Localizing Organomercury Uptake and Accumulation in Zebrafish Larvae at the Tissue and Cellular Level

Mercury is a well-known poison, but it is perhaps at its most dangerous when bound by organic groups to form organo mercury compounds (1). Such compounds are highly neurotoxic to mammals, but nevertheless have seen use as pesticides and also are made by microbial metabolism of mercuric ions. Several devastating mass-poisonings of human populations have been caused by organo mercury compounds. Organo mercury compounds exhibit an insidious latency in the development of toxic effects. Exposed humans may only develop toxic symptoms after a delay of several months. Adults are affected, but exposure *in utero* results in particularly severe consequences such as microcephaly, cerebropalsy, seizures, mental retardation, and other cruelly averse effects. Despite its toxicity and widespread occurance, many aspects of how organomercury compounds cause such deadly effects remains unknown.

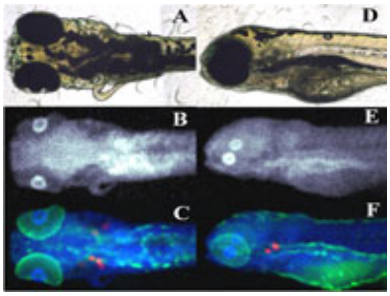
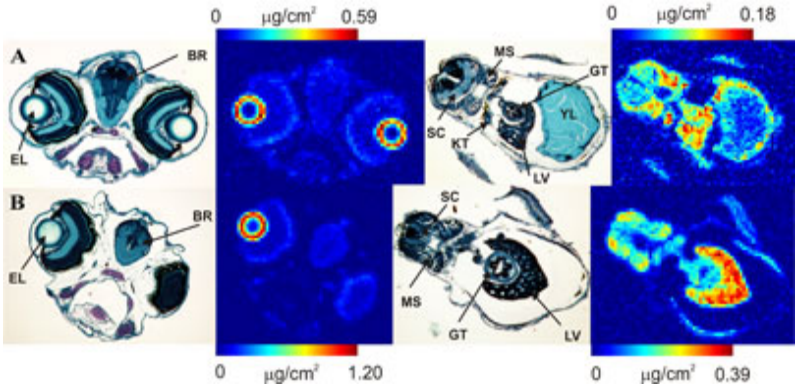


Figure 1:
X-ray fluorescence images of anaesthetized zebrafish larvae. A & D optical micrograph; B & E Hg; C & F Hg (blue), Zn (green) and Ca (red).

In an article published in the *Proceedings of the National Academy of Sciences USA*, Malgorzarta Korbas and co-workers have brought new insight into molecular mechanisms underlying organo mercury's toxicity. The team used X-ray fluorescence imaging conducted on SSRL beamlines 9-3 and 2-3 to study mercury accumulation in zebrafish (*Danio rerio*) larvae. Newly hatched zebrafish are relatively underdeveloped – for example, their eyes have yet to properly develop, and they still have a yolk sac attached – and they are therefore much used as a model organism for the study of vertebrate embryonic development and toxicology (2). Korbas and co-workers raised newly hatched zebrafish larvae in water containing low levels of methylmercury cysteinate (from 200 nm to 100 μM). The team first examined whole tricaine-anaesthetized larvae, using X-ray fluorescence imaging to obtain the distribution maps of mercury and other elements within the live fish. The researchers then investigated Hg distributions in sequential sections, with alternate sections being prepared for histology and X-ray fluorescence imaging (Figure 2). Strikingly, the greatest accumulation of methylmercury compounds was observed in the rapidly dividing layer of the lens epithelium, visible as rings at the periphery of the eye lenses (Figure 2,3). Lower levels of methylmercury were observed in brain, optic nerve and various other organs (Figure 3). The data suggest that the reported impairment of visual processes by mercury may arise not only from previously reported neurological effects, but also from direct effects on the ocular tissue.

Figure 2:
Experimental procedure for sectioning organomercury treated zebrafish. Fish were prepared for X-ray fluorescence imaging by using adjacent 6-μm sections, one section mounted on a glass slide and stained for histology (S1), while the other section, for X-ray fluorescence imaging, was placed on a plastic cover slip (S2) with no further processing.

Figure 3:
Head and liver sections from larvae treated with methylmercury cysteinate: (*A*) 2 µM for 36 h and (*B*) 200 nM for 84 h. The figure shows histological (left) and Hg (right) images. (BR)-brain, (EL)-eye lens, (LV)-liver, (GT)-gut, (KT)-kidney tubule, (MS)-skeletal muscle, (SC)-spinal cord.



Control fish (Figure 4) showed no mercury signal, but displayed significantly higher calcium levels in the outer layers of the retina than the mercury exposed fish, suggesting that methylmercury might interfere with the natural calcium distribution as has previously been suggested (3).

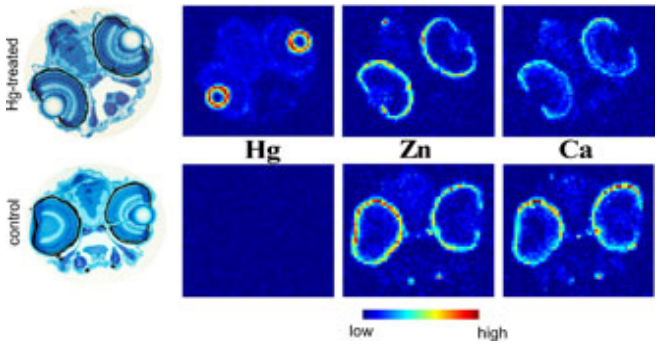


Figure 4:
Elemental distributions in methylmercury exposed and unexposed zebrafish. Comparison of elemental distributions for Hg, Zn and Ca in sections of fish heads from larvae exposed for 24 hours to 100 µM methylmercury cysteineate (upper) with those from control fish (lower), measured using X-ray fluorescence imaging.

This novel approach demonstrates synchrotron X-ray fluorescence imaging of zebrafish to be a powerful tool for investigating molecular toxicology of heavy metals. The method is equally applicable to the study of other elements of concern, such as arsenic, selenium, thallium and lead. The technique also provides an ideal tool for investigating drugs such as chelation agents (4) and can be applied to the study of essential metals and other elements of interest during normal development.

Primary Citation

Korbas M, Blechinger SR, Krone PH, Pickering IJ, George GN (2008) Localizing organomercury uptake and accumulation in zebrafish larvae at the tissue and cellular level. *Proc Natl Acad Sci* USA **105:**12108-12112.

References

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