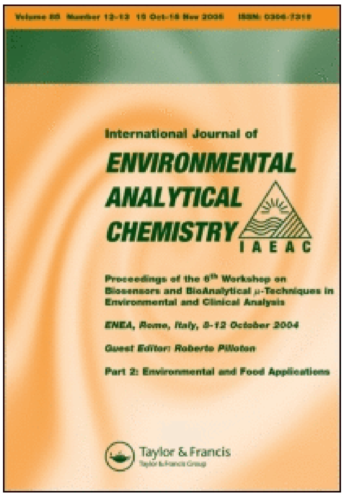

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The Cumulation of Methylmercury in Fish‡ (Poecilia reticulata)

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Methylmercury labelled with mercury-203 was used for the investigation of the uptake and the release of methylmercury in fish. It has been found that methylmercury compounds adsorbed on fish food remain completely in fish and that they are released with the biological half-time of 110 ± 20 days. The cumulation of methylmercury from water is very rapid: the cumulation constant is 237 ± 67 days^{-1}. Equations for the calculation of the concentration of methylmercury in fish were derived and compared with the uptake of phenylmercury and inoganic mercury.

KEY WORDS: Radioanalytical methods, methylmercury, fish, Poecilia reticulata

INTRODUCTION

In our previous papers the radiochemical methods for the determination of highly toxic methylmercury compounds have been developed.[1,2] These methods were used for the determination of methylmercury in surface waters,[3] fish[4] and natural sediments.[5] The study of mercury circulation in aquatic environments has shown that inorganic mercury was rapidly accumulated in algae cells, however, the methylation of mercury has not been observed during 14 days of incubation.[6] On the contrary, methylmercury and phenylmercury compounds were transformed by the action of algae to the inorganic form.[7] Similar results were obtained in our experiments with zooplankton.[5] It is therefore evident that phyto- and

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zooplankton are not responsible for the production of organic mercury in aquatic biocenoses.

The formation of methylmercury from inorganic mercury has been proved in natural sediments.[5,8–10]. In sediments the mineralisation of organic mercury also takes part and for this reason the equilibrium concentration of inorganic and organic mercury depends on the rate of the formation of methylmercury and the rate of its mineralisation.

In the continuation of these studies the uptake by fish (Poecilia reticulata) of methylmercury from water or sorbed on food and the release of this compound has been studied in order to determine the concentration of methylmercury in fish at different experimental conditions.

EXPERIMENTAL

Reagents and apparatus

Unless otherwise stated, all reagents were of analytical reagent grade purity.

Hydrochloric acid, suprapure (Merck) Washing solution was prepared by the dissolution of 90 g of disodium salt of ethylenediaminotetraacetic acid in 500 ml of 0.5 M sodium hydroxide solution.

Methylmercury-203 hydroxide (initial specific activity 15–30 GBq/g Hg) was prepared from $^{203}HgCl_2$ (Amersham, England) using an isotope exchange method.[11]

Labelled fish food, containing 40 ppm of mercury as methylmercury, was prepared as follows: an appropriate amount of methylmercury-203 hydroxide (neutralized with sulphuric acid) was mixed with commercial fish food (dry zooplankton containing Daphnia sp. and Cyclops sp.) and air dried at the room temperature. Fish Poecilia reticulata were of age of 3–10 months. The scintillation counter with the NaI(T1) crystal was used for the radioactivity measurements.

Procedures

Uptake of methylmercury from water: Two fishes were always placed into a glass vessel containing 500 ml of distilled water to which an appropriate amount of methylmercury-203 hydroxide · (neutralized with hydrochloric acid) and one drop of 2 M sodium chloride solution were added (in these conditions methylmercury is present as a chloride complex). After a certain time of exposition (during this time the fishes were not fed), the fishes were washed for 15 min. in distilled water, placed in vivo into a glass test tube containing 3 ml of distilled water and their radioactivity was measured. The concentration of methylmercury dropped during the experiment because of the uptake of methylmercury by fish and the mineralisation of methylmercury in the presence of fish (the decrease of the concentration of methylmercury in control vessels was very low). For this reason, the analysis of the aqueous solution for the content of methylmercury during the experiment was carried out and the mean value of the methylmercury concentration was used for the calculation of the exposition.

Uptake of methylmercury sorbed on fish food: Two series of experiments have been carried out: in the first series two fishes were always placed into a glass vessel containing 500 ml of distilled water and fed for 1–2 hours with the fish food labelled with methylmercury-203. After washing the fishes for 15 min. in distilled water, they were placed into a 5,000 ml glass vessel and fed by the non-active fish food. The radioactivity of fishes in vivo was measured for 20–50 days as described above.

In the second series, about 50 fishes were placed into a glass vessel filled with 5,000 ml of fresh water and bubbled with fresh air and fed each day with 0.2 g of fish food labelled with methylmercury-203. After a certain time of the exposition, 5–10 fishes were mechanically killed, weighted, washed for some minutes in washing solution and homogenized with 5 ml of distilled water using the glass homogenizator. The sample was transferred into a centrifugation glass tube and its radiactivity was measured to determine the total concentration of mercury in the fish. Then 10 mg of copper sulphate, 2 ml of conc. hydrochloric acid and 5 ml of benzene were added and the sample was shaken for 60 min. The phases were separated by centrifugation and the radioactivity of aliquots of both phases were measured and the content of methylmercury was determined. Under these conditions the distribution ratio of methylmercury chloride is equal to 7.0, whereas inorganic mercury remains quantitatively in the aqueous phase.[1,11]

Release of methylmercury by fish: Fishes containing sufficient radioactivity (more than 10,000 cpm) were placed into glass vessels with 1,000–5,000 ml of distilled water and fed by the non active fish food. After a certain period of exposition, the radioactivity of fishes in vivo was measured as described above.

RESULTS AND DISCUSSION

Uptake of methylmercury from water and its release

A typical example of the accumulation of methylmercury in fish from water is given on Fig. 1., from which it follows that the concentration of methylmercury in fish, c_f (in ppb) increases linearly with the exposition c_w, t, where c_w is the concentration of methylmercury in water (in ppb) and t is the time of exposition (in days). Thus for the short period of exposition, the following equation is valid:

$$c_f = c_a + K_w . c_w . t \tag{1}$$

where K_w is the cumulation constant for the uptake from water, c_a is the concentration of methylmercury adsorbed on the surface of the fish.

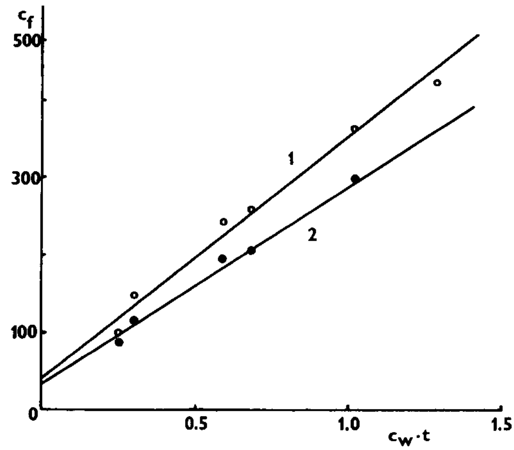

FIGURE 1 The dependence of the concentration of methylmercury in fish c_f on the exposition c_w, t (1-Sample No. 1, 2-sample No. 2).

The value of the cumulation constant for individual fishes has been calculated using both least squares method and the graphical evaluation. A survey of data for 9 fishes are summarized in Table 1. From this Table it is evident that the cumulation constant is practically independent of sex, weight (age), concentration of methylmercury in water and time of exposition under the conditions studied. The mean value of this constant is 237 ± 67 days^{-1} which means that after one day of exposition the concentration of methylmercury in fish is about 237 times higher than that in water.

The release of methylmercury by fish is rather slow. The concentration of methylmercury dropped within a few days by several percent (the desorption of methylmercury sorbed on the surface on fish) and then decreased very slowly with the biological half-time T=90–140 days; the mean value from 6 fishes measured longer than 30 days is 110 ± 20 days (the release constant $\lambda = \ln 2/T = 0.0063$ days^{-1}). The fish accumulates methylmercury from water and simultaneous releases it and for this reason the concentration of methylmercury in fish can be generally expressed as (for longer exposition time the value of c_a can be neglected)

$$c_f = \frac{K_w \cdot c_w}{\lambda} (1 - e^{-\lambda t}) \tag{2}$$

TABLE I

The determination of the cumulation constant K_w for methylmercury (f-female, m-male)

Sample no	Sex	Weight g	c_w ppb	Incubation days	K_w days^{-1}
1	f	0.156	0.35	4	303 ± 31
2	m	0.258	0.35	4	241 ± 46
3	f	0.117	0.36	5	188 ± 16
4	f	0.140	0.36	5	192 ± 22
5	f	0.073	3.5	4	366 ± 38
6	m	0.105	3.5	4	290 ± 66
7	f	0.091	3.7	5	163 ± 33
8	f	0.390	3.7	5	186 ± 34
9	m	0.230	20	0.15	208 ± 23
				mean	237 ± 67

From eq. (2) it follows that after 110 days of exposition the concentration of methylmercury in fish will be about 2×10^4 ($e^{-\lambda t} = 0.5$) higher than that in water and the maximum concentration (for $e^{-\lambda t} \to 0$) will be about $4 \times 10^4 c_w$. Considering the mean concentration of methylmercury in surface waters about 0.01 ppb[12] the maximum concentration of methylmercury in fish will be about 0,4 ppm.

Uptake of methylmercury sorbed on food and its release

The experiments with fishes fed by food containing methylmercury-203 have shown that more than 99% of this compound remained in fish which allowed us to appreciate the mean weight of consumed food (in g) consumed daily per g of fishes (K_m). The mean concentration of methylmercury in fishes was 5 ppm after 11 days of feeding in our experimental conditions which means that about 0.01 g of dry food was consumed daily per g of fish which corresponds to about 0.1 g of fresh food. Because the concentration of methylmercury in fish decreases with the half-time T equal to 110 ± 20 days (even after 60 days of the exposition more than 99% of radioactivity corresponds to the methylmercury compounds) we can write for the concentration of methylmercury in fish (in ppb) fed by food containing methylmercury the following expression

$$c_f = \frac{K_m \cdot c_m}{\lambda} (1 - e^{-\lambda t}) \tag{3}$$

where c_m is the concentration of methylmercury (in ppb) in food.

The maximum concentration of methylmercury (taken up from food) in fish is about $160 K_m \cdot c_m$ and for $K_m = 0.1$ days^{-1} it equals to $16 c_m$. The cumulation factor of methylmercury in phyto- and zooplankton[6,7] is about 10^4; then the maximum concentration of methylmercury in fish can be as high as 1.6 ppm assuming its concentration in natural waters to be equal to 0.01 ppb. However, methylmercury is rapidly transferred by the action of algae into the inorganic form and for that reason we can expect that the concentration of methylmercury will be lower in natural conditions.

Comparison with phenylmercury and inorganic mercury

The cumulation of phenylmercury compounds in fish is substantially lower.[13] The cumulation constant is also very high $K_w = 212 \pm 49$ days^{-1}, however, phenylmercury in fish is transformed into inorganic mercury (but not to methylmercury) with a half-time equal to 2.4 ± 0.3 days; along with this reaction, phenylmercury is directly released into water with a half-time 6 ± 1 days. Taking into account half-times of the release of inorganic mercury, the maximum concentration of total mercury in fish will be about 5,000 times higher than that in water and about 3 times higher than that in food.[13,14]

Inorganic mercury is practically not cumulated by fish directly from water.[13,14] The release of inorganic mercury from fish fed by food containing $^{203}HgCl_2$ is rather complicated: about 82% of mercury consumed is released with a biological half-time about 0.3 days, 12% of mercury with a half-time 7.0 ± 1.5 days and 6% with a half time 60 ± 15 days. The maximum concentration of mercury can be about $0.7 c_m$, assuming that $K_m = 0.1$ days^{-1}. The total concentration of mercury can be a little higher because about 0.7% of inorganic mercury consumed can be slowly transferred in fish into methylmercury compounds.[13,14]

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