

Uptake from Water, Internal Distribution and Bioaccumulation of Selenium in *Scenedesmus obliquus*, *Unio mancus* and *Rattus norvegicus*: Part B

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Abstract ^{75}Se -selenite transfer was investigated in a phytoplankton-mussel-rat food chain model consisting of *Scenedesmus obliquus* (Turpin) Kützing, *Unio mancus* Lamark and *Rattus norvegicus* Berkenhout. ^{75}Se -metabolized forms were investigated in order to identify potential critical steps in the food chain, as well as its relative bioavailability looking also at intracellular, cellular and organ partitioning. Tissue and intracellular distribution of ^{75}Se in mussels fed with ^{75}Se -*S. obliquus* was different compared

to those exposed only to inorganic ^{75}Se -selenite. The intracellular distribution of ^{75}Se in the hepatopancreas and mantle of mussels fed ^{75}Se -microalgae was similar to hepatic and renal distributions in rats, suggesting that their stomach dissociated larger ^{75}Se -containing molecules. The ^{75}Se partitioned from water (culture medium) to microalgae showing a bioconcentration factor of 435. The bottleneck in the trophic transfer of ^{75}Se occurred between *S. obliquus*–*U. mancus*. From microalgae to mussels and subsequently to rats no bioaccumulation was verified.

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In recent years, the problem of elevated levels of selenium (Se) in certain areas has become a major concern, especially to wildlife. Mainly, body burdens of Se accumulate either through food or dissolved Se uptake. Dietary exposure represents the most relevant pattern of Se uptake in terrestrial predators (Ohlendorf et al. 1998; Chapman et al. 2010; Poletti et al. 2014). Simplified laboratory food chain models have been used to study Se bioavailability and biomagnification. These have included food chains involving bacteria and the ciliated protozoan *Paramecium putrinum* (Sanders and Gilmour 1994), the alga *Scenedesmus obliquus* and the cladoceran *Daphnia magna* (Yu and Wang 2004), and the insect *Acheta domestica* and lizard *Sceloporus occidentalis* (Hopkins et al. 2005). The transfer of Se has also been investigated in various fish species fed invertebrates containing radiolabelled Se (Reinfelder and Fisher 1994; Baines et al. 2002; Xu and Wang 2002). Studies about the sensitivity and effects of Se to organisms are limited to a small number of animal groups (Chapman et al. 2010).

The aim of the present study was to investigate the nature of the transfer of Se through a simplified phytoplankton-mussel-rat food chain model, mainly focusing on its absorption, retention, intracellular and protein distribution in tissues and excreta. Mammals were included, thus providing data to integrate existing ecosystem-scale modeling activities (Presser and Luoma 2010). Exposure scenarios took into consideration elemental Se as selenite [Se(IV)] (Boisson et al. 1995; Takayanagi 2001), starting from the considerations reported in Poletini et al. (2014). The combined use of radiotracers and biochemical techniques supported the analytical characterization within and between the various identified trophic levels.

Materials and Methods

An alga (*S. obliquus*), mussel (*Unio mancus*), and rat (*Rattus norvegicus*) were used in the various exposure scenarios of the study. Spiking conditions and analyses were the same described in Poletini et al. (2014). Microalgae or mussels were spiked with ^{75}Se -selenite from water; mussels were contaminated with water-spiked-microalgae and rats per os (p.o.) with both spiked microalgae and mussels (spiked in both ways from water and microalgae). A 200 mL culture of freshwater green alga *S. obliquus* was prepared and spiked ($105 \text{ ng Se mL}^{-1}$ at pH 7.14 ± 0.01 and $24.5 \pm 0.5^\circ\text{C}$). After 4 days of continuous culture growth, the whole culture was centrifuged at $6,000\times g$ for 5 min and the residue washed twice with NaHCO_3 solution ($15 \mu\text{g L}^{-1}$). After suspending cells in 10 mL of the same solution, microalgae were counted for radioactivity.

A population of five freshwater mussels (*U. mancus*) was maintained in a 10 L tank containing 7.5 L aerated and filtered (Millipore GS 0.22 μm) Lake Maggiore (Italy) water (pH 7.2 ± 0.1 , conductivity $142.0 \pm 1.5 \mu\text{S cm}^{-1}$, temperature $22.0 \pm 0.5^\circ\text{C}$). Mussels were fed with the above ^{75}Se -spiked algal suspension containing about 1.9 mg of *S. obliquus* cells and a total content of 19.8 ng Se dry wt. The mussel tank water was continuously and gently stirred with a mixer to ensure a uniform treatment during the 24 h of exposure. After the exposure period, mussels were taken out for dissection. Removed tissues were rinsed in demineralized water, wet weighed and counted for ^{75}Se content. The hepatopancreas and mantle of all five animals were pooled and homogenized. Subcellular fractions, including nuclei, mitochondria, lysosome and microsomal cytoplasmic fractions were obtained as previously described (Poletini et al. submitted). The soluble cytoplasmic fraction (cytosol) of hepatopancreas and mantle were chromatographed on Sephadex G-150 columns ($1 \times 100 \text{ cm}$), previously calibrated with

proteins of known molecular weights and equilibrated with 10 mM ammonium acetate buffer (pH 7.1 ± 0.1). The UV profile of the eluate was continuously monitored using a Lambda 25 spectrometer (Perkin-Elmer, Waltham, MA, USA), and the ^{75}Se content was measured in all biological fractions.

The Sprague–Dawley male albino rat (*R. norvegicus*, 350–380 g) was considered as the top consumer. Three exposure scenarios were investigated: (1) rats fed p.o. with microalgae grown in ^{75}Se -spiked water; (2) rats fed with mussels exposed to ^{75}Se -spiked water; (3) Rats fed with mussels exposed to ^{75}Se -spiked water fed with ^{75}Se contaminated microalgae. Three rats that had been held without food for 24 h were treated p.o. with a ^{75}Se -spiked microalgae suspension obtained as previously mentioned. Each suspension contained about 0.7 mg of cells and 44.9 ng Se in 0.75 mL NaHCO_3 . The animals were separately placed in metabolic cages with free access to food and water for urine and feces collection. A population of six mussels (*U. mancus*) were exposed to $105 \text{ ng Se mL}^{-1}$. After 3 days of exposure, they were dissected and their soft parts taken out and counted for radioactivity. They were divided into three groups of relatively uniform weight and ^{75}Se content, and used to feed the three groups of rats. Thus, each rat received a range of 8–10 g of mussel tissue containing about 2.000 ng Se. A volume of 400 mL of microalgae culture was prepared as described above with a ^{75}Se -selenite concentration of $105 \text{ ng Se mL}^{-1}$. After 4 days of algal growth in this medium, the cells were separated, resuspended in 20 mL NaHCO_3 and counted for radioactivity. An aliquot of about 9.7 mg cells corresponding to 443.1 ng of Se was distributed in a tank containing 50 L of lake water and nine mussels (*U. mancus*). After 24 h exposure, the mussels were removed; their soft parts were taken out, weighed and counted for radioactivity. They were grouped in three parts with a relative uniform weight ($13.8 \pm 0.3 \text{ g}$) and Se content ($28.0 \pm 0.5 \text{ ng}$). Three rats were fed with one group of mussel tissue each and separately placed for 24 h in metabolic cages for separate collection of their urine and feces. After 24 h, the rats were sacrificed by heart puncture under ether anaesthesia. The analysis took into account various direct and indirect organisms' components (tissues and excreta): kidney, liver, testis, epididymis, brain, heart, lung, spleen, pancreas, stomach, small intestine, large intestine, blood, plasma, red blood cells (RBC), feces and urine. Blood was taken with heparinized syringes. The tissues were then dissected out, washed, weighed and counted for ^{75}Se radioactivity. Blood was centrifuged at $2,500\times g$ for 15 min to separate plasma and RBC. To obtain the respective intracellular distribution of liver and kidney, they were homogenized in 10 mM ammonium acetate buffer solution (pH 7.1). These subcellular fractions were

differently centrifuged as previously mentioned. The cytosols of liver and kidney were chromatographed under the same conditions used for mussels. Statistical analysis was performed using the Student's *t* test or the analysis of variance (ANOVA) considering a significance threshold level always set at 5 %. When ANOVA revealed significant differences among treatments, post hoc analyses were conducted using Dunnett's and Tukey's methods. Statistical analyses were performed using Microsoft® Excel 2013/XLSTAT©-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA).

Results and Discussion

The distribution of ^{75}Se in mussel tissues (wet weight) following uptake of microalgae that were exposed to ^{75}Se followed the decreasing order (in ng g^{-1}): hepatopancreas (20.5 ± 7.5), visceral residue (6.4 ± 1.6), mantle (4.2 ± 1.0), gills (2.5 ± 0.6), foot (1.9 ± 0.7), adductor muscle (1.4 ± 0.2) and shell (0.2 ± 0.1). The hepatopancreas showed the highest Se concentration, followed by the whole soft parts, with the shell having the lowest amount. The intracellular ^{75}Se distribution in hepatopancreas and mantle was displayed in Fig. 1. $^{75}\text{Selenium}$ occurred in all subcellular fractions, but the hepatopancreas cytosol showed the highest amount (about 40 % of ^{75}Se in the total homogenate) followed by nuclei and mitochondria (20 % total relative concentration). Less than 10 % was found in lysosomes and microsomes. In the mantle, more than 50 % of ^{75}Se was found in the nuclear fraction and about 28 % in the cytosol. ^{75}Se content in mantle mitochondria was much

lower than in the hepatopancreas, accounting for only 5 % of the total homogenate.

The ^{75}Se content in rat tissues (wet wt, $n = 3$) after p.o. treatment with ^{75}Se -water spiked *S. obliquus* was as follows in decreasing order in ng g^{-1} or ng mL^{-1} : kidney (0.71 ± 0.07), small intestine (0.35 ± 0.05), liver (0.35 ± 0.02), pancreas (0.17 ± 0.03), spleen (0.15 ± 0.01), large intestine (0.14 ± 0.02), stomach (0.14 ± 0.02), testis (0.14 ± 0.02), lung (0.13 ± 0.02), heart (0.09 ± 0.01), epididymis (0.08 ± 0.01) and brain (0.03 ± 0.01), blood (0.21 ± 0.03) (mainly composed of plasma and RBC), plasma (0.16 ± 0.01) and RBC (0.05 ± 0.02). For excreta (rat wet wt, $n = 3$), the ^{75}Se levels were $2.97 \pm 0.96 \text{ ng g}^{-1}$ in feces and $0.23 \pm 0.05 \text{ ng mL}^{-1}$ in urine. The highest ^{75}Se concentration was in the kidneys, whereas most of the elimination occurred via the feces. Similar trends were observed between the rat liver (Fig. 2) and mussel hepatopancreas (Fig. 1) in the distribution of ^{75}Se in the cellular compartments, with the cytosol containing the highest levels in both species. The rat liver cytosol contained about 45 % of the ^{75}Se that was present in the total homogenate. $^{75}\text{Selenium}$ was equally distributed between nuclear and mitochondrial fractions ($\approx 20 \%$). Compared to its distribution in the liver, its concentration in kidneys increased up to 50 % in the nuclear fraction and decreased to 20 % in the cytosolic fraction.

$^{75}\text{Selenium}$ content in rat tissues after feeding them with mussels that had accumulated ^{75}Se from ^{75}Se -spiked water ($n = 3$, doses per rat: $2,050 \pm 97 \text{ ng Se}$ in $8.63 \pm 0.83 \text{ g}$ mussel flesh) exhibited the following decreasing concentrations (ng g^{-1} , wet wt, or ng mL^{-1}): kidney (18.8 ± 0.52), small intestine (10.4 ± 1.52), liver (7.82 ± 0.74), lung (3.15 ± 0.04), stomach (2.74 ± 0.24), spleen

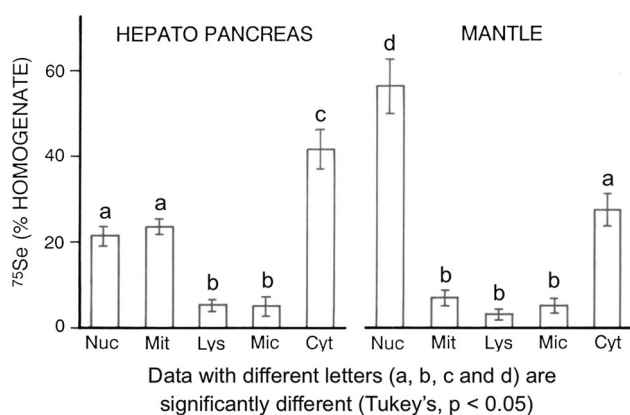


Fig. 1 Intracellular distribution of ^{75}Se in the hepatopancreas and mantle of mussels 24 h after the administration of *S. obliquus* grown in ^{75}Se -spiked water. Results expressed as % of radioactivity of the total respective homogenate \pm SD. Nuc nuclear fraction, Mit mitochondrial fraction, Lys lysosomal fraction, Mic microsomal fraction, Cyt cytosolic fractions

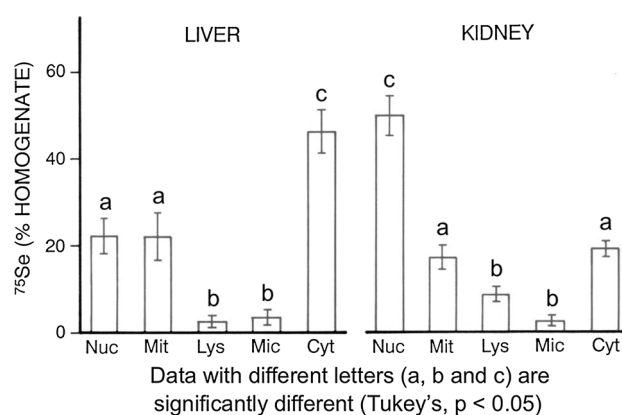


Fig. 2 Intracellular distribution of ^{75}Se in the liver and kidney of rats 24 h after p.o. administration of *S. obliquus* grown in ^{75}Se -spiked water. Results expressed as % of radioactivity of the total respective homogenate \pm SD. Nuc nuclear fraction, Mit mitochondrial fraction, Lys lysosomal fraction, Mic microsomal fraction, Cyt cytosolic fractions

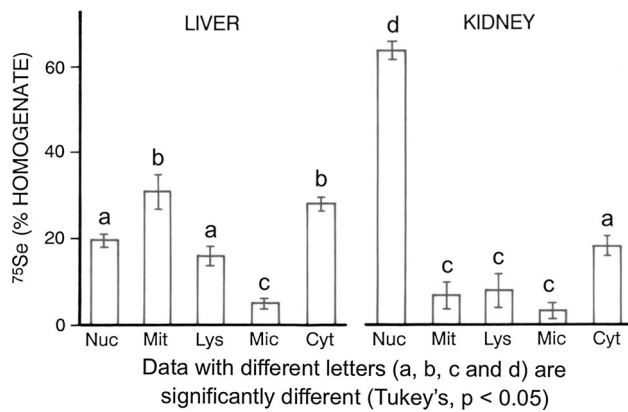


Fig. 3 Intracellular distribution of ⁷⁵Se in the liver and kidney of rats 24 h after the administration of *U. mancus* exposed to ⁷⁵Se-spiked water. Results expressed as % of radioactivity of the total respective homogenate ± SD. Nuc nuclear fraction, Mit mitochondrial fraction, Lys lysosomal fraction, Mic microsomal fraction, Cyt cytosolic fractions

(2.67 ± 0.08), testis (2.50 ± 0.09), heart (1.94 ± 0.08), epididymis (1.48 ± 0.12), pancreas (1.43 ± 0.75), large intestine (0.63 ± 0.32) and brain (0.36 ± 0.02), blood (5.96 ± 1.13), plasma (3.6 ± 0.67), and RBC (1.22 ± 1.14). Feces and urine contained 65.6 ± 5.6 ng g⁻¹ and 18.9 ± 1.74 ng mL⁻¹, respectively. Kidney and brain showed the highest and the lowest ⁷⁵Se content, respectively. It was verified that feces represented the main route of excretion. The intracellular ⁷⁵Se distribution in liver (Fig. 3) was slightly different from the one in rats fed with microalgae, since the amounts found in nuclei and cytosol were smaller than previously. Its distribution in the kidney was similar for both exposure routes, being lower only in the mitochondrial fraction (10 % of total homogenate) in Fig. 3.

The ⁷⁵Se content (ng g⁻¹ or ng mL⁻¹) in rat tissues 24 h after being fed with mussels contaminated by *S. obliquus* exposed to ⁷⁵Se-spiked water (105 ng Se mL⁻¹) was as follows in decreasing order: kidney (0.48 ± 0.02), small intestine (0.18 ± 0.04), liver (0.18 ± 0.02), lung (0.07 ± 0.01), stomach (0.08 ± 0.01), spleen (0.09 ± 0.02), testis (0.08 ± 0.01), heart (0.04 ± 0.01), epididymis (0.05 ± 0.02), pancreas (1.43 ± 0.75), large intestine (0.07 ± 0.01) and brain (0.01 ± 0.01), blood (0.12 ± 0.02), plasma (0.10 ± 0.02), and RBC (0.02 ± 0.01). For excreta, the feces and urine contained 1.02 ± 0.63 ng g⁻¹ and 0.29 ± 0.04 ng mL⁻¹, respectively. As previously found, kidney tissue showed the highest ⁷⁵Se concentration followed by liver, small intestine, testis and lung. Although the administered ⁷⁵Se doses were different in the three experimental treatments involving rats, its tissue uptake expressed as dose percentage did not significantly vary, presenting little standard deviation values as well. The

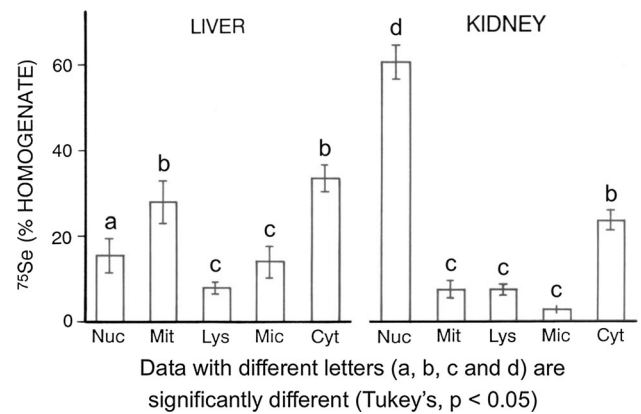


Fig. 4 Intracellular distribution of ⁷⁵Se in the liver and kidney of rats 24 h after the administration of *U. mancus* previously fed with *S. obliquus* grown in ⁷⁵Se-spiked water. Results expressed as % of radioactivity of the total respective homogenate ± SD. Nuc nuclear fraction, Mit mitochondrial fraction, Lys lysosomal fraction, Mic microsomal fraction, Cyt cytosolic fractions

intracellular distribution pattern as displayed in Fig. 4 was similar to the one obtained in Fig. 3.

After feeding mussels with ⁷⁵Se-microalgae, their hepatopancreas showed the highest Se concentration. This is in contrast to the observation on mussels exposed to inorganic ⁷⁵Se as simple selenite ions (Polettini et al. 2014). Indeed, the gills were the main targets presenting the highest content, suggesting the possible existence of an indirect interaction on how ⁷⁵Se would be incorporated in gills after intestinal absorption of the element, differentiating the manner by which it is taken up. Water and dietary exposures were shown to accumulate in distinct target tissues differently, indicating variable ⁷⁵Se bioavailability. In mussels, the ⁷⁵Se intracellular distribution in both hepatopancreas and mantle (Fig. 1) showed that it was distributed among the fractions in a different way than after exposure to ⁷⁵Se in the form of simple inorganic selenite ions (Polettini et al. 2014). In the hepatopancreas, the mitochondrial fraction contained nearly twice the amount. In the mantle, the ⁷⁵Se content of the nuclear fraction was above 50 % of total homogenate, whereas it was about 30 % when considering the exposure just to the inorganic element (Polettini et al. 2014). This could suggest that the element in the mussel mantle may be bound more strongly when it is taken in via the diet, such as in this case of ⁷⁵Se-*S. obliquus*.

Data from Table 1 showed that in rats fed with ⁷⁵Se-microalgae (water exposed) or with ⁷⁵Se-mussels (exposed via feeding with ⁷⁵Se-microalgae), the distributions of ⁷⁵Se were in both cases similar to that of inorganic Se (Polettini et al. 2014). Thus, rats seemed to be able to metabolize various forms of Se, as it was additionally demonstrated by its presence in urine.

Table 1 Selenium content in rat tissues and excreta 24 h after treatment with: (A) inorganic ^{75}Se ($5 \mu\text{g rat}^{-1}$); (B) *S. obliquus* after contamination with ^{75}Se -spiked water ($45 \text{ ng Se-microalgae rat}^{-1}$); (C) *U. mancus* contaminated with ^{75}Se -spiked water ($2 \mu\text{g Se-mussel rat}^{-1}$); (D) *U. mancus* fed microalgae grown in ^{75}Se -spiked water ($28 \mu\text{g Se-mussel rat}^{-1}$)

Tissue/excreta	% Dose g^{-1} or mL^{-1} wet weight (mean \pm SD)			
	A ^a	B ^a	C ^a	D ^a
Kidney	1.8 \pm 0.5	2.1 \pm 0.4	0.1 \pm 0.2	1.8 \pm 0.5
Liver	2.5 \pm 1.2	1.3 \pm 0.8	0.4 \pm 0.1	0.7 \pm 0.1
Testis	0.30 \pm 0.01	0.40 \pm 0.02	0.10 \pm 0.01	0.30 \pm 0.02
Epididymis	0.20 \pm 0.01	0.30 \pm 0.02	0.10 \pm 0.01	0.30 \pm 0.02
Brain	0.10 \pm 0.01	0.10 \pm 0.01	0.02 \pm 0.01	0.10 \pm 0.01
Heart	0.30 \pm 0.02	0.20 \pm 0.02	0.10 \pm 0.02	0.10 \pm 0.02
Lung	0.50 \pm 0.01	0.30 \pm 0.01	0.20 \pm 0.01	0.30 \pm 0.01
Spleen	0.50 \pm 0.05	0.30 \pm 0.01	0.10 \pm 0.02	0.30 \pm 0.01
Stomach	0.5 \pm 0.1	0.5 \pm 0.1	0.10 \pm 0.03	0.3 \pm 0.1
Small intestine	0.7 \pm 0.1	0.8 \pm 0.1	0.5 \pm 0.1	0.6 \pm 0.1
Large intestine	0.6 \pm 0.3	0.3 \pm 0.1	0.4 \pm 0.2	0.3 \pm 0.9
Blood ^b	0.6 \pm 0.3	0.5 \pm 0.3	0.3 \pm 0.1	0.4 \pm 0.4
Plasma ^c	63.7 \pm 4.3	82.0 \pm 6.2	83.5 \pm 5.3	89.5 \pm 5.6
RBC ^c	36.3 \pm 4.3	18.0 \pm 6.2	16.1 \pm 5.3	10.5 \pm 5.6
Feces	1.8 \pm 0.7	6.6 \pm 2.2	3.3 \pm 1.0	3.6 \pm 1.5
Urine	0.1 \pm 0.1	0.5 \pm 0.2	1.0 \pm 0.3	1.0 \pm 0.3

^a Mean of: (A) 5; (B) 3 (C); 3; and (D) 9 animals

^b % in the total blood

^c % of the blood

In mussels fed with ^{75}Se -microalgae, the intracellular Se distribution in both hepatopancreas and mantle was very similar to the concentration in liver and kidney of rats fed with the same mussels. It could be supposed that rats were able to dissociate larger ^{75}Se -containing molecules at the stomach pH, so that after intestinal absorption these compounds would have similar metabolic pathways in the body. In this case, its amount was lower than in the case of exposure to by ^{75}Se -spiked water, indicating that ^{75}Se incorporated into mussels was not readily available to rats. This could also be appraised by its intracellular distribution in both liver and kidney, showing a different pattern mainly in the nuclear and cytosolic fractions of the liver, where the ^{75}Se amount was lower than before. Interestingly, the relative distribution of ^{75}Se between RBC and plasma was different in the case of exposure of rats directly to inorganic selenite compared to those of animals fed with metabolized ^{75}Se (Table 1). A significantly higher amount of Se within 10–18 % was recovered in RBC of rats treated with the inorganic form (more than 35 % of Se in whole blood), compared to that of animals treated with metabolized ^{75}Se . This suggested that the dissociation in the stomach of the ^{75}Se in its metabolized form(s) might lead to bioavailable Se species, which would interact with blood components in a different way from the Se absorbed after exposure of rats to the simple inorganic selenite form.

From a quantitative point of view, the ^{75}Se transfer up the model food chain was summarized in Fig. 5 considering the average whole content in water per unit volume and the following average uptake per unit mass (wet wt) in *S. obliquus*, *U. mancus* and *R. norvegicus* including all tested

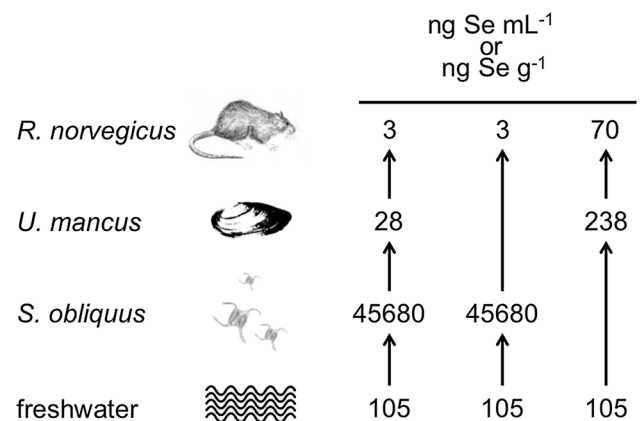


Fig. 5 Uptake of ^{75}Se -selenite in the selected phytoplankton-mussel-rat food chain model; numbers represent the average whole amount of ^{75}Se present in water per unit volume or uptaken by the target biological model per unit mass (wet wt)

scenarios. The trophic transfer of Se presented its main critical step between *S. obliquus*–*U. mancus*. The ^{75}Se partitioned from water (culture medium) to microalgae showing a bioconcentration factor of 435. Conversely, from microalgae to mussels and subsequently to rats no bioaccumulation was verified. Rats fed with mussels exposed to ^{75}Se -spiked water presented a higher rate of ^{75}Se accumulation compared to its uptake through the whole food chain (microalgae-mussel-rat). This study evidenced the existence of some preferential target organs in rats for Se bioaccumulation such as liver, kidney, intestine, lung and spleen (Table 1). Selenite gastro-intestinal absorption in rats has been shown to be up to 92 %

(WHO 1987). Thus, when it is taken up through the diet, Se is readily bioavailable and can partition into specific target organs. However, ^{75}Se was not subjected to bioaccumulation/biomagnification in the modelled food chain system.

According to Fig. 5, it was evidenced that the bioconcentration of Se is more likely to occur than bioaccumulation. As reported by Luoma and Presser (2009), trophic transfer factors can be more variable among invertebrate species than among fishes mainly due to physiological distinctions in assimilation efficiency and variable elimination rate constants for Se. Thus, this makes the ecological risk characterization of Se in aquatic environments difficult, as may be seen in the regulatory requirements for water quality of various countries worldwide (Luoma and Presser 2009). This study has confirmed observations from previous studies (e.g., Lemly 1985; Luoma et al. 1992; Wang et al. 1996) that Se uptake in animals through bioaccumulation is slow, but faster with aquatic microalgae due to Se bioconcentration from water (Poletini et al. 2014). As suggested by Luoma and Presser (2009), the environmental impact of Se is really species-specific. High exposures of organisms in the environment are determined more by their feeding habits and the ability of potential aquatic or terrestrial species that serve as food to bioconcentrate Se, rather than bioaccumulation of the element through the food chain.

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