Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Material B

Supplementary Table B1: Metal concentrations in digestion blanks in human cohorts

Run	23 Na (µg/L)	24 Mg (µg/L)	39 K (µg/L)	44 Ca (µg/L)	55 Mn (µg/L)	56 Fe (µg/L)	63 Cu (µg/L)	66 Zn (µg/L)	78 Se (µg/L)
Manchester Cohort									
Run 1									
Digestion Blank 1	<0.00	0.37	0.34	<0.00	0.02	1.18	0.37	0.27	0.01
Digestion Blank 2	<0.00	0.69	11.05	1.23	0.03	1.47	0.38	0.90	0.001
Lowest Sample	6304.68	352.33	5770.44	212.75	0.66	76.76	5.93	42.14	0.42
% highest blank/ lowest sample	**<0.0000**	**0.20**	**0.19**	**0.58**	**4.37**	**1.91**	**6.47**	**2.14**	**1.21**
Run 2									
Digestion Blank 1	184.40	0.87	6.06	9.18	0.04	1.47	0.61	0.73	0.003
Digestion Blank 2	<0.00	0.36	2.48	5.02	0.02	0.69	0.27	0.71	<0.00
Lowest Sample	6127.83	317.39	5920.04	206.36	0.89	126.80	5.12	24.85	0.19
% highest blank/ lowest sample	**3.01**	**0.27**	**0.10**	**4.45**	**4.48**	**1.16**	**11.89**	**2.94**	**1.77**
Run 3									
Digestion Blank 1	2.06	<0.00	0.36	<0.00	0.02	0.59	0.21	0.66	<0.00
Digestion Blank 2	6.23	1.65	0.42	<0.00	0.03	2.87	0.44	0.56	<0.00
Lowest Sample	7208.63	405.63	7457.42	172.51	0.74	99.45	4.13	24.70	0.24
% highest blank/ lowest	**0.09**	**0.41**	**0.006**	**<0.0000**	**4.24**	**2.89**	**10.56**	**2.68**	**<0.0000**

sample

<0.000 indicates that concentration was lower than that of the calibration blank. The Manchester cohort was analysed across three runs. Each run included two digestion blanks, as well as tubes containing nitric acid and standards but not samples in order to determine background metal levels.

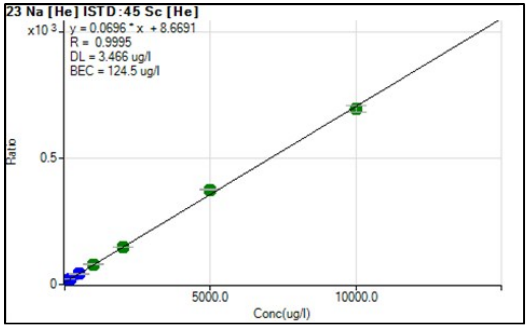
Supplementary Table B2: Detection limit, limit of quantitation and background equivalent concentration for each physiological metal measured in the human cohorts

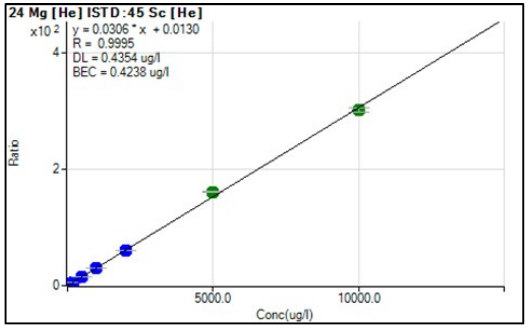
	23 Na (µg/L)	24 Mg (µg/L)	39 K (µg/L)	44 Ca (µg/L)	55 Mn (µg/L)	56 Fe (µg/L)	63 Cu (µg/L)	66 Zn (µg/L)	78 Se (µg/L)
Lowest Standard	50	50	50	50	0.5	50	0.5	0.5	0.5
Manchester Cohort Run 1									
DL	3.47	0.44	6.04	12.79	0.02	0.39	0.01	0.06	0.01
LOQ	50	50	50	100	0.5	50	2	4	0.5
BEC	124.54	0.42	51.56	8.31	0.02	0.46	0.07	0.57	0.02
Lowest Sample	6304.68	352.33	5770.44	212.75	0.66	76.76	5.93	42.14	0.42
Run 2									
DL	3.08	0.12	2.74	8.36	0.01	0.17	0.02	0.21	0.01
LOQ	100	100	100	100	1	100	0.5	4	1
BEC	59.81	0.42	46.02	6.86	0.01	0.24	0.09	0.41	0.01
Lowest Sample	6127.83	317.39	5920.04	206.36	0.89	126.80	5.12	24.85	0.19
Run 3									
DL	2.64	0.45	2.58	6.25	0.01	0.08	0.02	0.13	0.02
LOQ	50	50	100	400	0.5	50	0.5	4	1
BEC	29.28	0.53	40.73	73.01	0.00	0.20	0.06	0.13	0.02
Lowest Sample	7208.63	405.63	7457.42	172.51	0.74	99.45	4.13	24.70	0.24

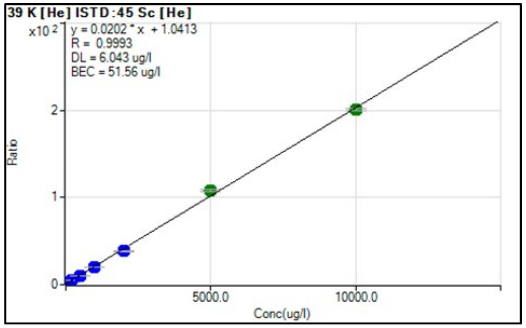
Lowest calibration standards analysed were 50µg/L for Na, Mg, K, Ca and Fe and 0.5µg/L for Mn, Cu, Zn, and Se. The software employed (Mass Hunter, Agilent) automatically calculated values for DLs (detection limits) and BECs (background equivalent concentrations) corresponding to each element analysed. LOQs (limits of quantitation) were calculated by comparison of calibration blanks and standards. Value shown for lowest sample are lowest raw measurements without correction for corresponding tissue mass. The lowest sample level of Se was lower than the LOQ in each run; however it remained well above the DL and so was retained for analysis. Average BEC was 3% of the lowest sample concentration. BECs were less than 10% for all metals in all runs with the exception of Ca in run three, which was not a significant finding in the analysis.

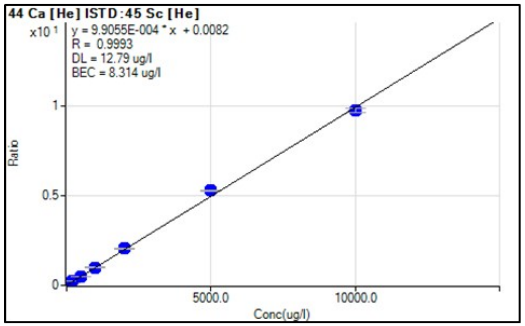
Supplementary Figure B1: Standard curves for each physiological metal measured in the human cohorts

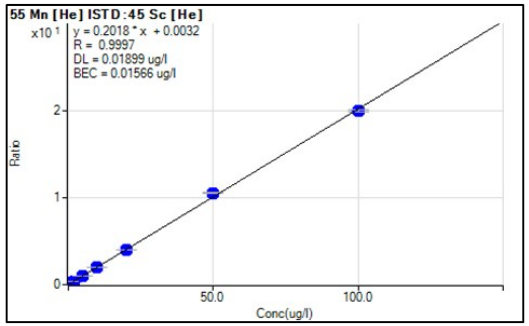
Manchester Cohort – Run 1

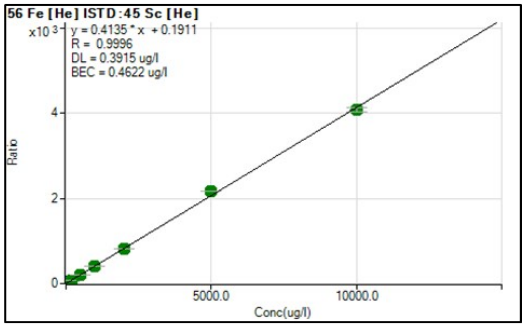


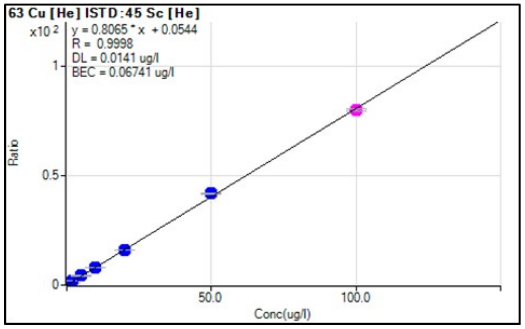


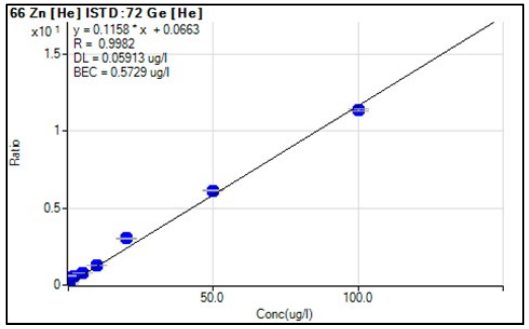


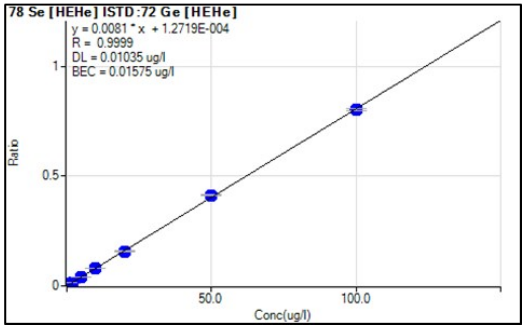




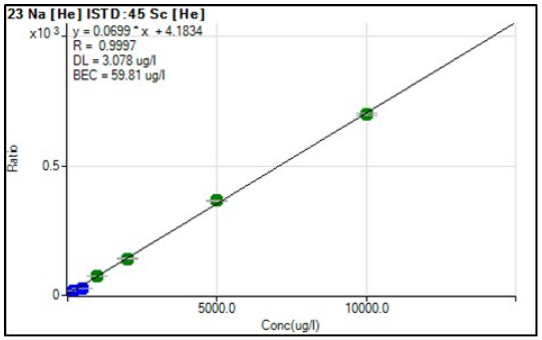


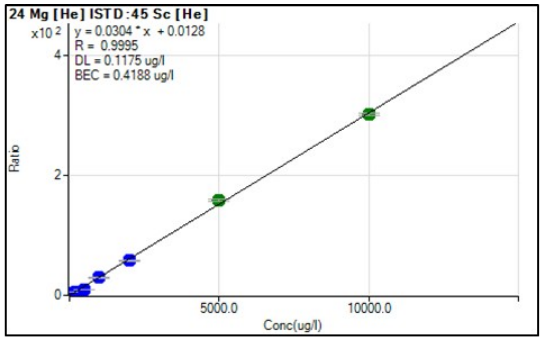


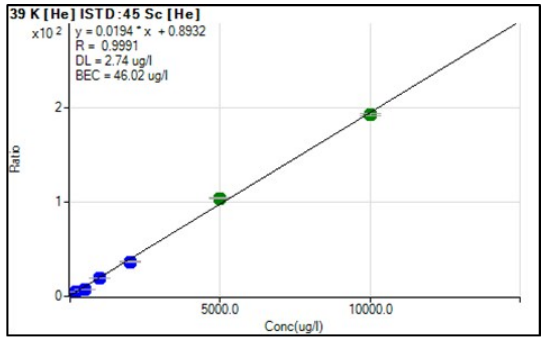


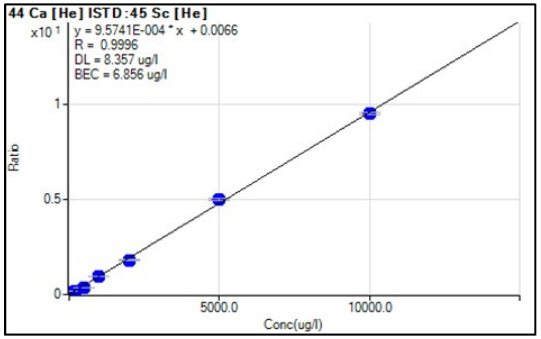


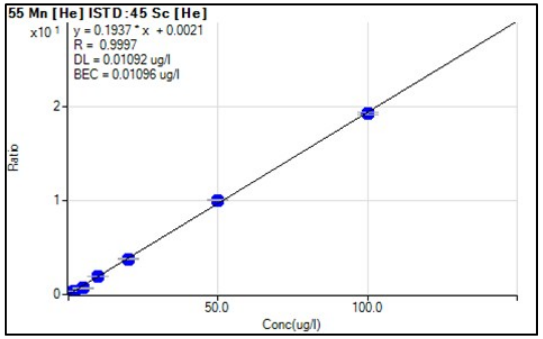
Manchester Cohort – Run 2

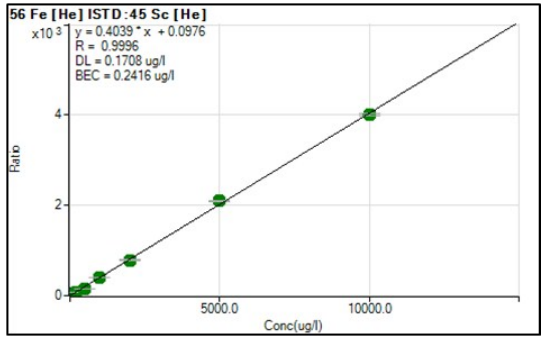


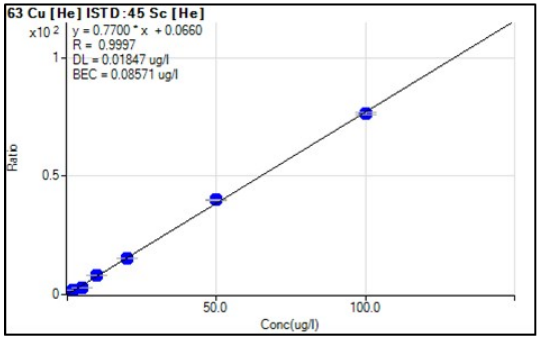


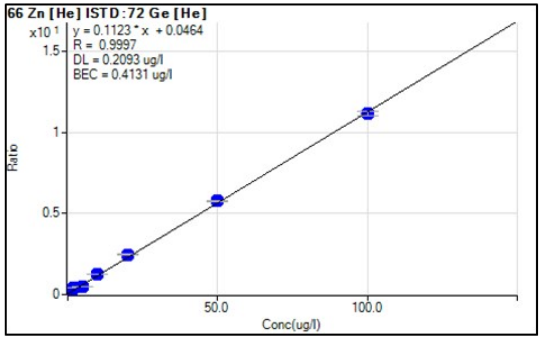


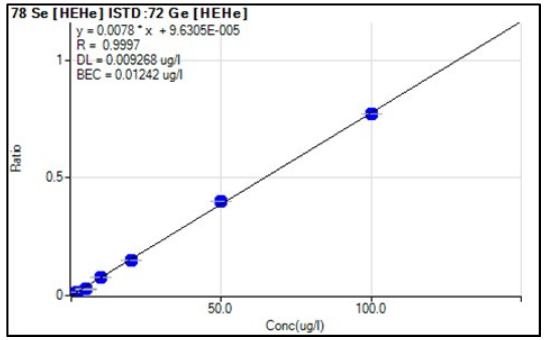




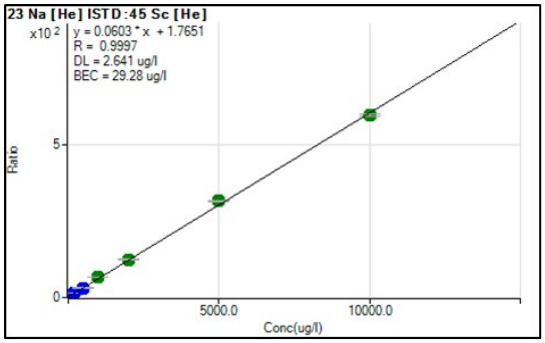


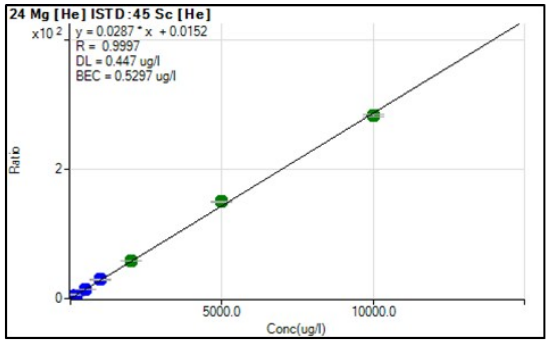


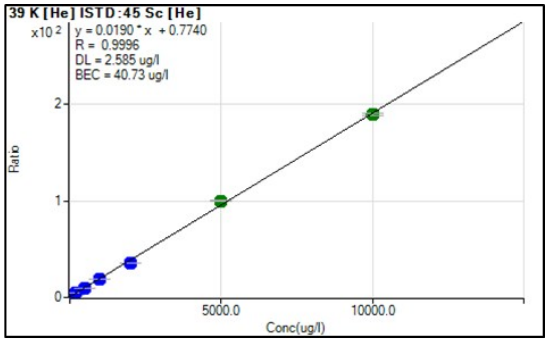


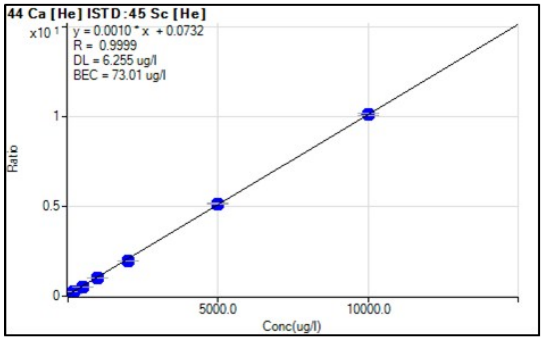


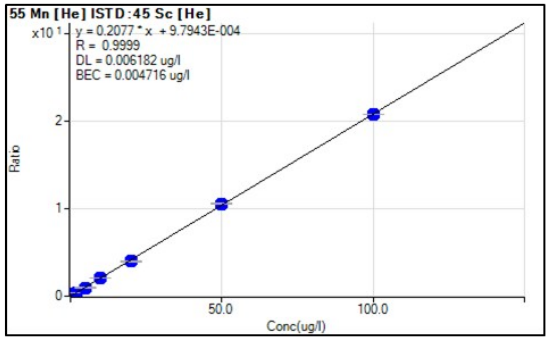
Manchester Cohort – Run 3

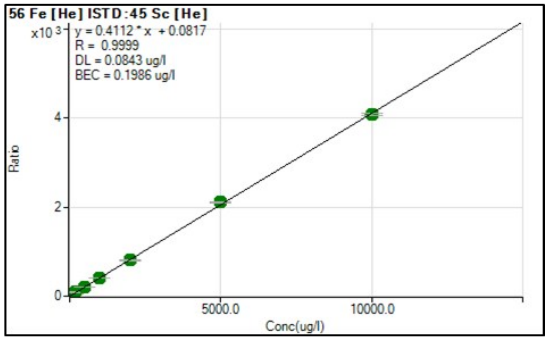


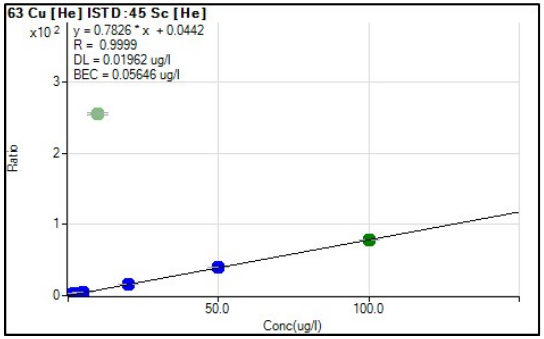


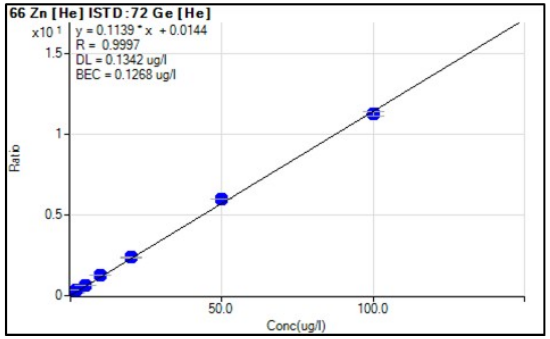


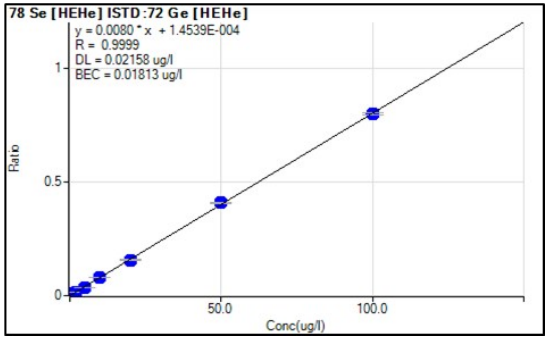












Supplementary Table B3: Metal concentrations in digestion blanks in rat cohort

Run	23 Na (µg/L)	24 Mg (µg/L)	39 K (µg/L)	44 Ca (µg/L)	55 Mn (µg/L)	56 Fe (µg/L)	63 Cu (µg/L)	66 Zn (µg/L)	78 Se (µg/L)
Rat Cohort									
Cortex									
Digestion Blank 1	<0.00	<0.00	6.19	<0.00	<0.00	<0.00	0.06	<0.00	0.01
Digestion Blank 2	<0.00	<0.00	14.94	<0.00	<0.00	<0.00	<0.00	<0.00	0.00
Digestion Blank 3	<0.00	<0.00	12.48	<0.00	<0.00	<0.00	0.09	<0.00	0.01
Lowest Sample	4560.41	587.18	14565.65	133.69	1.11	59.10	9.06	53.98	0.75
% highest blank/ lowest sample	**<0.00**	**<0.00**	**0.10**	**<0.00**	**<0.00**	**<0.00**	**0.95**	**<0.00**	**1.60**
Cerebellum									
Digestion Blank 1	16.27	0.29	20.99	12.41	0.03	1.94	<0.00	0.39	0.04
Digestion Blank 2	18.09	0.78	30.19	17.79	0.06	1.64	0.02	0.43	0.04
Digestion Blank 3	18.12	1.27	28.03	35.19	0.13	2.56	<0.00	0.71	0.03
Lowest Sample	5057.44	667.15	14064.77	194.52	1.84	88.51	8.53	42.46	0.76
% highest blank/ lowest sample	**0.36**	**0.19**	**0.21**	**18.09**	**7.28**	**2.89**	**0.19**	**1.67**	**5.70**

				Hippocampus					
Digestion Blank 1	<0.00	0.46	<0.00	13.81	<0.00	1.01	<0.00	<0.00	0.04
Digestion Blank 2	<0.00	0.51	4.54	33.25	<0.00	2.11	<0.00	0.07	0.04
Digestion Blank 3	<0.00	7.20	7.69	17.19	<0.00	0.86	<0.00	<0.00	0.04
Lowest Sample	4841.09	670.15	16212.70	212.22	1.74	69.26	8.90	49.61	0.85
% highest blank/ lowest sample	**<0.00**	**1.07**	**0.05**	**15.67**	**<0.00**	**3.05**	**<0.00**	**0.15**	**5.17**

<0.000 indicates that concentration was lower than that of the calibration blank. The Manchester cohort was analysed across three runs. Each run included two digestion blanks, as well as tubes containing nitric acid and standards but not samples in order to determine background metal levels. The highest concentration in a blank compared to the lowest concentration in a sample was for Ca in the rat cerebellum at 18.09%. In all but three incidences, blank concentrations were <5 % of the lowest sample concentration for all metals.

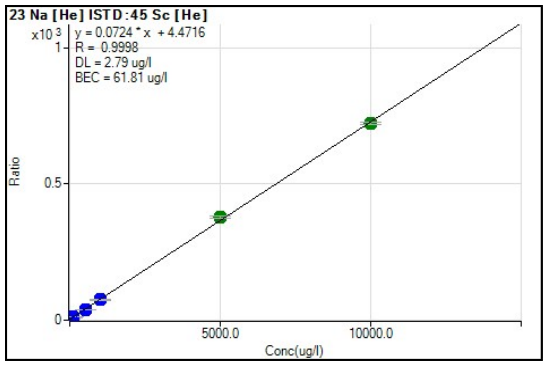
Supplementary Table B4: Detection limit, limit of quantitation and background equivalent concentration for each physiological metal measured in this study

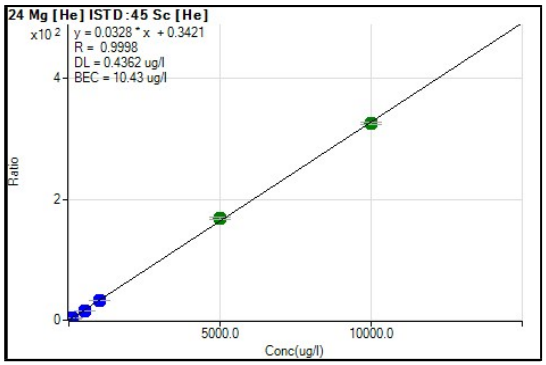
	23 Na (µg/L)	24 Mg (µg/L)	39 K (µg/L)	44 Ca (µg/L)	55 Mn (µg/L)	56 Fe (µg/L)	63 Cu (µg/L)	66 Zn (µg/L)	78 Se (µg/L)
Lowest Standard	50	50	50	50	0.5	50	0.5	0.5	0.5
Rat Cohort Cortex									
DL	2.79	0.44	4.38	33.94	0.18	1.06	0.04	0.11	0.01
LOQ	50	100	50	400	4	100	1	4	1
BEC	61.81	10.43	62.95	80.98	0.44	8.79	0.34	0.98	0.01
Lowest Sample	4560.41	587.18	14565.65	133.69	1.11	59.10	9.06	53.98	0.75
Cerebellum									
DL	3.55	0.82	5.77	4.21	0.01	0.62	0.06	0.04	0.05
LOQ	100	100	100	100	0.5	50	1	1	1
BEC	34.10	0.61	76.38	13.10	0.06	0.77	0.23	0.51	0.08
Lowest Sample	5057.44	667.15	14064.77	194.52	1.84	88.51	8.53	42.46	0.76
Hippocampus									
DL	2.72	0.06	4.48	9.64	0.07	0.47	0.06	0.07	0.02
LOQ	50	50	50	200	1	50	1	2	1
BEC	75.61	0.84	73.78	11.11	0.13	1.17	0.73	0.78	0.02
Lowest Sample	4841.09	670.15	16212.70	212.22	1.74	69.26	8.90	49.61	0.85

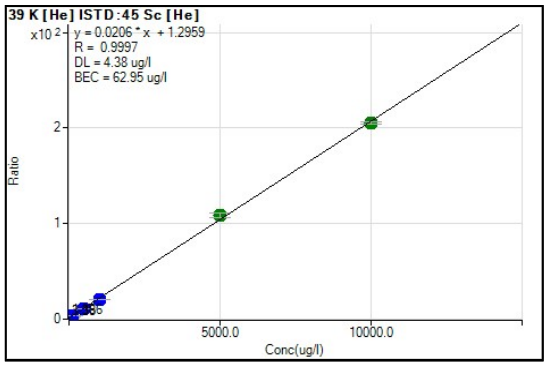
Lowest calibration standards analysed were 50µg/L for Na, Mg, K, Ca and Fe and 0.5µg/L for Mn, Cu, Zn, and Se. The software employed (Mass Hunter, Agilent) automatically calculated values for DLs (detection limits) and BECs (background equivalent concentrations) corresponding to each element analysed. LOQs (limits of quantitation) were calculated by comparison of calibration blanks and standards. Value shown for lowest sample are lowest raw measurements without correction for corresponding tissue mass. The lowest sample level of Se was lower than the LOQ in each run; however it remained well above the DL and so was retained for analysis. Average BEC was 7% of the lowest sample concentration. BECs were lower than 10% for all metals in all regions, with the exceptions of Ca, Mn, and Fe in the cortex, and Se in the cerebellum.

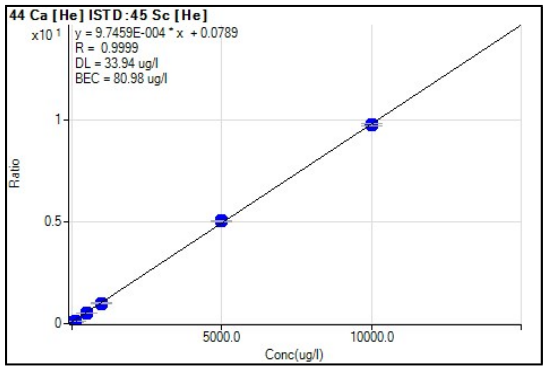
Supplementary Figure B2: Standard curves for each physiological metal measured in the rat cohort

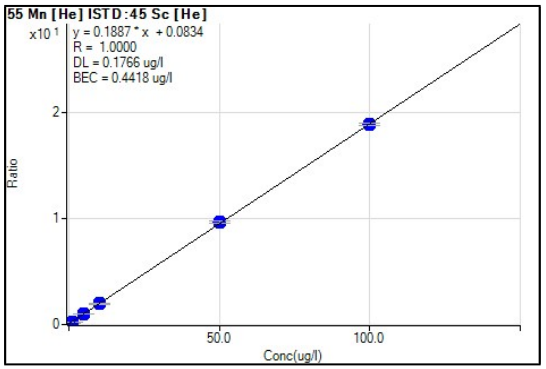
Rat Cortex

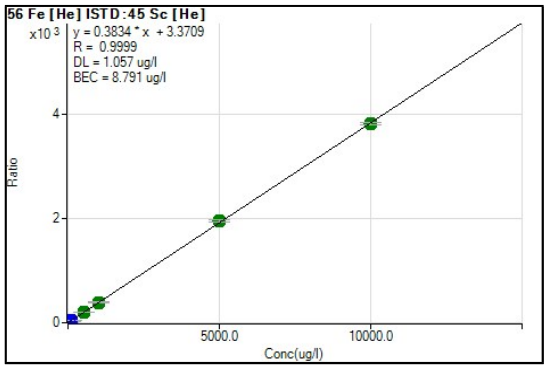


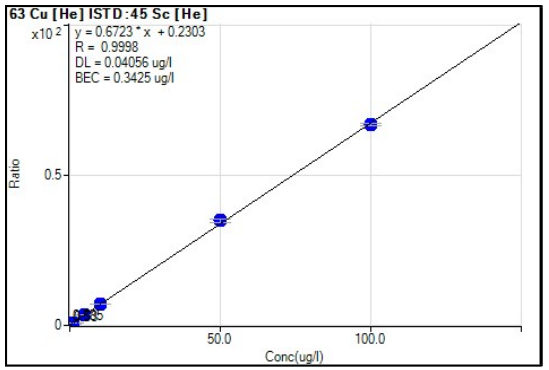


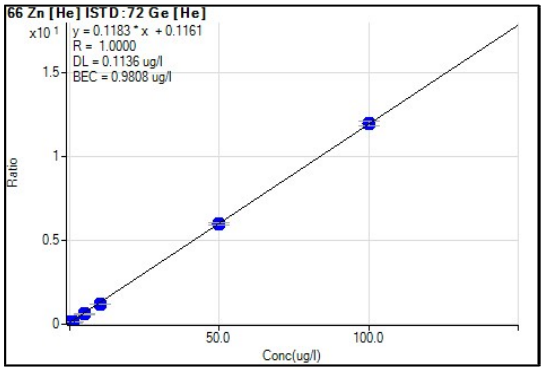


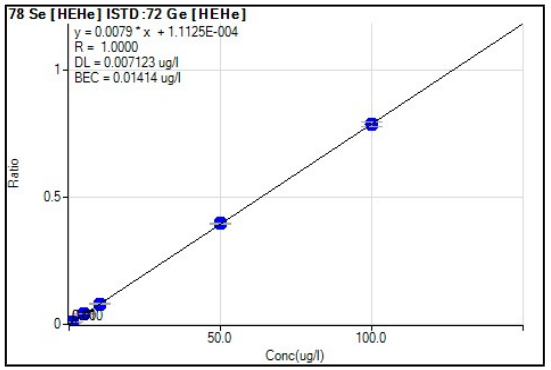




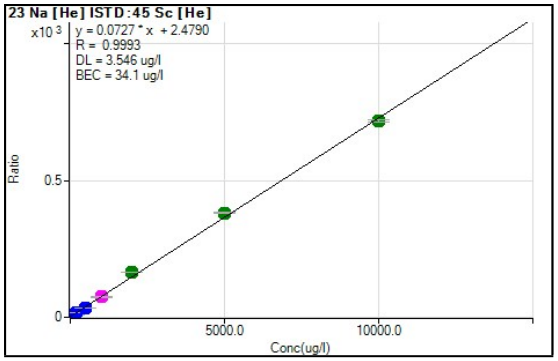


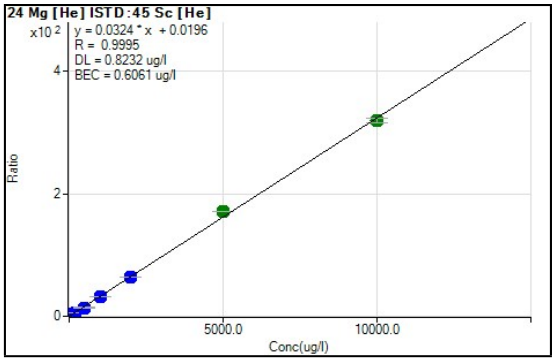


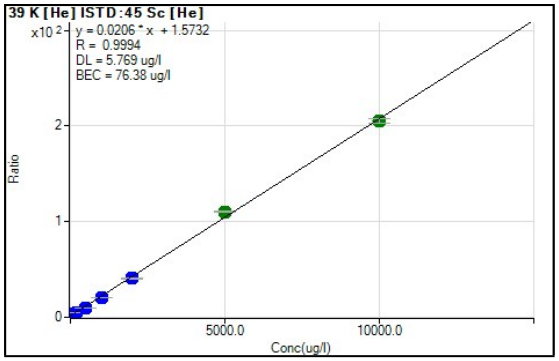


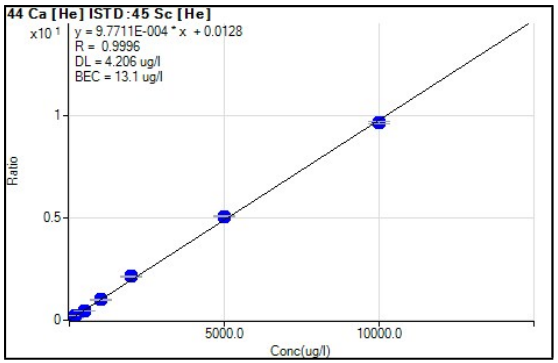


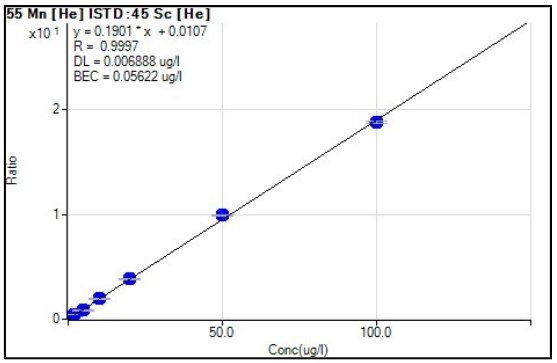
Rat Cerebellum

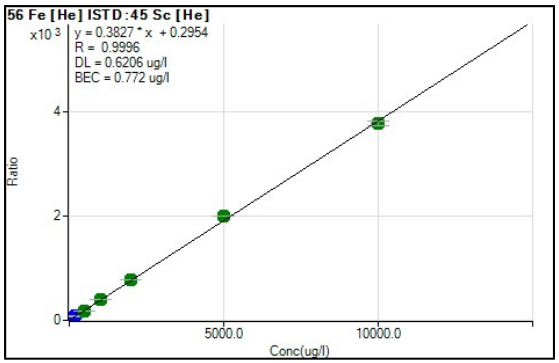


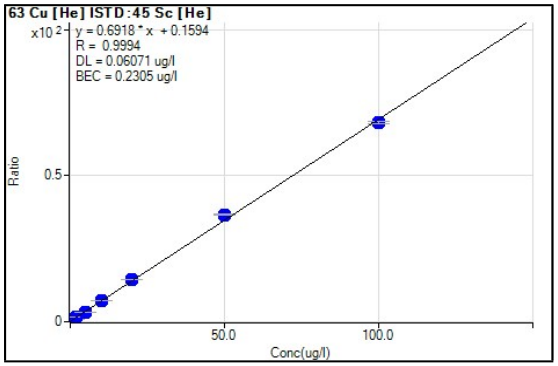


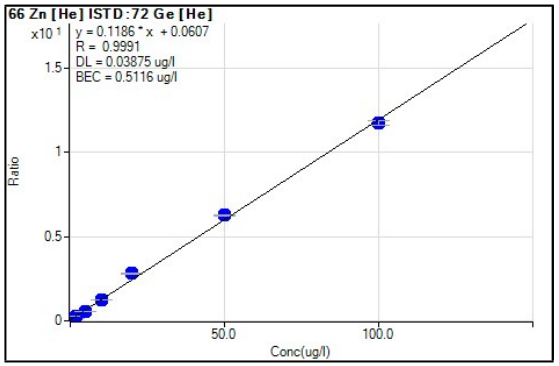


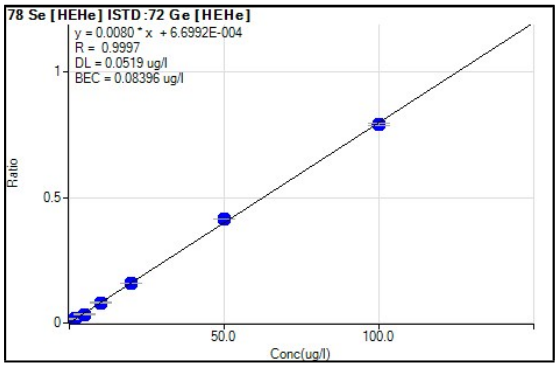




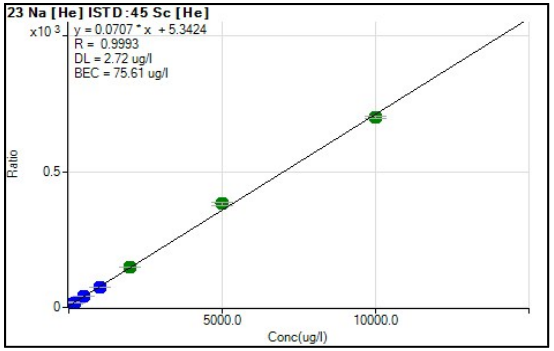


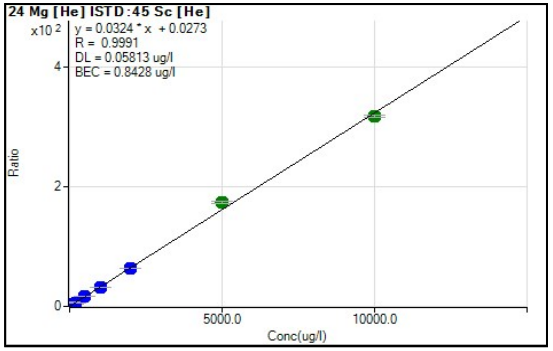


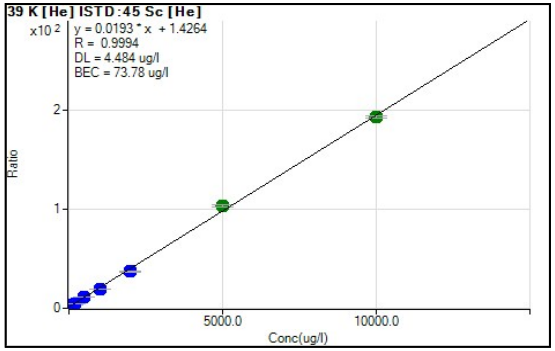


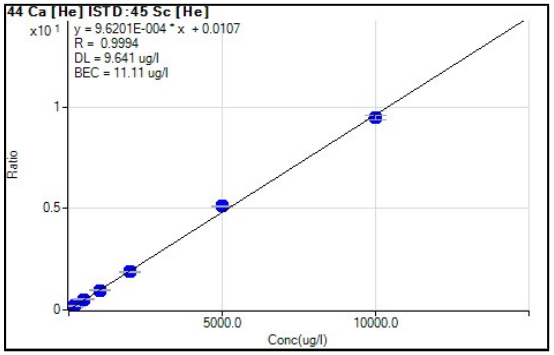


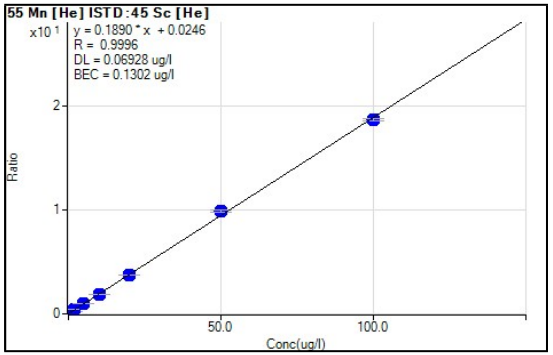
Rat Hippocampus

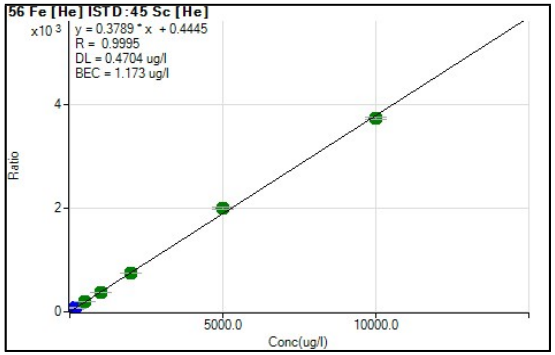


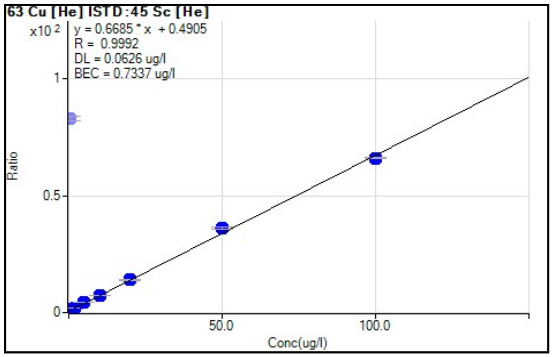


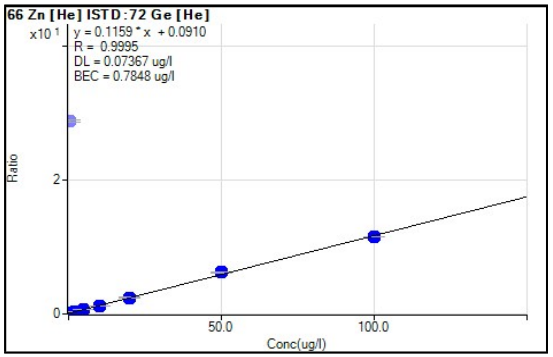


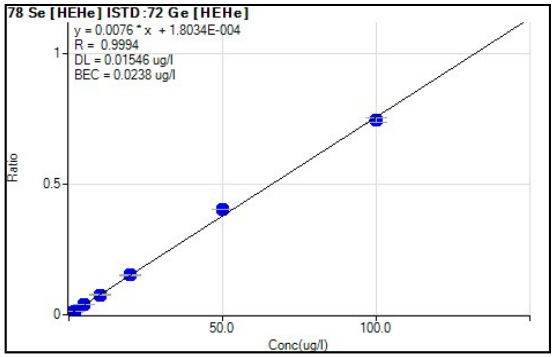












Supplementary Table B4: Metal concentrations in consumables & reagents

Consumable / Reagent	Concentration (µg/L)		
	23 Na [He]	63 Cu [He]	66 Zn [He]
1.5 ml Eppendorf tube	48.4	0.2	17.8
2 ml Eppendorf tube	**54.0**	**0.0**	**38.9**
50 ml falcon tube	6.6	-0.3	2.7
15 ml falcon tube	**39.3**	**2.1**	**11.0**
LC-MS grade water	**5.1**	**-0.5**	**-1.2**
Blue Nitrile Gloves	2978.2	0.3	2598.0
Orange Nitrile Gloves	**221.8**	**0.3**	**48.1**
Purple Nitrile Gloves	217.3	1.6	761.2
Red Nitrile Gloves	1540.3	0.6	6613.3
Pipette tip blue (1000 µl)	**36.9**	**2.2**	**12.2**
Glass vial	1441.0	1.9	108.1
Cyro vial	**59.0**	**2.5**	**9.0**

Values were obtained as follows: tubes/vials: 2% nitric acid placed in tubes/vials for three hours then analysed for Na, Cu, and Zn levels. Gloves: fingertip of gloves removed and placed in autosampler vial of 2% nitric acid for five minutes, followed by measurement of Na, Cu, and Zn in nitric acid. Pipette tips: pipette tips placed in 2% nitric acid in autosampler vial for five minutes, followed by measurements of Na, Cu, and Zn in nitric acid. These measurements constitute far higher exposure than regular usage of these consumables, but resultant measurements are still generally far lower than sample numbers (usually less than 10%). These tests were used to select orange nitrile gloves and cryo vials as optimal for use in the experiments.