work is needed to experimentally measure the free  $Ln^{3+}$  concentration in exposure  
534 medium and to determine confidence intervals to  $EC_{50}$  values based on TWM or free  $Ln^{3+}$ .

535



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

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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 VisualMinteq (see section 3.3 for details). Calculations for free lanthanum (La<sup>3+</sup>) are presented using two different La<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> solubility products to invite caution when making ecotoxicological inference using modelled La<sup>3+</sup> values (see text for details).

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Considering the important quantities of LN salts added to the test medium (Table S3) and the tendency of test medium to get more acidic at high levels of added LN salts, it is important to verify the possible contribution of these two factors (pH and counterions added along with LNs) onto the survival of *D. magna*. Available reports (Chen et al., 2012) and online article abstracts (El-Deeb et al., 2011; Zhuang, 1994) suggest that effect of low pH become visible at values below 5.5 units, lower than those observed in the present study. In the case of the NO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> ions, the maximum concentration added along with LN were 76 mg/L and 46 mg/L, respectively (information on the specific counterion for each LN salt can be found in section 2.1). In the case of nitrates, LC<sub>50</sub> reported in the literature for *D. magna* are in the range 323–611 mg/L N-NO<sub>3</sub><sup>-</sup>, i.e., 1,430–2,705 mg/L NO<sub>3</sub><sup>-</sup> (Scott & Crunkilton, 2000). In the case of chlorides, LC<sub>50</sub> depends on the specific salt added to the test medium. Potassium chloride (KCl) has the lowest reported 48h LC<sub>50</sub> of 660 mg/L, which corresponds to 314 mg/L of Cl<sup>-</sup> (Mount et al., 1997). Based on this information, we estimated the toxic units (TU = exposure concentration / LC<sub>50</sub>) for each LN and the appropriate counteranion at the maximum concentrations added to the test medium. A worst-case scenario was used for the counteranion contribution; i.e., the lowest reported LC<sub>50</sub> reported in the literature was divided by the maximum added Cl<sup>-</sup> or NO<sub>3</sub><sup>-</sup> concentrations used in the present study. The TU approach allows to compare the potential contribution of LN and counteranions to the overall ecotoxic effect for the maximum added LN levels. The TU values for counteranions were below 5% of those for LN in all cases except for Eu in one of the test series (Table S13). Overall, the contribution of counteranions to the observed ecotoxicity should therefore be secondary compared with that of the added LN. Depending on the added LN concentrations, future studies may consider including experimental verification of counterion ecotoxicity.

The EC<sub>50</sub> values based on nominal values (Fig. 4 and Table S10) agree with those observed in previous studies with daphnids and other crustaceans (see the recent data compilation reported in Table S5 of Lachaux et al. (2022)). Comparison based on measured LN concentrations or on modelled free ion

565 concentrations are less straightforward because of the methodological differences among the studies  
 566 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  
 567 95% confidence intervals of the values reported by Lachaux et al. (2022) who used the same test medium  
 568 and analogous methodologies. However, our estimations for EC<sub>50</sub> based on modelled free-ion  
 569 concentrations (Fig. 6) are three orders of magnitude lower than those of Lachaux et al. (2022) because of  
 570 different assumptions in model calculations. In particular, we allowed the precipitation of Ln<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> solids  
 571 in the test medium and subtracted the precipitated concentrations from the total ones before calculating  
 572 the free-ion concentrations. Both approaches can be acceptable depending on the experimenter's choice  
 573 as to the complexation and solubility constants in the speciation model (see sections 2.x and 3.9 for further  
 574 discussion). Blinova et al. (2018) did observe a marked decrease in La, Ce, Pr, No, and Gd concentrations in  
 575 standard artificial freshwater (OCDE, 2004), but did not attempt to calculate 48h EC<sub>50</sub> based on measured  
 576 concentrations. Vukov et al. (2016) reported a 48 h EC<sub>50</sub> of 0.49 mg/L Dy for *D. pulex* based on the  
 577 concentration measured at the end of the test. This value is 10- to 20-fold lower than the 48h TWM EC<sub>50</sub> of  
 578 the present study (Table S11). However, it compares favorably with the filterable Dy concentration of 2  
 579 mg/L that remained in our exposure medium after 48 hours (Table S9). Shu et al. (2023) reported 24 h and  
 580 48 h EC<sub>50</sub> of 16.2 and 2.3 mg/L, respectively, for *D. magna* exposed to La according to OCDE guideline 202  
 581 (OCDE, 2004). These values referred to analytically measured filterable La concentrations at t = 0 (after 2h  
 582 of equilibration following the initial La spike). They are higher than our EC<sub>50</sub> based on TWM (Table S11), but  
 583 difficult to compare with nominal values considering the tendency of La concentration to rapidly decrease  
 584 upon addition to the test medium (Fig. 1). Finally, Ma et al. (2016) substituted NaHCO<sub>3</sub> with NaOH in the  
 585 test medium for *D. magna* and successfully avoided carbonate precipitation when testing the ecotoxicity of  
 586 Ce. As a result, the filterable (0.22 μm) Ce concentration showed little variation between the beginning and  
 587 the end of the test. Their 48h EC<sub>50</sub> of 1.5 mg/L (based on measured concentration) is in excellent agreement  
 588 with our 48h TWM EC<sub>50</sub> of 1.11 mg/L (1st series) and 1.64 mg/L (2nd series) (Table S11), showing that  
 589 correction for concentration decrease can improve data comparability across studies. We are not aware of  
 590 other studies providing EC<sub>50</sub> based on free ion concentrations for *D. magna* or other daphnids, apart from  
 591 the mentioned comparison with Lachaux et al. (2022).

#### 592 4.5. Trends in EC<sub>50</sub> along the lanthanides series

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

597 In the present study, nominal EC<sub>50</sub> values did not show any clear trend along the LN series after 24 or  
 598 48h of exposure (Fig. 4). On the other hand, linear relationships could be derived between the atomic mass  
 599 of lanthanides and the EC<sub>50</sub> values expressed as TWM (equations 9 to 13, see Table S14 for the statistical  
 600 significance of regression coefficients and calculation details). For 24 h exposure in the first series of  
 601 experiments, two relationships were derived; one using TWM values calculated from equation 2 with  
 602 analytically measured concentration at 0 and 24 h as inputs (equation 9) and one using TWM estimated  
 603 from equation 4 (equation 10). On average, the predicted values differed by 30–40 % from the input TWM  
 604 values, with differences larger than 50% for many elements (Table S15).

$$605 \quad (9) \quad TWM_{24h(1stSeries\_Exp)} = (0.2582 \pm 0.115) \text{ atomic mass} + (-32.0 \pm 18.1) \quad R^2 = 0.29$$

$$606 \quad (10) \quad TWM_{24h(1stSeries\_Mod)} = (0.3010 \pm 0.0898) \text{ atomic mass} + (-37.5 \pm 14.1) \quad R^2 = 0.48$$

$$607 \quad (11) \quad TWM_{48h(1stSeries)} = (0.136 \pm 0.0413) \text{ atomic mass} + (-17.4 \pm 6.5) \quad R^2 = 0.48$$

$$608 \quad (12) \quad TWM_{24h(2ndSeries)} = (0.4516 \pm 0.1013) \text{ atomic mass} + (-56.9 \pm 15.9) \quad R^2 = 0.62$$

$$609 \quad (13) \quad TWM_{48h(2ndSeries)} = (0.1720 \pm 0.0489) \text{ atomic mass} + (-21.3 \pm 7.7) \quad R^2 = 0.51$$

614 Expressing TWM EC<sub>50</sub> in terms of free ion concentrations resulted a clear M-shaped pattern at both 24  
 615 and 48 h (Fig. 6). This pattern resembles the *tetrad effect* linked to the progressive filling of *f* orbitals along



616 the lanthanides series (McLennan, 1994) and is also similar to the changes in the energy of electronic  
617 configuration from La to Lu (Schinzel et al., 2006). In particular, La, Gd and Lu have the largest differences  
618 between the energy of the highest occupied and lowest occupied molecular orbital and form the most  
619 stable complexes, which agrees with their higher ecotoxic potential (lower  $\text{Ln}^{3+}$   $\text{EC}_{50}$ ) compared with  
620 adjacent elements (Fig. 6). However, the solubility products used in the present study (Fig. S3) also showed  
621 a M-shaped trend when plotted against the atomic mass of the corresponding elements. Otherwise stated,  
622 the pattern observed in  $\text{Ln}^{3+}$   $\text{EC}_{50}$  may simply be a model artifact linked to speciation calculation.  
623 Methodological developments are clearly necessary to obtain experimental measurements of LN speciation  
624 in ecotoxicological test media and verify the true meaning of this M-shaped pattern in  $\text{Ln}^{3+}$   $\text{EC}_{50}$ .

625 Few studies examined the ecotoxic effects of the whole LN series and they reached contrasting results  
626 (Table 1). Data from Blinova et al. (2018) provide the most relevant comparison for the present study and  
627 suggest a linear decreasing trend for 48 h  $\text{LC}_{50}$  to *D. magna* based on nominal values (La > Ce > Pr > Nd  $\approx$   
628 Gd). However, a strong decrease in LN concentration was reported over the test duration and the trend  
629 based on measured concentration could not be validated based on the information provided in the paper.  
630 Similarly, 21 d chronic  $\text{LC}_{50}$  did not show any trend (La  $\approx$  Gd > Ce  $\approx$  Pr  $\approx$  Nd). Decreasing ecotoxicity along  
631 the LN series has been reported for *Hyalella azteca*, *Hydra attenuata* and *Danio rerio* based on nominal  
632 concentrations (Table 1). However, the heaviest lanthanides did not conform to the observed trends for *H.*  
633 *azteca* and *H. attenuata* (see footnotes in Table 1). These findings (*i.e.*, a decrease in ecotoxicity along the  
634 LN series) agree with the observations of the present study, but the comparisons cannot be considered fully  
635 conclusive due to the missing analytical verification of exposure concentrations in these previous works  
636 (Table 1).

637 At the opposite, increasing ecotoxicity along the LN series was reported for the charophyte alga  
638 *Nitellopsis obtusa* (Manusadzianas et al., 2020) and the bacterium *Aliivibrio fischeri* (Weltje, 2002) (Table  
639 1). In the very simple medium (0.355 M  $\text{NaNO}_3$ ) used for testing with *A. fischeri*, free  $\text{Ln}^{3+}$  were predicted  
640 to be the dominant species and showed the same trend as total dissolved concentrations (nominal values).  
641 However, total dissolved and free  $\text{Ln}^{3+}$  concentration will likely have different patterns in more complexing  
642 media, especially those containing phosphates or carbonates. Gonzalez et al. (2014) warned about  
643 establishing trends in the absence of detailed and complete information on LN speciation in the various test  
644 media used for different organisms. Until minimum analytical requirements are adopted by the whole  
645 ecotoxicological community, it will remain elusive to establish meaningful and generally valid ecotoxicity  
646 trends along the LN series.

647 In the same vein, it is instructive to examine if differences exist in the ecotoxicity of LN after grouping  
648 them into light rare earth elements (LREE – from La to Gd) and heavy rare earth elements (HREE – from Tb  
649 to Lu); an approach that is widely used by the scientific community (Rétif et al., 2024). We are specifically  
650 interested in differences between LREE and HREE at any given time (24 or 48 h) and for each exposure  
651 metric (nominal  $\text{EC}_{50}$ , TWM  $\text{EC}_{50}$  and  $\text{EC}_{50}$  based on free ion concentrations; Figs. 4 and 6). On average, LREE  
652 appear more toxic than HREE ( $p < 0.05$ ) except when  $\text{EC}_{50}$  are expressed as nominal concentrations after 48  
653 h of exposure (Fig. S4). The use of TWM increases the probability of LREE being more toxic than HREE in all  
654 cases; in agreement with the larger decrease in the exposure concentrations for many LREE (see section 4.1  
655 and 4.2; Fig. S4). Although similar results have been reported (Lin et al., 2022), these observations confirm  
656 the need to reach a consensus on which metric to use for LN exposure in order to correctly interpret their  
657 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.

§, Tm, Yb, Lu do not follow the trend

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## 5. RECOMMENDATIONS FOR FUTURE STUDIES ON LN ECOTOXICITY

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

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

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

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

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

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

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

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

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

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

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

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

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

766 Identifying the possible role of precipitates in LN ecotoxicity is related, but not equivalent, to  
767 understanding the ecotoxicity of nanoparticulate LN forms. LN-bearing nanoparticles are engineered  
768 materials of known composition and primary particle-size distribution. This contrasts with the formation of  
769 precipitates following addition of soluble LN salts into the test medium. LN precipitates have variable  
770 composition, *i.e.*, different types of precipitates can be present at the same time, and unknown particle size  
771 distribution. Their concentration may also change over the test duration depending on the stability of  
772 exposure conditions and on the solubility of the precipitates. Specific experimental approaches are required  
773 to identify the presence of precipitates and their possible contribution to LN ecotoxicity in standard  
774 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 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 metrics	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 remains 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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789 Alternatively, exposure media could be aged after spiking with soluble LN forms and the precipitates  
790 removed by filtration, ultrafiltration or centrifugation before ecotoxicity testing in analogy to approaches  
791 proposed in the ecotoxicity of engineered nanoparticles (Sørensen & Baun, 2015). Results from the present  
792 study suggest that formation of particles larger than 0.45  $\mu\text{m}$  (nominal cut-off) becomes progressively less  
793 important along the LN series (Figs. 1 and 2). However, the time to reach stable filterable concentrations  
794 varies among LN (Fig. 2). The choice of the appropriate filter cut-off also requires further information on  
795 the particle size distribution of the precipitates. Such considerations may equally apply to chronic  
796 ecotoxicity testing because theoretical calculations suggest that the solubility limits could be as low as a  
797 few tens of micrograms per liter depending on specific elements and medium pH (Fig. 3 and Table S12).  
798 These solubility limits are comparable to, or lower than, the lowest chronic ecotoxicity values reported by  
799 Shu et al. (2023) and Lüring and Tolman (2010) for La (59  $\mu\text{g/L}$  and 100  $\mu\text{g/L}$ , respectively in artificial media),  
800 but higher than Ce and Er concentrations of 1  $\mu\text{g/L}$  used by Galdiero et al. (2019) to study multigenerational  
801 effects. Comparison with the chronic values determined by Blinova et al. (2018) for La, Ce, Pr, Nd and Gd  
802 (range 300 – 490  $\mu\text{g/L}$ ) is hindered by the use of a natural lake water at pH 7.5 and containing 10.6 mg/L of  
803 dissolved organic carbon.

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

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

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

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

#### 838 *Caveat # 6. Identifying meaningful trend along the LN series*

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

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

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

852 Risk assessment is based on the comparison between the EC<sub>50</sub> derived from laboratory studies (hazard  
853 assessment) with the measured ambient concentrations in the case of site-specific investigations or  
854 probable environmental concentrations to establish environmental quality standard on a wider scale  
855 (European Commission, 2019). At the current state of knowledge, the utmost care is required to avoid both  
856 overestimation and underestimation of the potential risks arising from (increasing) concentrations of LN  
857 following anthropogenic impacts (Table 2). According to the present study, laboratory exposure metrics  
858 derived from nominal concentrations (or concentrations measured at the beginning of the tests) would  
859 clearly underestimate risk. At the opposite extreme, comparing properly derived EC<sub>50</sub> with total elemental  
860 concentrations in natural water would likely overestimate the actual risk because LN bound to  
861 environmental particles will be considered equivalent to the dissolved (bioavailable) fraction. Studies  
862 attempting risk assessment should therefore be germane as to the analytical details of the concentrations  
863 being compared. An intriguing possibility would be to base risk assessment on comparisons of time-  
864 averaged, DGT-labile concentrations measured in both laboratory media and natural freshwater by  
865 extending approaches proposed for in situ ecological risk in sediments (Gu et al., 2020; Gu et al., 2022;  
866 Paller et al., 2019). Extrapolation of laboratory-based results to real-field situations also needs more  
867 information on how the variable composition of natural aqueous matrices in terms of major ions (e.g., Ca,  
868 Mg) or organic matter content affects LN uptake by and ecotoxicity to biota. Protective effects of Ca and  
869 organic matter against LN ecotoxicity have already been reported (Aharchaou et al., 2020; El-Akl et al.,  
870 2015; Lachaux et al., 2022; Vukov et al., 2016), but generally accepted models to predict such effects still  
871 have to be developed.

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

873 Reliable exposure vs. response relationships are the foundation of hazard and risk assessment.  
874 Understanding the dynamic and complex speciation of LN is therefore a missing cornerstone in standardized  
875 ecotoxicity tests for these elements. In the absence of such information, it is not possible to choose the  
876 appropriate metric to link exposure to LN with the measured biological even in controlled laboratory  
877 conditions. Given the difficulties in obtaining stable exposure concentrations of LN in many test media, one  
878 may even question the ability of standardized methods to provide reliable results on the ecotoxicity of LN  
879 to aquatic organisms. However, similar issues would equally apply to New Approach Methodologies (NAM)  
880 such as in vitro screening, and to any test method unless they adopt test media allowing a thorough control  
881 of LN speciation. In our opinion, addressing the caveats discussed in section 5 will provide stronger basis for  
882 mutual acceptance of data across studies and help to reconcile standardized tests with the much more  
883 complex environmental reality (Vignati, 2021). Similarly, ecotoxicity trends along the LN series will remain  
884 conditional (i.e., dependent on the chosen exposure metric and exposure time, among the other variables)  
885 until precise information becomes available as to the LN form(s) capable of interacting with test organisms.  
886 Alternatively, approaches based on LN bioaccumulation may be pursued to determine the critical body  
887 residues (CBR) capable of inducing adverse effects in *D. magna*. Such approaches are becoming increasingly  
888 attractive thanks to new techniques allowing a detailed mapping of element distribution across *D. magna*  
889 tissues. In this case, measurements of LN accumulation on a mass base (i.e., µg LN per gram tissue) should  
890 be completed by more complex approaches allowing to map and, ideally, quantifying the LN distribution  
891 across *D. magna* tissues.

892

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899

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

900 Data and supplementary information are available online: <https://doi.org/10.57745/NW0GF9>  
901 (*Vignati et al., 2024*)

902

## Conflict of interest disclosure

903 The authors declare that they comply with the PCI rule of having no financial conflicts of interest in  
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910 **References**

- 911 Aharchaou, I., Beaubien, C., Campbell, P. G. C., & Fortin, C. (2020). Lanthanum and Cerium Toxicity to the  
912 Freshwater Green Alga *Chlorella fusca*: Applicability of the Biotic Ligand Model. *Environmental Toxicology  
913 and Chemistry*, 39(5), 996-1005. <https://doi.org/https://doi.org/10.1002/etc.4707>
- 914  
915 Barry, M. J., & Meehan, B. J. (2000). The acute and chronic toxicity of lanthanum to *Daphnia carinata*.  
916 *Chemosphere*, 41(10), 1669-1674. [https://doi.org/https://doi.org/10.1016/S0045-6535\(00\)00091-6](https://doi.org/https://doi.org/10.1016/S0045-6535(00)00091-6)
- 917  
918 Bau, M., & Dulski, P. (1996). Anthropogenic origin of positive gadolinium anomalies in river waters. *Earth and  
919 Planetary Science Letters*, 143(1), 245-255. [https://doi.org/https://doi.org/10.1016/0012-821X\(96\)00127-  
920 6](https://doi.org/https://doi.org/10.1016/0012-821X(96)00127-6)
- 921  
922 Blaise, C., Gagné, F., Harwood, M., Quinn, B., & Hanana, H. (2018). Ecotoxicity responses of the freshwater  
923 cnidarian *Hydra attenuata* to 11 rare earth elements. *Ecotoxicology and Environmental Safety*, 163, 486-  
924 491. <https://doi.org/https://doi.org/10.1016/j.ecoenv.2018.07.033>
- 925  
926 Blinova, I., Lukjanova, A., Muna, M., Vija, H., & Kahru, A. (2018, 2018/11/15/). Evaluation of the potential hazard of  
927 lanthanides to freshwater microcrustaceans. *Science of The Total Environment*, 642, 1100-1107.  
928 <https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.06.155>
- 929  
930 Blinova, I., Lukjanova, A., Vija, H., & Kahru, A. (2022). Long-Term Toxicity of Gadolinium to the Freshwater  
931 Crustacean *Daphnia magna*. *Bulletin of Environmental Contamination and Toxicology*, 108(5), 848-853.  
932 <https://doi.org/10.1007/s00128-021-03388-0>
- 933  
934 Blinova, I., Muna, M., Heinlaan, M., Lukjanova, A., & Kahru, A. (2020). Potential Hazard of Lanthanides and  
935 Lanthanide-Based Nanoparticles to Aquatic Ecosystems: Data Gaps, Challenges and Future Research  
936 Needs Derived from Bibliometric Analysis. *Nanomaterials*, 10(2), 328. [https://www.mdpi.com/2079-  
937 4991/10/2/328](https://www.mdpi.com/2079-4991/10/2/328)
- 938  
939 Bobba, S., Carrara, S., Huisman, J., Mathieux, F., & Pavel, C. (2020). *Critical raw materials for strategic technologies  
940 and sectors in the EU : a foresight study, 2020*. <https://doi.org/doi/10.2873/58081>
- 941  
942 Borgmann, U., Couillard, Y., Doyle, P., & Dixon, D. G. (2005). Toxicity of sixty-three metals and metalloids to  
943 *Hyalella azteca* at two levels of water hardness. *Environmental Toxicology and Chemistry*, 24(3), 641-652.  
944 <https://doi.org/10.1897/04-177r.1>
- 945  
946 Campbell, P. G. C., & Fortin, C. (2013). Biotic Ligand Model. In J.-F. Féraud & C. Blaise (Eds.), *Encyclopedia of  
947 Aquatic Ecotoxicology* (pp. 237-246). Springer Netherlands. [https://doi.org/10.1007/978-94-007-5704-  
948 2\\_23](https://doi.org/10.1007/978-94-007-5704-2_23)
- 949  
950 Chen, L., Fu, X. e., Zhang, G., Zeng, Y., & Ren, Z. (2012). Influences of Temperature, pH and Turbidity on the  
951 Behavioral Responses of *Daphnia magna* and Japanese Medaka (*Oryzias latipes*) in the Biomonitor.  
952 *Procedia Environmental Sciences*, 13, 80-86.  
953 <https://doi.org/https://doi.org/10.1016/j.proenv.2012.01.007>
- 954  
955 Deng, L., Shu, J., & Wang, Z. (2022). Toxic Effects of Rare Earth Element Ytterbium and Neodymium on *Daphnia  
956 magna*. *Asian J. Ecotoxicology*, 17(3), 445-453. <https://doi.org/10.7524/AJE.1673-5897.20210604003>

957  
958 Dushyantha, N., Batapola, N., Ilankoon, I. M. S. K., Rohitha, S., Premasiri, R., Abeysinghe, B., Ratnayake, N., &  
959 Dissanayake, K. (2020). The story of rare earth elements (REEs): Occurrences, global distribution, genesis,  
960 geology, mineralogy and global production. *Ore Geology Reviews*, 122, 103521.  
961 <https://doi.org/https://doi.org/10.1016/j.oregeorev.2020.103521>

962  
963 El-Akl, P., Smith, S., & Wilkinson, K. J. (2015). Linking the chemical speciation of cerium to its bioavailability in  
964 water for a freshwater alga. *Environmental Toxicology and Chemistry*, 34(8), 1711-1719.  
965 <https://doi.org/10.1002/etc.2991>

966  
967 El-Deeb, M., Ghazy, Habashy, M., & Mohammady, E. (2011). Effects of pH on Survival, Growth and Reproduction  
968 Rates of The Crustacean, *Daphnia Magna*. *Australian Journal of Basic and Applied Sciences*, 5(11), 1-10.  
969 <https://ajbasweb.com/old/ajbas/2011/November-2011/1-10.pdf>

970  
971 European Commission. (2019). *WFD-CIS Guidance document No. 38. Technical Guidance for implementing*  
972 *Environmental Quality Standards (EQS) for metals*.

973  
974 Firsching, F. H., & Mohammadzadei, J. (1986). Solubility products of the rare-earth carbonates. *Journal of Chemical*  
975 *& Engineering Data*, 31(1), 40-42. <https://doi.org/10.1021/je00043a013>

976  
977 Galdiero, E., Carotenuto, R., Siciliano, A., Libralato, G., Race, M., Lofrano, G., Fabbricino, M., & Guida, M. (2019,  
978 2019/11/01/). Cerium and erbium effects on *Daphnia magna* generations: A multiple endpoints  
979 approach. *Environmental Pollution*, 254, 112985.  
980 <https://doi.org/https://doi.org/10.1016/j.envpol.2019.112985>

981  
982 Gonzalez, V., Vignati, D. A. L., Leyval, C., & Giamberini, L. (2014). Environmental fate and ecotoxicity of  
983 lanthanides: Are they a uniform group beyond chemistry? *Environment International*, 71(0), 148-157.  
984 <https://doi.org/http://dx.doi.org/10.1016/j.envint.2014.06.019>

985  
986 González, V., Vignati, D. A. L., Pons, M.-N., Montarges-Pelletier, E., Bojic, C., & Giamberini, L. (2015). Lanthanide  
987 ecotoxicity: First attempt to measure environmental risk for aquatic organisms. *Environmental Pollution*,  
988 199(0), 139-147. <https://doi.org/http://dx.doi.org/10.1016/j.envpol.2015.01.020>

989  
990 Gu, Y.-G., Gao, Y.-P., Huang, H.-H., & Wu, F.-X. (2020). First attempt to assess ecotoxicological risk of fifteen rare  
991 earth elements and their mixtures in sediments with diffusive gradients in thin films. *Water Research*,  
992 185, 116254. <https://doi.org/https://doi.org/10.1016/j.watres.2020.116254>

993  
994 Gu, Y.-G., Huang, H.-H., Gong, X.-Y., Liao, X.-L., Dai, M., & Yang, Y.-F. (2022). Application of diffusive gradients in  
995 thin films to determine rare earth elements in surface sediments of Daya Bay, China: Occurrence,  
996 distribution and ecotoxicological risks. *Marine Pollution Bulletin*, 181, 113891.  
997 <https://doi.org/https://doi.org/10.1016/j.marpolbul.2022.113891>

998  
999 Hall, G. E. M., Pelchat, J. C., Pelchat, P., & Vaive, J. E. (2002). Sample collection, filtration and preservation  
1000 protocols for the determination of 'total dissolved' mercury in waters [10.1039/B110491H]. *Analyst*,  
1001 127(5), 674-680. <https://doi.org/10.1039/B110491H>

1002

- 1003 Hissler, C., Stille, P., Iffly, J. F., Guignard, C., Chabaux, F., & Pfister, L. (2016). Origin and Dynamics of Rare Earth  
1004 Elements during Flood Events in Contaminated River Basins: Sr–Nd–Pb Isotopic Evidence. *Environmental*  
1005 *Science & Technology*, 50(9), 4624-4631. <https://doi.org/10.1021/acs.est.5b03660>
- 1006  
1007 Horowitz, A. J. (2013, 2013/03/19). A Review of Selected Inorganic Surface Water Quality-Monitoring Practices:  
1008 Are We Really Measuring What We Think, and If So, Are We Doing It Right? *Environmental Science &*  
1009 *Technology*, 47(6), 2471-2486. <https://doi.org/10.1021/es304058q>
- 1010  
1011 IEA. (2021). *The Role of Critical Minerals in Clean Energy Transitions*. IEA. [https://www.iea.org/reports/the-role-of-](https://www.iea.org/reports/the-role-of-critical-minerals-in-clean-energy-transitions)  
1012 [critical-minerals-in-clean-energy-transitions](https://www.iea.org/reports/the-role-of-critical-minerals-in-clean-energy-transitions)
- 1013  
1014 IEA. (2023). *Critical Minerals Market Review 2023*. IEA. [https://www.iea.org/reports/critical-minerals-market-](https://www.iea.org/reports/critical-minerals-market-review-2023)  
1015 [review-2023](https://www.iea.org/reports/critical-minerals-market-review-2023)
- 1016  
1017 ISO. (2006). *Water quality - Guidelines for algal growth inhibition tests with poorly soluble material, volatile*  
1018 *compounds, metals and waste water (norm 14442)*.
- 1019  
1020 ISO. (2012). *Water quality — Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera,*  
1021 *Crustacea) — Acute toxicity test (norm 6341)*.
- 1022  
1023 Janot, N., Groenenberg, J. E., Otero-Fariña, A., & Pinheiro, J. P. (2021). Free Eu(III) Determination by Donnan  
1024 Membrane Technique with Electrochemical Detection: Implementation and Evaluation. *Aquatic*  
1025 *Geochemistry*. <https://doi.org/10.1007/s10498-021-09392-4>
- 1026  
1027 Judge, W. D., Ng, K. L., Moldoveanu, G. A., Koliopoulos, G., Papangelakis, V. G., & Azimi, G. (2023, 2023/04/01/).  
1028 Solubilities of heavy rare earth sulfates in water (gadolinium to lutetium) and H<sub>2</sub>SO<sub>4</sub> solutions  
1029 (dysprosium). *Hydrometallurgy*, 218, 106054.  
1030 <https://doi.org/https://doi.org/10.1016/j.hydromet.2023.106054>
- 1031  
1032 Kang, S., Guo, C., Ma, C., Mu, H., Liu, Z., & Sun, L. (2022). Assessment of the biotoxicity of lanthanides (La, Ce, Gd,  
1033 and Ho) on zebrafish (*Danio rerio*) in different water environments. *Ecotoxicology and Environmental*  
1034 *Safety*, 246, 114169. <https://doi.org/https://doi.org/10.1016/j.ecoenv.2022.114169>
- 1035  
1036 Kulaksız, S., & Bau, M. (2013). Anthropogenic dissolved and colloid/nanoparticle-bound samarium, lanthanum and  
1037 gadolinium in the Rhine River and the impending destruction of the natural rare earth element  
1038 distribution in rivers. *Earth and Planetary Science Letters*, 362(0), 43-50.  
1039 <https://doi.org/http://dx.doi.org/10.1016/j.epsl.2012.11.033>
- 1040  
1041 Lachaux, N., Catrouillet, C., Marsac, R., Poirier, L., Pain-Devin, S., Gross, E. M., & Giamberini, L. (2022). Implications  
1042 of speciation on rare earth element toxicity: A focus on organic matter influence in *Daphnia magna*  
1043 standard test. *Environmental Pollution*, 307, 119554.  
1044 <https://doi.org/https://doi.org/10.1016/j.envpol.2022.119554>
- 1045  
1046 Le Faucheur, S., Mertens, J., Van Genderen, E., Boulemant, A., Fortin, C., & Campbell, P. G. C. (2021).  
1047 Development of Quantitative Ion Character–Activity Relationship Models to Address the Lack of  
1048 Toxicological Data for Technology-Critical Elements. *Environmental Toxicology and Chemistry*, 40(4),  
1049 1139-1148. <https://doi.org/https://doi.org/10.1002/etc.4960>

1050  
1051 Leguay, S., Campbell, P. G. C., & Fortin, C. (2016). Determination of the free-ion concentration of rare earth  
1052 elements by an ion-exchange technique: implementation, evaluation and limits. *Environmental*  
1053 *Chemistry*, 13(3), 478-488. <https://doi.org/http://dx.doi.org/10.1071/EN15136>

1054  
1055 Lerat-Hardy, A., Coynel, A., Dutruch, L., Pereto, C., Bossy, C., Gil-Diaz, T., Capdeville, M.-J., Blanc, G., & Schäfer, J.  
1056 (2019). Rare Earth Element fluxes over 15 years into a major European Estuary (Garonne-Gironde, SW  
1057 France): Hospital effluents as a source of increasing gadolinium anomalies. *Science of The Total*  
1058 *Environment*, 656, 409-420. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.11.343>

1059  
1060 Li, Y. X., Zhou, X. Z., Wang, Y., & You, X. Z. (2004). Preparation of nano-sized CeO<sub>2</sub> by mechanochemical reaction of  
1061 cerium carbonate with sodium hydroxide. *Materials Letters*, 58(1), 245-249.  
1062 [https://doi.org/https://doi.org/10.1016/S0167-577X\(03\)00454-3](https://doi.org/https://doi.org/10.1016/S0167-577X(03)00454-3)

1063  
1064 Lin, Y.-T., Liu, R.-X., Audira, G., Suryanto, M. E., Roldan, M. J. M., Lee, J.-S., Ger, T.-R., & Hsiao, C.-D. (2022).  
1065 Lanthanides Toxicity in Zebrafish Embryos Are Correlated to Their Atomic Number. *Toxics*, 10(6), 336.  
1066 <https://www.mdpi.com/2305-6304/10/6/336>

1067  
1068 Louis, P., Vignati, D. A. L., Pontvianne, S., & Pons, M.-N. (2023). Spatial distribution of Rare Earth Elements in a  
1069 transnational watershed: The case of the Danube River. *Science of The Total Environment*, 164368.  
1070 <https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.164368>

1071  
1072 Luo, Y.-R., & Byrne, R. H. (2004). Carbonate complexation of yttrium and the rare earth elements in natural waters.  
1073 *Geochimica et Cosmochimica Acta*, 68(4), 691-699. [https://doi.org/http://dx.doi.org/10.1016/S0016-](https://doi.org/http://dx.doi.org/10.1016/S0016-7037(03)00495-2)  
1074 [7037\(03\)00495-2](https://doi.org/http://dx.doi.org/10.1016/S0016-7037(03)00495-2)

1075  
1076 Lürling, M., & Tolman, Y. (2010). Effects of lanthanum and lanthanum-modified clay on growth, survival and  
1077 reproduction of *Daphnia magna*. *Water Research*, 44(1), 309-319.  
1078 <https://doi.org/https://doi.org/10.1016/j.watres.2009.09.034>

1079  
1080 Ma, Y., Wang, J., Peng, C., Ding, Y., He, X., Zhang, P., Li, N., Lan, T., Wang, D., Zhang, Z., Sun, F., Liao, H., & Zhang, Z.  
1081 (2016). Toxicity of cerium and thorium on *Daphnia magna*. *Ecotoxicology and Environmental Safety*, 134,  
1082 226-232. <https://doi.org/https://doi.org/10.1016/j.ecoenv.2016.09.006>

1083  
1084 MacMillan, G. A., Clayden, M. G., Chételat, J., Richardson, M. C., Ponton, D. E., Perron, T., & Amyot, M. (2018).  
1085 Environmental Drivers of Rare Earth Element Bioaccumulation in Freshwater Zooplankton. *Environmental*  
1086 *Science & Technology*. <https://doi.org/10.1021/acs.est.8b05547>

1087  
1088 Malhotra, N., Hsu, H.-S., Liang, S.-T., Roldan, M. J. M., Lee, J.-S., Ger, T.-R., & Hsiao, C.-D. (2020). An Updated  
1089 Review of Toxicity Effect of the Rare Earth Elements (REEs) on Aquatic Organisms. *Animals*, 10(9), 1663.  
1090 <https://www.mdpi.com/2076-2615/10/9/1663>

1091  
1092 Manusadzianas, L., Vitkus, R., Gylyté, B., Cimmperman, R., Dziugelis, M., Karitonas, R., & Sadauskas, K. (2020).  
1093 Ecotoxicity responses of the macrophyte algae *Nitellopsis obtusa* and freshwater crustacean  
1094 *Thamnocephalus platyurus* to 12 rare earth elements. *Sustainability* 12(17), 7130.

1095

- 1096 Marsac, R., Catrouillet, C., Davranche, M., Bouhnik-Le Coz, M., Briant, N., Janot, N., Otero-Fariña, A., Groenenberg,  
1097 J. E., Pédrot, M., & Dia, A. (2021). Modeling rare earth elements binding to humic acids with model VII.  
1098 *Chemical Geology*, 567, 120099. <https://doi.org/https://doi.org/10.1016/j.chemgeo.2021.120099>
- 1099  
1100 Martin, L. A., Vignati, D. A. L., & Hissler, C. (2021). Contrasting distribution of REE and yttrium among particulate,  
1101 colloidal and dissolved fractions during low and high flows in peri-urban and agricultural river systems.  
1102 *Science of The Total Environment*, 790, 148207.  
1103 <https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.148207>
- 1104  
1105 McLennan, S. M. (1994). Rare earth element geochemistry and the “tetrad” effect. *Geochimica et Cosmochimica*  
1106 *Acta*, 58(9), 2025-2033. [https://doi.org/https://doi.org/10.1016/0016-7037\(94\)90282-8](https://doi.org/https://doi.org/10.1016/0016-7037(94)90282-8)
- 1107  
1108 Moldoveanu, G. A., Koliopoulos, G., Judge, W. D., Ng, K. L., Azimi, G., & Papangelakis, V. G. (2024, 2024/01/01/).  
1109 Solubilities of individual light rare earth sulfates (lanthanum to europium) in water and H2SO4 solutions  
1110 (neodymium sulfate). *Hydrometallurgy*, 223, 106194.  
1111 <https://doi.org/https://doi.org/10.1016/j.hydromet.2023.106194>
- 1112  
1113 Mount, D. R., Gulley, D. D., Hockett, J. R., Garrison, T. D., & Evans, J. M. (1997). Statistical models to predict the  
1114 toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead  
1115 minnows). *Environmental Toxicology and Chemistry*, 16(10), 2009-2019.  
1116 <https://doi.org/10.1002/etc.5620161005>
- 1117  
1118 Nduwayezu, I., Mostafavirad, F., Hadioui, M., & Wilkinson, K. J. (2016). Speciation of a lanthanide (Sm) using an ion  
1119 exchange resin [10.1039/C6AY02018F]. *Analytical Methods*, 8(37), 6774-6781.  
1120 <https://doi.org/10.1039/C6AY02018F>
- 1121  
1122 OCDE. (2004). *Test No. 202: Daphnia sp. Acute Immobilisation Test*.  
1123 <https://doi.org/doi:https://doi.org/10.1787/9789264069947-en>
- 1124  
1125 OCDE. (2012). *Test No. 211: Daphnia magna Reproduction Test*.  
1126 <https://doi.org/doi:https://doi.org/10.1787/9789264185203-en>
- 1127  
1128 Paller, M. H., Harmon, S. M., Knox, A. S., Kuhne, W. W., & Halverson, N. V. (2019). Assessing effects of dissolved  
1129 organic carbon and water hardness on metal toxicity to *Ceriodaphnia dubia* using diffusive gradients in  
1130 thin films (DGT). *Science of The Total Environment*, 697, 134107.  
1131 <https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.134107>
- 1132  
1133 Pell, R., Tijsseling, L., Goodenough, K., Wall, F., Dehaine, Q., Grant, A., Deak, D., Yan, X., & Whattoff, P. (2021).  
1134 Towards sustainable extraction of technology materials through integrated approaches. *Nature Reviews*  
1135 *Earth & Environment*, 2(10), 665-679. <https://doi.org/10.1038/s43017-021-00211-6>
- 1136  
1137 Pinheiro, J. P., & Rotureau, E. (2023). Electroanalytical Trace Metal Cations Quantification and Speciation in  
1138 Freshwaters: Historical Overview, Critical Review of the Last Five Years and Road Map for Developing  
1139 Dynamic Speciation Field Measurements. *Molecules (Basel, Switzerland)*, 28(6), 2831.  
1140 <https://www.mdpi.com/1420-3049/28/6/2831>
- 1141

1142 Pourret, O., Davranche, M., Gruau, G., & Dia, A. (2007, 2007/08/30/). Rare earth elements complexation with  
1143 humic acid. *Chemical Geology*, 243(1), 128-141.  
1144 <https://doi.org/https://doi.org/10.1016/j.chemgeo.2007.05.018>

1145  
1146 Rétif, J., Briant, N., Zalouk-Vergnoux, A., Le Monier, P., Sireau, T., & Poirier, L. (2024, 2024/04/20/). Distribution of  
1147 rare earth elements and assessment of anthropogenic gadolinium in estuarine habitats: The case of Loire  
1148 and Seine estuaries in France. *Science of The Total Environment*, 922, 171385.  
1149 <https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.171385>

1150  
1151 Revel, M., Medjoubi, K., Charles, S., Hursthouse, A., & Heise, S. (2024, 2024/04/01/). Mechanistic analysis of the  
1152 sub chronic toxicity of La and Gd in *Daphnia magna* based on TKTD modelling and synchrotron X-ray  
1153 fluorescence imaging. *Chemosphere*, 353, 141509.  
1154 <https://doi.org/https://doi.org/10.1016/j.chemosphere.2024.141509>

1155  
1156 Revel, M., Medjoubi, K., Rivard, C., Vantelon, D., Hursthouse, A., & Heise, S. (2023). Determination of the  
1157 distribution of rare earth elements La and Gd in *Daphnia magna* via micro and nano-SXRF imaging  
1158 [10.1039/D3EM00133D]. *Environmental Science: Processes & Impacts*.  
1159 <https://doi.org/10.1039/D3EM00133D>

1160  
1161 Rodríguez de San Miguel, E., González-Albarrán, R., & Rojas-Challa, Y. (2023). Conditional Equilibrium Constants  
1162 Reviewed. *Critical Reviews in Analytical Chemistry*, 53(4), 775-797.  
1163 <https://doi.org/10.1080/10408347.2021.1977609>

1164  
1165 Romero-Freire, A., Joonas, E., Muna, M., Cossu-Leguille, C., Vignati, D. A. L., & Giamberini, L. (2019). Assessment of  
1166 the toxic effects of mixtures of three lanthanides (Ce, Gd, Lu) to aquatic biota. *Science of The Total  
1167 Environment*, 661, 276-284. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.01.155>

1168  
1169 Roth, N., & Ciffroy, P. (2016). A critical review of frameworks used for evaluating reliability and relevance of  
1170 (eco)toxicity data: Perspectives for an integrated eco-human decision-making framework. *Environment  
1171 International*, 95, 16-29. <https://doi.org/http://dx.doi.org/10.1016/j.envint.2016.07.011>

1172  
1173 Schinzel, S., Bindl, M., Visseaux, M., & Chermette, H. (2006). Structural and Electronic Analysis of Lanthanide  
1174 Complexes: Reactivity May Not Necessarily Be Independent of the Identity of the Lanthanide Atom – A  
1175 DFT Study. *The Journal of Physical Chemistry A*, 110(39), 11324-11331.  
1176 <https://doi.org/10.1021/jp060876d>

1177  
1178 Scott, G., & Crunkilton, R. L. (2000). Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales  
1179 promelas*), *Ceriodaphnia dubia*, and *Daphnia magna*. *Environmental Toxicology and Chemistry*, 19(12),  
1180 2918-2922. <https://doi.org/10.1002/etc.5620191211>

1181  
1182 Shu, J., Chen, W., Wang, Z., Jiang, D., Xiao, Y., & Li, Z. (2023). Two-phase effects of environmentally relevant  
1183 lanthanum on life-history traits of *Daphnia magna* and transgenerational bioenergetic profiles:  
1184 Implications for nutritional and environmental consequences. *Aquatic Toxicology*, 255, 106380.  
1185 <https://doi.org/https://doi.org/10.1016/j.aquatox.2022.106380>

1186  
1187 Siekierski, S., & Salomon, M. (1985, 1985/07/01). Thermodynamics of saturated lanthanide nitrate-water systems.  
1188 *Journal of Solution Chemistry*, 14(7), 473-484. <https://doi.org/10.1007/BF00646979>

1189



- 1190 Sørensen, S. N., & Baun, A. (2015). Controlling silver nanoparticle exposure in algal toxicity testing – A matter of  
1191 timing. *Nanotoxicology*, 9(2), 201-209. <https://doi.org/10.3109/17435390.2014.913728>
- 1192  
1193 Susanti, Y., Pratiwi, H., Sulistijowati, S., & Twenty, L. (2014). M estimation, S estimation, and MM estimation in  
1194 robust regression. *International Journal of Pure and Applied Mathematics*, 91, 349-360.  
1195 <https://doi.org/10.12732/ijpam.v91i3.7>
- 1196  
1197 Tesfa, M., Duval, J. F. L., Marsac, R., Dia, A., & Pinheiro, J.-P. (2022). Absolute and Relative Positioning of Natural  
1198 Organic Matter Acid–Base Potentiometric Titration Curves: Implications for the Evaluation of the Density  
1199 of Charged Reactive Sites. *Environmental Science & Technology*, 56(14), 10494-10503.  
1200 <https://doi.org/10.1021/acs.est.2c00828>
- 1201  
1202 Town, R. M., van Leeuwen, H. P., & Duval, J. F. L. (2019). Rigorous Physicochemical Framework for Metal Ion  
1203 Binding by Aqueous Nanoparticulate Humic Substances: Implications for Speciation Modeling by the  
1204 NICA-Donnan and WHAM Codes. *Environmental Science & Technology*, 53(15), 8516-8532.  
1205 <https://doi.org/10.1021/acs.est.9b00624>
- 1206  
1207 Venables, W. N., & Ripley, B. D. (1999). *Modern Applied Statistics with S-plus, 3rd ed.* Springer-Verlag.
- 1208  
1209 Vignati, D., Martin, L., Poirier, L., Zalouk-Vergnoux, A., Fouque, C., Bojic, C., Hissler, C., & Cossu-Leguille, C. (2023).  
1210 *Supporting information for: Behavior and speciation of lanthanides during standardized ecotoxicity tests*  
1211 *with Daphnia magna: implications and recommendations for hazard and risk assessment* Version V1)  
1212 [Dataset Image Other]. Recherche Data Gouv. <https://doi.org/doi:10.57745/NW0GF9>
- 1213  
1214 Vignati, D. A. L. (2021). Reconciling standard procedures and environmental realism in ecotoxicology: conceptual  
1215 and practical challenges. Habilitation à Diriger des Recherches. Université de Lorraine.  
1216 <https://hal.science/tel-03484547/>
- 1217  
1218 Vignati, D. A. L., Loizeau, J.-L., Rossé, P., & Dominik, J. (2006). Comparative performance of membrane filters vs.  
1219 high-surface area filtration cartridges for the determination of element concentrations in freshwater  
1220 systems. *Water Research*, 40, 917-924. <https://doi.org/https://doi.org/10.1016/j.watres.2005.12.024>
- 1221  
1222 Vukov, O., Smith, D. S., & McGeer, J. C. (2016). Acute dysprosium toxicity to *Daphnia pulex* and *Hyaella azteca* and  
1223 development of the biotic ligand approach. *Aquatic Toxicology*, 170, 142-151.  
1224 <https://doi.org/https://doi.org/10.1016/j.aquatox.2015.10.016>
- 1225  
1226 Weltje, L. (2002). *Bioavailability of lanthanides to freshwater organisms. Speciation, accumulation and toxicity.*  
1227 [Delft University of Technology]. Delft (The Netherlands).
- 1228  
1229 Wen, Y., Liu, P., Wang, Q., Zhao, S., & Tang, Y. (2024, 2024/04/25). Organic Ligand-Mediated Dissolution and  
1230 Fractionation of Rare-Earth Elements (REEs) from Carbonate and Phosphate Minerals. *ACS Earth and*  
1231 *Space Chemistry*. <https://doi.org/10.1021/acsearthspacechem.4c00009>
- 1232  
1233 Weyman, G. S., Rufli, H., Weltje, L., Salinas, E. R., & Hamitou, M. (2012). Aquatic toxicity tests with substances that  
1234 are poorly soluble in water and consequences for environmental risk assessment. *Environmental*  
1235 *Toxicology and Chemistry*, 31(7), 1662-1669. <https://doi.org/https://doi.org/10.1002/etc.1856>
- 1236

- 1237 Zhuang, D. (1994). The effects of low pH value on survival, growth and reproduction of *Daphnia magna* (Crustacea:  
1238 Cladocera). *Zhongguo Huanjing Kexue*, 14(2), 107-111. <http://europepmc.org/abstract/CBA/273135>
- 1239
- 1240