Electronic Supplementary Material (ESI) for Metallomics

Electronic Supplementary Information

Absorption, distribution, metabolism and excretion of selenium following oral administration of elemental selenium nanoparticles or selenite in rats

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Table S1 Instrumental settings for chromatographic separations of Se species

Anion exchange HPLC	
Column	ICSep ION-120, 120 mm x 4.6 mm x 5 µm (Transgenomic, San Jose, CA, USA)
Mobile phase	5 mM salicylate, 3% MeOH in water, pH 9.5 (isocratic)
Flow rate (mL/minute)	1.1
Injection volume (µL)	25
Reversed-phase HPLC	
Column	Hypersil Gold, 250 mm x 2.1 mm x 5 µm
Mobile phase	200 nM ammonium acetate in 5 % MeOH in water
Flow rate (mL/minute)	0.2
Injection volume (µL)	5
Cation exchange HPLC	
Column	Dionex IonPac CG5, 4 x 50 mm and Dionex IonPac GS5A, 4 x 250 mm
Mobile phase	10 mM oxalic acid, 20 mM pottasium sulfate in 2 % MeOH in water (pH 3)
Flow rate (mL/minute)	0.3
Injection volume (µL)	5
Heparin-affinity HPLC	
Column	Shodex AF pak HR-894, 50 mm x 10 mm x 10 µm
Mobile phase A	50 mM ammonium formate in 3 % MeOH in water
Mobile phase B	1.5 M ammonium formate in 3 % MeOH in water
Gradient	100 % B: 0-1 minute and 100 % A: 2-7 minutes
Flow rate (mL/minute)	1.0
Injection volume (µL)	25
Post-column isotope dilution:	
^{77}Se abundance (%)	99.2
Se concentration (µg/L)	10
Flow rate (g/minute)	0.3 (determined daily by weighing)

Table S2 The relative amounts of *Se*-methylseleno-*N*-Acetyl-galactosamine (SeGalNAc) and trimethylselenonium-ion ($TMSe^+$) in rat urine determined as the ratio of chromatographic peak areas to the total area of chromatogram. nd means not detectable

	0.05 mg/kg bw/day Se^0NP	0.5 mg/kg bw/day Se^0NP	0.05 mg/kg bw/day Se(IV)	0.5 mg/kg bw/day Se(IV)	Control (BSA)	Control (water)
% SeGalNAc						
	68.6	5,7	62.1	3.5	70.1	77.5
	74.0	30.1	79.1	54.8	73.7	71.7
	75.7	16.5	74.2	12.4	77.4	77.5
Mean ± s.d.	72.8 ± 3.7	17.4 ±12.2	71.8 ± 8.7	23.6 ± 27.4	73.7 ± 3.7	75.6 ± 3.4
% $TMSe^+$						
	nd	51.5	nd	74.1	nd	nd
	nd	39.1	nd	23.4	nd	nd
	nd	68.4	nd	66.8	nd	nd
Mean ± s.d.		53.0 ± 14.7		54.8 ± 27.4		

Fig S0. Antion exchange HPLC-ICP-DRC-MS chromatogram of $SeSO_3^{2-}$ yielded from a sulfite-treated rat liver from a high-dosed animal (0.5 mg/kg bw/day). Se(VI) was spiked to sample homogenate as calibrant.

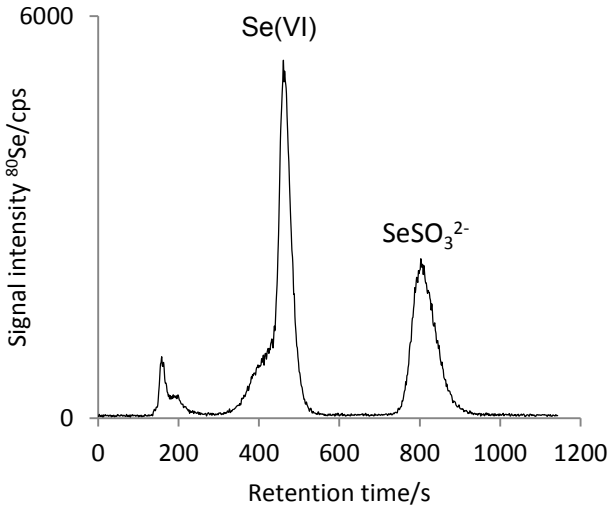

Fig. S1 (A) Effect of treatment time on yield of $SeSO_3^{2-}$ by reaction between sulfite and a range of Se^0NPs concentrations, and (B) Relative recovery of Se^0 as $SeSO_3^{2-}$ (150 ng Se) at different times after ended treatment of biological samples with sulfite for 20 minutes at 50°C. See Materials and Methods section for further method details.

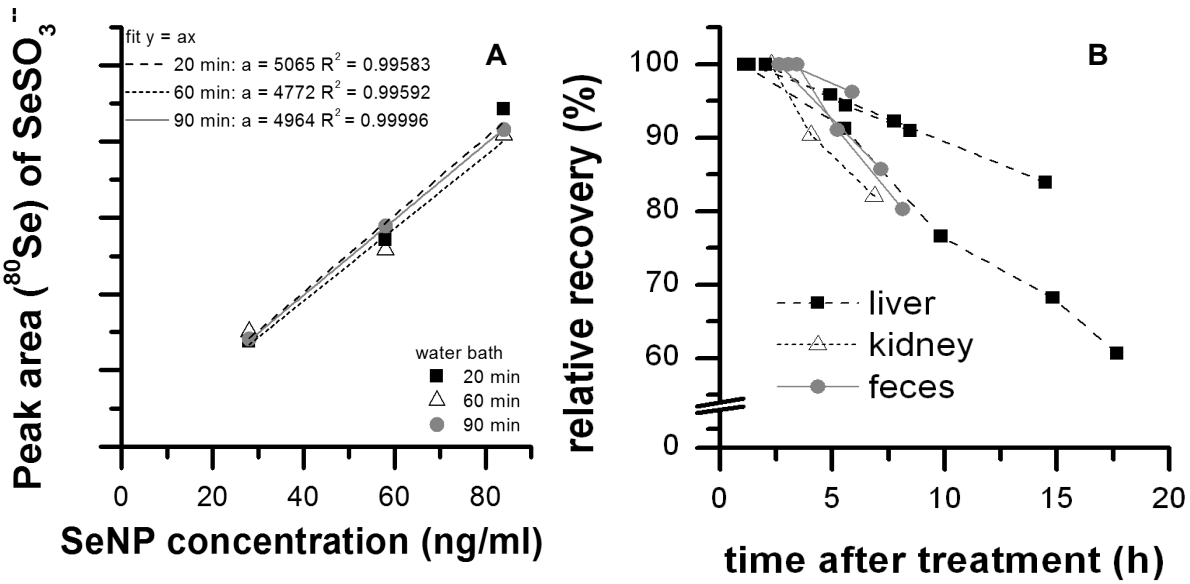

Fig. S2. Peak areas of the anion-exchange HPLC-ICP-MS peak corresponding to $SeSO_3^{2-}$ for Se^0NPs spiked to ultra-pure water, liver homogenates from animals from the control or low-dose groups. The absolute recovery of $SeSO_3^{2-}$ (as Se) using calibration against co-chromatographed Se(VI) standards following spiking with 150 ng of SeNPs to liver, kidney and feces homogenates were (mean ± s.d.) 64 ± 12% (N=22) for liver, 72 ± 13 % (N=9) for kidney and 68 ± 12 % (N=7) for feces homogenates.

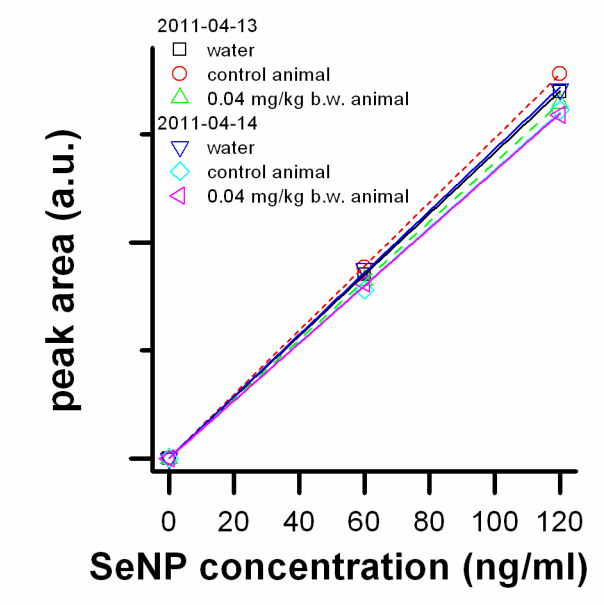

Fig. S3 Heparin-affinity HPLC-ICP-DRC-MS chromatogram of rat plasma using isotope dilution quantification by enriched ^{77}Se and using ^{80}Se as reference isotope

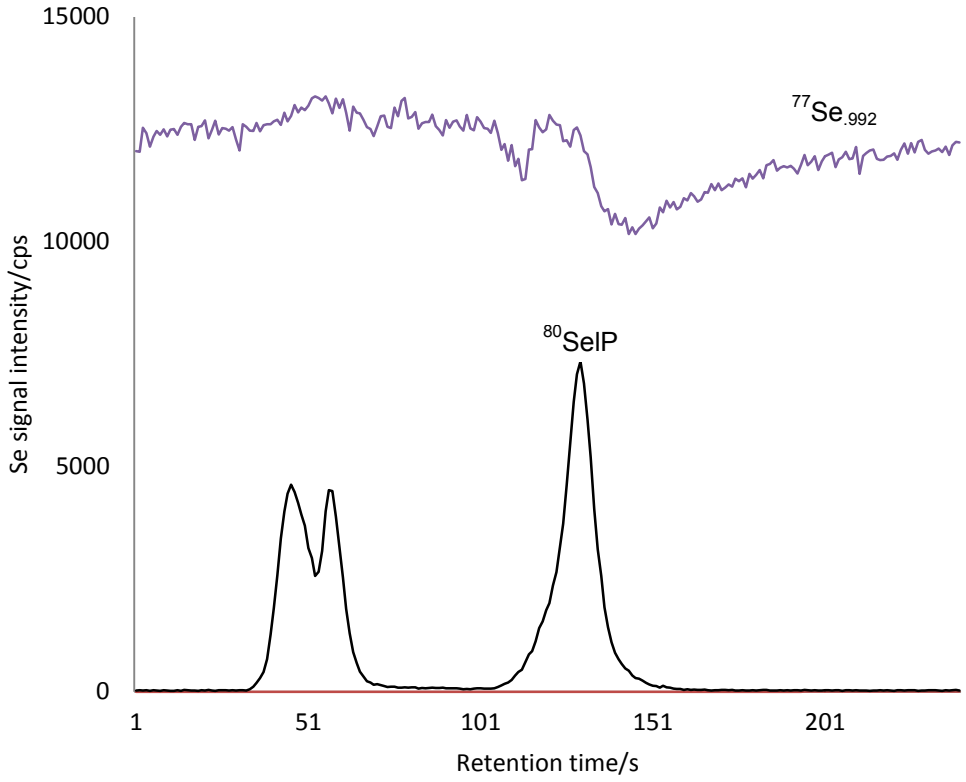

Fig. S4. HPLC-ICP-MS chromatograms of rat urine collected from animals receiving Se^0NPs (0.5 mg/kg bw/day). Upper: RP-HPLC-ICP-MS, lower: CX-ICP-MS. Peak identities: *Se*-methylseleno-*N*-Acetyl-galactosamine (SeGalNAc) and trimethylselenonium-ion ($TMSe^+$).