Supporting information

Bioaccumulation of ionic titanium and titanium dioxide nanoparticles in zebrafish eleutheroembryos.

A. López-Serrano Oliver, R. Muñoz Olivas, J. Sanz Landaluze, S. Rainieri, C. Cámara

Toxicokinetics and statistics

A first order two-compartment (water and aquatic organism) model, which describes the uptake and depuration process through Eq. 1, was used to calculate bioconcentration factor when a steady stage is not reached.

$$\frac{dC_f}{dt} = k_1 \cdot C_w - k_2 \cdot C_f \quad \text{(uptake)} \quad \frac{dC_f}{dt} = -k_2 \cdot C_f \quad \text{(depuration)} \quad \text{Equation 1}$$

where C_f is the concentration in fish (in $\text{ng} \cdot \text{g}^{-1}$), t is the exposure time (h), k_1 is the first-order uptake constant (liter per kilogram dry weight per hour), C_w is the concentration of the compound in the exposure media ($\text{ng} \cdot \text{mL}^{-1}$), and k_2 is the first-order elimination rate constant (per hour). Assuming that at t_0 , the concentration of the test substance in fish is negligible and the concentration of the tested compound in exposure media is constant, Eq. 2 is obtained:

$$C_f = \frac{k_1}{k_2} \cdot C_w (1 - e^{-k_2 t}) \quad \text{(uptake)} \quad C_f = C_{f,0} \cdot e^{-k_2 t} \quad \text{(depuration)} \quad \text{Equation 2}$$

Where $C_{f,0}$ denotes the analyte concentration in the organism when the depuration phase begins. k_1 and k_2 values can be obtained if the experimentally determined concentration values in the bioconcentration test fit to this equation. When the equilibrium is reached (steady-state), equation 2 may be reduced to equation 3.

$$C_f/C_w = \text{BCF}_k = k_1/k_2 \quad \text{Equation 3}$$

The software NONLIN 5.1 was used for kinetic calculations

Figure 1 SI. Total titanium content in the exposure medium where eleutheroembryos were incubated with TiO₂ NPs (75% anatase/25%rutile) a) Nominal concentration 2 mg·L⁻¹. b) Nominal concentration 10 mg·L⁻¹.

Figure 1a)

Figure 1b)

Figure 2 SI. Total titanium content in the exposure medium where eleutheroembryos were incubated with ionic titanium in presence and absence of eleutheroembryos. (a) Nominal concentration $0.1 \mu\text{g}\cdot\text{L}^{-1}$. (b) Nominal concentration $0.9 \mu\text{g}\cdot\text{L}^{-1}$. *Square markers: in absence of eleutheroembryos; triangular markers: in presence of eleutheroembryos*

Figure 2a)

Figure 2b)

Table 1 SI. Toxicokinetic parameters and bioconcentration factors (BCF_{48h} , BCF_k) obtained from the adjustments of the experimental data at different media concentration (C_w).

	Ionic Ti		TiO ₂ NPs	
C_w (mg·L ⁻¹)	0.11±0.03	0.90±0.10	1.8±0.2	9.1±2.1
C_f (ng·g ⁻¹)	7367	2931	5000	26802
k_1 (mL·ng·h ⁻¹)	4.6	0.2	0.051	0.12
$k_{2\text{ (acum)}}$ (ng·mL ⁻¹)	0.06	0.06	0.012	0.04
$k_{2\text{ (dep)}}$ (ng·mL ⁻¹)	0.05	0.06	0.032	0.04
BCF_{48h}	73	3.3	2.5	2.9
BCF_k	77	3.3	4.2	3.0

Table 2 SI. Statistical data of the fit of the experimental data to the model

	Final sum of squared deviations	Standard error of estimate	Average deviation	Proportion of variance explained (R ²)
Ti 0.1 mg·L ⁻¹	5.9	0.71	0.68	71.98%
Ti 0.9 mg·L ⁻¹	7.6 10 ⁻¹	0.38	0.29	95.79%
TiO ₂ NPs 2 mg·L ⁻¹	5.6 10 ⁻¹	0.33	0.226	96.96%
TiO ₂ NPs 10 mg·L ⁻¹	1.4	0.56	0.41	89.63%