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1 Ecotoxicity of lanthanides to *Daphnia magna*: 2 insights from elemental behavior and 3 speciation in a standardized test medium

4
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16 17 18 **ABSTRACT**

19 Lanthanides (LNs) are a group of 15 elements with steadily increasing economical importance
20 due to their multiple uses in technologies essential for sustainable ecological, digital and
21 energetic transitions. Although knowledge on LN ecotoxicology has greatly improved over the
22 last decade, uncertainty persists with regard to their actual hazard and risk in freshwater
23 environments. In particular, only limited information is available on i) the actual relationships
24 between LN speciation vs. ecotoxicological responses in standardized laboratory tests and ii)
25 the existence of regular and predictable patterns in LN ecotoxicity (expressed as *e.g.*, EC₅₀)
26 along the LN series. The present paper provides the first report on the ecotoxicity of all
27 lanthanides (except Pm) for the freshwater crustacean *Daphnia magna* along with an
28 unprecedented level of detail on LN speciation in the exposure medium.

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

41
42 **Keywords:** aquatic ecotoxicity, technologically critical elements, exposure metrics, hazard assessment,
43 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.,

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

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

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

115 2. METHODS

116 2.1. Chemicals

117 All lanthanides salts (chloride or nitrates) were purchased from Sigma-Aldrich and had a minimal purity
118 of 99% (Table S1). Chemicals for preparing the test medium for acute tests with *Daphnia magna* were also
119 purchased from Sigma-Aldrich. Concentrated hydrochloric acid (HCl Suprapur 30%) and concentrated nitric
120 acid (HNO₃ 67%, Prolabo) were from VWR. Ultrapure water for washing/preconditioning of plasticware and
121 preparing test medium and LN stock/working solutions was obtained from a Veolia Purelab® Chorus
122 apparatus.

123 2.2. Ecotoxicity testing with *Daphnia magna*

124 Parthenogenetic specimen of *D. magna* were reared in Lefebvre-Czarda (LC)-Volvic medium (Table S2)
125 at 20 °C and under a 16h light : 8 h dark photoperiod (light intensity 35 μmol/s/m²). The growth medium
126 was changed three times a week and daphnids were fed an algal mixture of *Chlorella vulgaris*,
127 *Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus* (2.5, 5, and 2.5 × 10⁶ cells/day/daphnia,
128 respectively) after each medium renewal.

129 Acute immobilization tests (48 h) were performed according to international guideline NF EN ISO 6341
130 (2012) at 20 ± 2 °C in the dark as per guideline specifications. Daphnids less than 24 h old were used in all
131 tests and were obtained from parent organisms aged from two to six weeks. Organisms were not fed during
132 the tests. The composition of the standardized ISO 6341 medium was: CaCl₂ × 2H₂O (294 mg/L), MgSO₄ ×
133 7H₂O (123 mg/L), NaHCO₃ (64.7 mg/L), and KCl (5.75 mg/L). Calculated medium hardness was approx 250
134 mg/L as CaCO₃.

135 In range-finding tests (data not shown), daphnids were exposed to a series of seven dilutions of all LN
136 (except the radioactive Pm) at concentrations ranging from 0.05 to 45 mg/L of the corresponding salt (see
137 Table S3 for details and the equivalent elemental concentrations). This range had to be extended up to
138 1,750 mg/L of LN salts to identify the appropriate test concentrations for the definitive tests with La, Nd,
139 Sm, Tb, Dy, Ho, Er and Tm (Table S3). Preliminary tests were performed at an initial pH of 7.8 units, as
140 recommended by the ISO 6341 norm, and of 6.5 units to reduce the visible precipitation of lanthanides
141 carbonate species without compromising organisms' survival. In the preliminary tests performed at the
142 initial pH value of 7.8, observed mortality did not span the 0–100% range required for optimal EC_x
143 estimations. Definitive tests were therefore carried out only at an initial pH of 6.5 units.

144 In definitive tests, minimum and maximum nominal exposure concentrations (expressed as elemental
145 values) were 0.7 to 15 mg/L and 20 to 70 mg L⁻¹, respectively. A dilution series of nine or ten concentrations
146 plus one control (test medium without added LN) were used for each element (Table S4). Test tubes were
147 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 EC₅₀ 24 h values between 0.6 and 2.1 mg/L of K₂Cr₂O₇ 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: ¹³⁹La, ¹⁴⁰Ce, ¹⁴¹Pr, ¹⁴⁶Nd, ¹⁴⁷Sm,
161 ¹⁵³Eu, ¹⁵⁷Gd, ¹⁵⁹Tb, ¹⁶³Dy, ¹⁶⁵Ho, ¹⁶⁶Er, ¹⁶⁹Tm, ¹⁷³Yb, ¹⁷⁵Lu. Rhenium (¹⁸⁷Re) 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 HNO₃ 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 EC₅₀, hereinafter C_{EC50}, 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_{min}) and maximum (C_{MAX})
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_{min} and C_{MAX} 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).

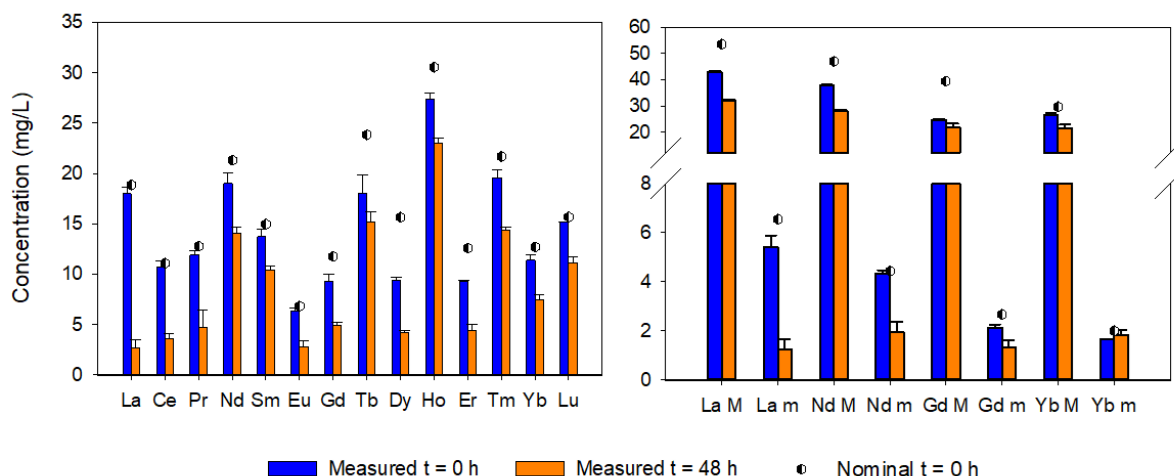
237 Effect concentrations (EC_x) were calculated with the Excel macro REGTOX (version EV7.0.7) using the
238 Hill model. The macro and the related documentation are available in French at:
239 https://www.normalesup.org/~vindimian/fr_index.html.

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).

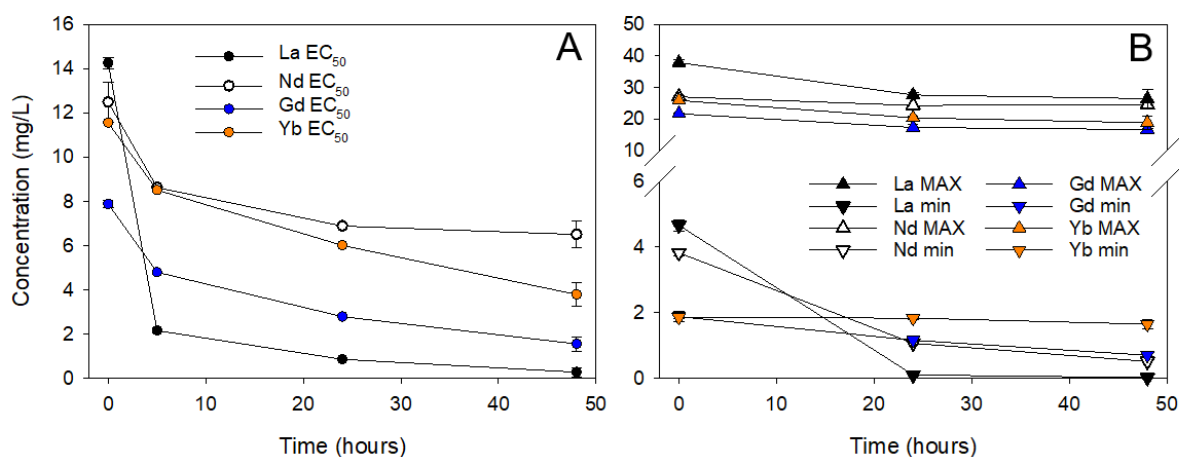


270 Figure 1. (A) Total concentrations of lanthanides (LN) measured at 0 and 48 hours in solutions initially
 271 amended with LN concentrations equal to the corresponding EC_{50} for *D. magna*. (B) Same as panel A
 272 for solutions initially amended with the maximum (LN M) and the minimum (LN m) concentrations 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 $t = 0h$ 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 μm 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 μm) of La, Gd, Nd, Yb for solutions initially amended with lanthanides concentrations equal to the corresponding EC₅₀ 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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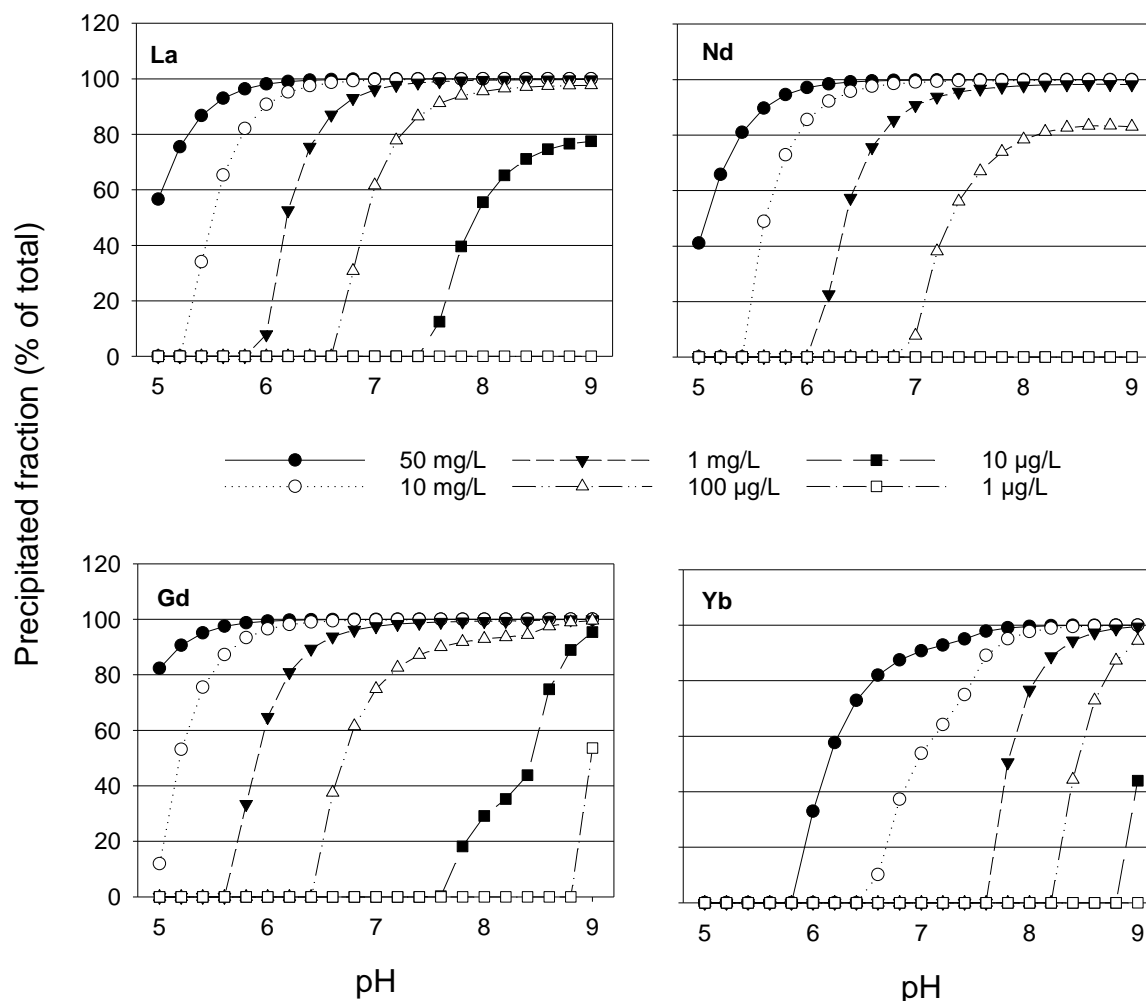
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The present section will focus on speciation results obtained using the carbonate solubility product (K_{SP}) established by Firsching and Mohammadzadei (1986), except for La and Ce. In the case of La, using the K_{SP} of Firsching and Mohammadzadei (1986) clearly overestimated La solubility compared with experimental results. The default K_{SP} in the VisualMinteq (VM) database was therefore preferred (Table S6). An indicative K_{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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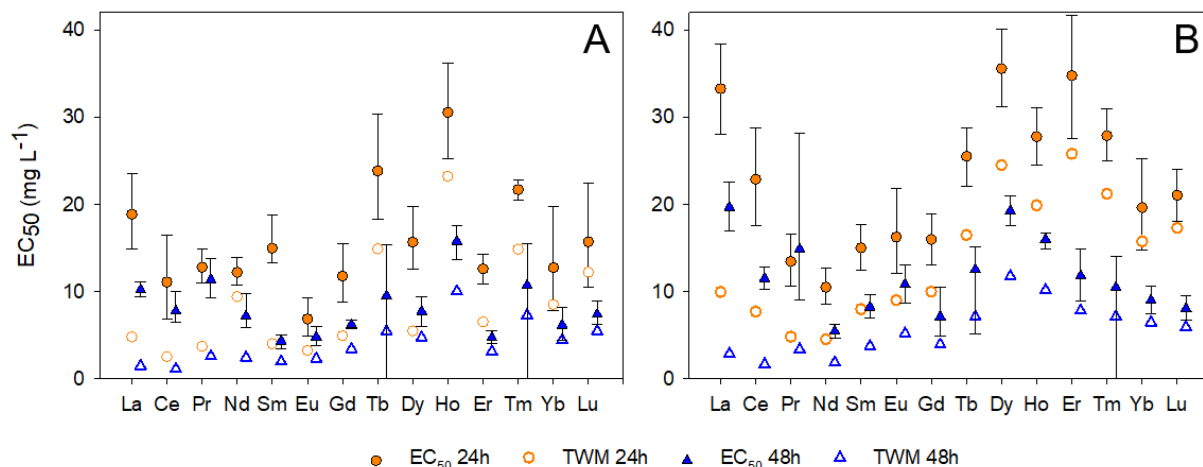
Figure 3. Theoretical solubility of La, Nd, Gd and Yb as a function of pH in ISO 6341 medium for acute toxicity testing with *Daphnia magna*. Model calculations were performed for theoretical concentration from 1 µg/L to 50 mg/L. No precipitation was predicted in theoretical solutions amended with 10 and 100 ng/L (plots not shown).

316 3.4. Lanthanides ecotoxicity

317 In the first round of definitive test, nominal EC₅₀ 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 EC₅₀ were calculated using equation 2. For the 24h TWM
320 EC₅₀, 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 EC₅₀, the nominal 48h EC₅₀ 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 EC₅₀. 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 EC₅₀ estimated using equation 4 (Table
329 S11).

330 The TWM 24h EC₅₀ calculated using experimental values differed from the nominal EC₅₀ by more than
331 2-fold for La, Ce, Pr, Sm, Eu, Gd and Dy (Fig. 4A). The 24h TWM EC₅₀ 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 EC₅₀ 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 EC₅₀ 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

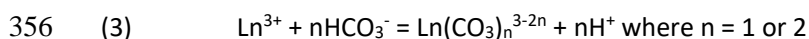
340 Figure 4. Nominal and Time-Weighted Mean (TWM) EC₅₀ values for specimen of *Daphnia magna*
 341 exposed to lanthanides for 24 or 48 h in the first (panel A) and second (panel B) series of definitive
 342 tests. Bars represent 95% confidence intervals for nominal EC₅₀ (estimation not possible for 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 Ln₂(CO₃)₃ 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 CO₂ (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 NaHCO₃ 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_{MAX} (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_{EC50} and C_{min} (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 (H₂CO₃ – HCO₃⁻ – CO₃²⁻) of the medium could equilibrate with atmospheric CO₂ 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 (H₂CO₃). The newly formed H₂CO₃ will dissociate
 370 into H₂O and CO₂, with CO₂ 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_{EC50}
 372 and C_{min}, 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).

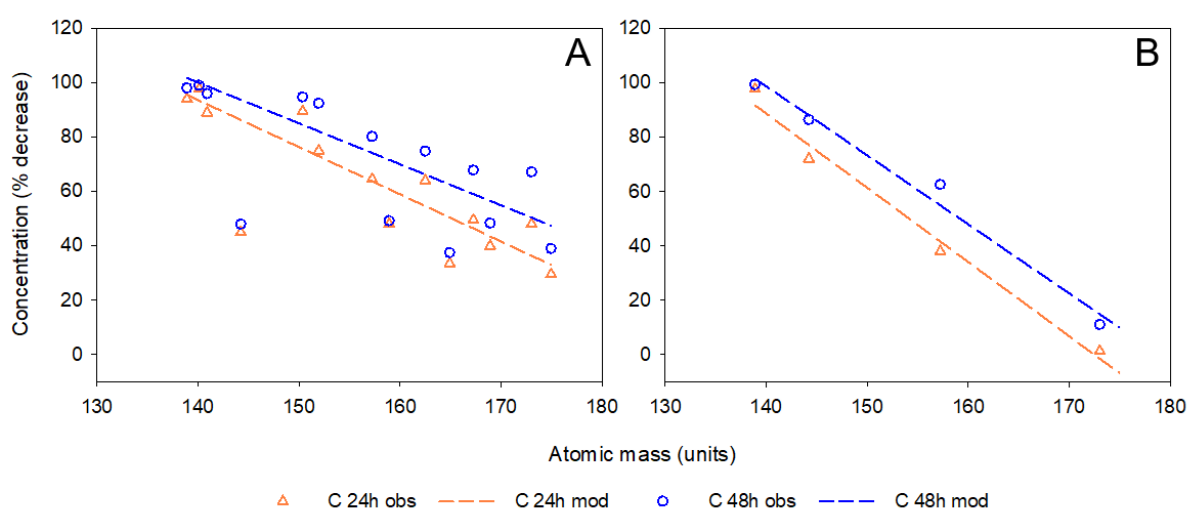
383 Assuming that our results are internally coherent (same filter type and batch, same procedures for all
 384 elements), the decrease in filterable concentrations was always more marked than the corresponding total
 385 ones, suggesting the presence of insoluble LN particles large enough to be retained 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_{EC50} and C_{min} (Fig. 5). Robust MM regressions showed a better fit compared with
 389 conventional partial least-squares regressions for the C_{EC50} scatterplots, while the performance of the two
 390 regressions was equivalent for C_{min} (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_{EC50}
 402 and (B) to the minimum tested concentration C_{min}. 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 μm 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)
$$\text{Average concentration}_{\text{period}} = \frac{\text{Conc}_{t(x)} - \text{Conc}_{t(x+1)}}{\text{Ln}(\text{Conc}_{t(x)}) - \text{Ln}(\text{Conc}_{t(x+1)})} * \text{Days}$$

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_{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).

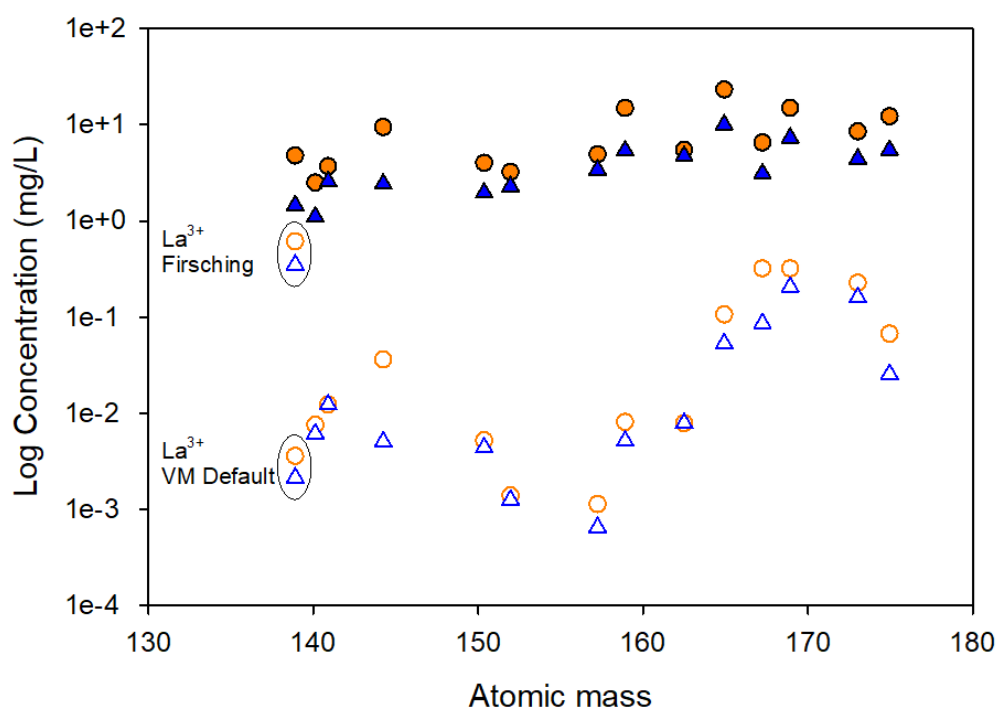
506 In both experiences, pH values were measured at $t = 0$ h in control solutions (assumed to be
507 representative of all exposure solutions at the beginning of the tests) and at 48 h for selected
508 concentrations (Table S7). Measurements of pH after 24 h of exposure were not systematically performed
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) ○ Ln³⁺ 24h (mg/L) △ Ln³⁺ 48h (mg/L)

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Figure 6. Comparison between 24h and 48h Time Weighed Mean (TWM) EC₅₀ expressed as total filterable concentrations and free Ln³⁺ concentrations. Free Ln³⁺ concentrations were estimated using VisualMinteq (see section 3.3 for details). Calculations for free lanthanum (La³⁺) are presented using two different La₂(CO₃)₃ solubility products to invite caution when making ecotoxicological inference using modelled La³⁺ 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 NO₃⁻ and Cl⁻ 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, LC₅₀ reported in the literature for *D. magna* are in the range 323–611 mg/L N-NO₃⁻, i.e., 1,430–2,705 mg/L NO₃⁻ (Scott & Crunkilton, 2000). In the case of chlorides, LC₅₀ depends on the specific salt added to the test medium. Potassium chloride (KCl) has the lowest reported 48h LC₅₀ of 660 mg/L, which corresponds to 314 mg/L of Cl⁻ (Mount et al., 1997). Based on this information, we estimated the toxic units (TU = exposure concentration / LC₅₀) 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 LC₅₀ reported in the literature was divided by the maximum added Cl⁻ or NO₃⁻ 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 EC₅₀ 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 EC₅₀ 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 EC₅₀ 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 Ln₂(CO₃)₃ 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 EC₅₀ based on measured
576 concentrations. Vukov et al. (2016) reported a 48 h EC₅₀ 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 EC₅₀ 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 EC₅₀ 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 EC₅₀ 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 NaHCO₃ 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 EC₅₀ of 1.5 mg/L (based on measured concentration) is in excellent agreement
588 with our 48h TWM EC₅₀ 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 EC₅₀ 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 EC₅₀ along the lanthanides series

593 Ecotoxicologists are constantly seeking for the presence of regular, predictable variations in EC₅₀ 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 EC₅₀ 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 EC₅₀ 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 EC₅₀ 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 Ln^{3+} 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 Ln^{3+} 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 Ln^{3+} 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 LC_{50} to *D. magna* based on nominal values ($\text{La} > \text{Ce} > \text{Pr} > \text{Nd} \approx$
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 LC_{50} did not show any trend ($\text{La} \approx \text{Gd} > \text{Ce} \approx \text{Pr} \approx \text{Nd}$). Decreasing ecotoxicity along
631 the LN series has been reported for *Hyaella 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 NaNO_3) used for testing with *A. fischeri*, free 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 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 EC_{50} , TWM EC_{50} and 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 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 [§] (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 [#] , ↓	N	Hydra medium CaCl ₂ , TES, pH 7	Blaise et al. (2018)
All LN	<i>Hyalella azteca</i>	Y, ↓ [§]	Y	Soft water	Borgmann et al. (2005)
All LN except Pr, Ho, Yb	<i>Vibrio fischeri</i>	Y, ↑	N	0.355 M NaNO ₃ , pH 5.5	Weltje (2002)
All LN except Ho, Tm, Yb	<i>Nitellopsis obtusa</i>	Y, ↑	N	Artificial Pond Water KCl, NaCl, CaCl ₂ and HEPES (pH 7.5)	Manusadžianas et al. (2020)
All LN except Ho, Tm, Yb	<i>Thamnocephalus platyurus</i>	N	N	Standard freshwater NaHCO ₃ , CaSO ₄ , MgSO ₄ 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 [§]	Y	Standard dilution water CaCl ₂ , MgSO ₄ , NaHCO ₃ , 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 ₂ HPO ₄ , KH ₂ PO ₄ , (NH ₄) ₂ SO ₄ , KNO ₃ (pH just below 7)	Den Dooren de Jong (1965)
All LN	<i>Skeletonema costatum</i>	N	N	f/2 medium without EDTA: NaNO ₃ , NaH ₂ PO ₄ , 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 LC₅₀ (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 μm 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 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 μm by substituting NaOH for 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 EC₅₀ 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 EC₅₀ 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 _x 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 ³⁺ 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 µm (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 µg/L and 100 µg/L, respectively in artificial media),
800 but higher than Ce and Er concentrations of 1 µg/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 µg/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 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 ΣREY 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 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 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 EC₅₀ 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 EC₅₀ 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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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*)

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Conflict of interest disclosure

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