

Supporting information:

Specific pathways of dietary methylmercury and inorganic mercury determined by mercury speciation and isotopic composition in zebra fish (*Danio rerio*).

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Experimental:

Reagents and standards

All solutions were prepared using ultrapure water (18 MΩ cm, Millipore). Optima acids (HNO₃ and HCl) from Fisher Scientific (Illkirch, France) were used for the preparation of all the samples, standards and blanks used in stable isotopic analyses. Mercury (Standard reference materials NIST-3133) and thallium standard solutions (Isotopic Standard for Thallium NIST-997)

were purchased from NIST (Gaithersburg, MD, USA). Tin chloride dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) was purchased from Sigma-Aldrich (Seelze, Germany).

Stock solutions ($1000 \text{ mg (Hg) L}^{-1}$) of iHg and MeHg of natural isotopic composition, used for speciation, were prepared by dissolving mercury (II) chloride (Strem Chemicals, USA) in 1% HNO_3 and MeHg chloride (Strem Chemicals, USA) in methanol (Sigma Aldrich, France), respectively. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions in 1% HNO_3 and stored at 4°C until use. $^{199}\text{HgCl}_2$ was prepared by dissolving ^{199}HgO (Oak Ridge National Laboratory, USA) in HCl (12 mol L^{-1}). $^{201}\text{MeHg}$ was synthesized from methylcobalamin and ^{201}HgO obtained from Oak Ridge National Laboratory (USA) according to the procedure previously described ⁴¹. The isotopic abundance of isotopes 199 and 201 in the ^{199}iHg and $^{201}\text{MeHg}$ tracers was 98 and 92%, respectively. In both cases the abundance of ^{202}Hg was lower than 1.5%. The exact concentrations of ^{199}iHg and $^{201}\text{MeHg}$ of the working solutions were determined by reverse isotope dilution analysis. Hg species were derivatized using sodium tetraethyl- or tetrapropyl-borate solutions prepared daily by dissolving NaBPr_4 (Merseburger Spezialchemikalien, Germany) in deionized water.

Hg stable isotopic analysis:

MDF is reported by using delta notation ($\delta^{202}\text{Hg}$) in per mil (‰) referenced to Hg standard NIST 3133, according to the following equation (Bergquist, et al, 2007):

$$\delta^{\text{xxx}}\text{Hg} = [({}^{\text{xxx}/198}\text{Hg}_{\text{sample}} / {}^{\text{xxx}/198}\text{Hg}_{\text{NIST 3133}}) - 1] \times 1000 \quad (1)$$

MIF signatures of ^{199}Hg and ^{201}Hg are calculated as follow:

$$\Delta^{199}\text{Hg} = \delta^{199}\text{Hg} - \delta^{202}\text{Hg} \times 0.252 \quad (2)$$

$$\Delta^{201}\text{Hg} = \delta^{201}\text{Hg} - \delta^{202}\text{Hg} \times 0.752 \quad (3)$$

Hg levels in pellets and aquarium's water

Hg concentration in water of the different aquariums after 7, 25 and 62 days of exposure is summarized in **Table S1**. Hg level in water of the low-MeHg aquarium ranged between $1.4\text{--}3.1 \text{ ng L}^{-1}$, meanwhile in the ones where Hg-enriched food was supplied the concentration increases

with time from 35 to 176 ng L⁻¹ and 15 to 75 ng L⁻¹, for MeHg and iHg conditions respectively. As expected, in aquariums where animals were fed with iHg and MeHg enriched diet, the Hg concentration in water was much higher than in the one with animals exposed to commercial food pellets. It can be attributed to a partial solubilization of Hg compounds from the food pellets and/or from the excrement.

A noticeable increase on Hg concentration in water of low-MeHg aquarium is produced at 62 days (**Table S1**). Although the same amount of food (3% of the fish weight) was supplied to the animals during the whole experiment, it was observed that in the last 15 days, it was not completely consumed. The food excess, as well as the excrements were daily removed at the moment of changing part of the aquarium's water. However, the permanence of the food excess during 24 hours seems to be reason of the Hg concentration rise at this moment of the experiment (62 days). This hypothesis is supported by the similar species distribution in the food pellets (80% MeHg) and in the mentioned Hg increase in the aquarium water (MeHg: 1.2 ng L⁻¹ and iHg 0.7 ng L⁻¹). Despite the increase of Hg concentration in the water of aquariums when Hg-enriched pellet were supplied, the comparison between the level in water and food evidences that diet is the main Hg source for the animals.

TableS1. Hg species concentration in water of the different aquariums

Exposure dietary conditions	time days	MeHg ng L ⁻¹	iHg ng L ⁻¹	THg* ng L ⁻¹	MHg/THg %
Low MeHg (Commercial pellets)	7	0.4±0.1	1.0±0.1	1.4±0.1	29
	25	0.3±0.1	0.9±0.2	1.2±0.3	26
	62	1.6±0.5	1.7±0.2	3.1±0.8	52
MHg-enriched	7	28±1	8.1±0.4	35.0±0.6	77
	25	49±2	10±0	60±2	83
	62	155±7	21±2	176±5	88
iHg-enriched	7	2.4±0.4	13±1	15±1	16
	25	1.0±0.0	53±1	54±1	2
	62	1.3±0.3	74±3	75±3	2

Results expressed as mean value ± SD of triplicate sample analysis (n=3). SD means standard deviation. *Values calculated by the sum of the Hg species concentration (iHg and MeHg).

Table S2. Hg speciation in certified reference materials. Comparision of certified and obtained values

CRM		iHg	MeHg		THg	
		Obtained Value	Certified Value	Obtained Value	Certified Value	Obtained Value
		ng g ⁻¹	ng g ⁻¹	ng g ⁻¹	ng g ⁻¹	ng g ⁻¹
Lobster	TOR-2	129±8	152±13	147±3	270±60	276±11
Dogfish Liver	DOLT- 4	1297±90	1330±120	1196±24	2580±220	2493±111
Tuna Fish	BCR 464	238±32	5120±170	4403±183	5240±100	4641±171

Results are expressed as mean value ± SD of triplicate sample analysis (n=3). SD means standard deviation. THg concentration is the sum of iHg and MeHg concentration.

Table S3. Hg isotopic composition in previously characterized reference materials and secondary standard used for method validation

Samples		$^{204}/^{198}\delta$ ‰	$^{202}/^{198}\delta$ ‰	$^{201}/^{198}\delta$ ‰	$^{200}/^{198}\delta$ ‰	$^{199}/^{198}\delta$ ‰	Δ^{201} ‰	Δ^{200} ‰	Δ^{199} ‰	n
UM-Almadén	This study	-0.80 ± 0.23	-0.52 ± 0.16	-0.42 ± 0.14	-0.26 ± 0.09	-0.16 ± 0.06	-0.03 ± 0.06	0.00 ± 0.05	-0.03 ± 0.06	31
	Epov et al, 2008	NR	-0.51 ± 0.17	-0.42 ± 0.15	-0.23 ± 0.13	-0.14 ± 0.08	-0.04 ± 0.07	0.02 ± 0.08	-0.01 ± 0.08	34
Fish BCR 464	This study	0.69 ± 0.24	0.54 ± 0.17	2.24 ± 0.14	0.34 ± 0.08	2.41 ± 0.05	1.84 ± 0.08	0.07 ± 0.03	2.27 ± 0.07	5
	Epov et al, 2008	NR	0.59 ± 0.20	2.23 ± 0.18	0.37 ± 0.14	2.33 ± 0.11	1.79 ± 0.08	0.07 ± 0.08	2.18 ± 0.08	7

Results expressed as mean value±2SD. SD means standard deviation. "NR" means not reported.

Table S4. Hg isotopic composition in food, skeletal muscle, liver, brain and feces of zebra fish after 0, 7, 25, and 62 days of different dietary conditions

Organs	Exposure Time (days)	$^{204}/^{198}\delta$ (‰)	$^{202}/^{198}\delta$ (‰)	$^{201}/^{198}\delta$ (‰)	$^{200}/^{198}\delta$ (‰)	$^{199}/^{198}\delta$ (‰)	Δ^{201} (‰)	Δ^{200} (‰)	Δ^{199} (‰)
Low MeHg-diet		0.06 ± 0.23	0.08 ± 0.16	0.99 ± 0.22	0.04 ± 0.12	1.15 ± 0.14	0.93 ± 0.17	0.00 ± 0.04	1.13 ± 0.12
MUSCLE	0	0.19 ± 0.23	0.12 ± 0.16	1.12 ± 0.14	0.12 ± 0.11	1.32 ± 0.12	1.03 ± 0.13	0.06 ± 0.10	1.28 ± 0.10
	7	0.31 ± 0.24	0.23 ± 0.16	1.19 ± 0.14	0.15 ± 0.09	1.30 ± 0.07	1.01 ± 0.06	0.04 ± 0.05	1.24 ± 0.06
	25	0.35 ± 0.23	0.23 ± 0.16	1.21 ± 0.14	0.16 ± 0.09	1.31 ± 0.10	1.04 ± 0.06	0.05 ± 0.06	1.25 ± 0.08
	62	0.14 ± 0.23	0.10 ± 0.16	1.22 ± 0.15	0.08 ± 0.09	1.42 ± 0.06	1.15 ± 0.06	0.03 ± 0.05	1.40 ± 0.06
LIVER	0	-0.09 ± 0.23	-0.04 ± 0.16	0.84 ± 0.14	0.00 ± 0.09	1.15 ± 0.06	0.87 ± 0.06	0.02 ± 0.05	1.16 ± 0.06
	7	0.07 ± 0.23	0.07 ± 0.16	0.95 ± 0.14	0.07 ± 0.09	1.15 ± 0.06	0.90 ± 0.06	0.04 ± 0.05	1.13 ± 0.06
	25	-0.05 ± 0.23	0.02 ± 0.16	0.88 ± 0.14	0.08 ± 0.09	1.08 ± 0.06	0.87 ± 0.06	0.07 ± 0.05	1.08 ± 0.06
	62	-0.12 ± 0.23	-0.06 ± 0.16	0.88 ± 0.14	0.01 ± 0.09	1.15 ± 0.06	0.93 ± 0.06	0.04 ± 0.05	1.17 ± 0.06
BRAIN	0	0.22 ± 0.23	0.08 ± 0.16	0.97 ± 0.14	0.05 ± 0.09	1.10 ± 0.06	0.91 ± 0.06	0.01 ± 0.05	1.08 ± 0.06
	7	ND	ND	ND	ND	ND	ND	ND	ND
	25	0.10 ± 0.23	0.15 ± 0.16	0.97 ± 0.14	0.11 ± 0.09	1.08 ± 0.06	0.86 ± 0.06	0.03 ± 0.05	1.04 ± 0.06
	62	0.00 ± 0.23	-0.01 ± 0.16	0.88 ± 0.14	0.02 ± 0.09	1.16 ± 0.06	0.89 ± 0.06	0.02 ± 0.05	1.16 ± 0.06
FECES	7	-1.75 ± 0.23	-1.16 ± 0.16	-0.68 ± 0.14	-0.59 ± 0.09	-0.03 ± 0.06	0.19 ± 0.06	-0.01 ± 0.05	0.26 ± 0.06
	25	-6.03 ± 0.23	-3.99 ± 0.16	-2.71 ± 0.14	-2.00 ± 0.09	-0.64 ± 0.06	0.30 ± 0.06	0.01 ± 0.05	0.37 ± 0.06
	62	-3.77 ± 0.23	-2.46 ± 0.16	-1.31 ± 0.14	-1.21 ± 0.09	0.05 ± 0.06	0.55 ± 0.06	0.02 ± 0.05	0.67 ± 0.06

MeHg-diet		-1.30 ± 0.26	-0.84 ± 0.11	-0.63 ± 0.17	-0.44 ± 0.02	-0.18 ± 0.06	0.00 ± 0.11	-0.02 ± 0.04	0.03 ± 0.07
MUSCLE	0	0.19 ± 0.22	0.12 ± 0.16	1.12 ± 0.17	0.12 ± 0.11	1.32 ± 0.12	1.03 ± 0.13	0.06 ± 0.10	1.28 ± 0.10
	7	-1.38 ± 0.23	-0.93 ± 0.16	-0.65 ± 0.14	-0.48 ± 0.11	-0.12 ± 0.09	0.05 ± 0.06	-0.02 ± 0.05	0.11 ± 0.06
	25	-1.29 ± 0.23	-0.87 ± 0.16	-0.60 ± 0.14	-0.42 ± 0.09	-0.15 ± 0.06	0.05 ± 0.06	0.01 ± 0.05	0.07 ± 0.06
	62	-1.34 ± 0.23	-0.89 ± 0.16	-0.63 ± 0.20	-0.46 ± 0.10	-0.17 ± 0.11	0.04 ± 0.09	-0.01 ± 0.05	0.05 ± 0.09
LIVER	0	-0.09 ± 0.23	-0.04 ± 0.16	0.84 ± 0.14	0.00 ± 0.09	1.15 ± 0.06	0.87 ± 0.06	0.02 ± 0.05	1.16 ± 0.06
	7	-1.19 ± 0.26	-0.79 ± 0.16	-0.57 ± 0.14	-0.41 ± 0.09	-0.13 ± 0.07	0.02 ± 0.07	-0.01 ± 0.05	0.07 ± 0.06
	25	-1.42 ± 0.23	-0.96 ± 0.16	-0.70 ± 0.14	-0.47 ± 0.10	-0.16 ± 0.06	0.03 ± 0.06	0.01 ± 0.08	0.08 ± 0.06
	62	-1.51 ± 0.23	-1.05 ± 0.16	-0.78 ± 0.14	-0.56 ± 0.09	-0.24 ± 0.06	0.01 ± 0.06	-0.03 ± 0.05	0.02 ± 0.06
BRAIN	0	0.22 ± 0.23	0.08 ± 0.16	0.97 ± 0.14	0.05 ± 0.09	1.10 ± 0.06	0.91 ± 0.06	0.01 ± 0.05	1.08 ± 0.06
	7	-1.30 ± 0.44	-0.86 ± 0.30	-0.56 ± 0.24	-0.42 ± 0.17	-0.09 ± 0.15	0.09 ± 0.06	0.01 ± 0.05	0.13 ± 0.07
	25	-1.43 ± 0.40	-0.90 ± 0.19	-0.67 ± 0.19	-0.47 ± 0.13	-0.15 ± 0.08	0.04 ± 0.06	0.01 ± 0.05	0.09 ± 0.08
	62	-1.44 ± 0.23	-0.94 ± 0.19	-0.67 ± 0.14	-0.47 ± 0.10	-0.16 ± 0.06	0.03 ± 0.06	0.00 ± 0.05	0.08 ± 0.06
FECES	7	-0.81 ± 0.23	-0.53 ± 0.16	-0.37 ± 0.14	-0.27 ± 0.09	-0.07 ± 0.06	0.03 ± 0.06	-0.01 ± 0.05	0.07 ± 0.06
	25	-1.18 ± 0.23	-0.87 ± 0.16	-0.59 ± 0.14	-0.47 ± 0.09	-0.21 ± 0.06	0.06 ± 0.06	-0.03 ± 0.05	0.01 ± 0.06
	62	-1.31 ± 0.23	-0.89 ± 0.16	-0.61 ± 0.14	-0.42 ± 0.09	-0.12 ± 0.06	0.06 ± 0.06	0.02 ± 0.05	0.11 ± 0.06
iHg-diet		-0.82 ± 0.23	-0.54 ± 0.16	-0.38 ± 0.26	-0.26 ± 0.15	-0.18 ± 0.04	0.03 ± 0.17	0.01 ± 0.09	-0.05 ± 0.06
MUSCLE	0	0.19 ± 0.23	0.12 ± 0.16	1.12 ± 0.17	0.12 ± 0.11	1.32 ± 0.12	1.03 ± 0.13	0.06 ± 0.10	1.28 ± 0.10
	7	0.19 ± 0.23	0.13 ± 0.16	1.24 ± 0.14	0.11 ± 0.09	1.42 ± 0.10	1.14 ± 0.09	0.04 ± 0.05	1.39 ± 0.12
	25	-0.23 ± 0.23	-0.14 ± 0.16	0.55 ± 0.14	-0.09 ± 0.09	0.76 ± 0.06	0.66 ± 0.06	-0.02 ± 0.05	0.80 ± 0.06
	62	-0.37 ± 0.23	-0.22 ± 0.16	0.32 ± 0.14	-0.08 ± 0.09	0.55 ± 0.08	0.48 ± 0.10	0.03 ± 0.07	0.60 ± 0.09
LIVER	0	-0.09 ± 0.23	-0.04 ± 0.16	0.84 ± 0.14	0.00 ± 0.09	1.15 ± 0.06	0.87 ± 0.06	0.02 ± 0.05	1.16 ± 0.06
	7	-1.20 ± 0.23	-0.79 ± 0.16	-0.51 ± 0.14	-0.40 ± 0.09	-0.10 ± 0.06	0.09 ± 0.10	0.00 ± 0.02	0.10 ± 0.06
	25	-0.62 ± 0.23	-0.42 ± 0.16	-0.26 ± 0.14	-0.23 ± 0.09	0.02 ± 0.08	0.05 ± 0.06	-0.02 ± 0.05	0.13 ± 0.10
	62	-0.25 ± 0.23	-0.22 ± 0.16	-0.09 ± 0.14	-0.11 ± 0.09	-0.02 ± 0.11	0.08 ± 0.06	0.00 ± 0.05	0.03 ± 0.13
BRAIN	0	0.22 ± 0.23	0.08 ± 0.16	0.97 ± 0.14	0.05 ± 0.09	1.10 ± 0.06	0.91 ± 0.06	0.01 ± 0.05	1.08 ± 0.06
	7	0.39 ± 0.23	0.29 ± 0.16	0.88 ± 0.14	0.14 ± 0.09	0.90 ± 0.06	0.66 ± 0.06	-0.01 ± 0.05	0.83 ± 0.06
	25	0.06 ± 0.23	0.04 ± 0.16	0.26 ± 0.14	0.03 ± 0.09	0.29 ± 0.06	0.24 ± 0.06	0.01 ± 0.05	0.28 ± 0.06
	62	-0.25 ± 0.23	-0.16 ± 0.16	-0.13 ± 0.14	-0.08 ± 0.09	0.04 ± 0.06	-0.01 ± 0.06	0.00 ± 0.05	0.08 ± 0.06
FECES	7	-0.92 ± 0.23	-0.60 ± 0.16	-0.45 ± 0.14	-0.34 ± 0.09	-0.14 ± 0.06	0.00 ± 0.06	-0.04 ± 0.05	0.01 ± 0.06
	25	-0.92 ± 0.23	-0.64 ± 0.16	-0.49 ± 0.14	-0.35 ± 0.09	-0.22 ± 0.06	-0.02 ± 0.06	-0.03 ± 0.05	-0.06 ± 0.06
	62	-0.81 ± 0.23	-0.49 ± 0.16	-0.38 ± 0.14	-0.24 ± 0.09	-0.17 ± 0.06	-0.01 ± 0.06	0.01 ± 0.05	-0.04 ± 0.06

Results expressed as mean value $\pm$ 2SD. SD means standard deviation. ND: not determined.

Table S5. MeHg, iHg and THg concentrations (ng g⁻¹) in food, skeletal muscle, liver, brain and feces of zebra fish after 0, 7, 25, and 62 days of different dietary conditions

Organs	Exposure Time (days)	THg by AMA-254 (ng g ⁻¹)			MeHg (ng g ⁻¹)			iHg (ng g ⁻¹)		
Low MeHg-diet		60	±	10	42	±	1	10	±	1
MUSCLE	0	295	±	38	339	±	2	24	±	6
	7	344	±	49	347	±	3	21	±	1
	25	356	±	27	344	±	10	27	±	3
	62	447	±	35	536	±	65	43	±	3
LIVER	0	149	±	17	164	±	19	52	±	12
	7	171	±	20	130	±	13	48	±	7
	25	168	±	9	146	±	21	70	±	12
	62	271	±	20	226	±	20	129	±	36
BRAIN	0	286	±	38	281	±	55	123	±	66
	7	213	±	9	257	±	44	56	±	6
	25	279	±	16	265	±	25	79	±	14
	62	481	±	67	453	±	97	175	±	22
FECES	7	115	±	9	ND			ND		
	25	211	±	64	ND			ND		
	62	133	±	20	ND			ND		
MeHg-diet		11580	±	450	12936	±	298	719	±	50
MUSCLE	0	295	±	38	339	±	2	24	±	6
	7	7490	±	476	7398	±	290	146	±	10
	25	22576	±	4606	22430	±	2498	1088	±	110
	62	28777	±	3739	31869	±	1638	762	±	193
LIVER	0	149	±	17	164	±	19	52	±	12
	7	12528	±	2181	6655	±	126	626	±	209
	25	30979	±	4980	41097	±	4743	1376	±	231
	62	46703	±	6825	60591	±	10005	3404	±	801
BRAIN	0	286	±	38	281	±	55	123	±	66
	7	11565	±	2444	13216	±	2728	1871	±	455
	25	42267	±	11127	39851	±	867	806	±	172
	62	82426	±	4957	110653	±	41090	3197	±	1228
FECES	7	1948	±	42	1390	±	102	1072	±	314
	25	13524	±	2896	12121	±	2509	1470	±	66
	62	18913	±	2321	12936	±	2717	1159	±	58
iHg-diet		11920	±	540	128	±	34	13461	±	311
MUSCLE	0	295	±	38	339	±	2	24	±	6
	7	424	±	41	444	±	38	69.6	±	0.3
	25	562	±	33	359	±	13	224	±	19
	62	780	±	35	369	±	27	419	±	43
LIVER	0	149	±	17	164	±	19	52	±	12
	7	2881	±	387	162	±	7	2067	±	304
	25	1079	±	165	179	±	19	1771	±	245
	62	3457	±	1069	187	±	32	3568	±	262
BRAIN	0	286	±	38	281	±	55	123	±	66
	7	432	±	83	365	±	7	260	±	29
	25	1013	±	269	288	±	53	835	±	81
	62	3028	±	99	296	±	26	2612	±	140
FECES	7	19398	±	1869	77	±	7	18645	±	3224
	25	30097	±	3531	174	±	31	33300	±	2818
	62	23383	±	1994	186	±	59	25172	±	1173

Results expressed as mean value ± SD of triplicate sample analysis (n=3). SD means standard deviation. ND: not determined.

Table S6. Bioaccumulation factor (BAF) for the different organs under different diet conditions

In this study, the BAFs were calculated as the THg concentration each organ and divided by the average Hg concentration in food pellets.

Dietary conditions	Organ	7 days	25 days	62 days
LowMeHg-diet	MUSCLE	7.1	7.2	11.2
	LIVER	3.4	4.2	6.9
	BRAIN	6.1	7.3	12.1
MeHg- diet	MUSCLE	0.6	1.7	2.4
	LIVER	0.5	3.1	4.7
	BRAIN	1.1	3.0	8.3
iHg- diet	MUSCLE	0.03	0.04	0.06
	LIVER	0.16	0.14	0.28
	BRAIN	0.04	0.08	0.21

Estimation of the species specific isotopic composition in iHg-diet

Considering that iHg-diet was prepared by spiking the commercial unamended pellets with a high purity iHg standard, the MeHg concentration as well as its isotopic composition ($\delta^{202}\text{MeHg}$ and $\Delta^{199}\text{MeHg}$) should remain unalterable in the iHg-diet. The MeHg concentration in organs of animals exposed to iHg-diet coincides with the values found in animals exposed to low-MeHg, supporting that it is originally from the food pellets.

Species specific isotopic composition in iHg-diet is estimated, as described for the unamended pellets (See main text) by solving the isotope mass balance equations:

$$\Delta^{199}\text{Hg}_{\text{iHgfood}} = \Delta^{199}\text{MeHg}_{\text{iHgfood}} \times f_{\text{MeHgfood}} + \Delta^{199}\text{iHg}_{\text{iHgfood}} \times (1 - f_{\text{MeHgfood}}) \quad (1)$$

$$\Delta^{199}\text{Hg}_{\text{muscle}} = \Delta^{199}\text{MeHg}_{\text{iHgfood}} \times f_{\text{MeHgmuscle}} + \Delta^{199}\text{iHg}_{\text{iHgfood}} \times (1 - f_{\text{MeHgmuscle}}) \quad (2)$$

Where f_{MeHg} represent the fraction of MeHg in the sample, $\Delta^{199}\text{MeHg}_{\text{iHgfood}}$ and $\Delta^{199}\text{iHg}_{\text{iHgfood}}$ are the MIF signature of MeHg and iHg in the iHg-pellets and $\Delta^{199}\text{Hg}_{\text{iHgfood}}$ and $\Delta^{199}\text{Hg}_{\text{muscle}}$ are the MIF signature measured in iHg-food and muscle samples.

$$\delta^{202}\text{Hg}_{\text{iHgfood}} = \delta^{202}\text{MeHg}_{\text{iHgfood}} \times f_{\text{MeHgfood}} + \delta^{202}\text{iHg}_{\text{iHgfood}} \times (1-f_{\text{MeHgfood}}) \quad (3)$$

$$\delta^{202}\text{Hg}_{\text{muscle}} = \delta^{202}\text{MeHg}_{\text{iHgfood}} \times f_{\text{MeHgmuscle}} + \delta^{202}\text{iHg}_{\text{iHgfood}} \times (1-f_{\text{MeHgmuscle}}) \quad (4)$$

Where $\delta^{202}\text{iHg}_{\text{iHgfood/muscle}}$ and $\delta^{202}\text{MeHg}_{\text{iHgfood/muscle}}$, are the $\delta^{202}\text{iHg}$ and $\delta^{202}\text{MeHg}$ in the iHg-food/muscle, respectively.

The estimated isotopic composition for iHg and MeHg was $\Delta^{199}\text{iHg}$ -0.09‰, $\delta^{202}\text{iHg}$ -0.54‰ and $\Delta^{199}\text{MeHg}$ 1.46‰, $\delta^{202}\text{MeHg}$ 0.18‰ respectively. As expected, the isotopic composition of MeHg in the iHg-enriched diet is similar to the one estimated in unamended pellets.

The MDF isotopic signature of MeHg in the iHg-diet can also be determined by the plot of the MeHg fraction as function of the $\delta^{202}\text{Hg}$ (**Figure S1**). The $\delta^{202}\text{MeHg}$ value is obtained by extrapolation from the best-fit line of the fish muscle. A strong correlation ($r^2=0.98$) of the percentage of MeHg and the $\delta^{202}\text{MeHg}$ in muscle is observed.

Figure S1. MDF ($\delta^{202}\text{MeHg}$) isotopic signature plotted as function of the fraction of MeHg in the muscle.

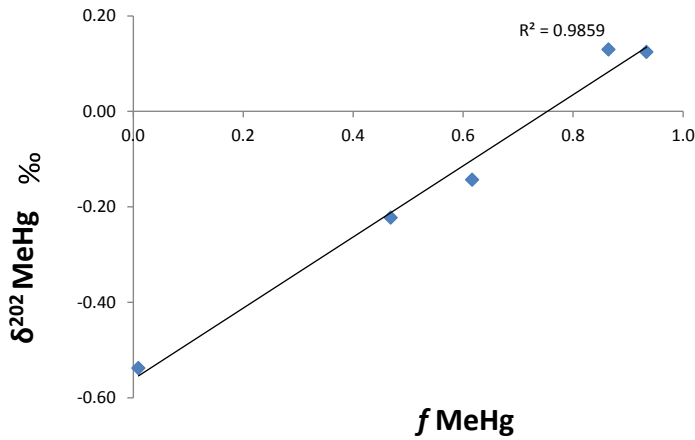


Figure S2. Comparison between modeled and measured $\Delta^{199}\text{Hg}$ in a) muscle, b) brain and c) liver of animals exposed to iHg.

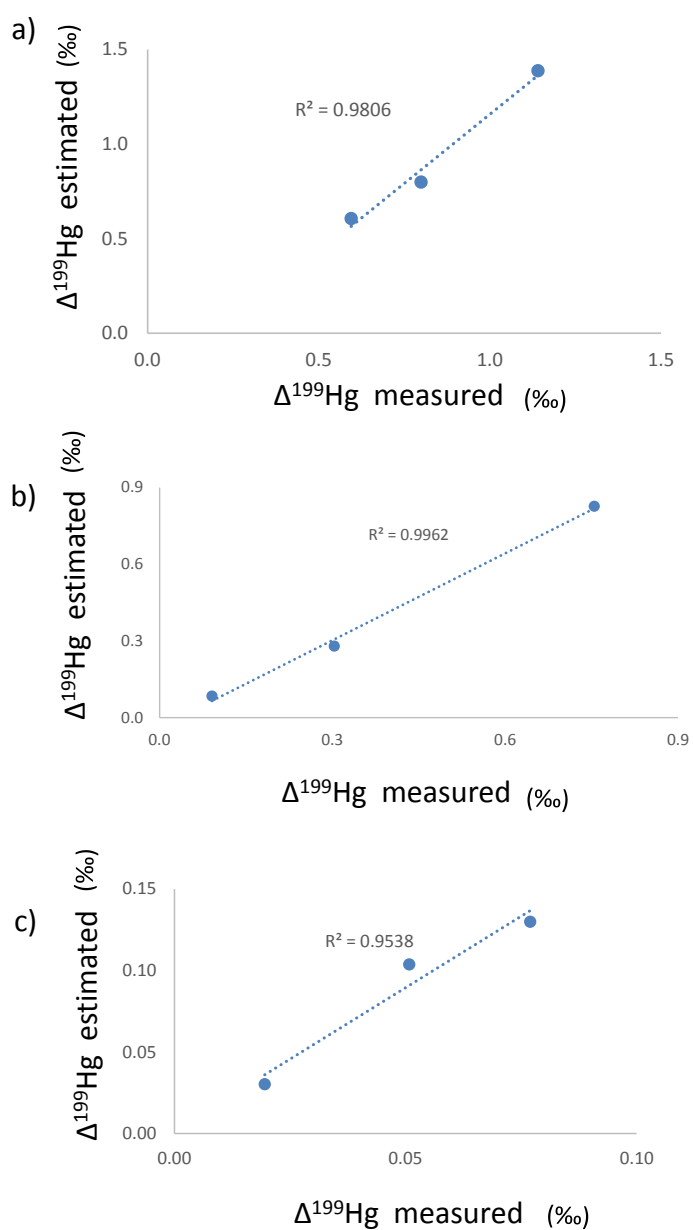


Table S7. Estimated $\delta^{202}\text{iHg}$ values in brain, liver and muscle of animals exposed to iHg-diet

	brain	liver	muscle
7 days	0.34	-0.87	-0.66
25 days	-0.03	-0.48	-0.78
62 days	-0.20	-0.24	-0.64