ACCU MULATION AND ELIMINATION OF LANTHANUM BY DUCKWEED
(LEMNA MINOR L.) AS INFLUENCED BY ORGANISM GROWTH AND LANTHANUM
SORPTION TO GLASS

LENNART WELTJE,* ANKE H. BROUWER, TONA G. VERBURG, HUBERT TH. WOLTERBEEK, and
JEROEN J.M. DE GOEIJ
Department of Radiochemistry, Interfaculty Reactor Institute, Delft University of Technology, Meke lweg 15,
NL-2629 JB Delft, The Netherlands

(Received 29 August 2001; Accepted 9 January 2002)

Abstract—Lanthanide emissions to the environment increase as a result of the growing industrial applications of these elements. However, robust data to evaluate the environmental fate of lanthanides are scarce. This article describes the accumulation and elimination of lanthanum (La) by common duckweed (Lemna minor L.). Speciation modeling was performed to assure that solubility products were not exceeded. It also showed that La was predominantly associated with ethylenediaminetetraacetic acid (EDTA). Lanthanum concentrations in plants and medium and the amounts sorbed to glass vessels were quantified by using the radioisotope 148La. The amount of La adsorbed on the glass reached values of 25% of the total La present. A model was formulated to describe La uptake in exponentially growing duckweed in the presence of an adsorptive surface. Growth-induced dilution appeared more efficient in lowering plant La concentrations than actual elimination. An elimination study revealed two compartments, of which the smallest eliminated 50 times faster than the bigger compartment, which eliminated mainly by growth dilution. The average bioconcentration factor was 2,000 L/kg fresh weight and 30,000 L/kg dry weight, comparable with those of other higher plants. At the applied concentration of 10 nM, no effects were observed on duckweed growth. However, the high bioconcentration factor warrants monitoring of lanthanide emissions.

Keywords—Lanthanides Rare-earth elements Bioavailability Speciation Radioisotope

INTRODUCTION
Lanthanides (57 ≤ atomic number ≤ 71) are elements with no known biological function, although beneficial effects on plants have been reported [1,2]. They comprise a chemically homogeneous group of 15 metals, one of which, promethium (Pm), has only radioactive isotopes (maximum 71Pm), has only radioactive isotopes (maximum t1/2 = 17.7 year) and hence no natural occurrence. The other 14 lanthanides are ubiquitous in most soils in reasonable quantities. For instance, the concentration of lanthanum (La), the first lanthanide element, in the earth’s crust is approximately equal to that of lead (Pb) and higher than that of cadmium (Cd) [1,3]. In nature, lanthanides exist mostly in a 3+ oxidation state and form slightly soluble complexes with, e.g., phosphate [4] and carbonate [5]. Lanthanides can also be found in practically all biota. Natural background concentrations of La in terrestrial plants range from 1.1 to 1.8 nmol/g dry weight [6] and for aquatic plants of the genus Lemna (duckweed) values of 0.93, 6.3, and 6.5 nmol/g dry weight are reported [7–9]. In physiological studies, lanthanides are frequently used to inhibit the uptake of free ionic calcium (Ca2+), which has a similar ionic radius but lower charge density than the trivalent lanthanide ion [10,11]. For duckweed, La3+ has also been shown to inhibit the uptake of Cd2+ and free ionic thallium (Tl+) [12].

Due to increasing industrial uses of lanthanides and lanthanide-containing ores, large amounts of these elements are emitted to the environment, particularly to the aquatic environment. The main sources of lanthanide emissions are the fertilizer and catalyst industries. The two main producers of artificial fertilizer in The Netherlands introduced about 100 tons of La in surface water in 1994 [13]. Nevertheless, lanthanides have received little attention in environmental studies so far. Because the final impact of the emissions is largely unknown, knowledge is required on the environmental fate of lanthanides in aquatic ecosystems. Currently, a lack of reliable data exists on availability, uptake, and effects of lanthanides in aquatic organisms. Some studies were performed, but they suffered from flaws, such as substantial precipitation as a result of exceeding lanthanide phosphate or carbonate solubility products in the applied media [14–16]. Although steady-state bioconcentration factors (BCFss) for lanthanides in aquatic plants from the field have been frequently measured, reaching values of up to 46,000 L/kg dry weight [17], little is known about the actual uptake and elimination processes. Knowledge of the behavior of one of the lanthanides can easily be extrapolated to other members of this series because their chemistry is quite similar. In addition, it can help to predict the environmental fate of the radioactive actinides since trivalent members of this series share a number of chemical characteristics with the lanthanides [18].

This work describes the uptake and elimination of La by common duckweed (Lemna minor L.). Lemna minor is a widespread, free-floating, higher freshwater plant, which is easy to handle in the laboratory, has a high vegetative growth rate [9], and is known for its accumulative behavior toward metals [19]. In our experiments, the mass balance for La was completed by quantifying the La concentrations in medium and plants and the amounts of La adsorbed onto glass vessels. All data were used to formulate a model with which the rates of La transport between the compartments could be described. Ex-
Table 1. Composition of *Lemna minor* nutrient medium, adapted from the Swedish Standards Institute [20]; concentrations in mol/L (M)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (M)</th>
<th>Component</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>5.00 × 10⁻⁴</td>
<td>CuSO₄·5H₂O</td>
<td>2.00 × 10⁻⁸</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>4.92 × 10⁻³</td>
<td>Co(NO₃)₂·6H₂O</td>
<td>3.44 × 10⁻⁸</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>3.04 × 10⁻⁴</td>
<td>NaVO₃</td>
<td>8.20 × 10⁻⁸</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>2.45 × 10⁻⁴</td>
<td>FeCl₃·6H₂O</td>
<td>3.11 × 10⁻⁶</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>1.62 × 10⁻³</td>
<td>Na₂EDTA·2H₂O</td>
<td>5.37 × 10⁻⁵</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>1.01 × 10⁻⁶</td>
<td>MES·H₂O</td>
<td>2.34 × 10⁻³</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>1.74 × 10⁻⁷</td>
<td>Glucose</td>
<td>1.25 × 10⁻⁴</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>4.13 × 10⁻⁸</td>
<td>LaCl₃·7H₂O</td>
<td>1.00 × 10⁻⁸</td>
</tr>
</tbody>
</table>

*2-Morpholinoethane sulfonic acid, a pH buffer.
Lanthanum component; not present in control treatment of experiment 1 and elimination phase of experiment 3.

Experiments were supported by speciation calculations to assure that no oversaturated solutions were used.

**MATERIALS AND METHODS**

**General**

To study La kinetics in duckweed, different exposure regimes were applied (described below). The following conditions apply to all experiments. Plants were cultured axenically and exposed in a climate chamber at a light intensity of 10,000 lux and a day:night regime of 16:8 h. Relative humidity was approximately 70% and the temperature 25 ± 2°C. The exposure substrate was a sterilized low-level nutrient medium, adapted from the Swedish Standards Institute (SIS) [20], with an initial pH of 5.05 ± 0.05. Its composition is given in Table 1. The applied La concentration was 10 nM, which is about 10 times above the natural background concentration for fresh water [3] but close to levels found in 0.45-µm filtered surface water of the Rotterdam harbor [21], which is polluted with lanthanides, and the river Rhine [22]. All chemicals used were of pro analysis quality. Before preparing test solutions, speciation calculations were performed with the software program CHEAQS (http://home.wish.net/~vdiest1) [23] to assure that La remained in solution even if the pH would rise half a unit during the experiments. This demonstrated the necessity of increasing the ethylenediaminetetraacetic acid (EDTA) concentration from the original 3.76 to 5.37 µM. Plants were exposed in 200 ± 1 ml medium in glass vessels, which were closed with a glass lid to minimize evaporation but allow limited gas exchange. Plant fresh weight was determined after spin drying at 3,000 rpm for 10 min at room temperature to remove attached and free-space water. This method yields highly reproducible measurements, contrary to dry blotting [24]. Fresh-weight measurements were used to construct growth curves.

**Experiment 1. La uptake from refreshed medium**

Twenty glass vessels with medium and 10 nM La and 20 glass vessels with La-free medium were inoculated with 12 duckweed fronds (3–5 colonies). Plants in vessels without La served as a control group to compare their growth rate with La-exposed plants and study possible toxicity or growth stimulation of La. At times 48, 96, 144, and 192 h, plants were transferred to vessels with fresh medium (with or without La). At times 0, 4, 8, 24, 45, 55, 72, 94, 124, 168, and 216 h, the pH of the medium was measured, fresh weight was determined, and La concentrations in media and plants and La amounts on glass vessels were measured. All measurements were performed in duplicate.

**Experiment 2. La uptake from nonrefreshed medium**

Twenty-seven glass vessels with medium and 10 nM La were inoculated with 12 duckweed fronds (3–5 colonies). In addition, three vessels with medium but no plants were followed in time to check for possible precipitation and constancy of pH. The first 48 h were thus the same as for experiment 1. At times 0, 1, 3, 5, 24, 48, 92, 120, and 167 h, pH of the media was measured, plant fresh weight was determined, and La concentrations in media and plants and La amounts on glass vessels were measured. All measurements were performed in triplicate.

**Experiment 3. La uptake from nonrefreshed medium and elimination in refreshed medium**

Twenty-eight glass vessels with medium and 10 nM La were inoculated with 12 duckweed fronds (3–5 colonies). In addition, three vessels with medium but no plants were followed in time to check for possible precipitation and constancy of pH. The first 48 h were the same as for experiments 1 and 2. To study elimination, plants from 15 vessels were gently lifted off the surface after 48 h of accumulation and transferred, without spin drying, to vessels with La-free medium. This medium was refreshed at times 55, 72, 120, and 144 h to keep the La concentration in medium as low as possible and thus prevent backflow of La to plants. At times 0, 5.5, 12, 24, 48, 55, 72, 96, 144, and 169 h, pH of the media was measured, fresh weight was determined, and La concentrations in media and plants and La amounts on glass vessels were measured. Measurements were performed in duplicate (accumulation) or triplicate (elimination).

La production and activity measurements

Lanthanum was spiked with the radionuclide ⁴¹⁰La (*t₁/₂* = 40.3 h), which emits β- and γ radiation (main Eγ are 329, 487, 816, and 1,596 keV). ⁴¹⁰La was prepared by irradiating a quartz vial containing a solution of 0.1 mM LaCl₃·7H₂O (Aldrich, Milwaukee, WI, USA; purity 99.999%) in Milli-Q water (Millipore®-Waters, Milford, MA, USA) at a neutron flux of 1.60 × 10¹⁷/(m²·s) for 24 h in the Hoger Onderwijs Reactor of our institute. The solution was allowed to decay for 3 h to eliminate the short-lived chloride isotope ³⁵Cl (*t₁/₂* = 37.2 min) and was subsequently added to the rest of the medium components to make up a final La concentration of 10 nM.

Activity of ⁴¹⁰La was measured in media and plants by γ-detection on a 1480 Wizard 3' gamma counter (Wallac Oy, Turku, Finland) using an energy window of 15 to 2,000 keV. Automatic background correction was applied. For measuring ⁴¹⁰La activity on glass vessels, a plane NaI(Tl) detector (type 12S12, Harshaw-Bicron, Newbury, OH, USA) of 7.62 cm × 7.62 cm was used, placed in a lead-shielded container. The detector was calibrated with a sample of known activity and the same geometry as the vessels. Lanthanum activities were directly converted to La amounts, which was justified because the La background concentration of the *L. minor* culture was about 9 pmol/g fresh weight (measured with inductively coupled plasma mass spectrometry, results not shown) and therefore had no effect on the initial specific activity.

**Data modeling**

Experimental data on duckweed growth and La concentrations in media, plants, and glass vessels were described ac-
According to the model depicted in Figure 1, assuming first-order kinetics for all processes. Duckweed growth, as fresh-weight increase, was described by an exponential growth curve (Eqn. 1) as

\[ W_t = W_0 e^{\mu t} \]  

in which \( t \) is time (h), \( W_t \) is fresh weight at time \( t \) (g), \( W_0 \) is fresh weight at \( t = 0 \) (g), and \( \mu \) is the exponential growth rate constant (h\(^{-1}\)). Lanthanum concentrations in plants (on a fresh wt basis), medium, and glass vessels were described by Equations 2 to 5 as follows, of which Equation 5 represents the mass balance:

\[ \frac{d[La]_{p1}}{dt} = k_1[La]_{g} - (k_2 + \mu)[La]_{p1} \]  

\[ \frac{d[La]_{p2}}{dt} = \frac{W_0}{V(k_2[La]_{g} - k_1[La]_{m})} + \frac{S}{V(k_5[La]_g - k_4[La]_{m})} \]  

\[ \frac{d[La]_{m}}{dt} = k_6[La]_{m} - k_5[La]_{g} \]  

\[ [La]_{m,t} = [La]_{p1}W_t + [La]_{m0}V + [La]_{g}S \]  

in which \([La]_{p1}\) is the La concentration in plants as a function of time (nmol/g), \([La]_{m}\) is the La concentration in medium as a function of time (nmol/m\(^2\)), \( k_1 \) is the uptake rate constant (L/[g·h]), \( k_2 \) is elimination rate constant (h\(^{-1}\)), \( k_3 \) is the adsorption rate constant (L/[m\(^2\)·h]), \( k_4 \) is the desorption rate constant (h\(^{-1}\)). The other variables have already been defined above.

Obviously, the model of Figure 1 simplifies our experimental system. For instance, duckweed is expected to consist of multiple compartments, e.g., cell walls, protoplasts, and vacuoles [24,27]. To obtain more information on compartmental behavior of La in duckweed, elimination was studied (experiment 3) since distinguishing compartments is much easier during an elimination phase [25,28]. In addition, the system was simplified by frequently refreshing the elimination medium, keeping its La concentration close to 0 nM, and thus preventing backflow from eliminated La to duckweed as well as La adsorption by glass. Two plant compartments could be distinguished and were described with the model depicted in Figure 2 and by Equations 8 to 10 as

\[ \frac{d[La]_{p1}}{dt} = k_4[La]_{p2} - (k_2 + k_5 + \mu)[La]_{p1} \]  

\[ \frac{d[La]_{p2}}{dt} = k_5[La]_{p1} - (k_6 + \mu)[La]_{p2} \]  

\[ [La]_{p1} = [La]_{p1}^0 + [La]_{p2} \]  

in which \([La]_{p1}^0\) and \([La]_{p2}\) are La concentrations in duckweed compartment \( p_1 \) and \( p_2 \) (nmol/g) as a function of time, respectively, and \( k_5 \) and \( k_6 \) are the rate constants describing La transport from the first to the second compartment and back, respectively (h\(^{-1}\)). The other variables have already been defined above.

Linear and nonlinear least squares regressions were performed with Prism, Ver 2.01 (GraphPad Software, San Diego, CA, USA) and numerical analyses with Scientist, Ver 2.01 (MicroMath, Salt Lake City, UT, USA).

RESULTS AND DISCUSSION

Growth and toxicity

During the course of the experiments, duckweed growth was purely exponential, indicating that growth limitation under the present conditions is not occurring. Exponential growth was one of the conditions for application of the proposed models. From Table 2, it can be seen that growth rate constants (\( \mu \)) are not significantly different between experiments, indicating the reproducibility of the experimental and culturing conditions. Differences in \( W_t \) reflect variations in duckweed fresh weight at the start of the experiments and do not influence \( \mu \) (Table 2).

Simultaneously tested control cultures in experiment 1, in which the most intensive exposure to La occurred, had growth rates equal to exposed cultures (Table 2). This leads to the concentrations in medium. The other variables have already been defined above.
conclusion that an initial medium concentration of 10 nM La, which is mainly associated with EDTA, has no adverse or beneficial effects on duckweed growth in 9 d. The associated maximum concentration of La in *L. minor* without effects was 12 nmol/g fresh weight.

**Speciation and availability**

Speciation calculations showed all La to be in solution up to pH 5.6 (initial pH 5.05) and for more than 99.9% associated with EDTA. They also showed that a substantially higher pH or an EDTA concentration below 5.37 μM would result in precipitation of LaPO₄ (solubility product, $K_{sp} = 10^{-25.7} \text{mol}^2/\text{L}^2$ at 25°C and infinite dilution [4]). The free La³⁺ ion concentration at pH 5.0 at the start of the experiment was calculated to be 12 pM, i.e., 0.12% of the total La concentration. While some researchers assume that, for *Lemna*, the free metal ion is the species available for uptake [29], there is also evidence for uptake of complexed metals by *Lemna* and other higher plants. The results of Buckley [30] for copper (Cu) and *L. minor* indicated that, next to the free Cu²⁺ ion, some Cu was taken up as organic complexes. For La and hydroponically grown *Triticum* ssp., it was shown that uptake of La by roots decreased in the presence of EDTA, while uptake in the shoots increased [31]. Vassil et al. [32] showed that uptake of Pb by *Brassica juncea* was directly correlated with EDTA uptake and also that Pb is transported in coordination with EDTA in xylem exudate, and Wolterbeek et al. [33] suggested simultaneous uptake of Cd and EDTA by *Solanium lycopersicum*, possibly as CdEDTA²⁻ complex. It must be noted that these observations on metal availability are of course subject to metal-complex lability. Because our experiments cannot discriminate between uptake of ionic La³⁺, which is supplied by dissociation of the LaEDTA complex, and the complex itself, both possibilities are considered here. Work is in progress to resolve this question.

**La in medium and on glass vessels**

Values for medium pH in treatments with or without plants were all within the desired range, between 5.0 and 5.5, thus assuring the abovementioned speciation calculations to be valid. Precipitation is therefore not expected. However, [La]ₘₐₙ in the vessels without plants decreased slightly (Fig. 3b), which is probably due to adsorption of La onto the glass vessels (see, e.g., Fig. 4b). The decrease of [La]ₘₐₙ in the vessels with plants (Fig. 3) is due to adsorption on the glass and, what is more, to La uptake by duckweed (Fig. 4). This underlines the importance of combining substance transport between (closed) compartments with a mass balance. While at the start, all La is in the medium, it can decrease to 20% at the end of the experiment (Fig. 4b). In the first experiment, where medium was renewed, it is clear that, with the increase of the transferred biomass, the decrease of the La medium concentration goes ever faster (Fig. 3a). Since the net La concentration in medium is decreasing at the applied refresh rate, it would make sense to increase either the refresh rate or the refreshed volume, corresponding to the amount of transferred biomass, and achieve a better semiconstant exposure. While the net La concentration in medium of experiment 1 is decreasing, the average exposure concentration (depicted by the solid horizontal line in Fig. 3a) was assumed constant. This means that, by applying Equation 7, the real exposure is underrepresented and overrepresented, respectively, at the beginning and end of this experiment.

The mass balances (Fig. 4) show that amounts of La on glass can measure up to 25% of total La present. From all measurements, La on glass was the most variable, which may be attributed to a lower γ-detection efficiency associated with sample geometry and differences in adsorptive capacity among glass vessels. Estimated values for the adsorption rate constant

### Table 2. Estimated values and standard errors (SE) for $W_i$, the initial fresh weight, and $\mu$, the exponential growth rate constant (Eqn. 1); units in parentheses. La = lanthanum.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$W_i$ ± SE (g)</th>
<th>$\mu$ ± SE (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 La exposed</td>
<td>0.009 ± 0.003</td>
<td>0.0191 ± 0.0016</td>
</tr>
<tr>
<td>1 control</td>
<td>0.011 ± 0.002</td>
<td>0.0185 ± 0.0009</td>
</tr>
<tr>
<td>2 La exposed</td>
<td>0.014 ± 0.002</td>
<td>0.0190 ± 0.0007</td>
</tr>
<tr>
<td>3 La exposed</td>
<td>0.021 ± 0.002</td>
<td>0.0186 ± 0.0007</td>
</tr>
</tbody>
</table>

![Fig. 3. Lanthanum concentrations (in nM) in media with plants (closed symbols) and without plants (open symbols) versus time (in h). Symbols are averages with standard errors ($n = 2–9$). If error bars are not visible, they fall within the symbols. Data from experiments 1, 2, and 3 are presented in a, b, and c, respectively. The vertical dashed lines in a and c indicate medium renewal and the solid lines are fits.](image)
Lanthanum uptake by duckweed

Environ. Toxicol. Chem. 21, 2002 1487

Fig. 4. Mass balances (in nmol) versus time (in h). Totals (closed dots) are the sum of La amounts in duckweed (squares), medium (asterisks), and glass vessels (open dots). Symbols are averages with standard errors (n = 2 or 3). If error bars are not visible, they fall within the symbols. Data from experiments 1, 2, and 3 are presented in a, b, and c, respectively.

Fig. 5. Lanthanum concentrations in Lemna minor (in nmol/g fresh wt) versus time (in h). Symbols are averages with standard errors (n = 2 or 3). If error bars are not visible, they fall within the symbols. Data from experiments 1, 2, and 3 are presented in a, b, and c, respectively. The solid lines are fits. The vertical dashed lines in a and c indicate medium renewal. The observation represented with an open symbol in a was considered an outlier and consequently excluded from fit.

$k_3$ in experiments 2 and 3 are $0.117 \pm 0.039$ and $0.055 \pm 0.007$ L/(m²·h), respectively. For the desorption rate constant $k_4$, a value of $0.019 \pm 0.009$/h was estimated in experiment 2, while in experiment 3, it took negative values and was consequently set to 0. This partly explains the lower value for $k_3$ in experiment 3, which, because desorption is not occurring, represents a net adsorption. Looser et al. [28] also incorporated a net adsorption rate in their bioaccumulation model but did not support it with data on metal amounts on the glass.

By using radionuclides, it is relatively easy to quantify the La amount on the glass vessel and thus complete the mass balance, which was typically $98 \pm 8\%$. With other analytical methods of metal detection, it would be much more elaborate to quantify the amount of metals lost, but as the present study shows, it can have a substantial influence on the applied concentration. To minimize this influence, containers with lower adsorption capacity for La should be applied.

**Modeling La in duckweed**

The accumulation of La in duckweed (Fig. 5b and c) was well described by the proposed model of Equations 1 to 5. This model assumes an exponentially growing organism in a closed system, accumulating a substance in the presence of an adsorptive surface. The resulting pattern is similar to the one described for uptake of nonpersistent chemicals [34]. As a result of uptake and adsorption, the medium concentration of La is going down. Due to the decreasing medium concentra-
tion, La in duckweed is not reaching equilibrium. However, this does not preclude calculation of a dynamic bioconcentration factor, $BCF_{\text{dyn}}$ (Eqn. 6, Table 3). Equation 7, which assumes constant exposure, did not describe La concentrations in duckweed satisfactorily because the net exposure concentration was not (semi-) constant but still decreasing and hence equilibrium was not reached. This explains the obvious misfit of Equation 7 with the experimental data (Fig. 5a). Refreshing the medium did not result in higher La concentrations in plants (compare Fig. 5a with Fig. 5b and c) but in fact made modeling of these discontinuous data less straightforward. Therefore, application of an unrefreshed medium is advocated, which enables the integral use of a mass balance in modeling the data, in turn leading to a better description of exposure. The model can be applied under the following conditions: growth must remain exponential during exposure, no substance is lost, and substance transport is satisfactorily described with first-order kinetics.

The first 5 h gave rise to a deviation from the model predictions (Fig. 5a through c), which slightly overestimated La concentrations in duckweed. The cause for this small delay in uptake, which was replicated in all experiments, is yet unknown. It may be related to transferring the plants at the beginning of the experiment from the culture to test solution.

The maximum concentration of La in duckweed in our experimental system, 12 nmol/g fresh weight, is reached at $t \approx 50$ h (Fig. 5b). At $t = 216$ h in experiment 1, the La amount in duckweed was 4 nmol (Fig. 4a), which equals the total amount of La present in 400 ml of medium (the contents of two vessels). At the end of experiment 2, about 60% of La in medium was taken up by duckweed (Fig. 4b).

The data of the elimination phase in experiment 3 were in excellent agreement with the proposed two-compartment model of Equations 8 to 10 (Fig. 5c). The second compartment, $p_2$, contained more La, 6.86 ± 1.50 nmol/g fresh weight, and appeared to eliminate slower than $p_1$, the first compartment, which contained 4.50 ± 1.53 nmol/g fresh weight. Values for the transport rate constants $k_3$ and $k_5$ were 0.157 ± 0.020 and 0.019 ± 0.005/h, respectively.

**Uptake and elimination rate constants and bioconcentration factors**

In Table 3, the calculated values for uptake and elimination rate constants ($k_1$ and $k_2$) and the resulting dynamic bioconcentration factor ($BCF_{\text{dyn}}$) with their respective standard errors are given. For reasons stated above, the rate constants from the accumulation phases of experiments 2 and 3 are considered more reliable than those from experiment 1. This can also be concluded from the calculated standard errors. The deviating value for $k_1$ from the elimination phase of experiment 3 will be discussed separately. Values for $k_1$ range from 0.049 to 0.056 L/(g·h) and are not significantly different. Furthermore, because $\mu > k_1$, it is concluded that growth-related dilution is more important than elimination sensu stricto in lowering the plant La concentration. This stresses the great importance of considering plant growth rates in uptake and elimination studies, something not always recognized [35]. However, in experiment 1, $k_1 > \mu$, but this is due to the fact that by applying Equation 7 to these data, the elimination rate constant must partly compensate for the decreasing medium concentration. The true elimination rate is smaller than the growth rate.

The elimination rate constant from the elimination phase of experiment 3 is about 50 times higher than the average $k_2$ value. This rate constant represents a small and fast eliminating compartment, which could not be distinguished in the accumulation phase. This fast elimination might be caused by transferring the plants, without spin drying, to medium without La and the presence of uncomplexed EDTA therein, which has a high affinity for La ($\log K = 17.92$ at 25°C and infinite dilution [5]). Judged by its value, it is likely that $k_2$ from the elimination phase represents a different elimination process than $k_1$ calculated from the accumulation phase.

Approximately 60% of the La taken up is only slowly eliminated by duckweed, mainly by growth dilution. This can be seen in Figure 4c, where the amount of La in plants remains almost constant in the second half of the elimination phase. The mass balances of the other experiments show that the amount of La in duckweed steadily increases, while concentrations decrease (compare Fig. 4a and c with Fig. 5a and c).

This is illustrative for growth-induced dilution. The calculated $BCF_{\text{dyn}}$ for La in duckweed ranges from 1.145 to 2.198 L/kg fresh weight (Table 3). Given that the fresh to dry weight ratio of duckweed is approximately 15 (results not shown), this means a $BCF_{\text{dyn}}$ between 17,175 and 32,970 L/kg on a dry weight basis, indicating the high accumulating potential of duckweed for La. This value for *L. minor* has the same order of magnitude as previously reported $BCF_{\text{a}}$ for La in other higher aquatic and terrestrial plants (for an overview, see L. Weltje [17]).

**CONCLUSIONS**

In summary, it can be stated that *L. minor* takes up La from medium as LaEDTA complex and/or as ionic La$^{3+}$, reaching a $BCF_{\text{dyn}}$ of 32,970 L/kg on a dry weight basis. Elimination study revealed a large (60%) slow and small (40%) fast compartment. The large compartment eliminates mainly by means of maintaining a high growth rate. The proposed model incorporating a mass balance rule and exponential growth of the exposed organism agreed well with the experimental data. Neither stimulating nor toxic effects of 10 nM complexed La on duckweed growth in 9 d were observed. However, considering the high $BCF_{\text{dyn}}$ values, effects may occur under constant exposure as uptake continues at the rate reported here. The use of radiotracers in uptake studies such as described here can reveal missing compartments of the mass balance, e.g., the glass vessels to which metals may adsorb. Support by speciation calculations in applying difficult substances in culture media reveals the possible exceeding of solubility products. Refreshing the medium did not result in higher La concentrations in plants but instead made the modeling of these data less straightforward.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$k_1 \pm SE$ (L/[g·h])</th>
<th>$k_2 \pm SE$ (h⁻¹)</th>
<th>$BCF_{\text{dyn}} \pm SE$ (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (accumulation)</td>
<td>0.050 ± 0.012</td>
<td>0.024 ± 0.012</td>
<td>1.145 ± 416</td>
</tr>
<tr>
<td>2 (accumulation)</td>
<td>0.049 ± 0.004</td>
<td>0.006 ± 0.003</td>
<td>1.917 ± 267</td>
</tr>
<tr>
<td>3 (accumulation)</td>
<td>0.056 ± 0.003</td>
<td>0.007 ± 0.003</td>
<td>2.198 ± 286</td>
</tr>
<tr>
<td>3 (elimination)</td>
<td>—</td>
<td>0.339 ± 0.049</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* Uptake not occurring, so $BCF_{\text{dyn}}$ is not calculable; see text for details.

*b* Value estimated with Equations 8 to 10.
Lanthanum uptake by duckweed

Acknowledgement—The authors are grateful to their colleagues W. den Hollander, J.J. Kroon, and J.J.H. Haftka for assistance with γ-detection, irradiation, and experiments, respectively, and to H. Heiderich (IHI, Zittau, Germany) for ICP-MS measurement of La in L. minor. The comments of B. Markert (IHI, Zittau, Germany) and two anonymous reviewers are gratefully acknowledged. This work was funded by the Delft University of Technology as Beek-Project 95-IRI-A-6. Part of it was presented at the Society of Environmental Toxicology and Chemistry—Europe conference in Leipzig, Germany, May 25–29, 1999.

REFERENCES