Maternal transfer of dietary methylmercury affects neurodevelopment in zebrafish embryos

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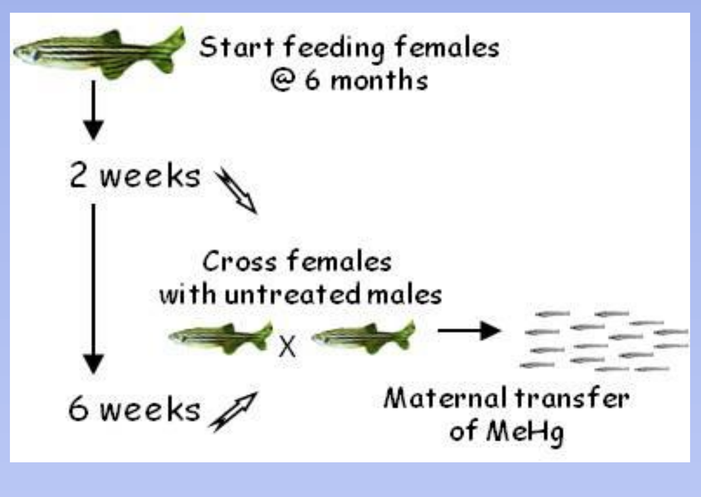
Introduction

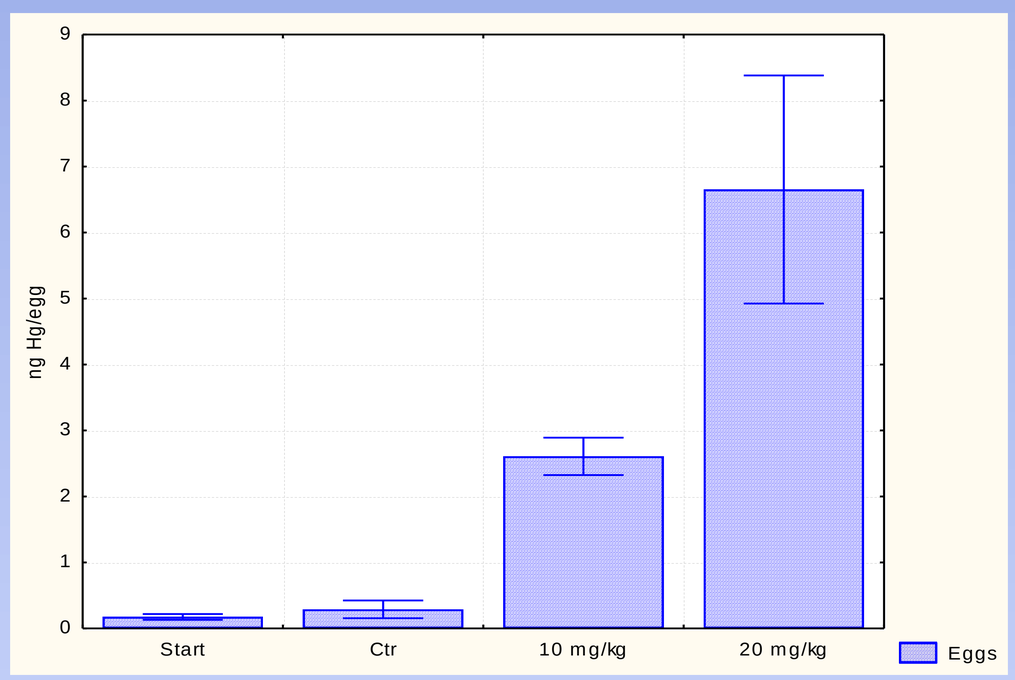
Methylmercury (MeHg) is an environmental contaminant that accumulates in the seafood chain and represents a significant risk to fish and human health. In Europe, the levels of contaminants in feed and seafood are controlled through the European feed and food legislation, which set statutory limits for a wide range of contaminants in feed ingredients, feed and food, including seafood. The current maximum level for mercury (Hg) 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. Understanding the underlying mechanisms of MeHg toxicity is important for future risk assessment improvements. *In vitro* and *in vivo* studies have shown that MeHg exposure affects many basic cellular processes, and maternal transfer of MeHg is frequently associated with neurobehavioral deficits during early life stages like altered motoric and cognitive functions. We have investigated the assimilation and depuration of dietary MeHg in adult zebrafish (*Danio rerio*) and the toxic effects in zebrafish embryos after maternal transfer of MeHg.

Experimental outline



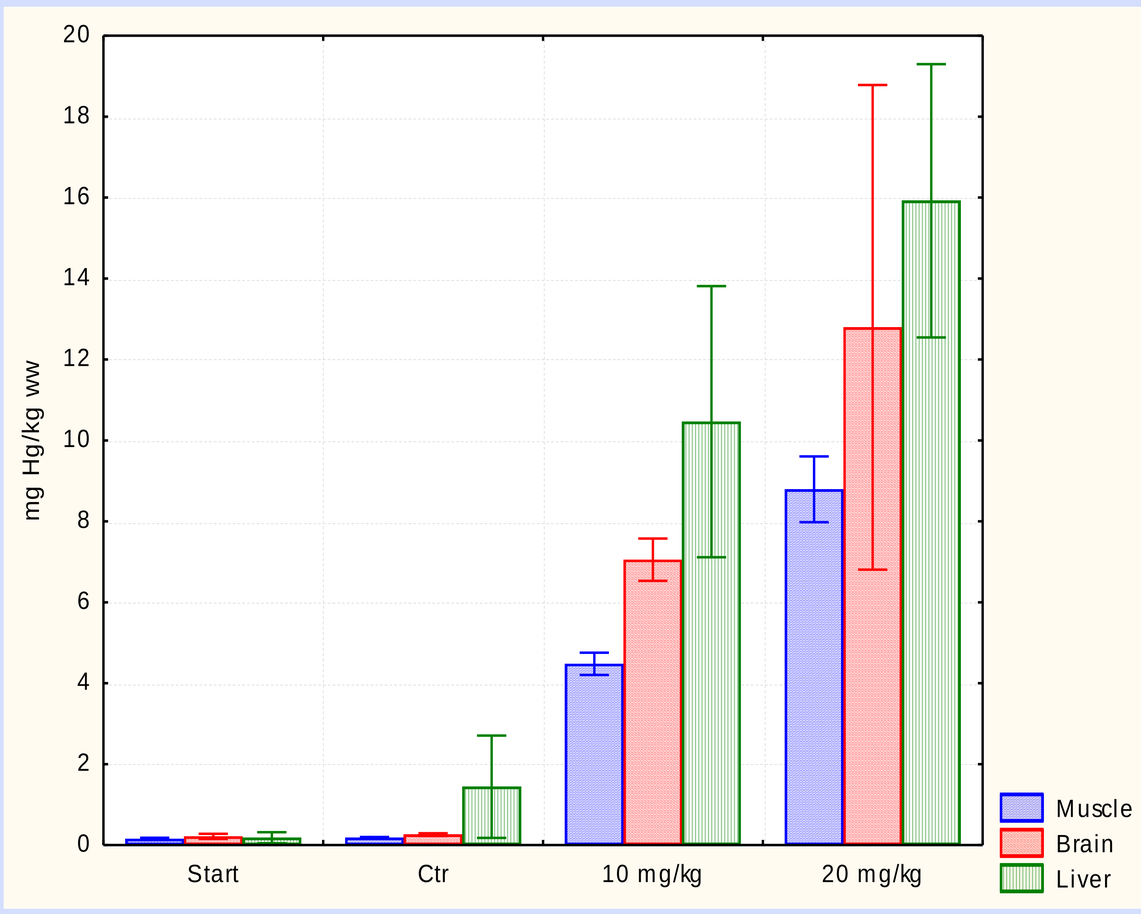
Quadruplicate groups of 25 female zebrafish were exposed to dietary MeHg for 6 weeks at nominal concentrations of 0, 10 and 20 mg Hg/kg. Methylmercury was added to a commercial zebrafish diet as methylmercury-cysteine. Exposed females were crossed against unexposed males and F1 progeny were collected for analysis. 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 from each tank were sampled for trace metal analysis.

Maternal transfer of dietary MeHg in zebra fish embryo



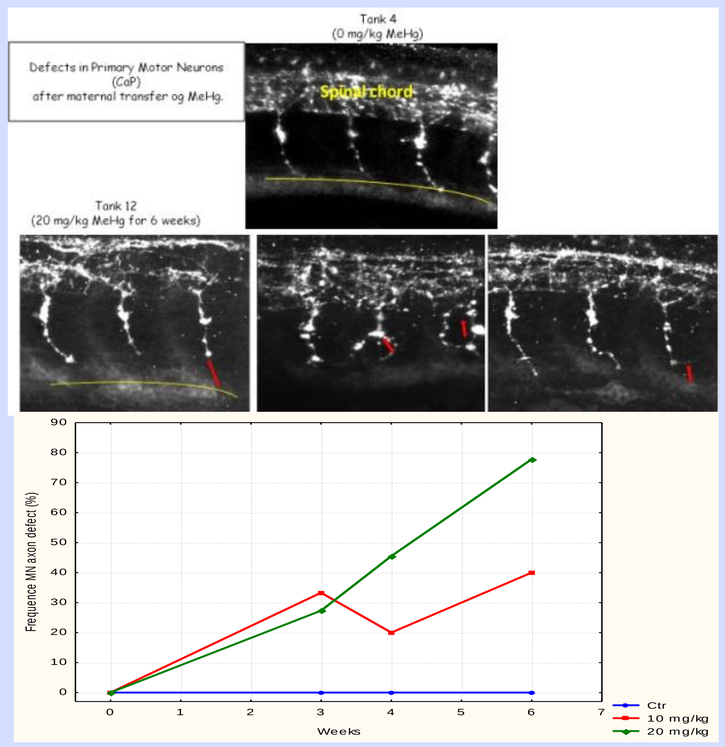
Batches of 100 eggs from crosses of female fish fed 0, 10 and 20 mg Hg/kg for 6 weeks were analyzed for their mercury content using a direct mercury analyzer (DMA-80). Amount of maternally transferred mercury was dose dependent, with the highest levels found in eggs from females fed the highest dietary level (20 mg Hg/kg). Amounts are given as ng Hg/egg.

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 ICPMS (inductively coupled plasma mass spectrometry). 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 leads to disturbance in motorneuron axon growth in zebrafish embryos



Spinal cord primary motorneurons were visualized by immunohistochemistry (Znp-1 antibody) on batches of 10 eggs from each diet. We observed a significant disturbance in caudal primary CaP axon growth in embryos from females fed the 20 mg Hg/kg diet. Their axons projected beyond the turning point, but then growth ceased, leading to a shortened axon, or in other cases led to abnormal branching and misguided axon growth.

Concluding remarks

- The accumulation of dietary MeHg (given as methylmercury-cysteine) in organs of 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.
- Maternally transferred MeHg affects normal growth of spinal cord primary motorneurons in zebrafish embryos.

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