Supplemental materials

The fate of methylmercury through formation of bismethylmercury sulfide as an intermediate in mice

Yumi Abiko[1], Yusuke Katayama[2], Wenyang Zhao[3,4], Sawako Horai[5], Kenji Sakurai[3,4], Yoshito Kumagai[1, 2, *]

[1] Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan

[2] Master's Program of Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan

[3] Doctoral Program in Materials Science and Engineering, Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-0006

[4] National Institute for Materials Science, Tsukuba, Ibaraki 305-0047, Japan

[5] Environmental Health Section, Dept. Environment and Public Health, National Institute for Minamata Disease, Minamata, Kumamoto 867-0008, Japan

[*]Address correspondence to Yoshito Kumagai, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan; Tel and Fax, +81-29-853-3133; E-mail, yk-em-tu@md.tsukuba.ac.jp

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Experimental procedure

Detection of CH_4 by GC/MS

Dimethyl mercury (DMeHg, 4 mM) or methyl mercury chloride (MeHgCl, 4 mM) in 0.5 N HCl-50% methanol was incubated for 3 h or 4 days at room temperature in a glass vial, and the sample was analyzed by GC/MS (GCMS-QP2020, Shimadzu, Kyoto Japan). Gas in the headspace of the sample tube was injected using a gas-tight syringe and separated by TC-BOND Q (30 m long, 0.32 mm i.d., 10 μm df; GL Sciences) with a PLOT column particle trap (2.5 m long, 0.25 mm i.d.; GL Sciences). The temperature was set as follows: 35°C for 5 min; a linear increase to 220°C (12°C/min); and hold at 220°C for 5 min. The eluted compounds were then transferred to the EI source (70 eV) of the system, and the control and analyses were performed using GCMSsolution (Shimadzu).

Detection of MeHg by EI-MS

DMeHg (4 mM) in 0.5 N HCl-50% methanol was incubated for 4 days at room temperature in a glass vial. An aliquot of sample (10 μL) was subjected to EI-MS (GCMS-QP2020), and the control and analyses were performed using GCMSsolution and direct-injection mode.

Detection of $(MeHg)_2S$ in the intestinal content of a mongoose

A wild small Indian mongoose (*Herpestes auropunctatus*) was collected at Goga, Nago city, Okinawa Prefecture, in 2017 as part of a wildlife damage-control program[1], and the intestinal content of the mongoose was mixed with a 5 volume of 50% methanol-10% formic acid to extract $(MeHg)_2S$. After centrifugation (20,000 *g*, 10 min, 4°C) of the mixture, the supernatant was analyzed by HPLC/AAS.

Figure S1

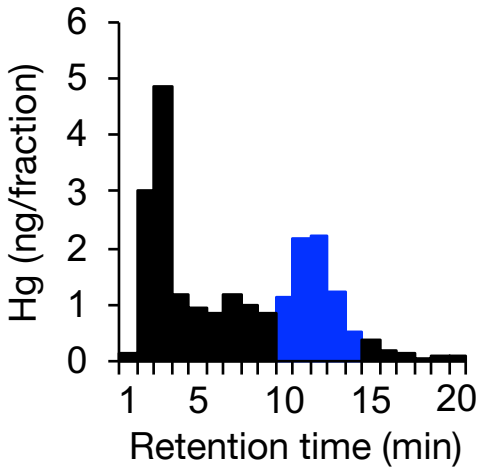

Figure S1. Analysis of $(MeHg)_2S$ in the intestinal content of mongoose. The blue bars indicate $(MeHg)_2S$. $(MeHg)_2S$ in the intestinal content of the mongoose was extracted by 50% methanol-10% formic acid, and then the supernatant was analyzed by HPLC/AAS. The blue bar indicates $(MeHg)_2S$, and representative data are shown.

Figure S2

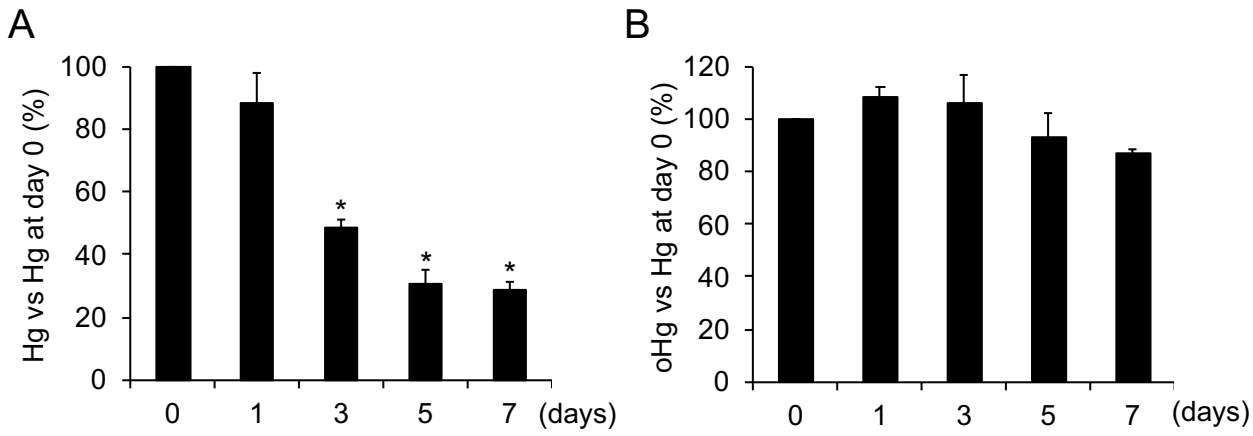

Figure S2. Detection of Hg during incubation with $(MeHg)_2S$ in mouse liver lysates.

(A) $(MeHg)_2S$ (100 μM) in 50 mM KPi (pH 7.5) was incubated for 0–7 days at 37°C, and the Hg content was measured by AAS. (B) MeHg in 50 mM KPi (pH 7.5) was incubated for 0–7 days at 37°C. After liquid-liquid extraction, the benzene layer and water layer were separately analyzed by AAS. The mercury content in the benzene layer was determined because the Hg level in the water layer was negligible. Each value is the mean ± SE of three determinations. *$P < 0.05$ vs. day 0.

Figure S3

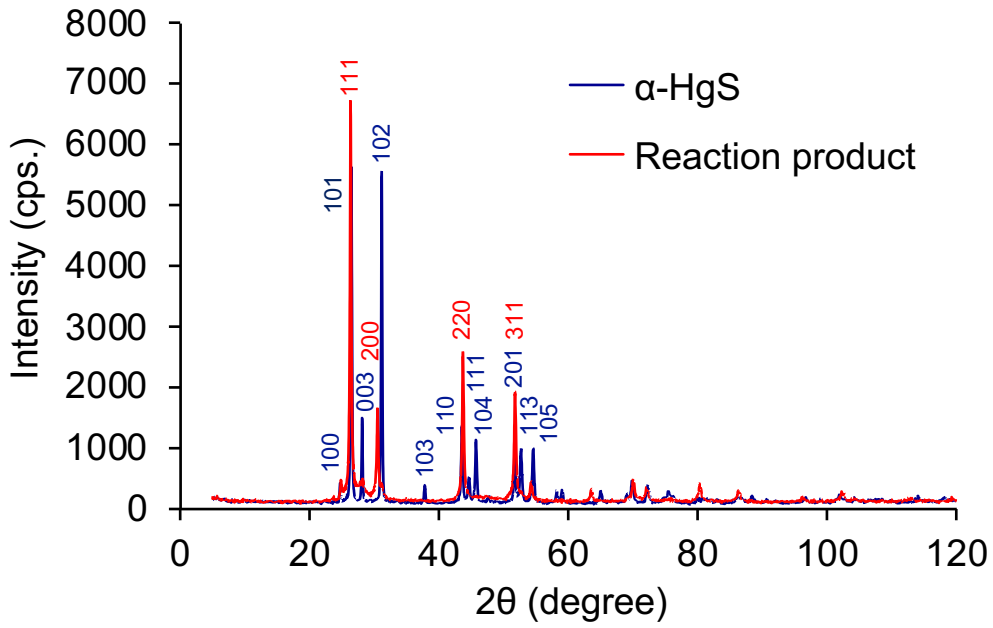

Figure S3. Analysis of insoluble mercury compounds during incubation with $(MeHg)_2S$. $(MeHg)_2S$ in 50 mM KPi (pH 7.5) was incubated for 7 days, and the reaction product in the water layer was purified. The reaction product (red) and authentic α-HgS (blue) were analyzed by X-ray diffraction, and the spectra are shown.

There are three small suspicious peaks at 24.84 deg, 28.16 deg, and 31.22 deg in the XRD pattern of the reference β-HgS (see Figure 3G) and the reaction product. One assumption could be that they are 100, 101, and 102 reflections of α-HgS. Under this assumption, α-HgS may be present in the black particles and even in the reference β-HgS sample as a secondary minor phase. However, considering the very weak peak intensities, this secondary minor phase is negligible. The conclusion of the present study would not be affected.

Figure S4

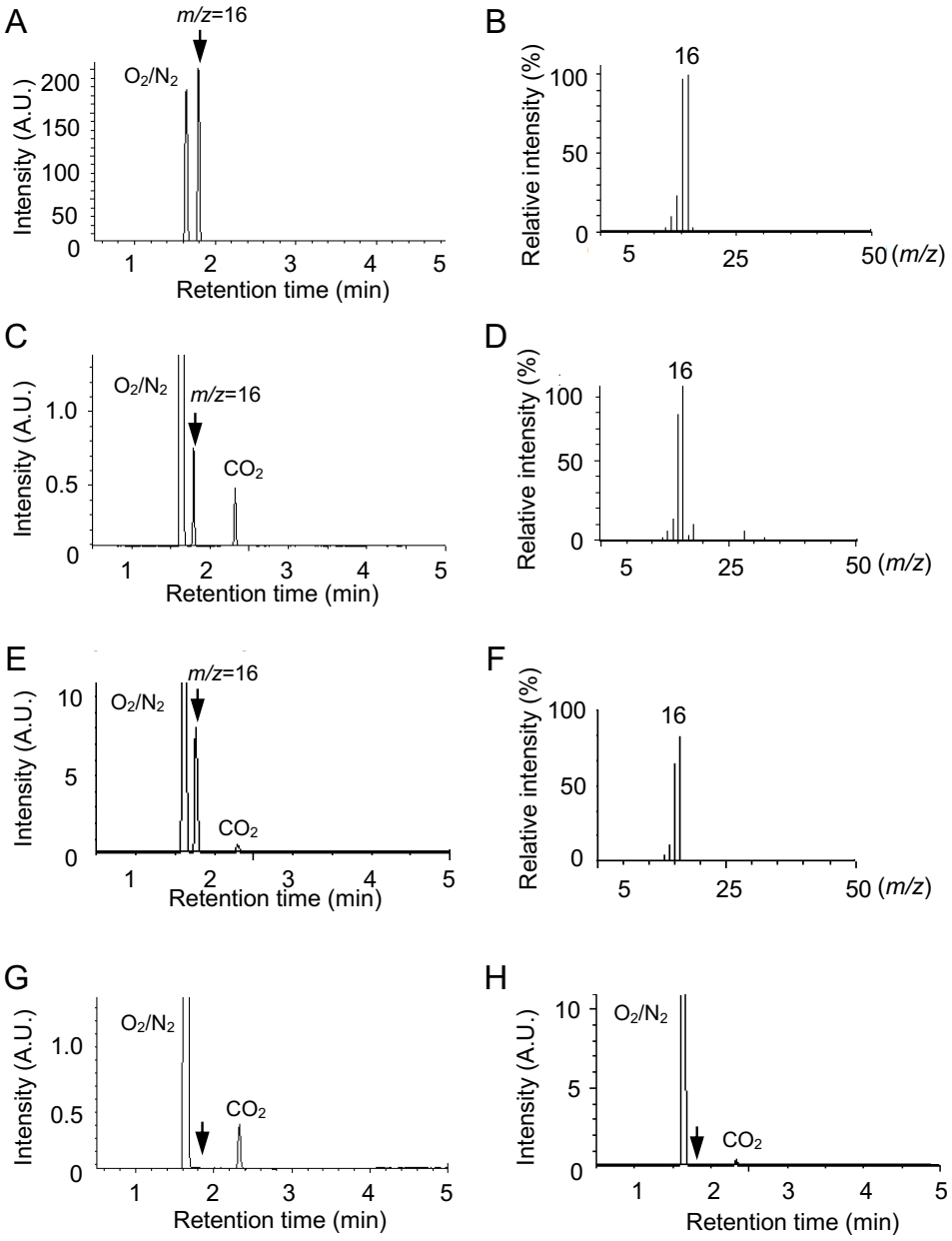

Figure S4. Detection of CH_4 during dimethylmercury or methylmercury incubation by GC/MS analysis. The arrows indicate CH_4. (A) Chromatogram of authentic CH_4 and (B) spectrum of the peak with a retention time of 1.79 min. Compounds in the head space of DMeHg solution after (C, D) 3 h or (E, F) 4 days of incubation were analyzed by GC/MS. (C, E) Chromatogram of the collected compounds and (D, F) spectrum of the peak with retention time of 1.79 min. Chromatogram of the collected compounds in the head space of MeHg solution after (G) 3 h or (H) 4 days of incubation.

Figure S5

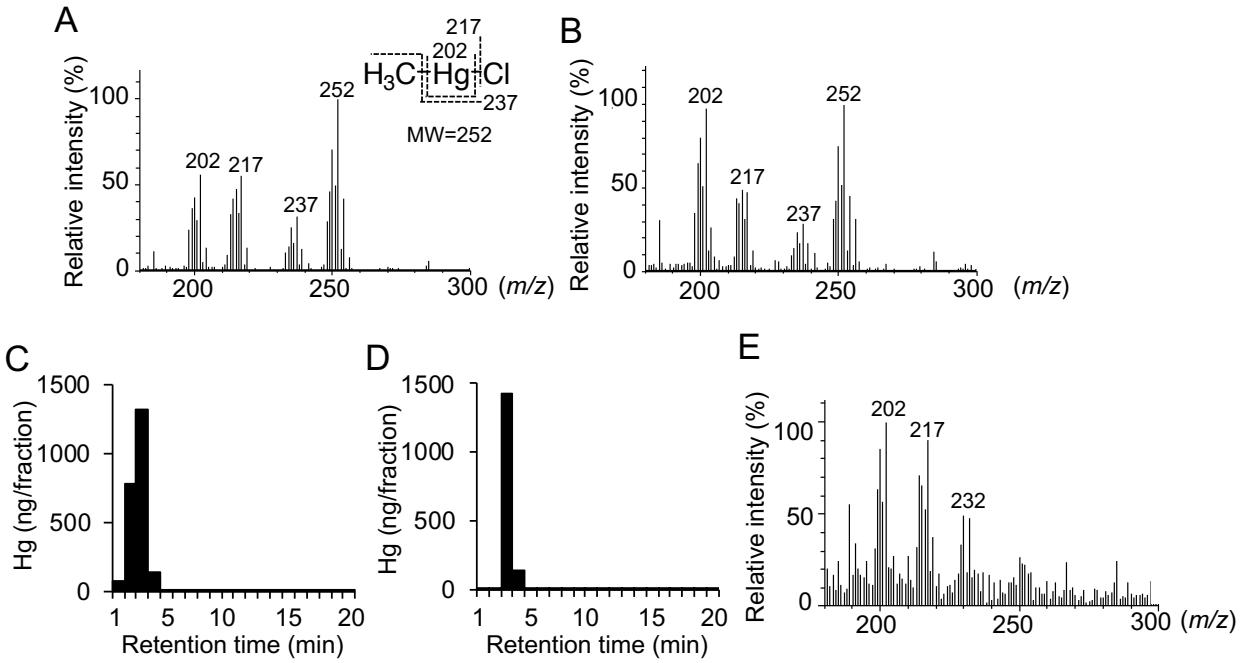

Figure S5. Detection of MeHg during incubation of dimethylmercury.

Spectra of (A) authentic MeHgCl and (B) DMeHg after 4 days of incubation. Hg levels of (C) MeHg and (D) DMeHg solution after 4 days of incubation detected by HPLC/AAS analysis. (E) EI-MS spectrum of DMeHg in methanol.

Figure S6

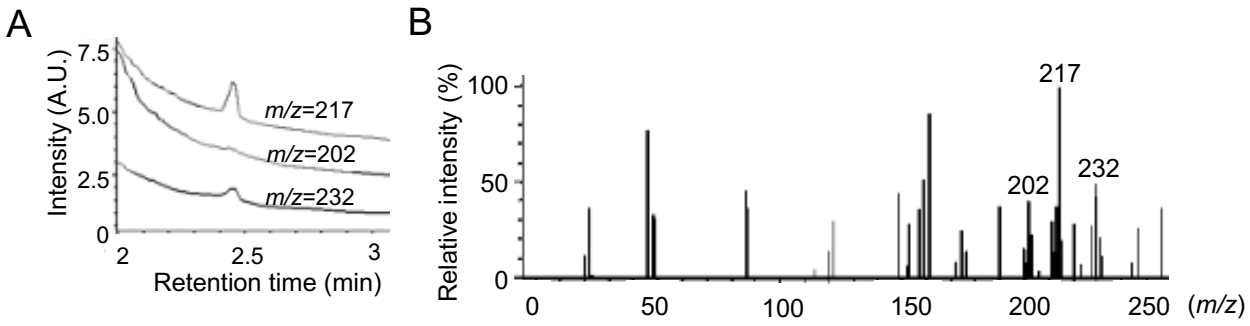

Figure S6. GC/MS analysis of mouse expiration after treatment with methylmercury. Expiration of mice given a single injection of 0.1 mmol/kg MeHg for 3 h was collected by XAD-4 resin, and the collected chemicals were extracted by acetone. The samples were analyzed by GC/MS, and the (A) chromatograms and (B) a spectrum are shown with representative data.

Figure S7

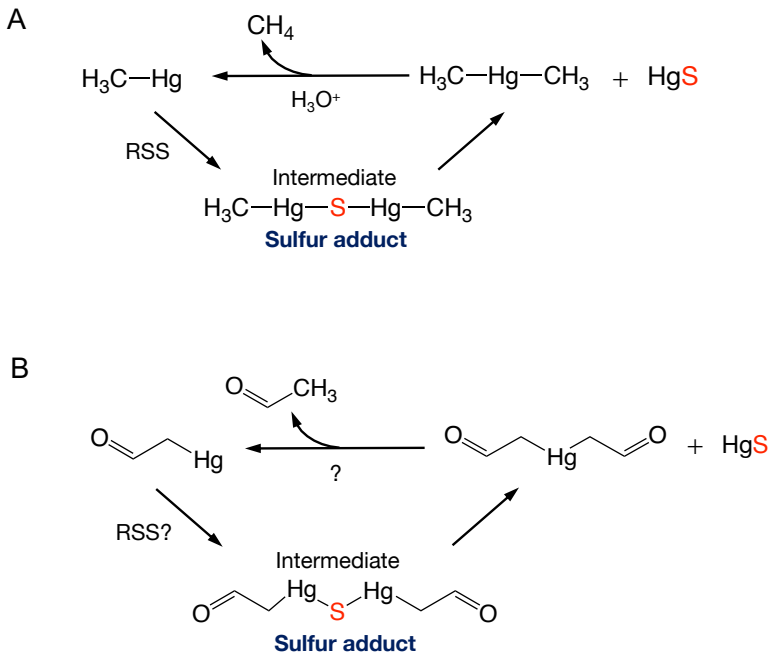

Figure S7. Formation of dialkylmercury and HgS through sulfur intermediates. RSS, reactive sulfur species. (A) MeHg reacts with RSS to yield $(MeHg)_2S$ and then gradually converts to DMeHg and HgS *in vivo* and *in vitro*. DMeHg is decomposed into CH_4 and MeHg under acidic conditions. (B) Alkylmercury species such as mercuri-acetaldehyde, similar to MeHg, may react with RSS, leading to the formation of bis-mercuri-acetaldehyde sulfide as an intermediate to form HgS and bis-mercuri-acetaldehyde, which is suggested to release acetaldehyde and mercuri-acetaldehyde in water.

References

1 Horai, S. *et al.* Establishment of a primary hepatocyte culture from the small Indian mongoose (Herpestes auropunctatus) and distribution of mercury in liver tissue. *Ecotoxicology* **23**, 1681-1689, doi:10.1007/s10646-014-1307-6 (2014).