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Developmental toxicity from exposure to various forms of mercury compounds in the medaka fish (*Oryzias latipes*) embryos

Wu Dong, Jie Liu, Lixin Wei, Yang Jingfeng, David E Hinton

Mercury is a toxic heavy metal that can become incorporated in marine fish and be a dietary concern for women of reproductive age. This study examined the developmental toxicity of different mercury compounds in medaka fish embryos. Medaka embryos were exposed to 0.001-10 μM concentrations of MeHg, $HgCl_2$, α-HgS (cinnabar), and β-HgS (*Zuotai*) from the Stage 10 (provide dpf also)to 10 days post fertilization (10 dpf). Of the forms of mercury, the methylated form (MeHg) is organic, readily crosses cell membranes and proved MeHg is highly toxic. Inorganic mercury ($HgCl_2$) was less toxic but produced embryo death and developmental toxicity in our fish model. This includes pericardial edema, elongated or tube heart, malformation of eye and failure of inflation in swim bladder. In contrast, both α-HgS, and β-HgS were much less toxic than MeHg and HgCl2 . Total RNA was extracted 3 days after exposure of embryos to MeHg (0.1 μM), $HgCl_2$ (1 μM), α-HgS (10 μM), or β-HgS (10 μM) to examine toxicity-related gene expression. MeHg and $HgCl_2$ markedly induced metallothionein and heme oxygenase-1, while α-HgS and β-HgS did not produce significant changes in either gene. In conclusion, chemical forms of mercury compounds are a major determinant of their developmental toxicity towards medaka embryos. The medaka embryo model proved capable of detecting effects rapidly and results differentiated between various forms of mercury. Engish needs improvement in underlined red areas - Native English speaker should revise.

Developmental toxicity from exposure to various forms of mercury compounds in the medaka fish (Oryzias latipes) embryos.

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Abstract

Mercury is a toxic heavy metal that can become incorporated in marine fish and be a dietary concern for women of reproductive age. This study examined the developmental toxicity of different mercury compounds in medaka fish embryos. Medaka embryos were exposed to 0.001-10 µM concentrations of MeHg, $HgCl_2$, α-HgS (cinnabar), and β-HgS (*Zuotai*) from the Stage 10 to 10 days post fertilization (10 dpf). Of the forms of mercury, the methylated form (MeHg) is organic, readily crosses cell membranes and proved MeHg is highly toxic. Inorganic mercury ($HgCl_2$) was less toxic but produced embryo death and developmental toxicity in our fish model. This includes pericardial edema, elongated or tube heart, malformation of eye and failure of inflation in swim bladder. In contrast, both α-HgS, and β-HgS were much less toxic than either of the above. Total RNA was extracted 3 days after exposure of embryos to MeHg (0.1 µM), $HgCl_2$ (1 µM), α-HgS (10 µM), or β-HgS (10 µM) to examine toxicity-related gene expression. MeHg and $HgCl_2$ markedly induced metallothionein and heme oxygenase-1, while α-HgS and β-HgS did not produce significant changes in either gene. In conclusion, chemical forms of mercury compounds are a major determinant of their developmental toxicity towards medaka embryos. The medaka embryo model proved capable of detecting effects rapidly and results differentiated between various forms of mercury.

Keywords: MeHg, $HgCl_2$, α-HgS (cinnabar), β-HgS (*Zuotai*), Medaka embryos, Developmental toxicity, Metallothionein

1.Introduction

Mercury is ubiquitous in the environment. Mercury vapor (Hg^0) evaporates from water, soil, volcano eruption/ash. Human activities such as coal-combustion, and metal mining industries are globally distributed. Eventually, the released mercury is oxidized to a water-soluble inorganic form (Hg^{2+}) and returns to the earth in rainwater. Portions of inorganic mercury are converted to methylmercury (MeHg) by anaerobic bacteria present in sediments of bodies of fresh and ocean water (Liu et al., 2008a). Recent studies found that two-gene clusters, hgcA and hgcB in microbes, are required for mercury methylation (Parks et al., 2013). Methylmercury is generally more toxic than inorganic mercury to human, aquatic organisms and marine ecosystems (Karimi et al., 2012). Indicate which Hg forms are released by those human activities...

Consumption of fish is the major route of human exposure to MeHg (Karimi et al., 2012; Sheehan et al., 2014). Inhalation of mercury vapor and exposure to inorganic mercury can occur from chloralkali industry, extraction of gold from amalgam, and industrial discharge as Hg^{2+}. Mercury was also a constituent of drugs for centuries as an ingredient in diuretics, antiseptics, skin ointments and laxatives, and was also used as dental amalgam and as a preservative in many vaccines such as Thimerosal (Clarkson et al., 2003; Liu et al., 2008b). Nowadays, mercury sulfides are still used in traditional medicines such as *Zhusha* (α-HgS, cinnabar) (Liu et al., 2008b) and *Zuotai* (β-HgS, metacinnabar) (Kan, 2013).

The early life stages of fish are sensitive to toxic chemcials. Fish, particularly small sized ones have been the most popular choice for vertebrate test organisms as they are the best-understood organism in the aquatic environment, Tests using these model fishes are short termed, requiring only a few days to weeks and could be a good substitute for life-cycle tests (Ismail and Yusof, 2011).

Medaka fish is an important model species among bony fish. They occupy the freshwater, brackish water and saltwater. The most established among the medaka fish is the Japanese medaka (*Oryzias latipes*). For example, medaka fish has been useful for developmental toxicity studies for saxitoxin (Tian et al., 2014), TCDD (Dong et al., 2010), silver nanoparticles (Chae et al., 2009), bisphenol A (Sun et al., 2014), and parking lot runoff retention (Colton et al., 2014). Thus, medaka embryos are an ideal model to study developmental toxicity of organic and inorganic mercurials.

Mercury exists in three major forms, i.e., the elementary mercury, inorganic mercury, and organic mercury, and their body disposition and toxicity should be distinguished (Klaassen, 2001). The present study was designed to compare the developmental toxicity of MeHg, $HgCl_2$, α-HgS and β-HgS in medaka fish embryos, thereby providing information on the relative toxicity of the various forms in a single study. The results showed the developmental toxicity produced by MeHg and $HgCl_2$, and also demonstrated differential toxicity among different mercury compounds.

Much of the languge in the Introduction is taken almost verbatim from Ismail and Yosof 2011 - this is plagarism....

2. Materials and Methods

2.1 Mercury compounds

$HgCl_2$, MeHg (in the form of $BrCH_3Hg$), and α-HgS were obtained from Sigma Chemical Company (St. Louis, MO). *Zuotai* (β-HgS) was provided from Northwest Institute of Plateau Biology, Chinese Academy of Sciences (Xining, China).

2.2. Medaka culture and embryo collection

Orange-red (OR) outbred-medaka (*Oryzias latipes*) were maintained at the Duke University Aquatic Research Facility under standard recirculating water conditions following approved animal care and maintenance protocols (Duke University Institutional Animal Care and Use Committee). Brood stocks were housed in a charcoal-filtrated and UV-treated recirculating aquatic system. Water temperature was monitored daily and maintained at 25°C ± 2°C with pH 7.4. The animal facilities were under a strict light:dark cycle of 16:8 h. Dry food (Otohime B1; Reed Mariculture, Campbell, CA) was fed several times per day via automated feeders with once daily supplementation of newly-hatched Artemia nauplii. Embryos were collected from this colony, examined under a dissecting microscope and selected only embryos of stage 10 (~6 h post fertilization) of development (Iwamatsu, 2004) for the experiments.

2.3. Experimental design

The experiment was initiated over a 10-day interval. When the experiment was initiated, the 15-18 embryos at the Stage 10 were incubated in 5 mL 0.1% NaCl solution in 6-well plates. Mercury compounds were dissolved in DMSO and added to the solution at 1:1000 dilutions to

obtain the final concentrations of 0.001, 0.01, 0.1, 1, and 10 µM. DMSO was used as control (what % DMSO?.
The plates were incubated in an environmental chamber at 25 ± 2°C on a 16:8 h light:dark cycle.
SHOULD HAVE A NaCl control to determine the effects of DMSO itself...

2.4. Mortality, hatching and teratogenesis

Embryos were observed daily for mortality, hatching and teratogenic effects including cranial-skeletal malformations, yolk-sac or pericardial edema, tube heart, eye malformations, failure to inflate the swim bladder, and other gross deformities. To quantify the timing of organogenesis and hatching, Stage 10 of development (Iwamatsu, 2004) WHY THIS STAGE? was selected for initiating the exposure
and time of onset for various alterations was based on the above scheme. Each day, the stage of development and hatching of each embryo was identified with the aid of a Nikon SMZ 1500 dissecting microscope. Mortality was recorded as any embryo with a brown, opaque shell or any embryo with heart but no heartbeat. Embryos that did not hatch by day 10 were considered dead. Hatching was defined as complete emergence from the chorion. The experiment was terminated on day 10 of fertilization (10 pdf). WHY DAY 10 FOR ENDING THE EXPERIMENT?
How were embryos euthanized?

2.5. RNA extraction and real-time RT-PCR

Total RNA was isolated from vehicle (DMSO), MeHg (0.1 µM), $HgCl_2$ (1 µM), α-HgS (10 µM), and β-HgS (10 µM) exposed embryos (n = 15-20) 3 days post-exposure. Embryos were homogenized with 1 ml of RNAzol using a stainless steel Polytron homogenizer (Kinematica, Newark, NJ). Following homogenization, total RNA was isolated as described in Dong et al. (2010). RNA quantity and 260/280 ratios were determined using a NanoDrop ND-1000 spectrophotometer. Total RNA (500 ng) was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Grand Island, NY). The following medaka-

specific real-time PCR primers were designed using Primer3 software and synthesized by Integrated DNA Technologies (Skokie, IL): Metallothionein (MT, AY466516, forward primer 5#- CTGCAAGAAAAGCTGCTGTG-3#, reverse primer 5#- GGTGGAAGTGCAGCAGATTC-3#; heme oxygenase-1 (Ho-1, AB163431, forward primer 5#- TGCACGGCCGAAACAATTTA-3#, reverse primer 5#- AAAGTGCTGCAGTGTCACAG-3#, and β-actin (S74868, forward primer 5#- GAGTCCTGCGGTATCCATGA-3#, reverse primer 5#-GTACCTCCAGACAGCACAGT-3#. The cDNA was amplified with SYBR Green PCR Master Mix (Applied Biosystems, Grand Island, NY). Real-time PCR reaction conditions were 95°C for 15 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. For each sample, the threshold cycle (Ct) was normalized with β-actin of the same sample, and the amplification was calculated using the $2^{-\Delta\Delta}$CT method (Dong et al., 2010).

2.6 Statistical analysis

Real-time PCR data were calculated as % of β-actin and expressed as mean ± SEM. All the data were analyzed using the SPSS 7.5 (SPSS Inc., Chicago, IL, USA). For all the measurements, one-way ANOVA followed by Tukey's test was used to assess the statistical significance between groups. $p \leq 0.05$ was considered to be statistically significant.

3. Results

3.1 Mortality of the medaka embryos after exposure to Hg compounds

Mekada embryos at the Stage 10 (Iwamatsu, 2004) exposed as above to 0.001-10 µM MeHg, $HgCl_2$, α-HgS (*Zhusha*, cinnabar) or β-HgS (*Zuotai*, metacinnabar) were observed daily till 10 days post-fertilization (pdf) (Fig. 1). MeHg proved to be highly toxic, at 1-10 µM concentration, all embryos died before 3 days post fertilization (dpf), and 0.1 µM produced some embryos died by 7 dpf and 30% mortality occurred at 10 dpf. $HgCl_2$ is also toxic to medaka embryos, At 10 µM of $HgCl_2$, all embryos died before 4 dpf, and at 1 µM of $HgCl_2$ 40% mortality was seen at 10 dpf, 0.1 µM $HgCl_2$ produced 12% mortality at 10 dpf. In contrast, 10 µM α-HgS and 10 µM β-HgS were much less toxic; all embryos survived 10 µM concentration, except for 5% mortality at 10 dpf, which is within the normal range.

Revise this paragraph with an English speaker - it is written in a very confusing manner

3.2 Developmental toxicity

Madaka embryos were exposed to mercury compounds from the Stage 10 (Early blastula stage, 6 h 30 min pf) (Iwamatsu, 2004) to 10 dpf. At 5 days of fertilization (5 dpf), pericardial edema, tube heart and malformation of the eye with reduced pigmentation occurred in both MeHg (0.1 µM) and $HgCl_2$ (1 µM)-treated embryos, with MeHg more severe. No developmental abnormalities at 5 dpf embryos treated with 10 µM α-HgS and β-HgS (data not shown). At the end of experiments (10 dpf), pericardial edema, tube heart, malformation of eye, and failure to inflate swim bladder were evident in MeHg (0.01 µM) and $HgCl_2$ (0.1 µM) treated embryos, while α-HgS (10 µM) and β-HgS (10 µM) did not show significant developmental toxicity.

Revise this section and cite the relevant FIGURES (e.g. Fig 2c) ....

Check spelling of medaka...sometimes it appears as mAdaka....throughout ms.

3.3 Metal toxicity-related gene expression

Madaka embryos were exposed to MeHg (0.1 µM), $HgCl_2$ (1 µM), α-HgS (10 µM) or β-HgS (10 µM) from the Stage 10 for 3 days. Total RNA was extracted and subjected to real-time RT-PCR

analysis for metallothionein (MT) and heme oxygenase 1 (Ho-1) expression. MeHg (4-fold) and $HgCl_2$ (5-fold) increased MT mRNA expression, while α-HgS (1.4-fold) and β-HgS (1.3-fold) had no appreciable effects. Ho-1 is a widely used biomarker for oxidative stress, and MeHg and $HgCl_2$ (2.5-fold) increased Ho-1 mRNA expression by 6-fold and 2.3-fold, respectively, while α-HgS (1.8-fold) slightly increased Ho-1 expression, while β-HgS (1.4-fold) had no significant effects.

First two sentences are METHODS and should be removed
Are the fold changes relative to CONTROLS? State that.

4. Discussion

The present study demonstrated the developmental toxicity of MeHg and $HgCl_2$ in medaka embryos, including mortality, teratogenic effects, and toxicity-related gene expressions. Many studies (Liao et al., 2007; Cuello et al., 2012; Gandhi et al., 2013) have been devoted to the toxicity of mercury to various marine animals; however most of them are focused on organic mercury (e.g. MeHg) toxicity, and relatively few on inorganic $HgCl_2$ toxicity (Ismail et al., 2011; Wang et al., 2011; 2013). The toxic potentials of inorganic α-HgS and β-HgS are largely unknown. This current study compared the developmental toxicity of the four mercury compounds in medaka embryos, and the results demonstrated that MeHg is the most toxic, about 10-times more toxic than $HgCl_2$, and at least 100 times that of α-HgS and β-HgS.

What is 10- and 100- fold based on? Be clear.

Methylmercury crosses the placenta and reaches the fetus where it is responsible for developmental toxicity in humans (Clarkson et al., 2003). Gestational exposure to MeHg produces developmental toxicity in rodents (Gandhi et al., 2013). In marine animals, MeHg exposure produced developmental toxicity in Zebrafish (Cuello et al., 2012) and toxicity in medaka fish at the early life (10-day old) (Liao et al., 2007). The teratogenic effects of MeHg in

medaka embryos are similar to that in Zebrafish in that diminished volume of swim bladder, remaining yolk, bent body axis and accumulation of blood in the heart, and others teratogenic effects occurred. MeHg exposure also produced de-regulation of 71 proteins (Cuello et al., 2012). In the present study, 0.1 µM MeHg produced sever pericardial edema, tube heart, malformation of the eye with reduced pigmentation and failure to inflate swim bladder (Fig. 2), and the metallothionein and heme oxygenase 1 expressions were de-regulated. Thus, MeHg is teratogenic not only to humans and mammals, but also to teleost embryos of marine or fresh water habitats.

When making comparisons to the findigns of others, it is critical that you provide the concentrations they tested, the life stage they tested, and the duration of exposure--also the endpoints. 'Toxicity' is too broad - was it survival? development? hatch success?

Compared to MeHg, the developmental toxicity of $HgCl_2$ is not a major concern, as $HgCl_2$ mainly induces kidney injury (Klaassen, 2001). Nonetheless, exposure of mouse embryos (van Maele-Fabry et al., 1996) sea urchin embryos (Marc et al., 2002), and medaka fish embryos (Ismail and Yusof et al., 2011) to $HgCl_2$ produces developmental toxicity. To date, we have not examined medaka embryo/larvae histologically for signs of renal alteration. $HgCl_2$ is acutely toxic to adult medaka fish (1000 µg/L for 8 hr) (Wang et al., 2011), but also chronically toxic to adult medaka fish (10 µg/L for 60 days) (Wang et al., 2013; 2015). Proteomic analysis revealed de-regulation of several dozens of proteins including oxidative stress-related proteins (Wang et al., 2011; 2013; 2015).

Mercury-based herbo-metallic preparations have been used in traditional medicines for thousands of years. Nowadays, in Pharmacopeia of China (2010) 26 recipes contain cinnabar (α-HgS), in Indian Ayurderic medicine *Rasasindura* that are primarily composed of mercuric sulfide (α-HgS or β-HgS) are included in over 20 recipes (Kamath et al., 2012), in Tibetan medicine, *Zuotai* that are mainly composed of β-HgS are included in a dozen of famous remedies (Kan, 2013; Li et al., 2014). Cinnabar and metacinnabar-based traditional medicines are used for

acute brain emergencies such as stroke, brain trauma, neuroinflammation, and high fever, but are also used to treat chronic ailments like syphilis, pneumonia, insomnia, nervous disorders, paralysis of the tongue, and even used as a rejuvenator to improve strength, stamina and energy (Kamath et al., 2012; Pharmacopeia of China, 2010; Kan, 2013; Li et al., 2014). The question becomes, are innabar and metacinnabar toxicologically similar to environmental mercurial such as MeHg and $HgCl_2$?

Little is known about developmental toxicity of mercuric sulfides, either α-HgS or β-HgS. Recent study in mice suggests that gestational exposure of α-HgS to mice (10 mg/kg, po x 4 week) can also cause developmental toxicity (Huang et al., 2012). No literature is available for the developmental toxicity of mercury sulfides towards marine animals. The present study showed that the developmental toxicity potentials of α-HgS and β-HgS are at least 100-fold lower than MeHg, and at least 10-fold lower than $HgCl_2$. At 10 dpf, only 5% medaka embryos are lost and morphologically no apparent teratogenic effects were evident. This coincides with much less toxicity of α-Hg (cinnabar) and cinnabar-containing traditional medicines in mice (Lu et al., 2011a; 2011b) and in rats (Shi et al., 2011) as compared to MeHg and $HgCl_2$. Similarly, β-HgS (*Zuotai*) is also much less toxic as compared to $HgCl_2$ (Zhu et al., 2013).

Mercury compounds display multiple organ toxicity (e.g. hepatotoxicity, nephrotoxicity and neurotoxicity) in humans and animals (Klaassen, 2001; Liu et al., 2008a). One of the most common mechanisms of mercury toxicity is ascribed to oxidative stress. Mercury induces oxidative stress and the production of reactive oxygen species (ROS) by binding to intracellular thiols (GSH and sulfhydryl proteins) and by acting as a catalyst in Fenton-type reactions, while concomitantly begetting oxidative damage [REF]. Metallothionein, a small, cysteine-rich, metal-binding protein is implicated in the detoxication of heavy metals including mercury (Klaassen et

al., 1999). Induction of metallothionein by mercury is a sensitive biomarker for Hg exposure and toxicity in Zebrafish (Chan et al., 2006), grey mullet (Oliveira et al., 2010), and in Javanese medaka (Woo et al., 2006). MT is increase 4-6 fold following MeHg and $HgCl_2$, but was basically unchanged following α-HgS and β-HgS. Thus, similar to our prior rodent studies (Lu et al., 2011a; Shi et al., 2011), induction of MT by MeHg and $HgCl_2$ in marine animals could be an important biomarker for developmental toxicity of mercury compounds. HAS MT expression been linked to development by others?

Heme oxygase-1 (Ho-1) is an oxidative stress biomarker, and is one of the most sensitive genes in response to toxic stimuli in zebrafish embryos acutely exposed to 14 different chemicals (Weil et al., 2009). In the present study, MeHg induced Ho-1 6-fold and $HgCl_2$ by 2.3-fold, suggesting that MeHg produced more oxidative damage to medaka embryos. Again, inability of Ho-1 induction by α-HgS and β-HgS coincides with their low developmental toxicity, and in good agreement with rodent study showing that exposure to α-HgS did not induce Ho-1 in the liver and kidney, in concord with much less hepatotoxicity (Lu et al., 2011a) and nephrotoxicity (Lu et al., 2011b) as compared to MeHg and $HgCl_2$, suggesting less oxidative damage.

5. Conclusions

When making comparisons to the findigns of others, it is critical that you provide the concentrations they tested, the life stage they tested, and the duration of exposure--also the endpoints. 'Toxicity' is too broad - was it survival? development? hatch success? These details are needed throughout the Discussion.

The current study showed the developmental toxicity of MeHg and $HgCl_2$ in medaka embryos, including pericardial edema, tube heart, eye malformation with reduced pigment precipitation, and failure to inflate the swim bladder. The developmental toxicity potential was MeHg > $HgCl_2$>>α-HgS=β-HgS, indicating that the chemical forms of mercury are a major determinant of mercury toxicity to medaka embryos. The present clearly demonstrated that medaka embryo is a rapid, economic model capable of showing early responses, mechanistic dissections, and excellent correlation with mammalian studies on differential toxicity of mercury compounds.

Author contributions

WD and JL conceived the experiments, DEH provided scientific guidance and resources for the experiments, WD and JL performed the experiments data analysis and WD, JL and DEH wrote the manuscript. Information onlne for this article indicates that WD, JL and DEH wrote the paper....

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Figure legends

Fig. 1. Mortality of mercury compounds in medaka fish embryos. Embryos were exposed to 0.001-10 µM MeHg, $HgCl_2$, α-HgS (*Zhusha*, cinnabar) and β-HgS (*Zuotai*, metacinnabar) from the Stage 10 to 10 days after fertilization (dpf). The mortality was recorded daily. Data are 18-20 embryos/exposure. Methods states 15-18 embryos were used.... Are the Data presented in the figure MEANS? If so, add the SD or SEM...

Fig. 2. Developmental toxicity of mercury compounds. Madaka embryos were exposed to mercury compounds from the Stage 10 to 10 dpf. A-C: embryo exposure at 5 dpf. A, control; B, 0.1 µM MeHg, and C, 10 µM $HgCl_2$. Arrows point to heart; e, eye; h, heart; S, swim bladder. Sever edema, tube heart and malformation of the eye with reduced pigmentation occurred in both MeHg and $HgCl_2$-treated embryos, with MeHg more severe. D-I: embryo exposure at 10 dpf. D, control, E and I, MeHg, F, $HgCl_2$, G, α-HgS (cinnabar), and H, β-HgS (*Zuotai*). Pericardial edema, tube heart, and failure to inflate swim bladder (S) were evident following MeHg (0.1 µM) and $HgCl_2$ (1 µM) treatments, while α-HgS (10 µM) and β-HgS (10 µM) did not show significant effects. Bar = 500 µm. do NOT put results into figure legends...This legend needs extensive revision.

Fig. 3. Gene expression analysis of mercury compounds in medaka embryos. Madaka embryos were exposed to MeHg (0.1 µM), $HgCl_2$ (1 µM), α-HgS (10 µM) and β-HgS (10 µM) from the Stage 10 for 3 days. Total RNA was extracted and subjected to real-time RT-PCR analysis for metallothionein(MT) and heme oxygenase 1 (Ho-1) gene expression. Data are mean ± **SD (n=3)**, *Significantly different from controls $p < 0.05$. Do not put methods into figure legend...Indi

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Figure 1

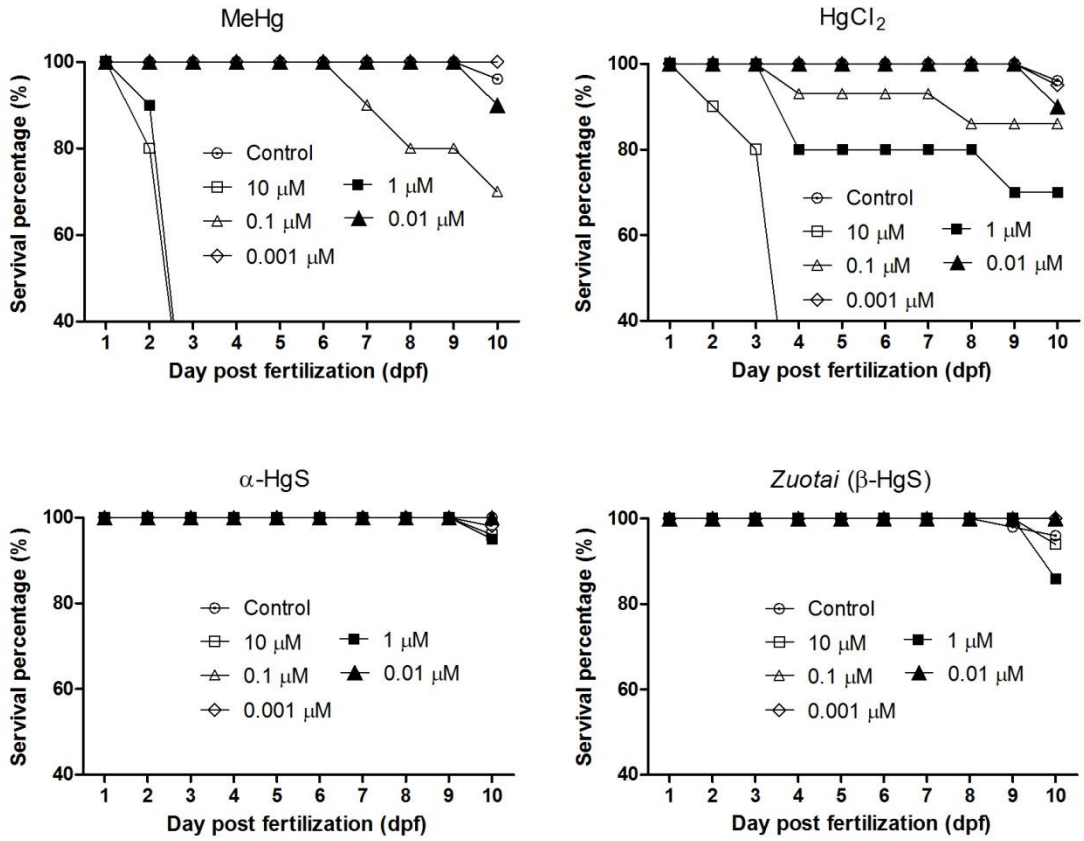
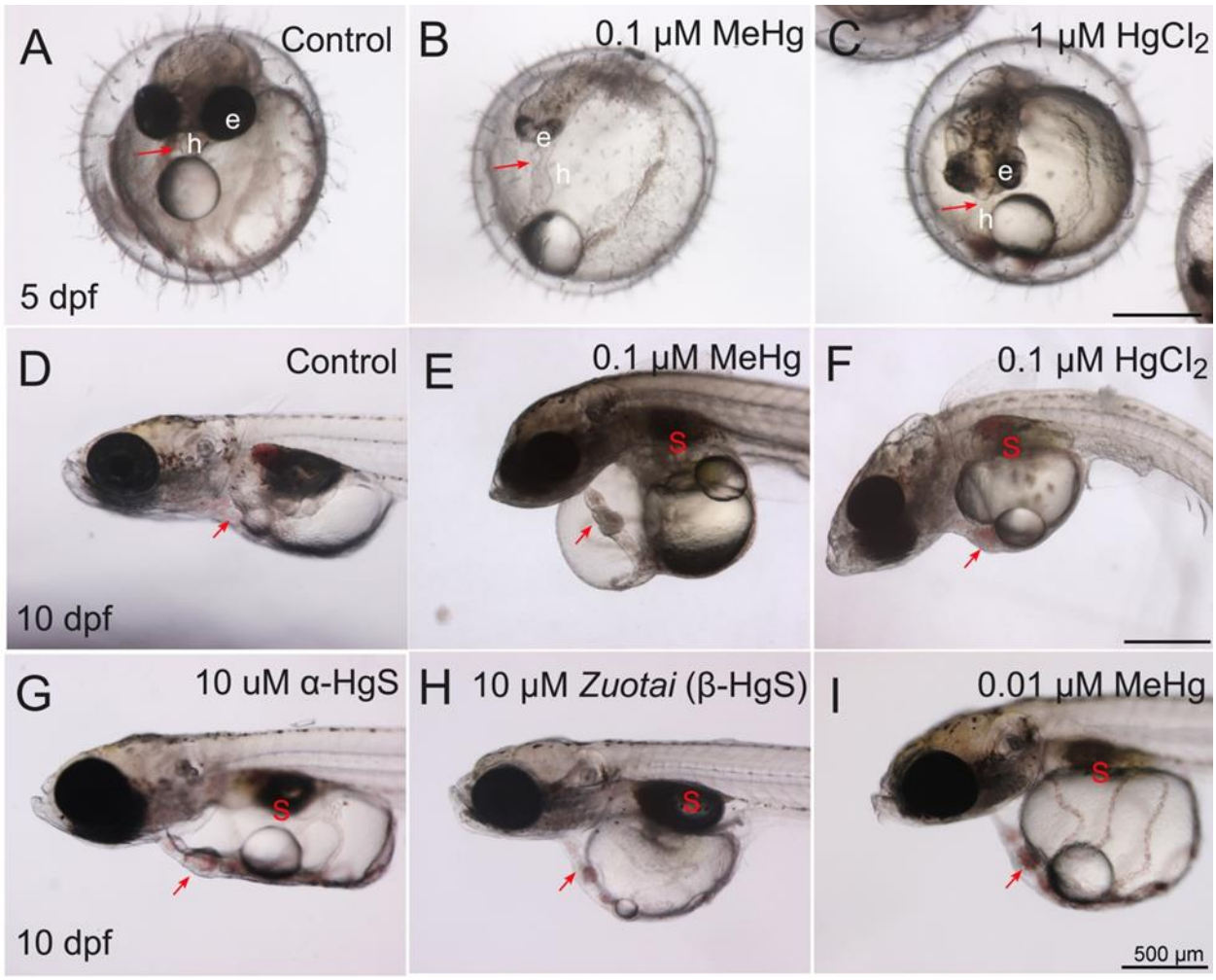
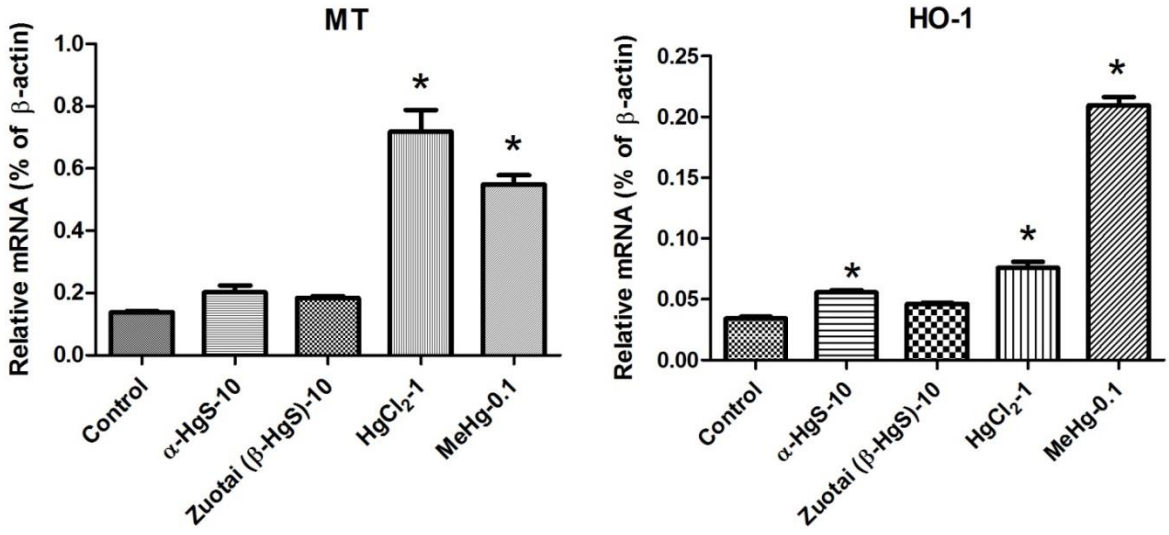

MeHg
HgCl2
α-HgS
Zuotai (β-HgS)
Servival percentage (%)
Day post fertilization (dpf)
Control
10 μM
1 μM
0.1 μM
0.01 μM
0.001 μM
100
80
60
40
1
2
3
4
5
6
7
8
9
10

Figure 2



Re-order the images to correspond with the order in which they are referred to in the text

Figure 3.


MT
Relative mRNA (% of β-actin)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Control
α-HgS-10
Zuotai (β-HgS)-10
HgCl2-1
MeHg-0.1
HO-1
Relative mRNA (% of β-actin)
0.25
0.20
0.15
0.10
0.05
0.00
*
*
*
Control
α-HgS-10
Zuotai (β-HgS)-10
HgCl2-1
MeHg-0.1