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REDUCTION AND METHYLATION OF MERCURY IN THE TERRESTRIAL ISOPOD *PORCELLIO SCABER* (CRUSTACEA) AND ITS ENVIRONMENT

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Abstract—Reduction and methylation of inorganic mercury in *Porcellio scaber* (Isopoda, Crustacea) and its environment were studied, using a purpose-built experimental setup where Hg cycling was followed using $^{203}Hg^{2+}$ tracer in experiments without and with isopods. In experiment without isopods, daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$ under sterile and nonsterile conditions was measured for three weeks to assess the contribution of bacteria to this process. In experiments with isopods, daily release of $^{203}Hg^{0}$ was measured for two weeks. Total mercury ($T^{203}Hg$) and monomethylmercury ($Me^{203}Hg$) in whole animals, gut, digestive glands (hepatopancreas), food (hazelnut leaves), and feces were measured to obtain the assimilation and distribution of mercury in the animals, to investigate the origin and fate of $Me^{203}Hg$, and, finally, to assess the mass balance of mercury in the experimental system. Experiment without isopods showed the important role of bacteria in reduction of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$, especially in the first day of the experiment. Experiments with isopods showed that formation of $^{203}Hg^{0}$ depended on the $^{203}Hg^{2+}$ concentration in the food. The contribution of the isopod's digestive flora in reduction of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$ was negligible. Approximately 3% of $T^{203}Hg$ and 2% of $Me^{203}Hg$ consumed was assimilated by the animals. Methylation of $^{203}Hg^{2+}$ occurred already in the leaves before they were consumed by the isopods. Assimilation of $Me^{203}Hg$ from the food surprisingly was low. Also, a loss of $Me^{203}Hg$ was noticed when comparing assimilated and excreted $Me^{203}Hg$ versus consumed $Me^{203}Hg$. This may be explained by the assumption that demethylation of MeHg prevailed over methylation of Hg^{2+}in the animal's digestive system, leading to excretion of ingested mercury as Hg^{2+}.

Keywords—Mercury reduction Mercury methylation *Porcellio scaber* Methylmercury Radiotracer

INTRODUCTION

Mercury (Hg) is an element that transforms into different chemical forms due to abiotic (humidity, temperature, pH, etc.) factors and biotic activity. In nature, it exists in four major forms: As elemental mercury (Hg^0), as the mercuric ion (Hg^{2+}), as alkylated mercury, and as a ligand with sulphide (HgS). Mercury is among the most toxic heavy metals in the environment [1–4]. According to Boening [5] and the World Health Organization [6], organic forms of Hg are 10 to 200 times more toxic than inorganic forms. Organic monomethylmercury (MeHg) is stable in association with tissue and is not degraded nor excreted from the body at significant rates [1]. As a result, it is accumulated by organisms for their lifetime, and biomagnified in food webs [1].

Methylation of inorganic mercury and demethylation of methylated mercury occur simultaneously due to abiotic factors or biotic activity in soil, sediment, water, and organisms. Monomethylmercury production is dependent on the concentration of bioavailable Hg and is promoted by low pH (<5), low salinity, moderate sulphide content, high temperature, the presence of decomposable organic matter, and the presence of other metals that act as catalysts in reducing environments [1,7,8]. Ninety percent of MeHg arises from biotransformations in anoxic environments. Generally it is believed that methylation occurs in anaerobic conditions (redox potential about -220 mV [9]) due to sulfate-reducing bacteria that are the main methylators of inorganic mercury in anaerobic sediments [1,8] and also can act as demethylators [10].

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In this work, we studied the transformations of Hg in the digestive system of the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea), which is one of the most-studied organisms in terrestrial ecophysiology and ecotoxicology [11,12]. The digestive system of *P. scaber* consists of a short stomach, a gut, and two pairs of tube-shaped digestive glands (hepatopancreas), that also act as a metal storage organ [13].

In previous work, methylation/demethylation processes of Hg in *P. scaber* were studied with the use of the radiotracer ^{203}Hg [14]. The results of that study indicated the occurrence of methylation and demethylation of mercury in *P. scaber*, but the organ where these processes take place was not determined. The mass balance of spiked mercury in the experimental system showed that some portion of added inorganic $^{203}Hg^{2+}$ was lost. It was presumed that losses occurred due to the formation of volatile Hg species, mainly Hg^0.

The aim of the work presented here was to study whether transformations of mercury occur in *P. scaber* or whether they exist here already in the environment (^{203}Hg spiked food). An experimental setup was constructed in which both reduction and methylation processes were followed with the radiotracer ^{203}Hg. To assess the amount of abiotic and biotic reduction of $^{203}Hg^{2+}$ in the spiked food, experiments under sterile and nonsterile conditions were carried out. In order to investigate the transformation of $^{203}Hg^{2+}$ to $Me^{203}Hg$ and possible assimilation sites in the body, $^{203}Hg^{2+}$ and $Me^{203}Hg$ were determined in the food, hepatopancreas, gut, and residue of *P. scaber*, as well as in the animal's feces.

MATERIALS AND METHODS

In this study, experiments without and with isopods were performed (Fig. 1). In both experiments, radiotracer $^{203}Hg^{2+}$ was

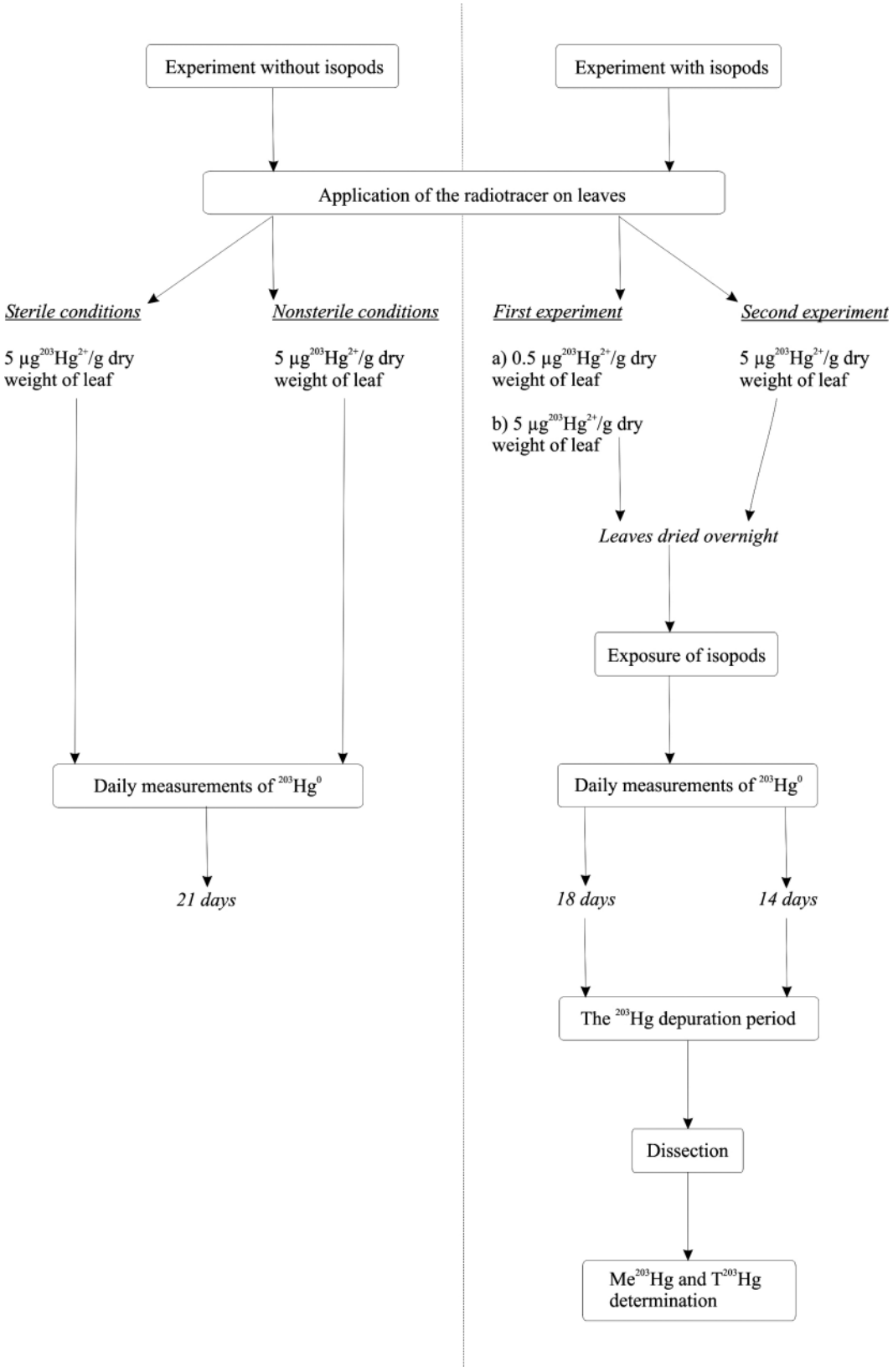

Fig. 1. Experimental scheme.

used. It should be pointed out that daily measurement of $^{203}Hg^0$ formed in the experiment without isopods started immediately after radiotracer was applied to the leaves. In experiments with isopods, measurements started with a delay of 12 h compared to the experiment without isopods, because leaves were dried overnight before being offered to the animals. This is a standard procedure for experiments with isopods [12,15,16]. The activity on the leaves could not be measured before they were offered to the animals, because the leaves would be damaged during the procedure (measurement geometry).

The basic idea of the experiment without isopods was to assess the contribution of bacteria present on the leaves to the reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$. Therefore, the amounts of $^{203}Hg^0$ released under sterile conditions (without bacteria) were compared to the amounts of $^{203}Hg^0$ formed under nonsterile conditions.

In experiments with isopods, daily measurements of $^{203}Hg^0$ were performed in vessels with and without animals exposed to different concentrations of radiotracer. At the end of the experiments, $T^{203}Hg$ and $Me^{203}Hg$ in animals, their food, and their feces were measured to study the transformations and distribution of different Hg species in the animals and their environment. In addition, mass balance calculations were performed. Because of the 12-h delay in $^{203}Hg^0$ measurements, data from the first day of the experiment without isopods were included in these calculations.

Experiment without isopods

Daily formation of $^{203}Hg^0$ was measured simultaneously in the vessels under sterile ($n = 3$) and nonsterile ($n = 3$) conditions for 21 d (Fig. 1). All vessels contained hazelnut leaves treated with same amounts and concentrations of radiotracer $^{203}Hg^{2+}$.

To ensure sterile conditions, glass vessels were sterilized together with leaves in an autoclave. Filtered $^{203}Hg^{2+}$ solution (pore size: 0.22 μm) was applied on the leaves in the sterile environment. The final concentration of tracer (stock solution of ^{203}Hg as $^{203}HgCl_2$ in 1 M HCl solution of 6.76 Ci/g Hg [November 15, 2001]; Isotope Products Laboratories, Burbank, CA, USA) was 5 μg $^{203}Hg^{2+}$/g dry weight of leaf (Fig.1). It was prepared with sterilized rainwater. The date in brackets is the date when the stock solution was prepared by the supplier and is important for further calculations of ^{203}Hg activity. Air entered the system through a filter with a pore size of 0.22 μm (to prevent the entrance of microorganisms). Air then passed through a brain–heart infusion (BHI) broth (sterility checking), which was prepared with 1.85 g of BHI powder (Biolife, Milano, Italy) dissolved in 50 ml of distilled water and sterilized in an autoclave for 20 min at 121°C. The BHI broth commonly is used for cultivation of a variety of fastidious and nonfastidious aerobic and anaerobic microorganisms [17]. Finally, the air passed through the system of vessels (experimental setup; Fig. 2).

During the experiment, $^{203}Hg^0$ was trapped on previously dry sterilized (3 h at 160°C) charcoal traps (activated charcoal for gas chromatography, 0.5–1.0 mm), placed on the top of the vessels (Fig. 2). Trapped $^{203}Hg^0$ was measured daily on a well-type high-purity germanium detector (Ortec, Oak Ridge, TN, USA).

At the end of the experiment, the sterility of the leaves was examined: Smears of the leaves were transferred to BHI agar plates (preparation: 37 g of BHI powder and 15 g of agar was dissolved in distilled water and autoclaved for 20 min at 121°C, then poured into Petri dishes at 50 to 60°C), incubated at 30°C for a few days, and examined for bacterial colonies. Vessels where no bacterial colonies were detected and where the BHI soup remained transparent were considered to be sterile.

Under nonsterile conditions, sterilization of material, solutions, and the experimental setup and sterility checking procedures were not performed.

Experiments with isopods

In order to follow uptake, distribution, and transformation processes of inorganic mercury in *P. scaber*, its food, and its feces, the radiotracer $^{203}Hg^{2+}$ was used. The half-life of the radiotracer ^{203}Hg is 46.6 d, which poses a problem in long-lasting experiments. We were not able to detect small amounts of newly formed $Me^{203}Hg$ in animal samples of the first experiment. For that reason, a second experiment with isopods was performed. These experiments are referred to in the following text as first and second experiment.

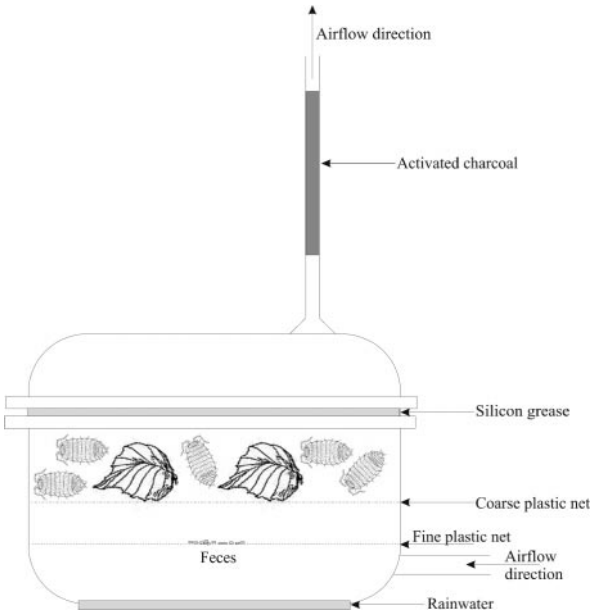

Fig. 2. Experimental setup (experimental vessel).

In the first experiment, a stock solution of ^{203}Hg as $^{203}HgCl_2$ in 1 M HCl solution of 6.76 Ci/g Hg (November 15, 2001; Isotope Products Laboratories, Burbank, CA, USA) and, in the second experiment, a stock solution with specific activity 9.47 Ci/g Hg (May 1, 2002) was used from the same supplier. The dates in parentheses are dates when the stock solutions were prepared by the supplier and are important for further calculations of ^{203}Hg activity. Our working solutions with defined mercury concentrations were prepared with rainwater to simulate natural conditions.

The hazelnut leaves were prepared and spiked with tracer with defined concentration and known activity following the protocol proposed by Drobne and Hopkin [15].

Individuals of the terrestrial isopod *P. scaber* (Isopoda, Crustacea) were collected in the vicinity of the University Campus in Rozna dolina in Ljubljana, Slovenia. Mature males were selected and held in the experimental setup (5 animals per vessel) with a controlled airflow (0.5–0.75 L/h) at room temperature (21 ± 2°C) under natural light and fed with hazelnut leaves (*Corylus avellana*) spiked with $^{203}Hg^{2+}$. High air humidity in the experimental vessels was maintained by the presence of 10 ml of rainwater on the bottom of each vessel (Fig. 2).

The duration of the first experiment was 18 d. In three experimental vessels, the concentration of $^{203}Hg^{2+}$ in the food was 0.5 μg $^{203}Hg^{2+}$/g dry weight of leaf and, in three other vessels, 5 μg $^{203}Hg^{2+}$/g dry weight of leaf. Two vessels at each Hg concentration contained leaves and animals. One vessel at each Hg concentration contained only spiked leaves.

The second experiment lasted 14 d. The concentration of $^{203}Hg^{2+}$ in the food was 5 μg $^{203}Hg^{2+}$/g dry weight of leaf in

all six vessels. Four vessels contained animals and two vessels contained only leaves.

The $^{203}Hg^0$ formed during the experiment was trapped daily on activated carbon traps and detected by gamma counting.

At the end of both experiments, animals, remaining leaves, and feces were weighed. Animals and feces were counted for gamma activity ($T^{203}Hg$). Then, after 48 h on untreated leaves (Hg depuration period), animals were counted for gamma activity again, and only then were dissected. In the first experiment, hepatopancreas, guts, and residues of all animals from one vessel ($n = 5$) were combined in one composite sample for $^{203}Hg^{2+}$ and $Me^{203}Hg$ determination. In the second experiment, hepatopancreas, gut, and residue were counted individually for gamma activity. However, for $^{203}Hg^{2+}$ and $Me^{203}Hg$ analysis, the organs of animals from two vessels ($n = 10$) were combined.

The $^{203}Hg^{2+}$ and $Me^{203}Hg$ in leaves and feces from each vessel and composite samples of organs were measured by a radiochemical method with specific separation of $^{203}Hg^{2+}$–dithizonate and $Me^{203}Hg$–dithizonate by thin-layer chromatography and gamma counting. This method was developed by Czuba et al. [18] and modified by Jereb et al. [14]. Some modifications in the first steps of the procedure were made during our experiment: Samples were decomposed in 4 M HNO_3 rather than in 1 M KOH [1] in Milli-Q® (Millipore, Billerica, MA, USA), at 90°C for 1 h. After that, 4 M KOH in Milli-Q water was added to achieve pH 2 and $NH_2OH \cdot HCl$ (12%) was added to destroy the oxidizing properties of the solution. In further steps, the procedure described in Jereb et al. [14] was followed. The $T^{203}Hg$ was measured after extraction of ^{203}Hg species in 0.1% dithizone in toluene with a well-type high-purity germanium detector (Ortec). For gamma counting, a p-type coaxial high-purity germanium detector (Canberra Industries, Meriden, CT, USA), and a well-type high-purity germanium detector were used.

RESULTS

Experiments without isopods

Experiments without isopods showed large differences in the daily reduction of $^{203}Hg^{2+}$ between sterile and nonsterile experimental conditions in the first few days of an exposure lasting 21 d (Fig. 3A). The most pronounced difference in the reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ between sterile and nonsterile conditions was observed in the first day of exposure. Under sterile conditions, almost four times less $^{203}Hg^{2+}$ was reduced as compared to reduction of $^{203}Hg^{2+}$ in the first day under nonsterile conditions. Of the total reduced mercury, about 55% was reduced in the first day under sterile conditions. About 80% of total reduced mercury was reduced in the first day under nonsterile conditions. After 21 d, on leaves with 5 μg of $^{203}Hg^{2+}$/g dry weight, about 49 ng of $^{203}Hg^{2+}$ was reduced under sterile conditions and about 136 ng of $^{203}Hg^{2+}$ was reduced under nonsterile conditions. As a percentage, about 5% of added $^{203}Hg^{2+}$ transformed under sterile and about 13% under nonsterile conditions.

Experiments with isopods

Comparison of the absolute values of daily released $^{203}Hg^0$ in the first experiment showed higher reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ in the vessels containing leaves with 5 μg $^{203}Hg^{2+}$/g dry weight than in the vessels with leaves with 0.5 μg $^{203}Hg^{2+}$/g dry weight (Fig. 3B).

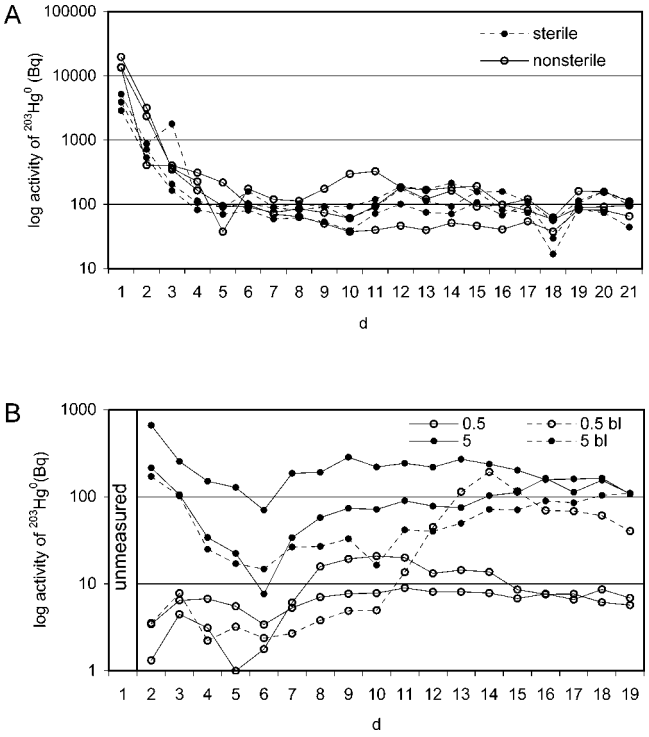

Fig. 3. (**A**) Experiment without isopods: Daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ under sterile ($n = 3$ vessels) and nonsterile ($n = 3$ vessels) conditions. Concentration of $^{203}Hg^{2+}$ on the leaves was 5 μg$^{203}Hg^{2+}$/g dry weight of leaf. (**B**) Experiment with isopods—first experiment: Daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ under normal conditions in the presence of the animals. Concentrations of $^{203}Hg^{2+}$ on the leaves in two vessels were 0.5 μg$^{203}Hg^{2+}$/g dry weight of leaf and, in the other two vessels, 5 μg$^{203}Hg^{2+}$/g dry weight of leaf. Vessels marked with 0.5 bl and 5 bl contained no animals. White bar (''unmeasured''): Reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ that occurred 12 h before measurements.

Different trends in reduction of $^{203}Hg^{2+}$ were observed at different concentrations of $^{203}Hg^{2+}$ spiked on the leaves. The daily reduction of $^{203}Hg^{2+}$ increased in the first few days on leaves with 0.5 μg $^{203}Hg^{2+}$/g dry weight and fell in the first few days on leaves with 5 μg/g of $^{203}Hg^{2+}$ (Fig. 3B). The trend of daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ on leaves with 5 μg $^{203}Hg^{2+}$/g dry weight was comparable with the reduction observed in the second experiment (data not shown) and in the experiment without isopods, performed at the same concentration (Fig. 3A).

No differences in reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ between vessels containing animals and vessels without animals were observed after 18 d (Fig. 3B). Similar results were obtained in the 14-d-long second experiment, where animals were exposed to leaves spiked with 5 μg $^{203}Hg^{2+}$/g dry weight (data not shown).

The white bar (''unmeasured'') in Figure 3B shows reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$, which occurred during preparation of leaves (spiking, drying overnight) before measurements. On the basis of the results of the experiment without isopods, it was concluded that about 80% of the total $^{203}Hg^0$ that was released during the experiment was formed in 12 h. This finding was the basis for subsequent mass balance calculations.

Mass balance and distribution of ^{203}Hg species

The mass balance of mercury ($T^{203}Hg$) in the 18-d-long first experiment and 14-d-long second experiment is shown in Table

Table 1. First experiment: Mass balance of $T^{203}Hg$ in ng. Values with asterisks (*) = excess of $T^{203}Hg$ in feces due to experimental error (it is possible that feces were measured together with some pieces of food or that feces were not completely dry) [a]

	First experiment				Second experiment			
	Concn. of Hg in leaves (μg/g)							
	0.5		5		5			
$T^{203}Hg$	ng	%	ng	%	ng		%	
O	153		1,524		1,335	1,280		
	152		1,514		1,250	1,335		
C	103	67	432	28	605	760	45	59
	66.0	43	496	33	830	895	66	67
U	50.0	33	1,092	72	730	523	55	41
	86.0	57	1,018	67	420	440	34	33
F	97.3	94	248	57	773*	942*	128*	124*
	65.5	100	311	63	816	1,018*	98	114*
A	2.50	2.4	13.3	3.1	5.05	4.72	0.8	0.6
	2.31	3.5	15.6	3.1	9.21	5.80	1.0	0.7
R	0.84	0.6	26.6	1.7	3.19	5.88	0.2	0.5
	1.16	0.8	11.0	0.7	22.4	21.4	1.8	1.6
R**	4.20	2.8	133	8.7	15.9	29.4	1.2	2.3
	5.80	3.8	55.1	3.6	112	107	9.0	8.0
(U+F+A+R)/O·100		98		91			113	115
		102		90			101	113
(U+F+A+R**)/O·100		100		98			114	117
		105		93			109	118

[a] Offered (O) = amount of $T^{203}Hg$ (ng) offered on food (hazelnut leaves); Consumed (C) (= assimilation + feces) = $T^{203}Hg$ consumed by animals; Unconsumed (U) = unconsumed $T^{203}Hg$; Feces (F) = $T^{203}Hg$ in feces (percentage of $T^{203}Hg$ in feces is calculated with regard to consumed food); Assimilation (A) = assimilation of $T^{203}Hg$ from consumed food; Reduction (R) = $^{203}Hg^{2+}$ reduced to $^{203}Hg^{0}$ during exposure of the animals; Reduction** (R**) = includes 80% of the total released $^{203}Hg^{0}$, which was released in the 12 h before exposure of the animals (estimated on the basis of results from the experiment without isopods). (U+F+A+R)/O·100 = comparison of input and output of $T^{203}Hg$ in experimental system; (U+F+A+R**)/O·100 = comparison of input and output of $T^{203}Hg$ in experimental system considering reduction of $^{203}Hg^{203}$ to $^{203}Hg^{0}$ 12 h before exposure of the animals.

1. In the first experiment, about 55% of mercury offered in the food with 0.5 $\mu g^{203}Hg^{2+}$/g dry weight of leaf was consumed by animals. The consumption was lower (about 30%) when the concentration of $^{203}Hg^{2+}$ in the food was 5 $\mu g^{203}Hg^{2+}$/g dry weight of leaf. Approximately 1 to 3% of consumed mercury was assimilated by isopods in both the first and second experiment. The majority of Hg consumed (~60 to 100%) was excreted in feces in the first experiment. In the second experiment, an excess of mercury in the feces was obtained (marked with an asterisk in Table 1), most probably due to experimental error. In the case of $Me^{203}Hg$, approximately 2% of $Me^{203}Hg$ consumed remained in the animals, and about 85% was excreted in feces. The deficit of $Me^{203}Hg$ (15%) was ascribed to demethylation (Fig. 4). Approximately 0.2 to 1.8% of $^{203}Hg^{2+}$ added to the system was reduced to $^{203}Hg^{0}$ during the exposure (marked in Table 1 as reduction). Some loss of mercury was observed in the mass balance calculations of the experimental system in the first experiment where animals were exposed to higher concentrations of $^{203}Hg^{2+}$ (U+F+A+R)/O·100; see Table 1 for further information regarding the equation). As mentioned before, about 80% of the total $^{203}Hg^{0}$ that formed during the experiment was released in the first day of the experiment without isopods (nonsterile conditions). For this reason, it was presumed that about 80% of $^{203}Hg^{0}$ was released during the overnight drying of the leaves in the experiments with isopods. After this finding was taken into consideration, the loss in the mass balance calculations was only about 5% (U+F+A+R**)/O·100). In the second experiment, an excess of $T^{203}Hg$ in the mass balance calculations was observed, due to the excess of $T^{203}Hg$ in the feces described below.

In the second experiment with isopods, samples of animal organs were combined in composite samples for $Me^{203}Hg$ and $^{203}Hg^{2+}$ determination. Due to one case of cannibalism during the mercury depuration period, unrealistic results were obtained in one of the combined gut samples. In this paper, we focus on the animals that ate the same food (leaves). For this reason, unrealistic results (due to cannibalism) are not shown in the following figures.

The relative distribution of assimilated total mercury ($T^{203}Hg$) in the animals, presented as the average ± standard

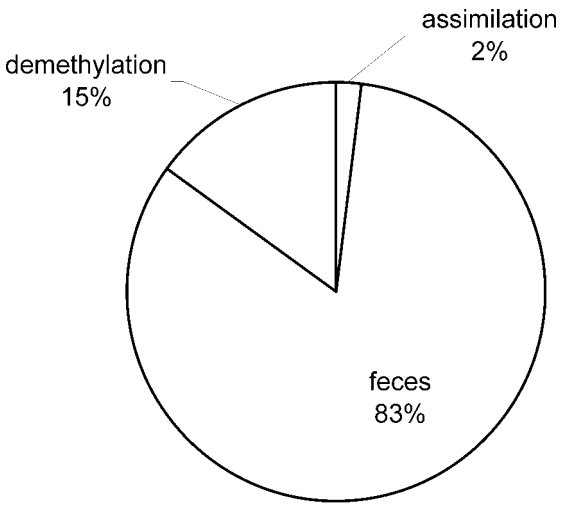

Fig. 4. Second experiment: Percentages of assimilated $Me^{203}Hg$ and $Me^{203}Hg$ in feces relative to $Me^{203}Hg$ consumed from food. Some loss of $Me^{203}Hg$ was noticed and marked with demethylation.

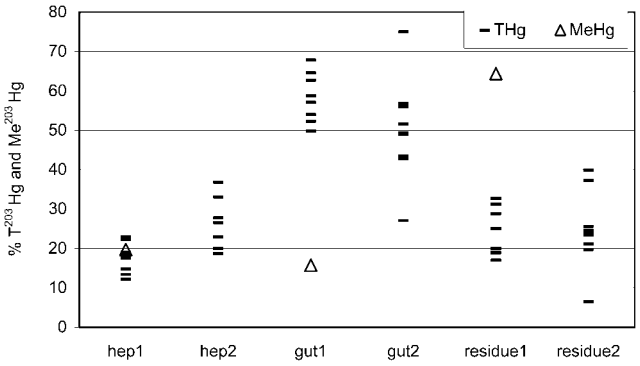

Fig. 5. Second experiment: Relative distribution in % of $T^{203}Hg$ (individual measurements of organs) and $Me^{203}Hg$ (composite samples of organs) in the animals exposed to 5 μg$^{203}Hg^{2+}$/g of dry weight of leaf. Animal organs: hep—hepatopancreas (digestive gland), gut, and residue. Hep1, gut1, residue1: Measurements of $T^{203}Hg$ and $Me^{203}Hg$ in animals' organs (n = 8) from the first and second experimental vessel. Hep2, gut2, residue2: Measurements of $T^{203}Hg$ in animal organs (n = 9) from the third and fourth experimental vessel.

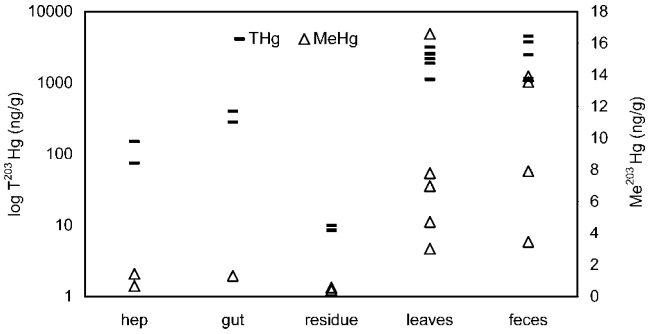

Fig. 7. Second experiment: $T^{203}Hg$ and $Me^{203}Hg$ concentrations in composite samples of animal organs: hep—hepatopancreas (digestive gland), gut, and residue, ($n1$ = 8, $n2$ = 9) and individual samples of leaves (n = 6) and feces (n = 4). The experiment was carried out with leaves treated with 5 μg$^{203}Hg^{2+}$/g dry weight of leaf. For calculations of $T^{203}Hg$ and $Me^{203}Hg$ concentrations in organs, a lyophilized mass of 1.23 mg for hepatopancreas, 0.98 mg for gut, and 12 mg for residue was used.

error, was as follows: In digestive gland–hepatopancreas, 17.6 ± 1.39% (hep 1) and 25.3 ± 2.14% (hep 2); in the gut, 58.4 ± 2.23% (gut 1) and 50.1 ± 4.30% (gut 2); and, in the residue, 24.1 ± 2.19 (residue 1) and 24.7 ± 3.25% (residue 2). The individual data are presented in Figure 5. Distribution of $Me^{203}Hg$ in the animals was different from that of $T^{203}Hg$. The highest percentage of $Me^{203}Hg$ relative to the total amount of $Me^{203}Hg$ in the animals was detected in the residue (65%); 25% was found in the hepatopancreas and 15% in the gut (Fig. 5).

Percentages of $Me^{203}Hg$ in organs with respect to $T^{203}Hg$ were very low. The highest percentage, about 5%, was found in the residue (Fig. 6). The percentages of $Me^{203}Hg$ with respect to $T^{203}Hg$ in leaves and feces were lower than in animals (~0.3%).

The concentrations of $T^{203}Hg$ and $Me^{203}Hg$ in leaves and feces are in the same range and higher compared to the concentrations of $T^{203}Hg$ and $Me^{203}Hg$ in the animals' organs (Fig. 7). Because $^{203}Hg^{2+}$ was applied on the leaves at the beginning of the experiment, the presence of $Me^{203}Hg$ in leaves indicates methylation of $^{203}Hg^{2+}$ during the experiment before the leaves were consumed by the animals. The concentrations of $T^{203}Hg$ were the highest in the gut and the lowest in residue. The concentration of $Me^{203}Hg$ was in the same range in both gut and hepatopancreas and higher than in the residue.

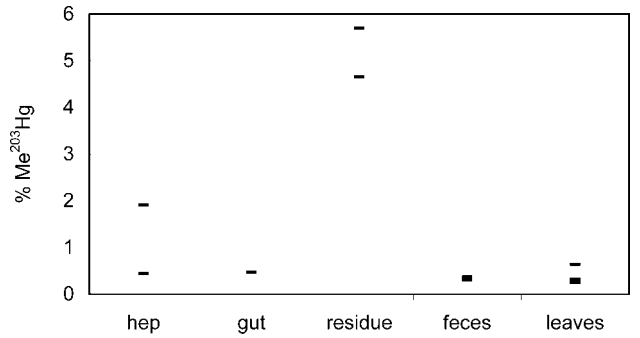

Fig. 6. Second experiment: % $Me^{203}Hg$ relative to $T^{203}Hg$ in different organs of animals, feces, and food. Animal organs: hep = hepatopancreas (digestive gland); gut; and residue.

DISCUSSION

The mass balance made in previous work by Jereb et al. [14] showed that some portion of spiked mercury in the experimental system was lost. It was presumed that losses occurred due to the formation of volatile Hg^0. Therefore, in our closed experimental setup, formation of $^{203}Hg^0$ during the experiments was measured.

The important finding from experiments without isopods was that reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ in nonsterile conditions (due to abiotic factors and bacteria) was almost four times higher than reduction under sterile conditions (due to abiotic factors) in the first day of the experiment. About 80% of total reduced mercury was transformed in the first day under nonsterile conditions and about 55% under sterile conditions. This indicated intensive activity of bacteria in transforming $^{203}Hg^{2+}$ to $^{203}Hg^0$ at the beginning of the experiment. The finding was in agreement with literature data, which report that mercury-resistant strains are able to reduce inorganic mercury to the volatile elemental form. These strains have genetic determinants for mercury resistance, known as the mer operon. Expression of the mer operon is correlated positively with concentrations of Hg^{2+} in the environment [10,19]. Barkay [1] described inducible concentrations of Hg^{2+} in the range of 0.2 to 20 μg/ml. In our experiments, solutions with 0.33 and 3.33 μg Hg^{2+}/ml were applied to the leaves. Also, results from experiments with *Escherichia coli* containing mer and glutathione *S*-transferase genes (important for mercury resistance) showed that up to 70% of the total mercury volatilization occurred in the first 4 h of the experiment [20].

In the experimental setup with isopods (first experiment), some loss of mercury at higher $^{203}Hg^{2+}$ concentrations was noticed after mass balance calculations. On the basis of the results from the experiment without isopods and literature data, we explained this loss as a loss of mercury due to microbiological activity during the 12-h-long preparation of the leaves. Thus, some portion of mercury was lost before animals were included in the experiment and measurements of $^{203}Hg^0$ started. When this was taken into the consideration, the mass balance calculation in the system with higher concentrations of Hg indicated a loss of only about 5%, which can be ascribed to measurement uncertainties.

No differences in Hg reduction levels between vessels that contained animals and vessels without animals were observed.

This indicates that reduction of $^{203}Hg^{2+}$ due to microflora in the digestive system of *P. scaber* was negligible compared to reduction of $^{203}Hg^{2+}$ in their environment (hazelnut leaves). Furthermore, the results showed that formation of $^{203}Hg^{0}$ was dependent on the concentration of $^{203}Hg^{2+}$ applied on the leaves. As shown in Figure 3B, at low concentrations of added $^{203}Hg^{2+}$ (0.5 μg/g), the amount of Hg^0 formed in the initial period increased, whereas the opposite trend was observed at higher $^{203}Hg^{2+}$ concentrations (5 μg/g). These results are insufficient to explain fully the processes involved but, probably at higher concentrations of added mercury ($^{203}Hg^{2+}$), activation of enzymes such as mercury reductase and organomercurial lyase occurred in bacteria present on the leaves [1].

At the end of the exposure, the majority of mercury in the animals and in the leaves still was present as $^{203}Hg^{2+}$. During animal exposure, only a small fraction (1–2%) of added inorganic mercury ($^{203}Hg^{2+}$) was transformed to the forms of $^{203}Hg^{0}$ and $Me^{203}Hg$.

The animals assimilated approximately 1 to 3% of total consumed mercury. Similar results were obtained by Jereb et al. [14] for mercury and by other authors working on other metals [15,16].

Our results showed a different distribution pattern of assimilated mercury in the animal compared to other metals. The hepatopancreas may contain more than 75% of assimilated zinc, 95% of cadmium, 80% of lead, and 85% of copper in the whole body [21]. The $T^{203}Hg$ in our experimental animals was distributed as follows: Approximately 20% was detected in hepatopancreas, 25% in residue, and the most, 55%, in the gut of the animal. The distribution of $Me^{203}Hg$ in the animal was different from that of $T^{203}Hg$: About 25% of total $Me^{203}Hg$ in the animal was detected in the hepatopancreas, 15% in the gut, and 65% in the residue. The highest portion of $Me^{203}Hg$ relative to the whole amount of assimilated $Me^{203}Hg$ in the animal was detected in the residue of the animal. This could be explained by deposition of organic mercury in chitin due to its strong affinity for −SH (sulfhydryl) groups [1]. Also, literature data report accumulation of MeHg in the neural nerve cord and gills of the grass shrimp (*Palaemonetes pugio*) [22] and in the hepatopancreas of Norway lobster (*Nephros norvegicus*) [23]. Assimilation of MeHg in *P. scaber* poses a potential danger to its predators and transfer through food chains.

The highest concentrations of $T^{203}Hg$ were detected in the gut, the lowest in the residue. The concentration of $Me^{203}Hg$ was in the same concentration range in gut and hepatopancreas and higher than in the residue.

Concentrations of $Me^{203}Hg$ were higher in leaves and feces compared to the animals. The presence of $Me^{203}Hg$ on the leaves showed that methylation of $^{203}Hg^{2+}$ was formed on hazelnut leaves, contributing to the $Me^{203}Hg$ consumed by the animals. Thus, methylation occurs on the leaves and in the digestive system of the animals, as reported by Jereb et al. [14]. However, based on the quantity of food consumed by the animals, relatively low amounts of $Me^{203}Hg$ (~2%) were assimilated. From literature data reporting high values of $Me^{203}Hg$ assimilation by animals (e.g., 70–80% assimilation of MeHg by the shrimp *Hyalella azteca* [24]), higher assimilation was expected in experimental isopods.

Mercury levels in sediments and soils are controlled both by methylation and demethylation. The relative importance of each process and the resulting net effect is dependent on environmental conditions and biological factors with spatial and temporal variations [7–10,25]. The isopod digestive system is aerated and its redox potential varies from 0 to +15 mV [26], making it an unfavorable environment for sulfate-reducing bacteria that have the prevailing role in methylation/demethylation of Hg. Some sulfate-reducing bacteria already have been isolated from the gut of *P. scaber* and were not found in their environment, indicating the possibility of Hg transformation both in the gut and in the food/environment or feces [27]. In a study of microbial pathways for Hg transformations in soils at Hg mining sites [28] and in lake sediments [25], lower mercury methylation potential rates were reported as compared to mercury demethylation potentials. Based on the facts described above, the deficit (15%) of $Me^{203}Hg$ obtained in our experiment (e.g., comparing assimilated and excreted vs. consumed $Me^{203}Hg$) also could be attributed to demethylation in the digestive system of the animal.

In other words, on the basis of the results from our study and literature data, demethylation of MeHg could prevail over methylation, leading to lower assimilation of MeHg and excretion of ingested mercury as Hg^{2+}. Further experiments are needed to verify this hypothesis, for example using $^{14}CH_3Hg$ [29] or stable mercury isotopes in combination with inductively coupled plasma mass spectrometry [30].

CONCLUSION

The radiotracer ^{203}Hg was used to follow transformations of mercury in a closed experimental system, where assimilation, distribution, and excretion of different mercury species in *P. scaber*, its food, and its feces were measured.

Biotic transformation of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$ was very pronounced at the beginning of the experiment without isopods: About 55% of total reduced $^{203}Hg^{2+}$ transformed under sterile (due to abiotic factors, e.g., humidity) and about 80% under nonsterile conditions (due to abiotic factors and bacterial activity) in the first day of the experiment. After these results were taken into consideration in the mass balance calculations of the experiment with isopods (measurements of $^{203}Hg^{0}$ started 12 h after $^{203}Hg^{2+}$ was applied on the leaves), only 5% loss of mercury was observed in the experimental setup with food treated with higher mercury concentrations.

The amount of $^{203}Hg^{2+}$ reduced to $^{203}Hg^{0}$ and the trend of reduction depended on the concentration of spiked $^{203}Hg^{2+}$. Transformation of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$ in the digestive system of *P. scaber* was negligible compared to reduction in its environment.

Methylation of $^{203}Hg^{2+}$ occurred on the hazelnut leaves before they were consumed by the animals. Based on the quantity of $Me^{203}Hg$ consumed, surprisingly low amounts of $Me^{203}Hg$ (~2%) were assimilated by the experimental animals. Also, a deficit of $Me^{203}Hg$ was observed when comparing assimilated and excreted $Me^{203}Hg$ versus consumed $Me^{203}Hg$. This was explained by the hypothesis that demethylation of MeHg could prevail over methylation in the animal's digestive system, resulting in excretion of ingested mercury as inorganic mercury.

Our results showed a different pattern of distribution of mercury in the body of *Porcellio scaber* than that found for other heavy metals, where the digestive glands are the most important metal storage organ. The highest portion of assimilated $T^{203}Hg$ was found in the gut (~55%) and the highest percentage of $Me^{203}Hg$ (~65%) was found in the residue. Concentrations of $T^{203}Hg$ were the highest in the gut, and concentrations of $Me^{203}Hg$ were in the same range in the gut and hepatopancreas, and lowest in the residue.

Mercury radiotracers are useful tools for following biolog-

ical and geochemical transformations of mercury in living organisms and their environments. However, in long-lasting experiments, their relatively short half-lives pose problems. In addition, they are not easily available and are very expensive. For long-term experiments, therefore, it is better to use stable mercury isotopes.

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