The fate of Hg in terrestrial isopod *Porcellio scaber* and its environment

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Abstract: In our work reduction and methylation of inorganic mercury in the *Porcellio scaber* (Isopoda, Crustacea) and its environment were studied using radiotracer ^{203}Hg. Total mercury ($T^{203}Hg$) and monomethylmercury ($Me^{203}Hg$) in the whole animals, gut, digestive glands (hepatopancreas), food (hazelnut leaves) and excrement were measured in order to: (1) to obtain the distribution of $T^{203}Hg$ and $Me^{203}Hg$ in animals, (2) to investigate the origin of $Me^{203}Hg$ and the site of its accumulation, and finaly, (3) to assess the mass balance of mercury in our experimental system. After two weeks of the experiment majority of mercury in animals and their environment remained as inorganic $^{203}Hg^{2+}$. The net formation of elemental mercury ($^{203}Hg^0$) and $Me^{203}Hg$ was detected at much lower concentrations as $^{203}Hg^{2+}$. Approximately 3 % of consumed mercury was assimilated by the animals and the majority of Hg was excreted by feaces. Approximately 20 % of $T^{203}Hg$ was detected in hepatopancreas, 55 % in gut and 25 % in residue of animal. About 25 % of $Me^{203}Hg$ was found in hepatopancreas, 15 % in gut and 65 % in animal residue. Concentrations of $Me^{203}Hg$ were higher on leaves and in faeces compared to the animals. Also, the amounts of $Me^{203}Hg$ found in animals were lower than expected. This suggests that demethylation of $Me^{203}Hg$ could prevail over mercury methylation in the digestive system of the animal.

Key words: Mercury transformations, reduction, methylation, radiotracer ^{203}Hg, *Porcellio scaber*

INTRODUCTION

In the framework of studies on mercury biogeochemistry in contaminated and polluted sites due to past mercury mining in Slovenia a study on the uptake, distribution and transformation of mercury in terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea) was initiated. In our work reduction and methylation of inorganic mercury in the *Porcellio scaber* and its environment was followed. For this purpose an experimental set up was build (Figure 1) where Hg uptake, distribution, retention and transformation was followed using a ^{203}Hg tracer.

During experiment daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^0$ was measured. Elemental mercury was trapped on activated carbon traps and the radiotracer $^{203}Hg^0$ was detected by gamma counting. In animals, animal organs, food (hazelnut leaves) and excrement $^{203}Hg^{2+}$ and $Me^{203}Hg$ were measured. For $Me^{203}Hg$ and $^{203}Hg^{2+}$ determination, a radiochemical method with specific separation of $^{203}Hg^{2+}$-dithizonate and $Me^{203}Hg$ - dithizonate by thin

layer chromatography described by JEREB ET AL.[1], and gamma counting was used.

RESULTS AND DISCUSSION

An the end of experiment majority of mercury in animals, their food and feaces remained as $^{203}Hg^{2+}$. $^{203}Hg^{0}$ and $Me^{203}Hg$ were detected at much lower amounts as $^{203}Hg^{2+}$. Approximately 0.60 – 1.88 % of $^{203}Hg^{2+}$ added to the system reduced to $^{203}Hg^{0}$ (Table 1). Results from daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$ from the experiment with animals showed that the reduction in the vessels containing leaves with 5 µg $^{203}Hg^{2+}$/g dry weight was higher than reduction on leaves with 0,5 µg $^{203}Hg^{2+}$/g of $^{203}Hg^{2+}$ (Figure 2), indicating that $^{203}Hg^{0}$ formed as a function of initial concentration of $^{203}Hg^{2+}$. Similar results were reported by LUDWICKI[2]. No differences in reduction of inorganic mercury between vessels containing animals and control vessels without animals were observed in 18 days, indicating that reduction of $^{203}Hg^{2+}$due to bacteria in digestive system of animals (*Porcellio scaber*) was negligible and mercury was most probably reduced due to humidity in the experimental set up and microorganisms on the leaves and feaces.

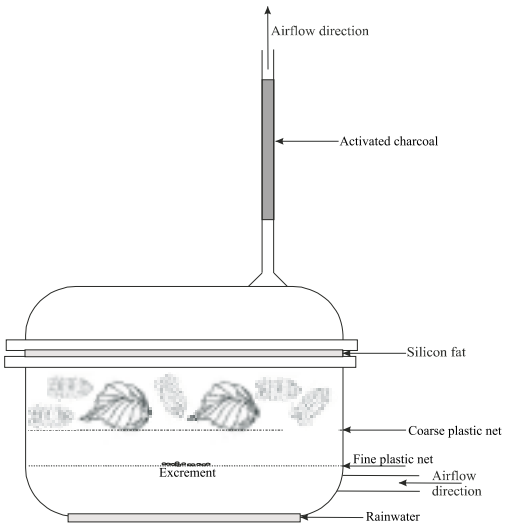

Figure 1. Experimental set up

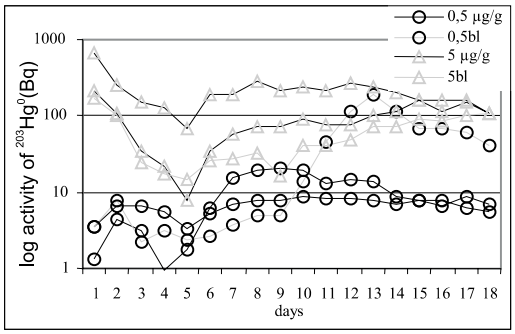

Figure 2. Daily reduction of $^{203}Hg^{2+}$ to $^{203}Hg^{0}$ under normal conditions, where animals were present. Concentrations of $^{203}Hg^{2+}$ were in two vessels 0,5 µg$^{203}Hg^{2+}$/g dry weight of leaf and in other two vessels 5 µg$^{203}Hg^{2+}$/g dry weight of leaf. In the vessels marked with 0,5bl and 5bl were no animals and were used as controls.

About 3 % of consumed $T^{203}Hg$ was assimilated by the animals, majority (60 to 100 %) of Hg was excreted by feaces (Table 1). The assimilated mercury was distributed in the animal as follows: hepatopancreas 12.2 to 36.8 %, the gut about 27.0 to 75.0 % and in the residue of the animals about 6.5 to 39.9 % of $T^{203}Hg$. For MeHg the following distribution was observed: 25 % of $Me^{203}Hg$ was detected in hepatopancreas, 15 % in gut and 65 % in residue (Figure 3). The percentages of $Me^{203}Hg$ compared to $T^{203}Hg$ in organs were very low, the highest, about 5 %, in animals residue (Figure 4). The results showed that the animal accumulated $Me^{203}Hg$ in some selective parts, which is in agreement with the data from the literature where accumulation of MeHg in nevtral nerve cord and gills in grass shrimp (*Palaemonetes pugio*)[6] was found.

Table 1. Mass balance of $T^{203}Hg$ in ng. O - amount of $T^{203}Hg$ (ng) offered on a food, C - amount of consumed $T^{203}Hg$ (ng) by animals, U - amount of unconsumed $T^{203}Hg$ (ng), F - amount of $T^{203}Hg$ in the feaces, % F - % of feaces production with regard to consumed food , A - amount of $T^{203}Hg$ assimilation from consumed food, % A - % of $T^{203}Hg$ assimilated from consumed food, R - amount of reduced $^{203}Hg^{2+}$ to $^{203}Hg^0$, % R...% of $^{203}Hg^0$ in the system. Factor (F+A)/C indicates mass balance of $T^{203}Hg$ in animal. Factor (U+F+A+R)/O indicates mass balance of $T^{203}Hg$ in experimental system.

Conc. Hg in leaves (µg/g)	O	C	U	F	% F	A	% A	R $^{203}Hg^0$	% R	(F+A) /C	(U+F+A +R)/ O
0,5	153	103	50	97	94.2	2.5	2.43	0.92	0.60	0.97	0.98
0,5	152	66	86	66	100	2.31	3.50	1.22	0.80	1.04	1.02
5	1524	432	1092	248	57.4	13.3	3.08	28.6	1.88	0.60	0.91
5	1514	496	1018	311	62.7	15.6	3.14	13.6	0.90	0.66	0.90

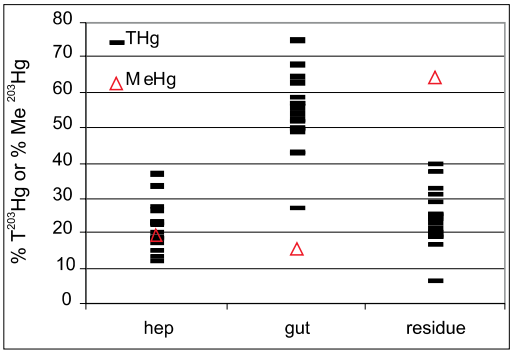

Figure 3. Distribution of $T^{203}Hg$ and $Me^{203}Hg$ in the animals exposed to 5 µg$^{203}Hg^{2+}$/g of dry weight of leaf.

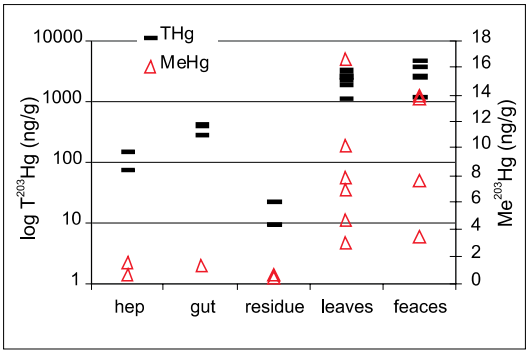

Figure 4. % $Me^{203}Hg$ regarding $T^{203}Hg$ calculated from TLC (Thin Layer Chromatography) in different organs of animals, feaces and food (hazelnut leaves).

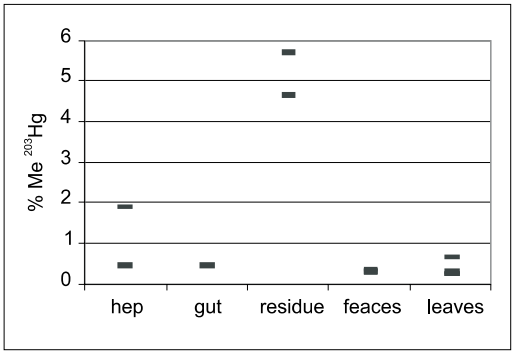

Figure 5. $T^{203}Hg$ and $Me^{203}Hg$ concentrations in composite samples of animal organs (n1=8, n2=9) and individual samples of leaves (n=6) and excrement (n=4) after acid digestion and extraction in Dz-tl. Anials were exposed to 5µg$^{203}Hg^{2+}$/g dry weight of leaf. For calculations of $T^{203}Hg$ and $Me^{203}Hg$ concentrations in organs liophylized mass 1,23 mg for hepatopancreas, 0,98 mg for gut and 12 mg for residue were used.

Precise values of $T^{203}Hg$ and $Me^{203}Hg$ concentrations in hepatopancreas, gut and residue are shown in Figure 5. Generaly, concentrations of $Me^{203}Hg$ in organs were very low, compared to $T^{203}Hg$ concentrations.

Concentrations of $Me^{203}Hg$ were higher in leaves and feaces compared to the animals (Figure 5), which shaws that the methylation already occurs on hazelnut leaves. With regard to quantity of $Me^{203}Hg$ in consumed food and knowing that greater part (95 %[5], 70-80 %[6]) of consumed MeHg should be absorbed in the gut of the animal, our experimental animals assimilated relatively low amounts of $Me^{203}Hg$ (app. 4 %). This sug-

gested that demethylation of $Me^{203}Hg$ could prevail over mercury methylation in the digestive system of the animal, leading to increased excretion of ingested mercury.

Loss of $T^{203}Hg$ from mass balance of system and animal were detected by higher concentrations of $^{203}Hg^{2+}$on leaves (Table 1). Some additional experiment (*data not shown*), where Hg reduction was measured immediately after $^{203}Hg^{2+}$ was aplied on leaves has shown that about 80 % of total reduced Hg formed during the first day of experiment. Therefore, most probably loss of Hg occured during leaves preparation (radiotracer aplication, dryig overnight), due to microbial reduction of inorganic mercury.

CONCLUSIONS

Our results have shown that $^{203}Hg^0$ formed as a function of initial concentration of $^{203}Hg^{2+}$, and that reduction of $^{203}Hg^{2+}$due to bacteria in digestive system of *Porcellio scaber* was negligible compared to reduction in its environment. Based on consumed $Me^{203}Hg$, relatively low amounts of $Me^{203}Hg$ in animals were found, therefore demethylation of $Me^{203}Hg$ could prevail over mercury methylation in the digestive system of the animal. Further experiments will therefore be needed with $^{14}CH_3Hg$ in order to verify this hypothesis. In the future, transformation processes of mercury in feaces should be addressed as part of the experiment.

Acknowledgements

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