Assimilation and depuration of dietary methylmercury in adult zebrafish (*Danio rerio*) – Implications for maternal transfer to eggs

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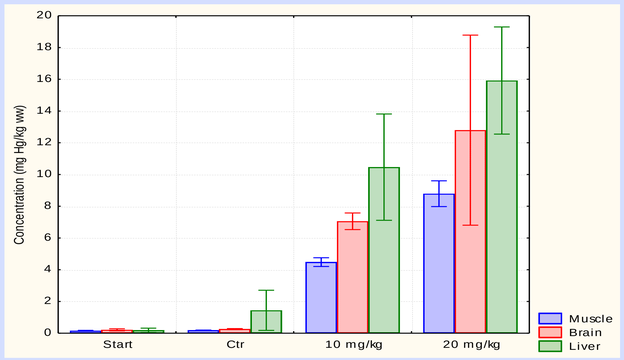
Introduction

Fishmeal is the dominant source of mercury, mainly as methylmercury (MeHg), in fish feed and consequently in farmed fish. In Europe, the levels of contaminants in feed and seafood are controlled through the European feed and food legislation, which sets statutory limits for a wide range of contaminants in feed ingredients, feed and food, including seafood. The current maximum level for mercury in fish feed is 0.2 mg/kg feed (Commission Directive 2010/6/EC); however knowledge regarding tolerable dietary mercury levels in fish, is limited. As part of a larger project studying toxic effects of mercury in fish, zebrafish (*Danio rerio*) was used as a model to characterize the assimilation and depuration of dietary MeHg in fish. Results gained from zebrafish will be used for a later study with Atlantic cod (*Gadus morhua* L.).

Experimental outline

Quadruplicate groups per treatment (n = 25) of adult female zebrafish were exposed to dietary MeHg for six weeks followed by a four week depuration period. Methylmercury was added to a commercial zebrafish diet as methylmercury-cysteine at nominal concentrations of 0, 10 or 20 mg Hg/kg. After exposure and depuration, eight females from each tank were transferred to individual spawning tanks and paired with a male (non-exposed). After spawning, eggs were collected (sub-samples of 100 eggs) and the females were sacrificed. Pooled samples of brain, liver and muscle from three fish per tank were sampled for mercury analysis and the activity of enzymes involved in detoxification of MeHg.

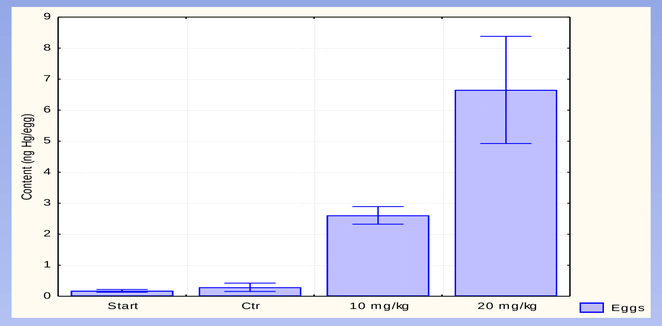
Accumulation of MeHg in liver, brain and muscle



Mean mercury levels (mg/kg ww) were determined in pooled samples of muscle, brain and liver (n = 4) of female zebrafish exposed to three levels (0, 10 and 20 mg Hg/kg) of dietary MeHg for six weeks. Samples were digested by microwave-assisted decomposition and the total mercury concentrations were determined by inductively coupled plasma mass spectrometry (ICPMS).

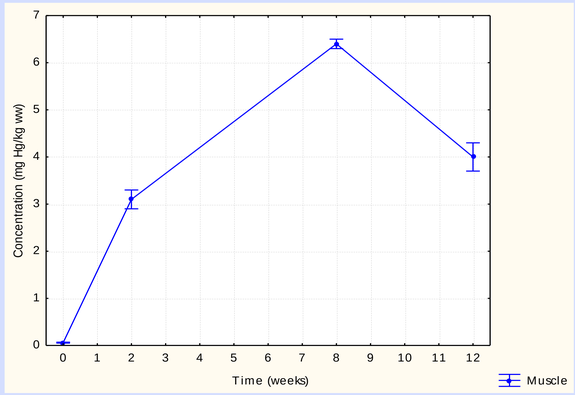
The accumulation of dietary MeHg was dose dependent in all investigated organs, with the highest tissue levels seen in zebrafish exposed to the highest dietary mercury level (20 mg Hg/kg). At both exposure levels the mercury concentration was higher in liver and brain than in muscle, indicative of the propensity of MeHg to cross the blood-brain barrier.

Maternal transfer of MeHg in zebra fish embryo



Batches of 100 eggs from crosses of female fish fed 0, 10 and 20 mg Hg/kg for six weeks were analyzed for their mercury content using a direct mercury analyzer (DMA-80). Maternal transfer of MeHg was also dose dependent, with higher mercury levels found in eggs of females fed the 20 mg Hg/kg diet compared to eggs of females fed the 10 mg Hg/kg diet, with implications for embryonic neurodevelopment. Amounts are given as ng Hg/egg.

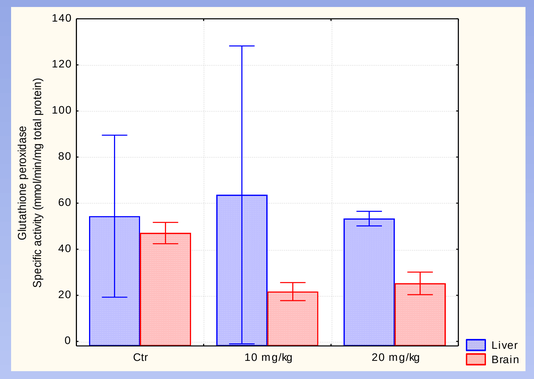
Depuration of dietary MeHg in muscle



Mean mercury levels (mg/kg ww) were determined in pooled samples of muscle (n = 4) of female zebrafish exposed to dietary MeHg (10 mg Hg/kg) for eight weeks followed by a four week depuration period. Samples were digested by microwave-assisted decomposition and the total mercury concentrations were determined by ICPMS.

A continuous accumulation of dietary MeHg was seen during the eight weeks of exposure leading to a final concentration of 6.4 ± 0.1 mg Hg/kg ww (n = 3). After the depuration period the mercury level in muscle decreased to 4.0 ± 0.3 mg Hg/kg ww (n = 3) suggesting that the elimination of MeHg from muscle is slow.

Effects of MeHg on enzyme activity



Total glutathione (GSH) concentration and total specific activity of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were measured in pooled samples of muscle, brain and liver (n = 4) of female zebrafish exposed to three levels (0, 10 and 20 mg Hg/kg) of dietary MeHg for six weeks.

The specific activity of GPx was similar in liver and brain of fish fed the control diet; the activity in liver was not affected by dietary treatment, while in brain the specific activity of GPx decreased in samples of fish fed the two mercury diets, indicating that dietary MeHg inhibits the activity.

Similar levels of GSH were found in liver and brain, and the levels were not affected by dietary treatment (data not shown). The GST activity was higher in liver than in brain, but the activity was not affected by dietary treatment in either organ (data not shown).

Conclusions

- The accumulation of dietary MeHg (given as methylmercury-cysteine) in organs of adult female zebrafish is dose dependent, with higher levels found in liver and brain than in muscle.
- The maternal transfer of dietary MeHg is dose dependent.
- The depuration of dietary MeHg from muscle appears to be slow.
- The specific activity of GPx decreased in brain of fish fed diets enriched with MeHg for six weeks.

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