

The effect of selenium and organic material in lake sediments on the bioaccumulation of methylmercury by *Lumbriculus variegatus* (oligochaeta)

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Abstract. The accumulation of methylmercury (MeHg) to an oligochaete worm *Lumbriculus variegatus* (Müller) was measured in two different lake sediments in the laboratory. ¹⁴C-labelled MeHg was added to sediments at the nominal concentration of 95 ng/g dw sediment. Groups of six oligochaete worms were exposed in glass beakers to 35 g of spiked sediment for 14 days. The two sediments had organic carbon concentrations of 3.4% and 9.9% and natural selenium concentrations of 1.45 and 0.28 mg/kg (dw), respectively. After two weeks exposure, both the accumulation rate of MeHg and the body residue in the worms were much lower in the sediment having a high organic carbon content. The effect of selenium concentration in the sediment on bioaccumulation of MeHg in *Lumbriculus variegatus* was measured in one sediment (organic carbon 3.4% and Se 1.45 mg/kg) by adding sodiumselenite (Na₂SeO₃) at different concentrations. The added amounts of selenium were 0, 0.1, 0.5, 2.5, 15.0, and 50.0 mg Se/kg dry sediment. In this exposure the nominal concentration of MeHg was 102 ng/g dw sediment. The two lowest selenium concentrations did not affect the bioaccumulation of MeHg. But, the dose of 2.5 mg Se/kg resulted in a 25% reduction in the body residue after two weeks exposure. When 15 and 50 mg Se/kg were added to the sediment the accumulation of MeHg in the organisms was decreased by 75% and 86%, respectively, as compared to the reference.

Introduction

It is well documented that sediments are a sink for hydrophobic organic pollutants as well as metals discharged into the aquatic environment (Eisenreich 1987; Baker et al. 1991; Armstrong et al. 1987). Although mostly bound by sediment particles, these pollutants can be bioavailable to benthic organisms. Thus, sediment-associated contaminants can be toxic to benthic organisms or reintroduced in significant levels into the aquatic food chains. Both total mercury (Hg) and methylmercury (MeHg) have a high affinity to particles and dissolved organic matter (Hintelman et al. 1995) and accumulate in lake sediments. For example, Verta (1984) estimated that about 99% of the Hg

in a 'typical freshwater lake' is present in the sediment, if 5 cm of surface sediment is included in the calculation. A similar budget was also reported by Wiener et al. (1990).

In many studies where analyses of Hg and MeHg in sediments have been performed, the aim has mainly been to quantify the concentrations and the methylation and demethylation dynamics, but not the possible bioavailability of the compounds. It has been shown that the bioaccumulation of sediment-bound MeHg by benthic organisms is 20 times higher compared to sediment-bound inorganic Hg (Odin et al. 1995a). However, if MeHg and inorganic Hg were dissolved in the water phase a similar kind of difference was not noticed (Odin et al. 1995a). This leads to the factors that affect the overall distribution and environmental fate of these compounds. Very little is known of MeHg distribution among the different phases or particle size fractions in different sediments and how this partitioning affects the bioavailability of MeHg. Besides chemical factors, the bioavailability of MeHg depends on biological factors like feeding behaviour, food quality and overall ecology of the organisms exposed, i.e. the factors that determine the possible and dominant uptake routes (Parkman & Meili 1993; Odin et al. 1995a, 1995b).

One element that has an interesting and not so well understood effect on the environmental behaviour of Hg is selenium. Selenium and mercury are both considered as environmental pollutants, although selenium has also been shown to be an essential element in human and animal nutrition. The interaction between these two elements was reviewed recently by Cuvin-Aralar and Furness (1991). The protective effect of selenium against Hg toxicity and vice versa has been observed in several different organisms. Also there is clear evidence that selenium affects the environmental fate and bioavailability of mercury in certain systems (Rudd et al. 1980; Turner & Rudd 1983; Paulsson & Lundberg 1991; Southworth et al. 1994). However, the mechanism of Se-Hg interaction in nature and in the organisms is unknown, and even less is known about the effects of selenium on the bioavailability of MeHg in aquatic systems and especially in sediments.

The objectives of this laboratory study were 1) to measure the accumulation kinetics and the body burden of added MeHg in an oligochaete worm *Lumbriculus variegatus* in different lake sediments and 2) to measure the effect of different selenium concentrations on the bioavailability of MeHg to *L. variegatus*.

Materials and methods

Organisms, chemicals, sediment and water

Cultures of the oligochaete worm, *L. variegatus*, were kept in 24 l glass aquaria containing artificial freshwater. The rearing temperature was $20 \pm 2^\circ\text{C}$ and the light rhythm 16 h light and 8 h dark. Presoaked shredded paper towels were used as a substrate in the aquaria. The overlaying water was replaced 2–3 times per week and the population was fed three times per week with Tetramin[®] fish food.

[¹⁴C]Methylmercuric iodide (specific activity 15.2 mCi/mmol, Amersham) was dissolved in methanol and ethanol (1:1). Sodium selenite-5-hydrate, $\text{Na}_2\text{SeO}_3 \times 5\text{H}_2\text{O}$ (Riedel-deHaën AG) was dissolved in deionized water.

Two different sediments were used in the exposures. Their characteristics are presented in Table 1. Lake Höytiäinen sediment was obtained by centrifugal pump fitted with a pipehose at 20–30 m depth and Lake Mekrijärvi sediment by Ekman grab at 2 m depth. The sediments were held at $+5^\circ\text{C}$ before dosing. Throughout the work we used phosphorous buffered artificial soft freshwater at pH 7.0. Artificial organic-free freshwater used for the experiments was made up of Milli-Q grade water and the following reagent-grade salts: $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 58.8 mg/l; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 24.7 mg/l; KCl, 1.1 mg/l; and NaHCO_3 , 13.0 mg/l (Ca+Mg hardness = 0.50 mM).

Sediment samples were characterized before experiments. Carbon and nitrogen content of the dry sediments was analysed with a Carlo Erba elemental analyzer (model 1106). For total Hg analysis sediment samples were digested with $\text{HNO}_3\text{-H}_2\text{O}_2$ (5:1, vol:vol) mixture using a high performance microwave digestion unit (Milestone mls 1200 mega) followed by reduction with SnCl_2 before determination with a gold film mercury analyzer (Jerome Instrument Corp. model 511). Fe and Cr were analysed from the same digestion with flame AAS. For total Se, the sediment samples were digested with the mixture of concentrated HNO_3 and HCl in microwave oven (CEM MDS-81D). Se-analyses were performed with graphite furnace AAS (Perkin-Elmer > Zeeman 5000). Palladium was used as a matrix modifier.

Sediment dosing and bioaccumulation assays

The sediment samples were prepared for all of the exposures by using the same procedure. First the sediments were stirred for one hour and then the dosing solutions were added dropwise to the sediment. After dosing the sediment was stirred for two hours at room temperature and kept overnight at 5°C .

Table 1. Some characteristics of the two lake sediments used in the exposures (mean \pm SD).

	Lake Höytiäinen	Lake Mekrijärvi	<i>n</i>
Dry weight (%)	21.8 \pm 0.4	22.3 \pm 1.5	4
LOI (%)	7.1 \pm 0.2	17.8 \pm 0.1	4
C%	3.47 \pm 0.07	10.01 \pm 0.26	20
N%	0.32 \pm 0.01	0.56 \pm 0.01	20
Total Se (mg/kg dw)	1.45 \pm 0.09	0.28 \pm 0.05	4
Total Hg (μ g/kg dw)	103.9 \pm 5.2	60.2 \pm 18.7	3
Total Cr (mg/kg dw)	57.1 \pm 1.0	27.9 \pm 3.0	3
Total Fe (g/kg dw)	64.2 \pm 0.1	22.0 \pm 0.1	3

The bioaccumulation of MeHg was measured in two different lake sediments in the laboratory (Table 1). The added nominal concentrations of radiolabelled MeHg were 90 ng/g dw sediment and 106 ng/g dw sediment in Lake Höytiäinen and in Lake Mekrijärvi sediment, respectively. The natural background concentrations of MeHg in these sediments were not measured.

For the exposure, 35 g of wet sediment was transferred to each of the 250 ml exposure beakers. Artificial freshwater was then added with minimal disturbance and the beakers were immersed in the aquaria containing artificial freshwater (pH 7.0, 20.0 \pm 1.5 °C). On the following day six test organisms were carefully added by a pipette to each beaker. The exposure was carried out with the water renewal system described by Zumwalt et al. (1994). For the Lake Höytiäinen experiment the sampling times were 24, 48, 96, 120 and 168 h and for Lake Mekrijärvi 24, 48, 96, 168, 240 and 336 h. At each time three replicate beakers were sampled. Thus the total number of beakers was 15 for Lake Höytiäinen and 18 for Lake Mekrijärvi.

For each beaker, duplicate 8 ml water samples were taken from the overlying water for radioisotopic analysis, all of the organisms were gently sieved from the sediment, rinsed in artificial freshwater, blotted dry, weighed, placed in scintillation cocktail (Lumac LSC, Belgium) and sonicated for 2 min. to ensure the extraction of MeHg from organisms to scintillation cocktail. ^{14}C -activity was counted after 2 days with a liquid scintillation counter (1217 Racbeta, Wallac LKB, Finland). Dosed wet sediment samples were taken in duplicate for determination of contaminant concentration and dry:wet weight ratios. The dry:wet weight ratios were obtained by weighing a wet sediment sample and drying it at 105 °C to constant weight. The contaminant concentration in the sediment was determined either by placing approximately 100 mg wet sediment into 12 ml scintillation cocktail, sonicating for 2 min and measuring ^{14}C activity 2 days after the sonication, or by solubilizing

100 mg wet sediment by LumaSolve (Lumac) and measuring the ^{14}C -activity in 12 ml of scintillation cocktail.

The effect of selenium concentration in the sediment on bioavailability of MeHg by *L. variegatus* was measured in Lake Höytiäinen sediment (Table 1.) by adding sodiumselenite (Na_2SeO_3) at different concentrations. Two different bioassays were performed. In the first experiment the effect of three selenium concentrations on accumulation kinetics was studied. The nominal concentration of MeHg in the sediment was about 93 ng/g dw and the added selenium concentrations were 0.45, 2.35 and 14.18 mgSe/kg dw sediment. The sampling times were 24, 48, 96, 168, 240 and 336 h. Thus the total number of beakers was 54 and animals 324. The sampling was similar to that described above. In the second selenium experiment we analysed only the accumulated body residue of MeHg in the worms after two weeks (336 hours) exposure period. The added concentrations of selenium were 0, 0.1, 0.5, 2.5, 15.0, and 50.0 mg Se/kg dw sediment. In this exposure the nominal concentrations of MeHg was 102 ng/g dw sediment. The total number of beakers was 30.

Calculations

The kinetics of MeHg accumulation were determined by fitting the data to a first-order rate-constant model:

$$C_a = \frac{k_u \cdot C_s \cdot (1 - e^{-k_e t})}{k_e}, \quad (1)$$

where k_u is the uptake clearance coefficient ($\text{g dry sediment g}^{-1} \text{ wet organism h}^{-1}$), C_s is the concentration in the sediment ($\text{ng g}^{-1} \text{ dw}$), t is time (h), k_e is the elimination rate constant (0.0005 h^{-1}), and C_a is the concentration in the organism ($\text{ng g}^{-1} \text{ ww}$). For some treatments, the model was simplified because the accumulation was apparently linear during the one or two week exposure period (Landrum 1989). In these cases, a linear regression model was applied.

Results and discussion

Sediment concentration of MeHg

Methylmercury concentration in the sediment showed a decrease during the two weeks exposure (Figure 1). This decrease was either due to degradation of MeHg or it became so tightly bound by sediment particles that our extraction method did not get into the scintillation cocktail. The measured overlaying

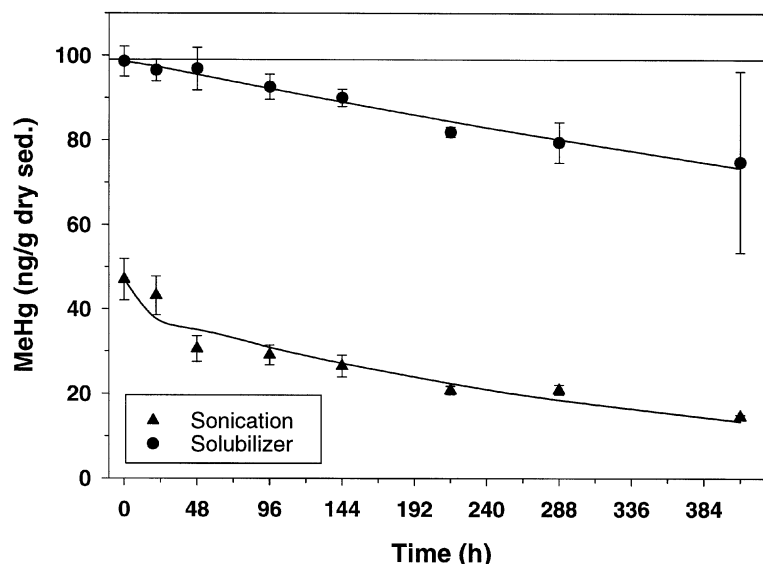


Figure 1. Recovery of added ^{14}C -methylmercury from Lake Höytiäinen sediment by two different methods. The nominal concentration (99 ng/g dw sediment) is indicated by line.

water concentration were negligible and the mass of organisms was extremely low compared to the amount of sediment. The MeHg was tightly bound to sediment particles and the method to measure the added radioactivity significantly affected recovery. A simple extraction and sonication with liquid scintillation cocktail was not sufficient to get a good recovery from the sediment. The use of a solubilizer gave much better results in analytical point of view (Figure 1) but it is hard to say whether this kind of tightly bound fraction of MeHg is available to organisms at all.

MeHg accumulation from different sediments

Animals were actively feeding in the sediments during the exposures. Both the accumulation rate of MeHg and the body residue after the exposure period in the worms were lower in the sediment having a high organic carbon content (Figure 2). The uptake rate ($k_u \pm \text{SE}$; $\text{g sed. g}^{-1}\text{org.h}^{-1}$) constant for MeHg accumulation by *L. variegatus* in Lake Höytiäinen sediment was 0.0089 ± 0.0004 and in Lake Mekrijärvi sediment 0.0032 ± 0.0002 . The MeHg concentration in the worms did not reach a steady-state during the two weeks exposure (Figure 2). This and the low k_u values indicate that MeHg is well bound by the particles and only slowly accumulates into the organisms as some lipophilic organic chemicals like pyrene or benzo(a)pyrene (Landrum et al. 1994; Kukkonen & Landrum 1995).

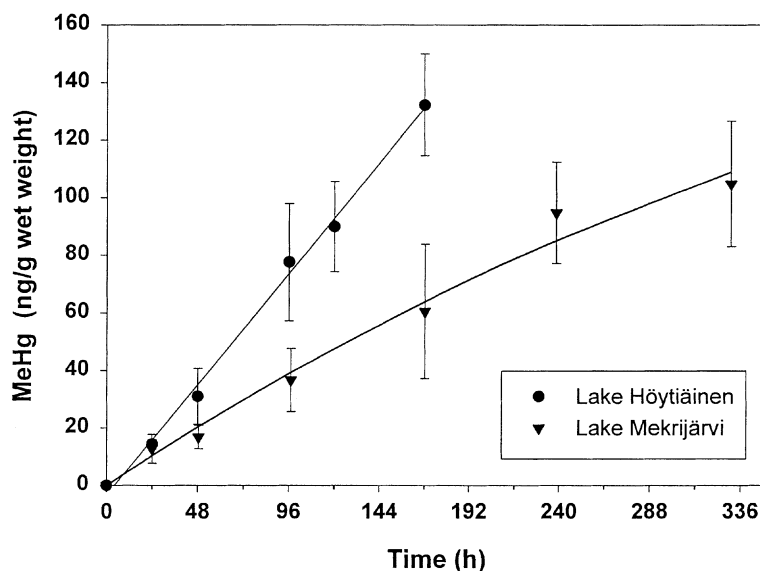


Figure 2. Effect of sediment organic carbon content on the accumulation of ^{14}C -methylmercury by *Lumbriculus variegatus* from two different sediments. The nominal concentrations of MeHg were 90 and 106 ng/g dw sediment for Lake Höytiäinen and Lake Mekrijärvi, respectively.

The sediment organic carbon content is an important factor in determining sorption properties of the sediment. In most cases, the sediment-water partition coefficient of organic non-polar chemicals is increasing with the increasing hydrophobicity of the xenobiotics and the amount of organic matter in sediment (Karickhoff et al. 1979; Means et al. 1980; Schwarzenbach & Westall 1981). In this series of exposures, we spiked the sediment and started the exposures two to three days later. However, it is noteworthy that the bioavailability of sediment-associated compounds may decrease with increased contact time between the sediment and the xenobiotic (Landrum 1989; Landrum et al. 1992; Kukkonen & Landrum 1997). For example, PAH compounds such as fluorene, phenanthrene, and pyrene were more available to organisms (as determined by uptake clearance) in dosed sediments aged less than one week than in that dosed and aged 60 to 150 days (Landrum et al. 1992; Harkey et al. 1994).

Increasing sorption to the particles reduces the bioavailability of the compounds. Some field studies have shown that the total mercury concentration of the lake sediments correlates strongly with the organic material content of the sediment (Wiener et al. 1990; Lucotte et al. 1994; Tremblay et al. 1995). However, the field data does not show that the bioavailability of mercury or MeHg is changed, it only indicates that the natural organic matter plays

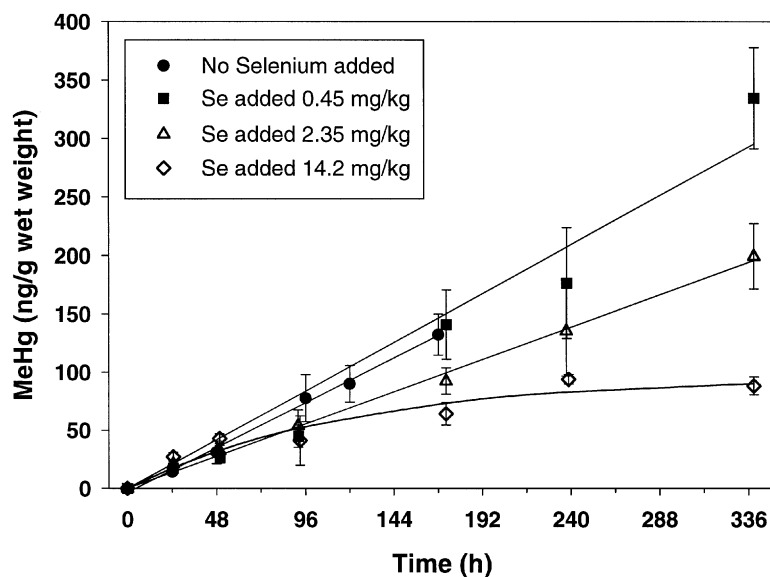


Figure 3. The accumulation of ^{14}C -methylmercury by *Lumbriculus variegatus* in sediments with different added selenium concentrations. The nominal concentrations of MeHg was 93 ng/g dw sediment. The “no added Se” exposure is the same as shown in Figure 2 for Lake Höytiäinen.

an important role in the environmental fate of mercury. The data presented here indicates that the bioavailability of MeHg is strongly affected by the sediment organic carbon content. Further, it is important to notice that sediment dwelling and sediment ingesting organisms, like oligochaetes, may be an important step introducing the sediment-bound MeHg back to the food webs, where concentration of MeHg has shown to increase from trophic level to the other even in benthic food webs (Tremblay et al. 1996).

Effect of selenium on bioaccumulation of MeHg

The selenium-MeHg exposures were performed in the Lake Höytiäinen sediment, with natural selenium concentration of $1.45 \mu\text{g/g}$ dw sediment (Table 1). The accumulation kinetics of MeHg was affected by the added selenium in this sediment (Figure 3, Table 2). The added selenium concentrations of $2.35 \mu\text{g/g}$ dw sediment and $14.2 \mu\text{g/g}$ dw sediment decreased the uptake rate as well as the accumulated body residue of methylmercury in the worms.

In the other bioassay with selenium, where we analysed only the accumulated body residue after two weeks exposure, the two lowest selenium concentrations (0.1 and $0.5 \mu\text{g/g}$ dw sediment) did not affect the bioavailability of methylmercury. The dose of $2.5 \mu\text{gSe/g}$ dw sediment resulted in

Table 2. The uptake rate ($k_u \pm \text{SE}$; $\text{g sed. g}^{-1} \text{ org. h}^{-1}$) constant for methylmercury accumulation by *L. variegatus* in the sediment from Lake Höytiäinen (natural selenium $1.45 \mu\text{g/g dw}$) with different selenium doses. The nominal concentration of MeHg was 93 ng/g dw sediment. The accumulation curves are plotted in Figure 3.

	k_u
No Se added	0.0089 ± 0.0004
Se added $0.45 \mu\text{g/g dw}$	0.0105 ± 0.0005
Se added $2.35 \mu\text{g/g dw}$	0.0062 ± 0.0003
Se added $14.2 \mu\text{g/g dw}$	0.0030 ± 0.0002

a 25% reduction in the body residue. When 15 and $50 \mu\text{gSe/g dw}$ sediment were used the accumulation of methylmercury in the organism was decreased to 25% and 14%, respectively, of the reference (Figure 4). There is a certain relationship between selenium concentration and reduction in bioavailability of MeHg. If these two selenium experiments are compared, the accumulated body residues of MeHg in worms are identical in similar selenium concentrations after a two weeks exposure. During these exposures we did not notice any toxic effect caused by selenium, i.e. all of the organisms survived in every selenium concentration and their feeding activity as well as behaviour was comparable to the organisms kept in the reference sediment. We did not performed any exact measurement on feeding or behaviour, but monitored the beakers on a daily basis.

Some previous studies indicate that elevated selenium concentrations may decrease the bioavailability of mercury to fish (Rudd et al. 1980; Turner & Rudd 1983; Southworth et al. 1994). In Sweden, field experiments of remedial measures involving selenium additions have been performed in lakes with high mercury concentration in biota and they were successful in reducing total mercury body residue in fish but at the same time high selenium concentrations caused adverse effects on fish populations (Paulsson & Lundberg 1991; Meili 1996). Based on this data, it appears that selenium has an effect in reducing the mercury accumulation. However, some other studies have shown that high selenium concentrations do not have any effect on the bioavailability of mercury (Pelletier 1986; Rouleau et al. 1992) or may even increase the assimilation of MeHg from the food (Bjerregaard & Christensen 1993). This inconsistency in the data may indicate that there is a certain molecular ratio of mercury and selenium needed to reveal the interaction, or different forms of mercury may interact differently with different forms of selenium. It is also possible that these interactions are different in different environments, for example freshwater versus marine, because the presence

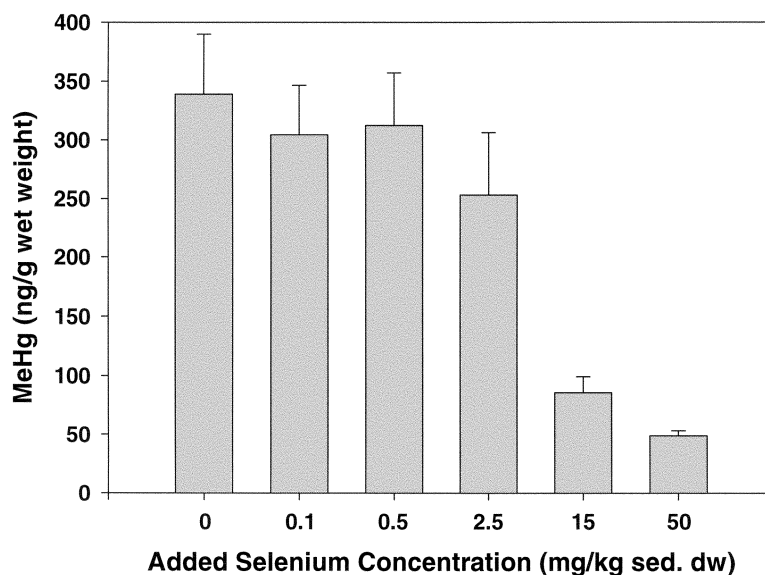


Figure 4. ^{14}C -methylmercury body burden in *Lumbriculus variegatus* after 14 days exposure at 102 ng/g dw sediment MeHg concentration and at different selenium concentrations.

of other elements and compounds at different concentrations can also modify these reactions. Unfortunately, there are few experimental data available on the bioavailability and bioaccumulation of MeHg by benthic organisms and the modifying factors in the sediments. However, this is considered as crucial information when we try to model the effect of benthic organisms on transferring the sediment-associated mercury and methylmercury back to the aquatic food webs.

Conclusions

The data presented here indicates that the bioavailability of MeHg is strongly affected by the sediment organic carbon content. Further, it is important to notice that sediment dwelling and sediment ingesting organisms may be an important step introducing the sediment-bound MeHg back to the food webs. Also, these results show that added selenium clearly decreases the bioaccumulation of methylmercury by an oligochaete worm *Lumbriculus variegatus* in sediments. Also, this effect depends on selenium concentration. Further research is needed to evaluate whether the effect is just a chemical reaction in the sediment, or whether selenium accumulates and affects the toxicokinetics of methylmercury inside the organisms.

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