Maternal transfer of methylmercury leads to disturbance in motorneuron axon growth in zebrafish embryos

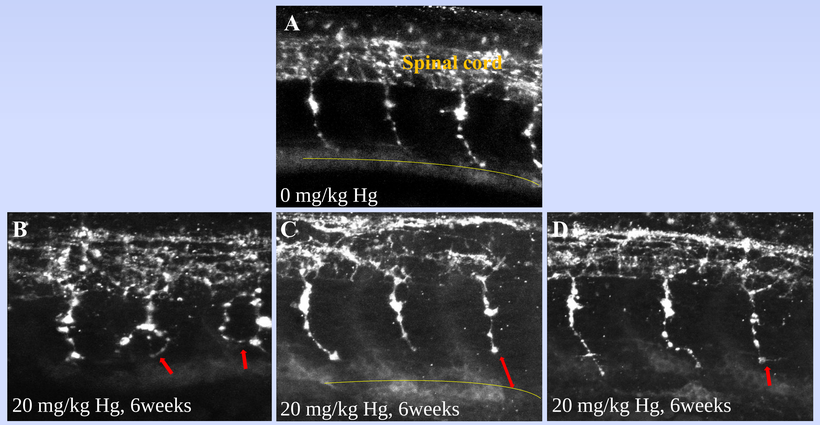
Staale Ellingsen[1], Heidi Amlund[1], Ane Grønsberg[1], David Boyle[2], Anne-Katrine Lundebye[1]

[1]National Institute of Nutrition and Seafood Research (NIFES), Bergen, NORWAY
[2]University of Plymouth, Plymouth, UNITED KINGDOM

Introduction

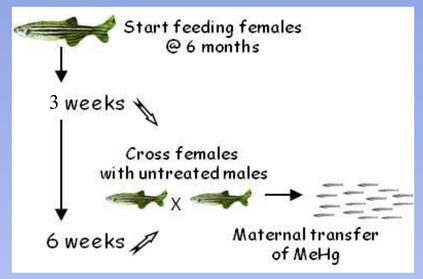
Methylmercury (MeHg) is an environmental contaminant that accumulates in the seafood chain and represents a risk to fish and human health. In Europe, the levels of contaminants in feed and seafood are controlled through the European feed and food legislation, which set statutory limits for a wide range 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. Maternal transfer of MeHg is frequently associated with neurobehavioral deficits during early life stages like altered motoric and cognitive functions. We have investigated neurotoxic effects in zebrafish embryos after maternal transfer of MeHg and identified disturbance of trunk motorneuron axon growth. Further analyses have shown reduced mRNA expression of a motorneuron axon guidance protein of the Ephrin family.

Maternal transfer of MeHg leads to disturbance in spinal cord motorneuron axon growth in zebrafish embryos



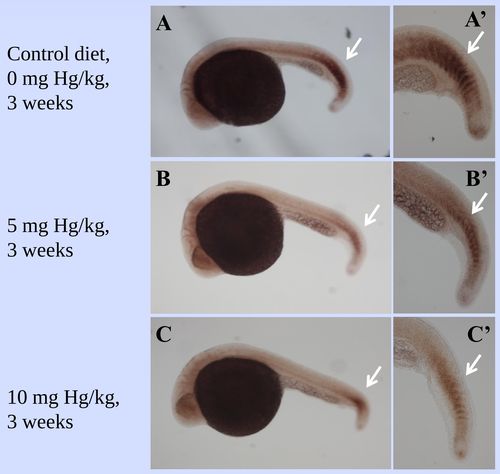
Spinal cord primary motorneurons were visualized by immunohistochemistry (Znp-1 antibody) on batches of 10 eggs from each diet. We observed a significant disturbance in caudal primary CaP axon growth in embryos from females fed the 10 mg and 20 mg Hg/kg diets (E). Their axons projected to the branching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

Experimental outline



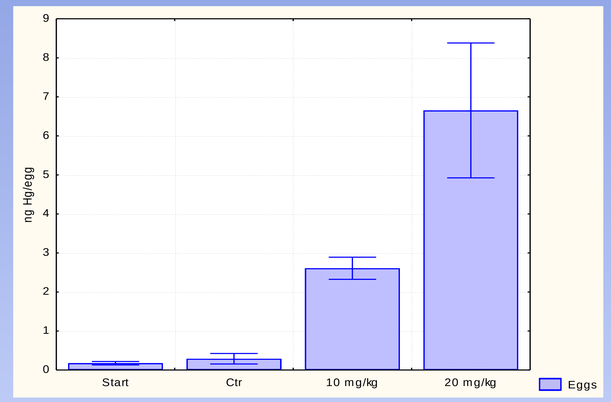
Quadruplicate groups of 25 female zebrafish were exposed to dietary MeHg at nominal concentrations of 0, 5, 10 and 20 mg Hg/kg. Methylmercury was added to a commercial zebrafish diet as methylmercury-cysteine. Exposed females were crossed against unexposed males at 3 and 6 weeks. After spawning, eggs were collected (sub-samples of 100 eggs) and the females were sacrificed.

Maternal transfer of MeHg reduces expression of Ephrina1b in developing muscles in zebrafish



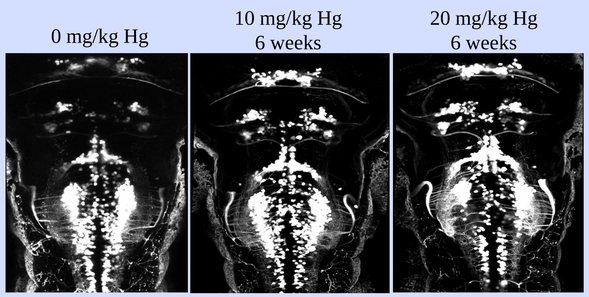
Whole-mount in situ hybridization of embryos detects ephrin1ab transcripts. Embryos were collected from females fed diets containing 0 mg MeHg/kg (A, A'), 5 mg MeHg/kg (B, B') or 10 mg MeHg/kg for 3 weeks. Lateral, whole mount views show expression of epha1b in embryos at 24 hours post fertilization (A, B, C). Magnification of trunk and tail show expression of epha1b in developing muscles (A', B', C'). Expression of epha1b is reduced in developing muscles (arrows) in embryos from crosses of females fed MeHg enriched diets (B', C').

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.

Maternal transfer of MeHg does not affect branchial motorneurons in zebrafish embryos



Branchial motorneurons were visualized by crossing female fish with males from the transgenic zebrafish line Tg:Isl1:GFP (Higashijima et al, J. Neurosci 2000), and embryos were imaged using confocal microscopy. We could not detect significant disturbance in branchial motorneurons or in axon growth from these neurons.

Concluding remarks

- Dietary MeHg is transferred from female zebrafish to progeny in a dose dependent manner.
- Maternally transferred MeHg affects growth of spinal cord primary motorneurons in zebrafish embryos that innervate trunk muscles, but does not affect branchial motorneurons.
- Maternally transferred MeHg reduces expression in developing trunk muscles of a motorneuron axon guidance protein of the Ephrin family.

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