Supplementary information for: Extraction, quantification and characterization of mercury and selenium containing nanoparticles in seal livers using single particle inductively coupled plasma time of flight mass spectrometry (spICP-ToF-MS)

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Table S1: Samples of marine mammals from Northern Quebec: Seal species information.

Sample ID	Age	Species	Sex	Location
Carole H1	3 years	Unknown	Unknown	3 km south of Puvirnituq, Hudson Bay
Carole H2	8-9 months	Unknown	Unknown	2 km north of Puvirnituq, Hudson Bay
Ringed	Young (age unknown)	Ringed seal	Female	Near Marralik River, Kangiqsualujjuaq, Ungava Bay
Bearded	Unknown	Bearded seal	Male	Near Marralik River, Kangiqsualujjuaq, Ungava Bay

Figure S1: Sampling location of the Bearded seal (*Erignathus barbatus*) near Marralik River, Kangiqsualujjuaq, Ungava Bay area.

Table S2: Operating conditions for the ICP-ToF-MS and for the ICP-MS. ICP-ToF-MS was run from 60-210 amu.

Technique	**Nebulizer gas flow rate** (mL min^{-1})	**Auxiliary gas flow rate** (mL min^{-1})	**Cooling gas flow rate** (mL min^{-1})	**Reaction/ collision gas**	**Sample flow rate** (µL min^{-1})	**Dwell time** (µs)	**Acquisition time** (s)	**Transport efficiency** (µL s^{-1})
ICP-ToF-MS	1050	2000	13000	He: 17 mL min^{-1} H_2: 5 mL min^{-1}	450	71.97-192	60	0.352± 0.120
ICP-MS	980	1200	16000	O_2: 600 mL min^{-1}	450	50000	20	-

Table S3: Recoveries of the certified reference materials (DOLT-5 for total Hg and total Se; SRM 2974a for MeHg). In addition, total mercury, total selenium and methylmercury (MeHg) concentrations were determined for selected seal livers

	Hg 202 (µg g^{-1})	**Se 82 (µg g^{-1})**	**MeHg (µg g^{-1})**
Recovery (%)	96.4	88.9	105
Carole H1	12.7±0.4	17.2±0.3	0.799
Carole H2	1.8±0.1	7.2±0.0	0.439
Ringed seal	4.8±0.2	6.6±0.2	1.217
Bearded seal	425.9±5.1	172.6±2.5	2.015

Text S1: Methylmercury (MeHg) analyses

Methylmercury (MeHg) analyses were conducted in the laboratory of Marc Amyot, Université de Montréal, Québec, Canada. Tissue samples were freeze-dried and homogenized before analysis following trace metal clean protocols. Liver tissue was analyzed by Cold Vapor Atomic Fluorescence Spectrometry (CV-AFS) with a Tekran 2700 using Environmental Protection Agency (EPA) method 1630[1] (Tekran Instruments Corporation, Seattle, WA, USA). To ensure analytical accuracy, the standard reference material SRM 2974a (mussel tissue, National Institute of Standards and Technology, USA) was analyzed alongside the samples. The measured recoveries of MeHg are reported in Table S3.

Table S4: Tested enzymatic and chemical digestion protocols.

Sample Mass	Solution Composition	Conditions	Post-treatment	References
20-100 mg	8 mL of 5 mM HEPES (pH 7.5), pancreatin and 1.5 mg mL^{-1} lipase (≥1,050 units mL^{-1})	15 min ultrasonication (21% amplitude), Pulse mode (50s on/10 s off), ice bath	Centrifuge 1000×g, 5 min	[2]
20-50 mg	3 mL of 50 mM ammonium bicarbonate (pH 7.4), 2 mg/mL proteinase K (≥ 60 units mL^{-1}) + 2 mL buffer (HEPES) with 4% SDS	2 min ultrasonication + 1 h sonication bath	Defatting with methanol, centrifuge 4400×g, 20 min	[3]
20-50 mg	5 mL of 50 mM ammonium bicarbonate (pH 7.4) + 5 mg mL^{-1} SDS, 1 mg mL^{-1} proteinase K (≥ 60 units mL^{-1})	37°C overnight (12-16 h)	Centrifuge 4000×g, 6 min	[4], [5]
20-50 mg	5 mL of 10% (v/v) TMAH + 0.01% Triton X-100	15 min ultrasonication	Centrifuge 1000×g, 5 min	[2]
250 mg	5 mL of 25% v/v TMAH + 20 mL Milli-Q water	Room temp 1h	Centrifuge 500×g, 3 min	[6]
20-50 mg	5 mL of 50% (v/v) formic acid+ 0.01% (v/v) Triton X-100	15 min ultrasonication	Centrifuge 1000×g, 5 min	[3]
75 mg	2 mL of 25% w/w TMAH + 8 mL (0.5% HNO_3 + 0.01% Triton X-100)	Room temp 12 h with constant mixing	Store at -20°C	[7]
20 mg	5 mL of Milli-Q water	15 minutes at 25% amplitude (pulse mode: 50s on, 10s off, ice bath)	Centrifuge 1000×g, 5 min	-
300 mg	300 mg Tris-HCl 10mM buffer + 2% SDS (3 mL)37°C for 1 hour Centrifuge 3500×g, 15 min	37°C for 1 hour	Centrifuge 3500×g, 15 min	[8], [9]
100 mg	0.1 M NaCl + 1 M 2-mercaptoethanol (10 mL)	Room temperature (~21°C) for 1 hour	Store at 4°C	[8]

Table S5: The number of mercury-containing particles that were obtained for each extraction method. Particle numbers (mL^{-1}) were determined using a transport rate of 0.3874 $\mu L\ s^{-1}$, with a total acquisition time of 180 s and a 100-fold dilution factor applied the samples.

Method	Hg particle	HgSe particle	Particle mL^{-1}	Se SDL (nm)	Hg SDL (nm)
HCOOH	2107	1707	$(5.5\pm0.1)\times10^6$	110.2	79.1
TMAH	246	77	$(4.6\pm0.8)\times10^5$	112.2	82.6
Proteinase & Lipase	50	99	$(2.2\pm0.1)\times10^5$	100.0	67.3
Lipase	280	178	$(6.5\pm0.2)\times10^5$	100.5	69.8
Pure water	254	112	$(5.3\pm0.1)\times10^5$	96.6	67.3

Text S2:

To validate the stoichiometric composition of the bulk HgSe nanoparticle powder used as a reference material, a total acid digestion was performed using a CEM Mars 5 microwave digestion system following EPA Method 3051. Approximately 80-90 mg of HgSe powder was accurately weighed and transferred into Teflon digestion vessels. A mixture of concentrated acids (3 mL HNO_3 + 1 mL HCl) was added to each vessel to ensure complete digestion of the material. Following digestion, the clear solutions were quantitatively transferred to 50 mL volumetric flasks and diluted with ultrapure water (18.2 MΩ·cm). Further dilutions were prepared as needed for instrumental analysis in order to bring the concentrations within optimal analytical ranges for each instrument. A dual-instrument validation confirmed the 1:1 stoichiometric composition of the HgSe reference material:

Instrument	Se:Hg molar ratio
ICP-ToF-MS (Nu Vitesse)	1.096
ICP-MS (NeXION 5000)	0.990

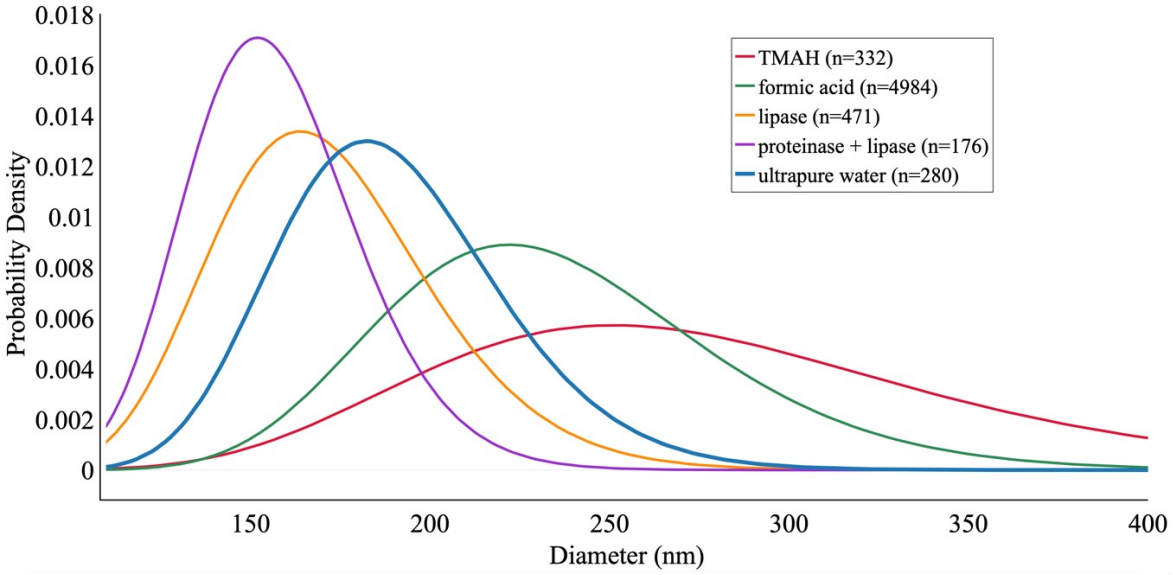

Figure S2: Size distribution of HgSe nanoparticles extracted from a Bearded Seal liver using five different extraction methods fitted to log-normal (TMAH (tetramethylammonium hydroxide): red, formic acid (HCOOH): green, lipase: orange, and proteinase + lipase: violet and ultrapure water: brown) (3 biological replicates, 3 analytical replicates per method). Under the assumption that the NP were spherical, particle size distributions were obtained by determining the diameter of a sphere (d) estimated from an assumed particle density (ρ) of 8.27 g cm^{-3} for HgSe with the particle mass determined from the sum of the detector counts. Numbers in parentheses represent the total particle numbers analyzed containing HgSe from all replicates.

Table S6: Sensitivity, limit of detection (LOD), limit of quantification (LOQ) for the ionic solution. Mass detection limits (MDL), mass quantification limits (MQL), size detection limits (SDL), and size quantification limits (SQL) of isotopes analyzed by spICP-ToF-MS from formic acid extraction.

Isotope	Sensitivity [cps/µg L^{-1}]	R^2	LOD [µg L^{-1}]	LOQ [µg L^{-1}]	MDL [fg]	MQL [fg]	SDL [nm]	SQL [nm]
^{80}Se	2530	0.9949	0.6	2.2	0.3	1.0	70.7	105.6
^{107}Ag	36428	0.9992	0.2	3.7	0.1	0.2	15.4	23
^{202}Hg	3230	0.9955	0.7	11.1	0.5	1.7	54.8	81.9
^{209}Bi	61100	1	0.12	8.09	0.1	0.2	20.7	31

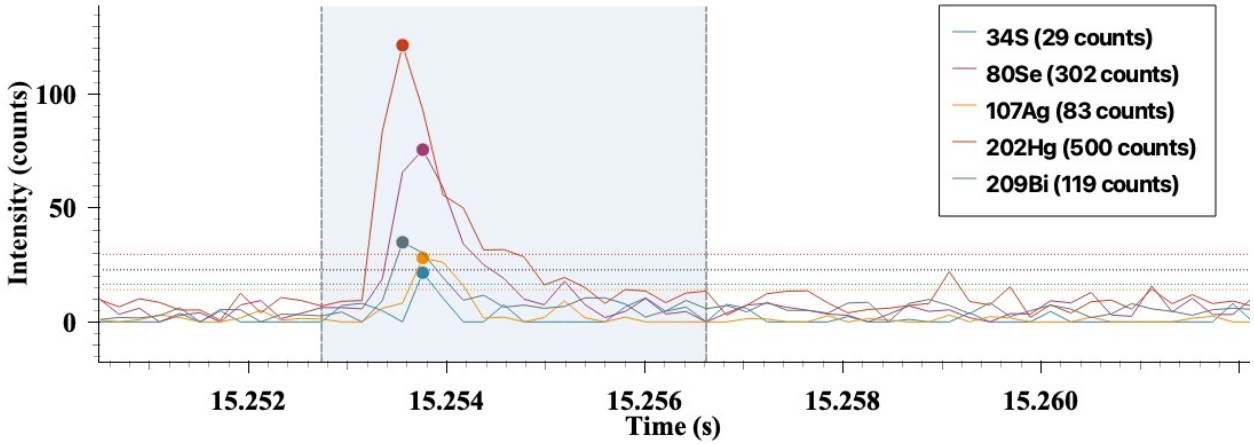

Figure S3: Transient mass spectrometry signal for sulfur (blue), selenium (purple), silver (orange), mercury (red), and bismuth (grey) containing nanoparticles by spICP-TOF-MS following an extraction using formic acid. Note ^{34}S, with a natural abundance of only 4.25%, was used for the detection of sulfur.

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