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Updated on 2nd November 2021: This supplementary information replaces the original version to rectify errors in Table SI-2 and the legend of Figure SI-7.

Supplementary Information to:

Methylmercury Speciation and Dimethylmercury Production in Sulfidic Solutions

Charlotte R. Kanzler[1], Peng Lian[2,3], Emma Leverich Trainer[1,4], Xiaoxuan Yang[1,5], Niranjan Govind[6], Jerry M. Parks[2], and Andrew M. Graham[1]*

[1]Grinnell College Department of Chemistry, Grinnell, Iowa 50112, United States

[2]Biosciences Division, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, Tennessee 37831-6309, United States

[3]University of Tennessee, Department of Biochemistry and Cellular and Molecular Biology, Knoxville, Tennessee 37996, United States

[4]Present Address: University of Wisconsin, Department of Civil and Environmental Engineering, Madison, Wisconsin 53706, United States

[5]Present Address: Duke University, Earth and Ocean Science, Durham, North Carolina 27708, United States

[6]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352, United States

*Corresponding Author (grahaman@grinnell.edu, 641-269-9813)

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Table SI-1. Summary of experimental conditions. Enriched $Me^{198}Hg$ is 92.78% ^{198}Hg; ambient MeHg refers to MeHg with natural isotopic abundance Hg. The total sulfide concentration ($[H_2S]_T$ (with standard deviation) was determined by repeated measures of sulfide concentration, generally within the first 48 hours of the experiment. All experiments were performed in a buffer consisting of 50 mM $NaNO_3$ and 25 mM of a pH buffer (MOPS = 3-(*N*-morpholino)propane sulfonic acid; HEPES = 4-(2-hyroxyethyl)-1-piperazineethanesulfonic acid; CHES = 2-(cyclohexylamino)ethanesulfonic acid).

$[MeHg]_0$ (nM)	Enriched $Me^{198}Hg$ or ambient MeHg	$[H_2S]_T$ (μM)	pH	Buffer	DMeHg measured (yes/no)
9.0	enriched $Me^{198}Hg$	3.91±0.99	7.14	MOPS	yes
9.0	enriched $Me^{198}Hg$	1.97±0.88	7.63	MOPS	yes
9.0	enriched $Me^{198}Hg$	6.72±0.67	8.14	HEPES	yes
9.0	enriched $Me^{198}Hg$	7.33±0.77	8.84	CHES	yes
9.0	enriched $Me^{198}Hg$	6.56±0.97	9.49	CHES	yes
2.5	enriched $Me^{198}Hg$	18.1±1.0	7.47	MOPS	yes
10.7	enriched $Me^{198}Hg$	18.0±0.01	7.51	MOPS	yes
29.3	enriched $Me^{198}Hg$	19.4±2.9	7.43	MOPS	yes
21.2	enriched $Me^{198}Hg$	0.22±0.03	7.43	MOPS	yes
21.2	enriched $Me^{198}Hg$	4.78±0.29	7.52	MOPS	yes
21.2	enriched $Me^{198}Hg$	19.3±0.5	7.53	MOPS	yes
21.2	enriched $Me^{198}Hg$	83.6±0.8	7.53	MOPS	yes
2.12	ambient	73.1±2.6	7.51	MOPS	yes
21.0	ambient	71.3±1.3	7.51	MOPS	yes
64.0	ambient	69.0±1.3	7.51	MOPS	yes
21.0	ambient	0.01±0.02*	7.51	MOPS	yes
2.50	ambient	50.8±8.6	7.48	MOPS	no
7.50	ambient	47.5±2.3	7.47	MOPS	no
29.0	ambient	48.4±8.5	7.47	MOPS	no
25.0	ambient	43.3±8.7	7.51	MOPS	yes
50.0	ambient	42.0±9.9	7.51	MOPS	yes
75.0	ambient	42.7±10.7	7.51	MOPS	yes
2.50	ambient	43.4±10.2	7.61	MOPS	no
2.50	ambient	155±19	7.60	MOPS	no
2.50	ambient	49.4±9.7	7.49	MOPS	no
2.50	ambient	615±135	7.60	MOPS	no
2.50	ambient	1300±83	7.62	MOPS	no

* Measured sulfide concentration below approximate method detection limit (0.1 μM).

Table SI-2. Equilibrium constants used for modeling MeHg speciation in sulfidic solutions. Equilibrium constants adjusted to *I* = 0.0 M using the Davies equation for activity coefficient calculation.

Reaction	**log K**	**Reference**
$CH_3Hg^+ + Cl^- = CH_3HgCl$	5.44	NIST CRITICAL[1]
$CH_3Hg^+ + H_2O = CH_3HgOH + H^+$	-4.55	NIST CRITICAL[1]
$CH_3Hg^+ + HS^- = CH_3HgSH$	14.5	Dryssen and Wedborg (1991)[2]
$CH_3Hg^+ + HS^- = CH_3HgS^- + H^+$	7.0	Schwarzenbach and Schellenberg (1965)[3]
$2CH_3Hg^+ + HS^- = (CH_3Hg)_2S + H^+$	23.5; 26.0	Schwarzenbach and Schellenberg (1965)[3]; this study
$CH_3Hg^+ + cysteine^{2-} = CH_3Hg\text{-}cysteine^{-1}$	17.27	Jawaid and Ingman (1981)[4]
$CH_3Hg^+ + H^+ + cysteine^{2-} = CH_3HgHcysteine$	26.2±0.3	Average of Simpson (1961)[5], Jawaid and Ingman (1981)[4], and Stary and Kratzer (1981)[6]
$CH_3Hg^+ + RS^- = CH_3HgSR$ (where RS^- is a generic thiol)	16.5	Skyllberg[7]
$H_2S = HS^- + H^+$	-7.02	NIST CRITICAL[1]
$HS^- = S^{2-} + H^+$	-18.5	NIST CRITICAL[1]
$RSH = RS^- + H^+$	-10.0	Skyllberg[7]
$cysteine^{2-} + H^+ = Hcysteine^-$	10.5	Skyllberg[7]
$cysteine^{2-} + 2H^+ = H_2cysteine$	19.0	Skyllberg[7]
$cysteine^{2-} + 3H^+ = H_3cysteine^+$	21.0	Skyllberg[7]

Table SI-3. Free energies[a] (kcal mol^{-1}) of stationary points relative to the reactant state (RS) calculated with various relativistic approximations.

Relativistic approximation	TS1	INT	TS2	PS (comb)[b]	PS (sep)[c]
Stuttgart RSC 1997 SC-ECP	22.2	22.0	26.8	24.7	27.5
SC-ZORA/Def2-TZVPP	27.4	27.2	31.4	29.5	30.4
cc-pVTZ-pp SC-ECP	26.4	26.2	31.1	28.6	28.9
cc-pVTZ-pp SO-ECP	25.7	25.5	30.2	27.9	29.7
cc-pVTZ-pp SO-ECP (M06-L)	22.5	22.2	27.0	25.5	26.4
cc-pVTZ-pp SO-ECP (PBE0)	28.7	28.4	31.9	30.9	31.7
cc-pVTZ-pp SO-ECP (BLYP)	22.9	22.9	29.1	26.0	28.9

[a] Geometries were optimized with the B3LYP density functional and the Stuttgart RSC 1997 scalar ECP and basis set for Hg and the 6-31G(d) basis for all other elements (See Methods). Free energies include single-point energies calculated with each approximation and thermodynamic corrections from frequency analysis performed at the same level of theory as the geometry optimizations. D3 dispersion was used in all cases, but Becke-Johnson damping was used only with the BLYP and B3LYP functionals. SMD solvation was included in all calculations.

[b] Calculated as a complex between DMeHg and HgS.

[c] Calculated as two infinitely separated species.

Table SI-4. Mass balance of MeHg and THg in variable pH experiments. Total mass of MeHg lost calculated as the cumulative mass of NaTEB-reactive MeHg lost from solution. MeHg recovered is the MeHg recovered from bottle walls after desorbing with 5% HCl. THg recovered is THg recovered after adding 2% BrCl to the 5% HCl MeHg-desorption solution.

pH	MeHg added (nmol)	MeHg lost (nmol)	MeHg recovered (nmol)	THg recovered (nmol)
7.14	0.45	0.207	0.126	0.167
7.63	0.45	0.1979	0.135	0.156
8.14	0.45	0.139	0.049	0.092
8.84	0.45	0.088	0.060	0.120
9.49	0.45	0.047	0.036	0.061

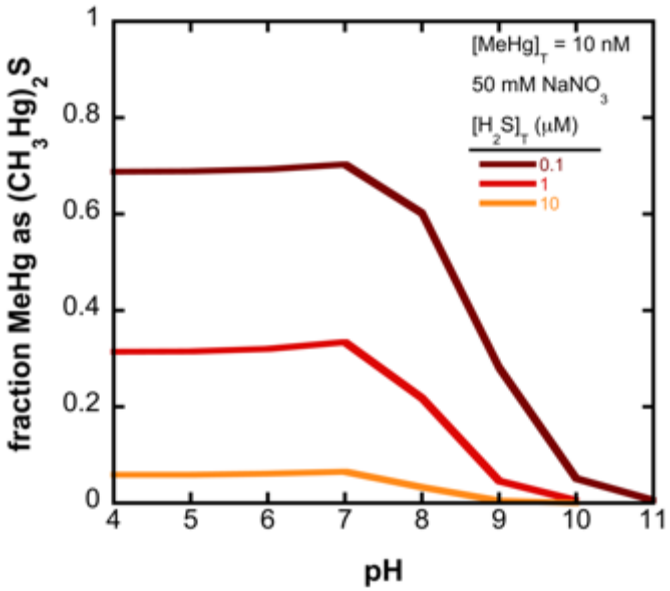

Figure SI-1. Fraction of total MeHg (10 nM) as bis(methylmercuric) sulfide ($(CH_3Hg)_2S$) as a function of pH and total sulfide concentration ($[H_2S]_T$). Calculation performed assuming log K = 23.5 for reaction: $2CH_3Hg^+ + HS^- = (CH_3Hg)_2S + H^+$ after Schwarzenbach and Shellenberg.[3] This calculation assumes that HS^- and OH^- are only ligands for MeHg, but addition of Cl^- up to 10 mM does not significantly alter the results.

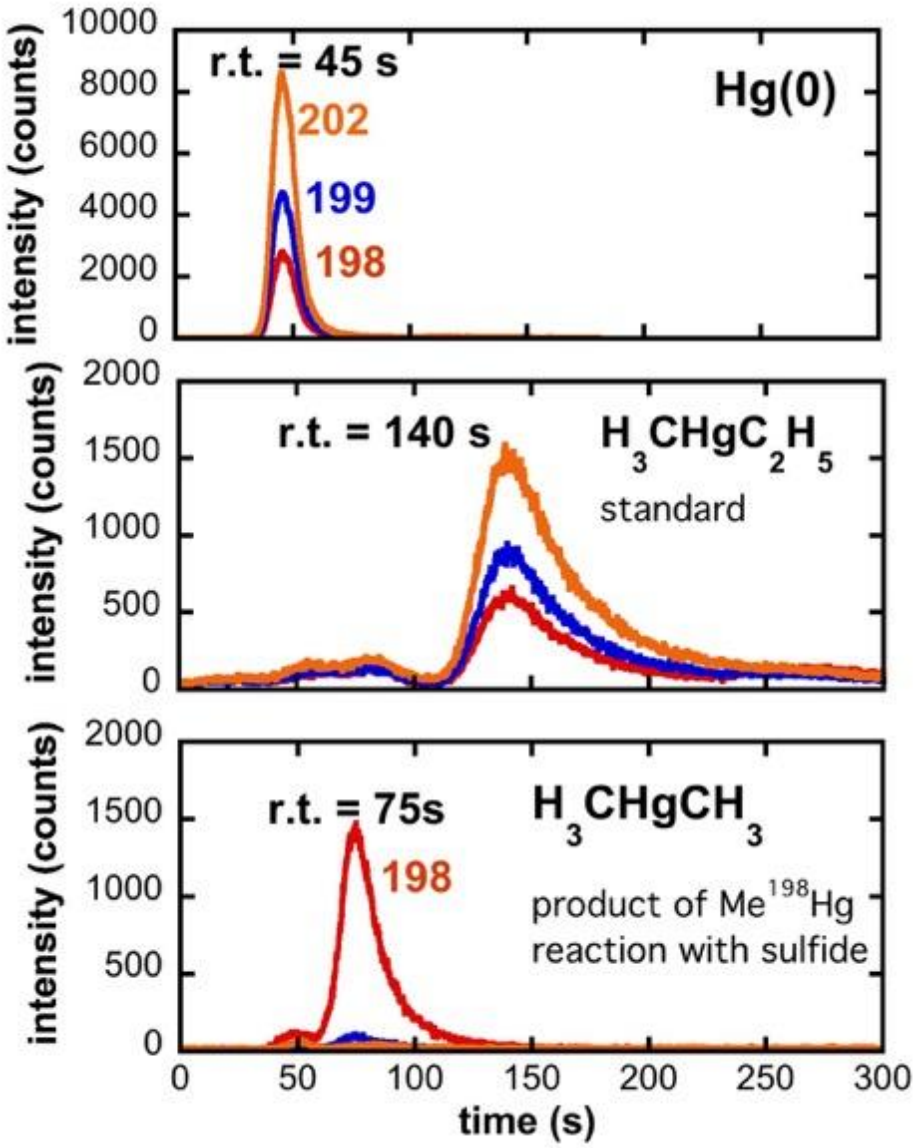

Figure SI-2. Chromatograms illustrating separation of elemental Hg, methylethyl Hg ($H_3CHgC_2H_5$) produced by reacting MeHg with sodium tetraethylborate), and dimethyl Hg (H_3CHgCH_3; produced by reacting stable isotope enriched $Me^{198}Hg$ with aqueous sulfide. Note that the product H_3CHgCH_3 has a retention time intermediate between Hg(0) and $H_3CHgC_2H_5$ and an isotopic distribution consistent with the $Me^{198}Hg$ reactant.

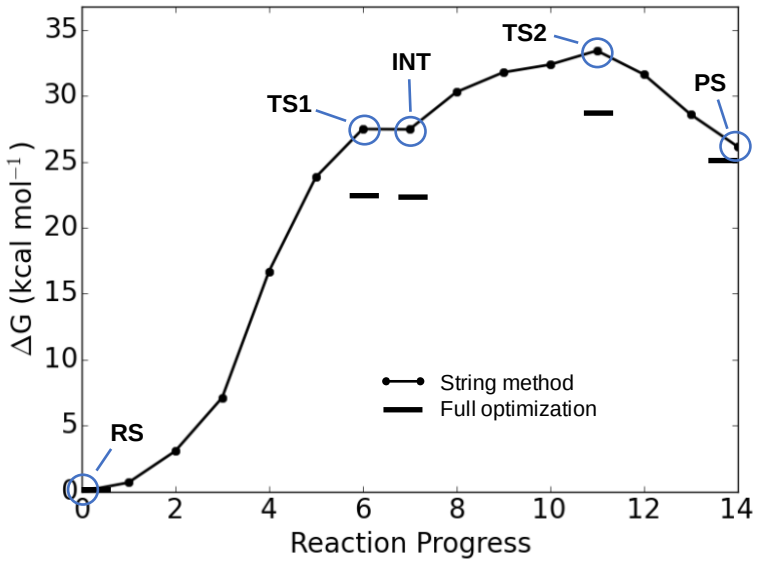

Figure SI-3. Minimum energy path for the decomposition of $(CH_3Hg)_2S$ to form DMeHg and HgS in aqueous solution computed with the zero-temperature string method and energies of fully optimized stationary points. All geometries were optimized with the B3LYP density functional in SMD continuum solvent (water). The Stuttgart RSC 1997 ECP was used for Hg and the 6-31G(d) basis set was used for all other elements (black line). See Methods for additional details of the calculations.

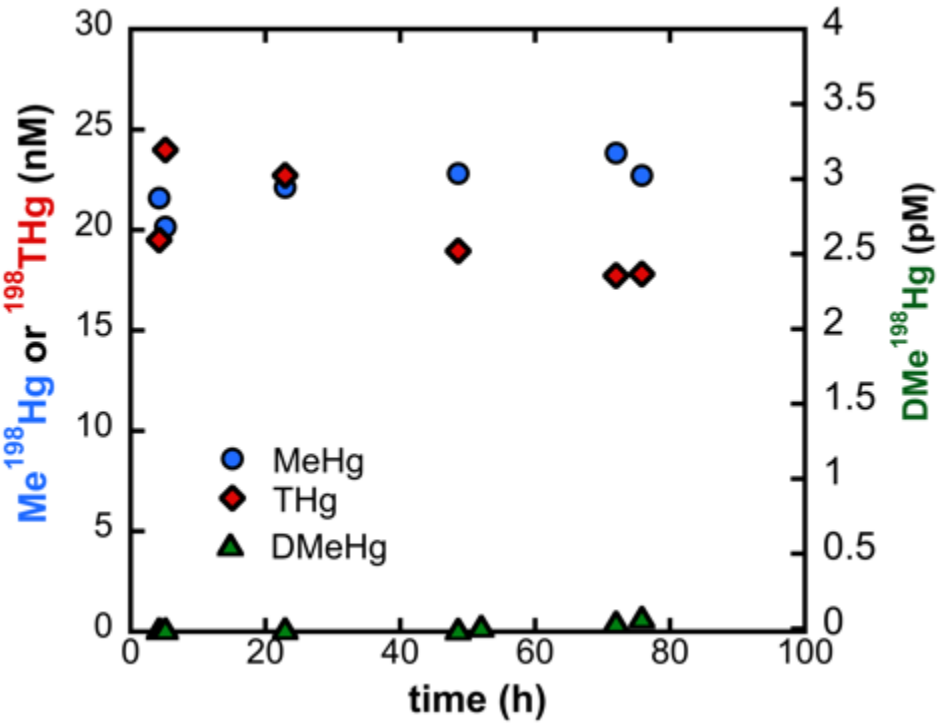

Figure SI-4. Time course of stable isotope-enriched methylmercury ($Me^{198}Hg$) and dimethylmercury ($DMe^{198}Hg$) in 50 mM NaCl solutions at pH 7.50 (buffered by 25 mM MOPS) containing no sulfide. No loss of $Me^{198}Hg$ was observed over the duration of the experiment.

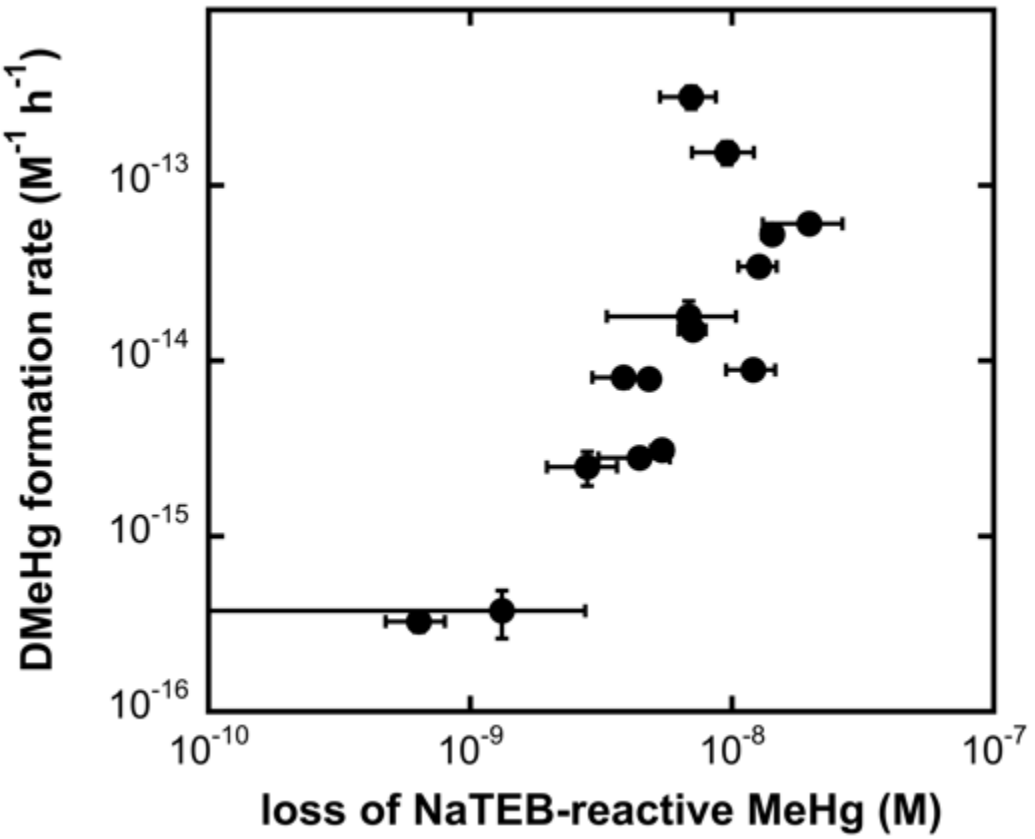

Figure SI-5. Relationship between inferred $(CH_3Hg)_2S$ based on loss of NaTEB-reactive MeHg and observed dimethylmercury (DMeHg) production rate. Error bars represent standard deviations of loss of MeHg calculated using MeHg concentrations measured during latter portion of experiment and standard errors of linear regression of DMeHg vs. time data used for determining DMeHg production rates.

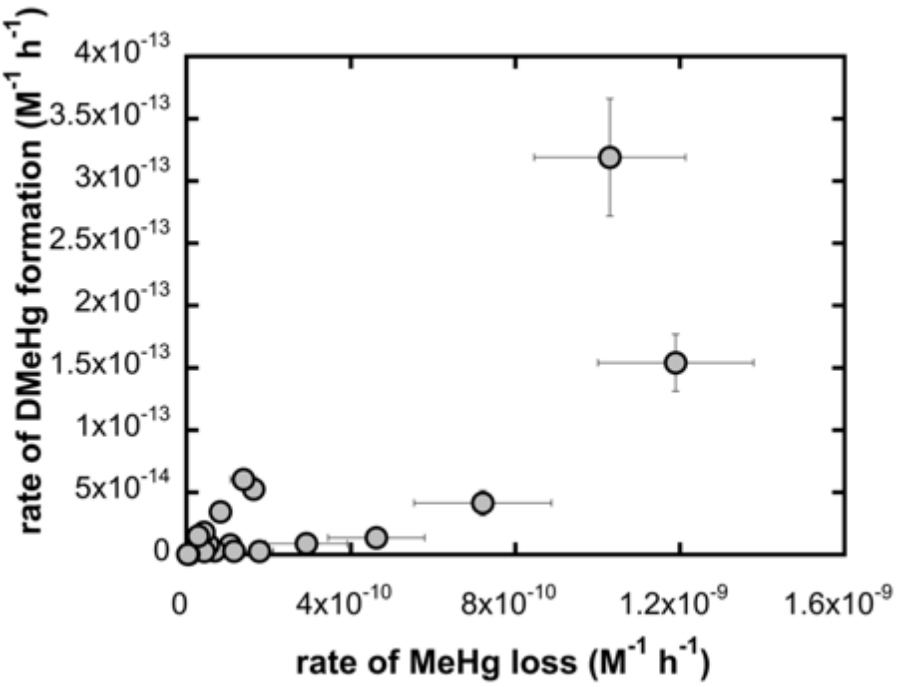

Figure SI-6. Relationship between the rate of MeHg loss and dimethylmercury (DMeHg) formation rate across all experiments (r^2 = 0.63, p < 0.001). Error bars represent standard errors of rates determined by linear regression.

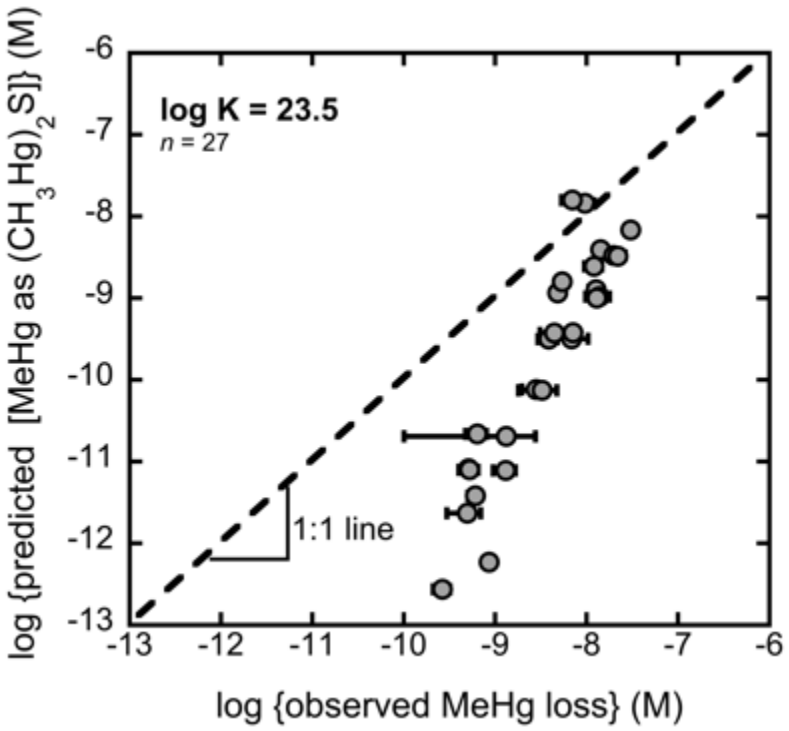

Figure SI-7. Relationship between observed loss of MeHg across all experiments and that predicted based on formation of $(CH_3Hg)_2S$ when log K = 23.5 for reaction: $2CH_3Hg^+ + HS^- = (CH_3Hg)_2S + H^+$.

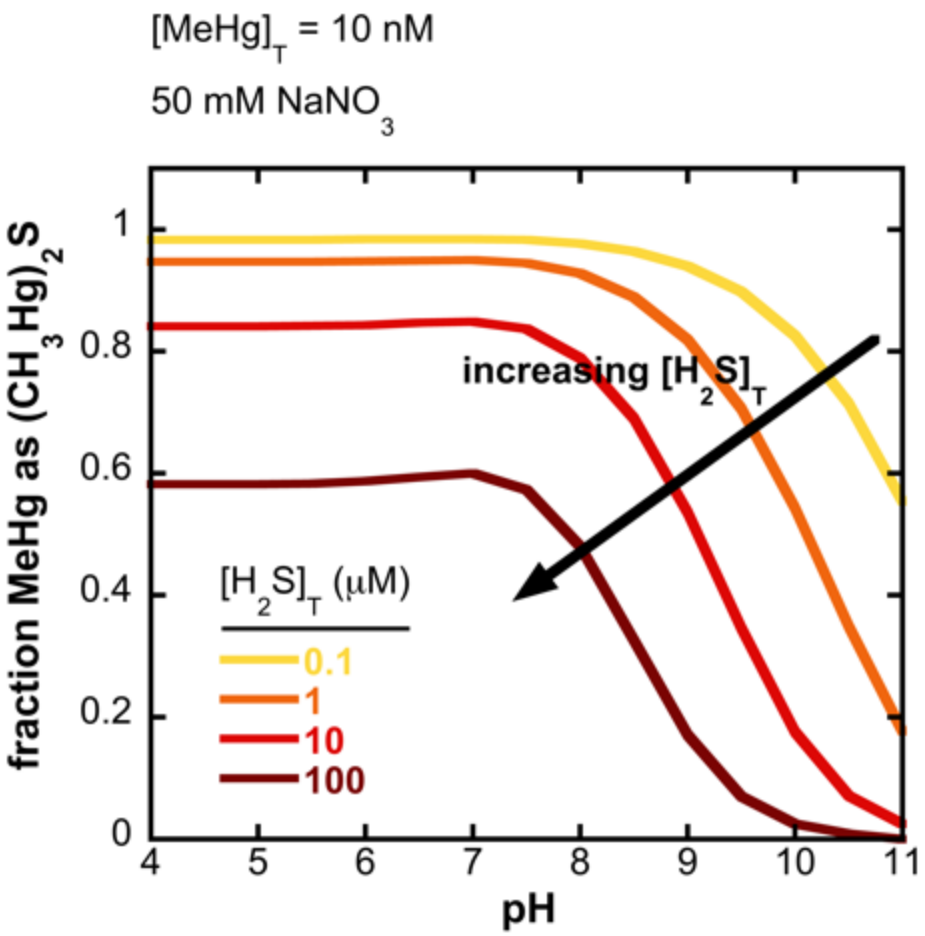

Figure SI-8. Effect of pH and sulfide concentration ($[H_2S]_T$) on the fraction of methylmercury (MeHg) as the binuclear complex $(CH_3Hg)_2S$. Equilibrium speciation calculations performed for $[MeHg]_T$ = 10 nM and ionic strength fixed by 50 mM $NaNO_3$. The experimentally determined log K for $(CH_3Hg)_2S$ was used in all calculations.

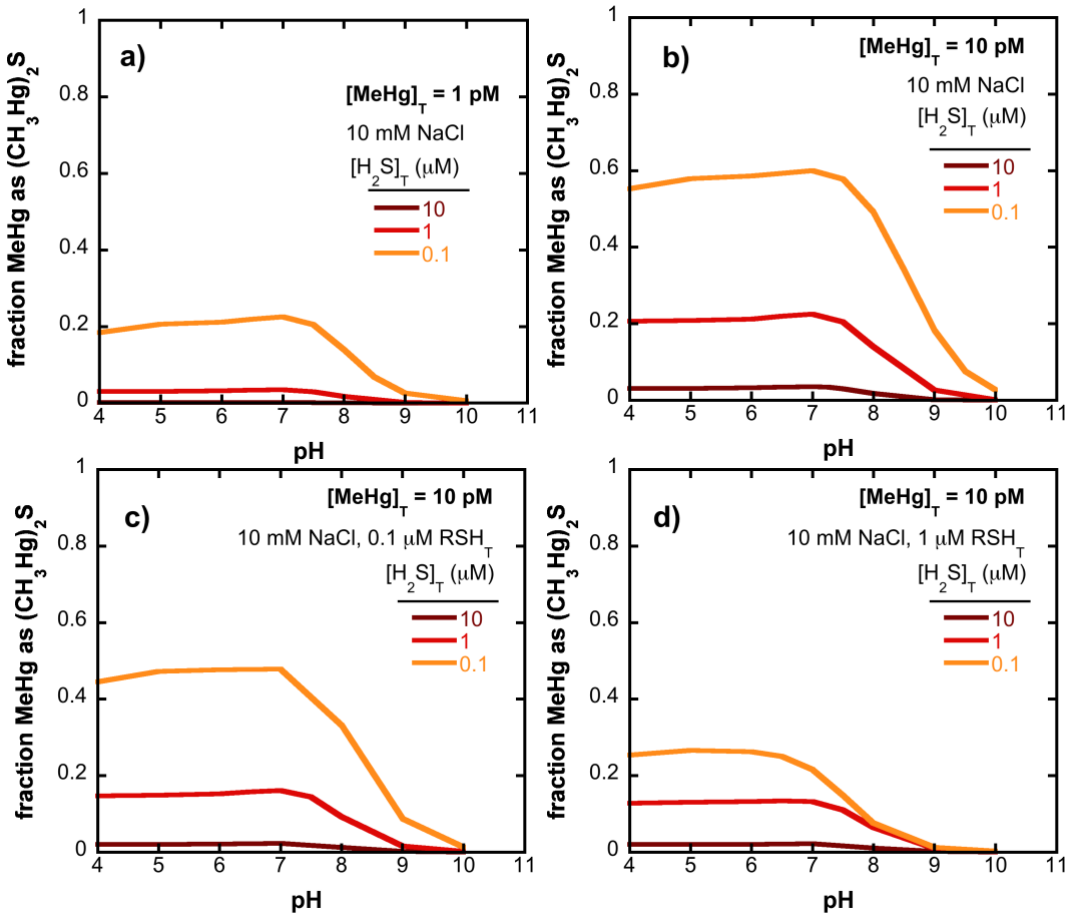

Figure SI-9. Effect of competitive ligands on $(CH_3Hg)_2S$ formation as a function of pH, sulfide, and MeHg concentration. **a)** Fraction of total MeHg as $(CH_3Hg)_2S$ under conditions typical of freshwater with elevated Cl^- (10 mM) and low (1 pM; 0.2 ng/L) MeHg concentration; **b)** Fraction of total MeHg as $(CH_3Hg)_2S$ under conditions typical of freshwater with high (10 pM; 2.0 ng/L) MeHg concentration; **c)** Fraction of total MeHg as $(CH_3Hg)_2S$ in freshwater with 0.1 μM thiol and high (10 pM) MeHg concentration; **d)** Fraction of total MeHg as $(CH_3Hg)_2S$ in freshwater with 1.0 μM thiol and high (10 pM) MeHg concentration.

Cartesian coordinates (in Å) for stationary point structures optimized at the SMD/B3LYP/RSC1997/6-31G(d) level of theory

RS

C	-1.988363070	1.565603280	-2.766112600
H	-1.709574610	2.603667330	-2.556252560
H	-3.069145260	1.519526450	-2.938887570
H	-1.473776500	1.239530880	-3.676414670
Hg	-1.445989940	0.305247100	-1.107491720
S	-0.847522740	-1.177780610	0.747907140
C	3.452887130	0.394487400	1.103799380
H	3.465903590	1.488766270	1.053116480
H	3.910616570	0.083363680	2.049169120
H	4.053456330	-0.001072500	0.277341550
Hg	1.428077770	-0.324348770	0.970128230

TS1

C	-1.827149100	1.535876410	-2.522808780
H	-1.465032730	2.530773470	-2.789494500
H	-2.860745670	1.584671350	-2.173156860
H	-1.747298090	0.858274940	-3.376311850
Hg	-0.614836090	0.731238570	-0.926728280
S	-0.861838390	-2.499335670	1.483770950
C	2.385826990	0.575500190	0.656347890
H	1.996696440	1.540098830	0.313356680
H	2.953758000	0.739897020	1.579691000
H	3.059490070	0.180916650	-0.111901720
Hg	0.791076720	-0.861263960	1.048318140

INT1

C	-1.820970530	1.468590660	-2.481011920
H	-1.497657980	2.440758600	-2.859190000
H	-2.847809580	1.524018330	-2.112981210
H	-1.737915270	0.708501660	-3.261444430
Hg	-0.548657720	0.867258770	-0.844190820
S	-0.850110440	-2.506217630	1.437851150
C	2.437891710	0.523433020	0.658832550
H	2.098232610	1.451998420	0.186864700
H	2.905609700	0.773184060	1.618155030
H	3.183191810	0.056291880	0.005843810
Hg	0.806036560	-0.879753620	1.008395160

TS2

C	-1.624605270	1.467730640	-2.256004330
H	-1.606116050	2.486443220	-2.658583960
H	-2.554729810	1.334241780	-1.691762170
H	-1.628282580	0.763876990	-3.096267660
Hg	0.060971540	1.078790640	-0.972065270
S	-0.813787420	-2.962025760	1.844700060
C	1.870383990	0.778371560	0.352897920
H	2.167828440	1.796464260	0.057885460
H	1.852781540	0.773371780	1.447335610
H	2.620031060	0.079052570	-0.021961550
Hg	0.139586130	-1.036518470	0.846563800

PS (DMeHg•HgS)

C	-1.381954830	1.483823550	-2.038726590
H	-1.866018510	2.458243760	-1.899381710
H	-2.092011290	0.706138850	-1.731669690
H	-1.176737340	1.349514860	-3.107938190
Hg	0.436211120	1.354718690	-0.882952750
S	-0.791647990	-2.686131940	2.164440970
C	2.201169560	1.112185190	0.365853310
H	2.885335690	1.953471830	0.191483130
H	1.932391020	1.104684520	1.427627330
H	2.735401670	0.183672790	0.137326180
Hg	-0.358194880	-1.030843790	0.525651560

DMeHg

C	-2.160367870	-0.064648440	0.000237380
Hg	-0.000075900	0.002447000	0.001652970
H	-2.585345890	0.786847830	-0.546122700
H	-2.561134910	-0.037094910	1.021343570
H	-2.529533190	-0.982206320	-0.475265810
C	2.160115210	0.065364660	0.000995600
H	2.570567500	-0.422061660	-0.892456110
H	2.573975760	-0.448092890	0.878098430
H	2.531871960	1.097598720	0.015358100

HgS

Hg	-0.270717210	0.071840710	-0.276008650
S	1.358750550	-0.360574040	1.385308650

References for Supplementary Information

1 National Institute of Standards and Technology. NIST Critically Selected Stability Constants of Metal Complexes; Gaithersburg, MD, 2013.

2 D. Dryssen and M. Wedborg, *Water Air Soil Poll*, 1991, **56**, 507–519.

3 G. Schwarzenbach and M. Schellenberg, *Helv. Chim. Acta*, **48**, 28–46.

4 M. Jawaid and F. Ingman, *Talanta*, 1981, **28**, 137-143.

5 R. B. Simpson, *J Am Chem Soc*, 1961, **83**, 4711-4717.

6 J. Stary and K. Kratzer, *J Radioan Nucl Ch Le*, 1988, **126**, 69–75.

7 U. Skyllberg, *J Geophys Res-Biogeo*, 2008, **113**, G00C03.