Embryotoxicity in medaka (*Oryzias latipes*) following exposure to select alkaline earth metals: a screening bioassay

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Abstract

Environmental exposure to radium, a radioactive alkaline earth metal, and barium, a chemically similar but non-radioactive earth metal, are of growing concern. Radium and barium levels in some groundwater aquifers exceed maximum contaminant levels, and future groundwater resources may be increasingly at risk. Surface waters may also be at risk from disposal of residual waters enriched in metals, including radium and barium, from the increased use of chemical filtration processes such as ion exchange or reverse-osmosis desalination to treat groundwater. Leaching of uranium mine tailings generated during mining activities and industrial uses of barium, including use in high-density oil and gas well drilling muds, serve as additional anthropogenic sources of these metals to surface waters. Currently, there is a lack of information on the effects of radium and barium on fish development. Such lack of data may complicate ecological risk assessment, as recruitment of young of the year fishes have been demonstrated to be major drivers of fish populations.

I employed a high throughput, screening level bioassay to experimentally characterize toxicity in developing medaka fish embryos and eleutheroembryos (an embryonic phase starting with hatching and ending with absorption of yolk sac) following exposure to radium (radium-226 chloride) and barium (barium chloride). The ability to follow individual embryos over time and view embryonic development through the transparent chorion were key design characteristics of this experiment. Two endpoints, time to hatch and mortality, were concurrently assessed.

Results of the bioassay failed to demonstrate evidence of embryotoxicity from exposure to radium at levels up to 60,000 pCi/L. Exposure to high levels of barium (100 ppm) resulted in earlier hatching time. Additionally, an increase in post-hatch mortality was observed, suggesting that the chorion may play a protective role with regard to alkaline earth metal exposure. Water chemistry appeared to affect the magnitude of post-hatch mortality, although results between different exposure scenarios used in the study were ambiguous. The lack of observed embryotoxicity from radium exposure does not definitively demonstrate that radium is not toxic, as other endpoints not assessed in this screening level bioassay may be more sensitive indicators of toxicity and effects from exposure during development may manifest themselves at later life stages. Results of the barium analysis suggest that young of the year fishes may be particularly sensitive to acute exposure to high levels of alkaline earth metals. This suggests that better management of radium and barium in the environment, including the disposal of brine solutions enriched in alkaline earth metals to surface waters, may be needed to reduce ecological risks to fish populations.

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1. Introduction

1.1 Study Objectives

The objective of this study was to experimentally characterize toxicity in developing 73medaka fish embryos and eleutheroembryos (an embryonic phase starting with hatching and ending with absorption of yolk sac) following exposure to radium (radium-226 chloride) and barium (barium chloride). To characterize toxicity, I used a high-throughput, screening level bioassay and assessed two endpoints, time to hatch and mortality. The null hypothesis was that developing medaka embryos and eleutheroembryos would exhibit no observable evidence of toxicity when exposed in vivo to radium at levels up to 60,000 pCi/L or barium at levels up to 100 ppm. Results of the study provided initial information to help assess ecological risk to fishes from exposure to radium and barium and focus future research efforts to further characterize toxicity of these metals to developing fishes.

1.2 Environmental Sources

Radium and barium, like all alkaline earth metals, are found naturally in the Earth's crust distributed in rock structures. Barium ranks seventeenth among elements in the Earth's crust (0.05%) and is found primarily in ores of barite ($BaSO_4$), a sulfate, and witherite ($BaCO_3$), a carbonate, and radium is even rarer, accounting for 0.6 parts per trillion (ppt) of Earth's crust (Winter 2008). The relative scarcity of radium in the environment is attributed to its intense radiation and geologically short half-life (ATSDR 1990). Radium compounds occur almost exclusively in uranium ores.

Industrial uses of radium and its compounds are limited. The Agency for Toxic Substances and Disease Registry (ATSDR) reports that radium has been used as a radiation source for treating neoplastic diseases, as a radon source, in radiography of metals, and as a neutron source for research (ATSDR 1990). Radium was a component of the luminous paints used for watch and clock dials, instrument panels in airplanes, military instruments, and compasses; however, this use ceased in the 1960's due to occupational exposures and resultant chronic toxicity in workers. Radium is also present in the wastes of uranium mining and refining processes. Industrial uses of barium and its compounds, on the other hand, are wide and varied. They are used in oil and gas drilling muds, automotive paints, stabilizers for plastics, case hardening steels, bricks, ties, lubricating oils, jet fuel, and various types of pesticides (ATSDR 2007).

1.3 Physical/chemical properties

Radium and barium are alkaline earth metals, which comprise group 2 of the periodic table and also include beryllium, magnesium, calcium, and strontium. The alkaline earth metals have well-characterized homologous behavior (www.webelements.com). Due to their high levels of reactivity, they are rarely found in elemental form in nature; rather, they are typically found combined with other elements as compounds, often as carbonates or sulfates. All alkaline earth metals have two electrons in their valence shell, occurring in the environment as cations with a +2 valence state.

Radium is a naturally-occurring radionuclide and is extremely radioactive relative to other radionuclides in the environment. For example, it is over one million times more radioactive than an equivalent mass of uranium. There are 25 known radium isotopes, four of which are found in nature. Radium-226, the sole radium isotope used in this study, is the most common. It is also the most stable isotope with a half-life of 1602 years. All four naturally occurring radium isotopes are generated in the decay of either uranium or thorium.

Radium-226 (subsequently referred to as radium) is an intermediate isotope generated in the decay of uranium-238. Radium decays into radon-222, its immediate daughter isotope, which is also radioactive and undergoes further decay. Many of the daughter isotopes of radium, including polonium-218 and lead-214, have much shorter half-lives typically measured in minutes. The division of daughter isotopes continues until a stable, non-radioactive daughter, lead-206, is formed.

Radium decays via alpha particle emission. Alpha particles consist of two protons and two neutrons bound together into a particle identical to a helium nucleus. Because alpha particles are relatively heavy and positively charged, they tend to have a short mean free path and quickly lose kinetic energy within a short distance of their source. As such, external exposure to alpha particles is not generally considered harmful. Due to their high Linear Energy Transfer (LET), however, alpha particles are more effective at causing cancer or cell-death relative to other forms of radiation once ingested or taken into the body, and internal contamination, therefore, is of concern.

In the United States, the standard unit of radioactivity is the curie (Ci), defined as the activity of one gram of radium-226. The International System of Units, the modern form of the metric system, quantifies radioactivity in units of becquerels (Bq), defined as the activity of a material in which one nucleus decays per second. One curie is equivalent to 3.7×10^{10} Bq. Radium levels in the United States are typically reported in units of picocuries per liter (pCi/L), where one picocurie is equivalent to one trillionth of a curie.

Barium is chemically similar to radium, with one major difference. Like all alkaline earth metals other than radium, it is not radioactive. Metallic barium is a silvery-white soft metal that oxidizes readily in air turning a silver-yellow color. Due to its reactivity with air, barium is never found in nature in its pure form. Both barium and radium are also chemically similar to calcium, an essential nutrient used by vertebrates for the growth and maintenance of bones, with important consequences discussed later in this report.

1.4 Environmental Fate and Transport

Environmental fate and transport of radium and barium and their related compounds plays an important role in determining human and ecological exposure and potential for toxicity. Two aspects of particular importance to this study, discussed below, include the solubility of various radium and barium compounds and the effects of water chemistry on radium and barium speciation.

Radium and barium compounds differ in their solubility in aquatic media and, as such, serve as variable sources of Ba^{2+} or Ra^{2+} ions (Table 1). The two most common naturally occurring barium minerals, barite and witherite, are relatively insoluble. Other barium compounds produced and used in industry, including barium chloride, acetate, cyanide, hydroxide, and oxide, are quite soluble in water. These more soluble barium compounds have generally been shown to be more toxic to humans and experimental animals than their less soluble counterparts (ATSDR 2007). Information regarding the solubility of various radium compounds is limited. Because the two alkaline earth metals are chemically similar, however, it is reasonable to assume that the solubilities of radium compounds are comparable to similar barium compounds.

Table 1. Solubility of various radium and barium compounds. Radium and barium chloride, emphasized in bold, were the two compounds used in this study. Quantitative solubility values were available for barium compounds (Lide 2005); however, only qualitative descriptions were found for various radium compounds (ATSDR 1990).

compound	solubility
Barite ($BaSO_4$)	0.00031 g/100 g water (at 20º C)
witherite ($BaCO_3$)	0.0014 g/100 g water (at 20º C)
barium chloride ($BaCl_2$)	**37.0 g/100 g water (at 25º C)**
radium chloride ($RaCl_2$)	**soluble**
radium bromide ($RaBR_2$)	soluble
radium carbonate (RaCO3)	insoluble

The chemical and physical parameters of the aquatic media in which organisms may be exposed have been shown to affect the speciation, bioavailability, and toxicity of various metals (Erickson et al. 1996; Burnison et al. 2006; McDonald et al. 1989). In the aquatic environment, chemical parameters of concern include pH and water hardness, and physical parameters include the presence of dissolved organic matter and suspended solids. In aquatic media, for example, barium is likely to precipitate out of solution as insoluble salts (i.e., as barite or witherite) or may adsorb to suspended particulate matter (Bodek et al. 1988; EPA 1984). When appropriate, these parameters were also of concern in this study. Table 2, below, summarizes the various ways in which the chemical and physical parameters previously described can affect speciation, bioavailability, and toxicity of radium and barium and their related compounds.

Table 2. Summary of important physical and chemical parameters of aquatic media that have been shown to affect speciation, bioavailability, and toxicity of metals.

Chemical/physical parameter	Effect
PH	As pH decreases, solubility of metal compounds (including barium and radium) increase
Water hardness	Toxicity of metals typically increase with decreased water hardness
Dissolved organic matter	Anionic sites on DOM serve as ligands that bind cationic metals and reduce their bioavailability
Suspended solids	Suspended solids may serve as sites of adsorption, reducing metal bioavailability

Radium has been shown to bioaccumulate in the aquatic environment. Bioaccumulation is a process by which living organisms, especially those living in water, can collect and concentrate chemicals both directly from the surrounding environment and indirectly from their food. Bioaccumulation is represented quantitatively in terms of a bioaccumulation factor, which is a numerical ratio of the concentration of a chemical inside an organism to the concentration in the surrounding environment. In a study assessing biological uptake of radium in Canadian lakes, Hesslein & Slavicek (1984) reported that bioaccumulation factors ranged from 30 to 80 in large adult lake trout, white sucker, and whitefish, and from 230 to 1200 in fathead minnows, pearl dace, and northern redbelly dace. Studies of the potential for barium to bioaccumulate in the environment were not found in the literature. Based on the chemical similarities of radium and barium, however, it is reasonable to suppose that barium too would have a tendency to bioaccumulate in the environment.

1.5 Exposure routes of concern

Radium and barium are primarily released into the environment from the leaching and eroding of aquifer rocks enriched in these metals. Levels of radium and barium in groundwater depend on the concentration of these elements in the aquifer rocks and chemical and physical processes that facilitate their transport across the water-rock interface. Sedimentary rocks, such as shale and phosphate, are particularly enriched in these metals (Vengosh 2006; Kojola et al. 1978). Groundwater salinity also affects the migration of these metals from rock to water. In general, in freshwater conditions, most of the radium (and barium) will remain in the aquifer rocks; in saline conditions, however, these metals will escape from the rocks and concentrate in groundwater (Vengosh 2006). Other chemical conditions that affect mobilization of radium and barium include acidity (pH), temperature, and oxidation (Vengosh 2006).

Consumption of well water containing high levels of radium or barium is a potential route for human exposure. In aquifers particularly enriched in these metals, well water may contain levels of radium and barium above maximum contaminant levels (MCLs) in drinking water established by the US Environmental Protection Agency (EPA). Location may be the biggest factor in determining if well water is likely to have high levels of radium or barium. Vengosh (2006) reported that a recent study conducted by the U.S. Geological Survey found groundwater with radium levels exceeding the MCL of 5 pCi/L in southern Minnesota, northern Illinois, Iowa, Missouri, and southern and eastern Wisconsin and that studies in the Southeastern US have also found similarly high levels. Levels of barium in certain areas of the country have been reported as high as 10 times the EPA maximum contaminant level of 2 ppm (ATSDR 2007).

Although barium and radium also occur naturally in most surface water bodies, surface waters are usually low in radium and barium relative to groundwater levels. Some surface waters, however, may be naturally enriched in these metals. Moore (1996) and Shaw et al. (1998), for example, attributed enrichment of radium and barium in certain coastal waters of the Southeastern United States to groundwater input. Similarly, Lauria and Godoy (2002) reported high concentrations of radium in surface waters of a coastal lagoon and attributed these levels to springs at the lagoon head with naturally high levels of radium-226 and –228. Anthropogenic inputs of radium and barium, discussed below, act as additional sources of these metals in surface waters.

Significant surface water related releases of radium include leaching of enriched uranium mine tailings and release of ore-processing effluents generated during mining activities (ATSDR 1990). In Canada, for example, it has been estimated that 10 million tons of uranium mine tailings are generated each year, and laboratory extraction studies have demonstrated that leachate from these mine tailings have contained radium-226 at levels ranging from 38 to 116 pCi/L (Swanson 1985). Compared to the natural occurrence of radium in the environment, however, the amount released through industrial use is considered to be insignificant (ATSDR 1990).

Industrial uses of barium and its compounds, on the other hand, are wide and varied. As such, these activities represent a significant source of anthropogenic release of barium and its compounds in the environment. The use of barite to make high-density oil and gas well drilling muds, for example, is a major anthropogenic source of barium into ocean waters. During offshore drilling operations there are periodic discharges of drilling wastes into the ocean (Ng & Patterson 1982). Phillips et al. (1998) reported that operations involving three drilling platforms in the Santa Maria Basin of the coast of central California released approximately 1.8 x 10^6 kg of barium to the ocean. An estimated 1.48 million pounds of barium and its compounds were released to surface waters by industry in 2004 alone (ATSDR 2007).

An emerging concern with regards to potential increases in exposure to radium and barium, particularly in surface waters, involves the increased use of chemical filtration processes such as ion exchange or reverse-osmosis desalination to treat groundwater. Large-scale desalination plants are becoming increasingly used to increase potable water supplies both domestically and internationally. Similarly, water softeners are used to improve water quality for domestic household uses. These processes are effective in improving the quality of water for its intended use; however, residual waters selectively accumulate and concentrate certain cationic metals, including radium and barium. Thus, disposal of these brine solutions and the potential environmental impacts resulting from such activities are of growing concern and served as a major reason for conducting this study.

1.6 Basis of study

Currently, there is a lack of information regarding the potential ecological effects of radium and barium in the environment. In particular, there is a total lack of information on the effects of these metals on fish development. Such lack of ecological data may complicate ecological risk assessment, as recruitment of young of the year fishes have been demonstrated as major drivers of fish populations (Jones 1990; Bennett & Moyle 1996). There exists a need, therefore, to obtain relevant data regarding potential embryo toxicity in developing fishes following exposure to radium and barium.

The experimental design I used was based on a high throughput approach for developmental toxicity testing described by Oxendine et al. (2006). It was also used in a study on differential toxicity of napthoic acid isomers on medaka embryo development by Carney et al. (2007). Initially, the focus of the study was to assess embryotoxicity in medaka fish following exposure to radium alone. However, a number of issues arose during the initial stages of the assessment that necessitated the incorporation of barium into the analysis. These issues are summarized in Table 3, below.

Table 3. Summary of factors that necessitated the incorporation of barium into the study.

Issue	Why important?
Chemical Similarity	Because radium and barium are chemically similar, exposure to barium, a non-radioactive alkaline earth metal, allowed for comparison of potential toxicity of radium (if observed) due to exposure to the radium cation itself (elemental exposure) versus exposure to daughter isotopes and radiation emitted as part of the decay process (radiation exposure).
Radium Standard	The radium standard used in this study also contained barium in the form of barium chloride.
Cost	The high cost and chemical composition of the radium standard limited exposure to low levels (in terms of a mass concentration amount). As such, use of barium allowed for exposure to higher levels of alkaline earth metals.
Environmental Concern	Exposure to barium is also of growing concern due to the increased use of chemical filtration processes previously described as well as from releases to surface waters from industrial use.

Several features of the experimental design were particularly attractive. As reported by Carney et al (2007), “the ability to follow individual embryos in the micro plate format and view developmental stages through the transparent chorions were key design characteristics of this experiment.” Additionally, the use of fish embryos in toxicity testing meets goals recently developed by the European Union (REACH legislation) of reducing, refining, and replacing current animal testing methods.

2. Methods

2.1 Radium and barium

Radium used in this assessment was in the form of a radium standard (Radium-226 Radioactivity Standard, Standard Reference Material 4967A, National Institute of Standards & Technology). The radium standard consisted of radioactive radium-226 chloride (and its decay products), non-radioactive barium chloride, and hydrochloric acid dissolved in 5 mL of distilled water and contained in a flame-sealed NIST borosilicate-glass ampoule. The Radiation Safety Division of the Occupational Environmental Safety Office at Duke University approved the procedures for use and disposal of radium-226 as part of this experiment. Barium used in this assessment was in the form of barium chloride ($BaCl_2$) (99.9% metal basis) purchased online from Sigma-Aldrich (www.sigma-aldrich.com).

2.2 Exposure scenarios

To explore potential toxicological effects at various stages of development and the protective role of the chorion, multiple exposure scenarios and water chemistry conditions were used in this study. The first exposure scenario consisted of embryonic exposure 3-5 hours after initiation of spawning and continuing for approximately 48-hours post hatch (termed Exposure Scenario #1) (Table 4). Total exposure time in this exposure scenario ranged from 8 to 14 days. The second exposure scenario consisted of embryonic development under normal husbandry conditions followed by 48-hour exposure post-hatch (termed Exposure Scenario #2) (Table 4). To examine effects of water chemistry on metal toxicity, embryos and eleutheroembryos were exposed to radium and barium in both deionized water (DI) and embryo rearing medium (ERM) (Yamamoto, 1975). In ERM, sodium chloride, potassium chloride, calcium carbonate, and magnesium sulfate were added to the DI water.

Table 4. Description of exposure scenarios used in this study. Each scenario included exposure in both DI water and ERM (described in Section 2.2)

Exposure Scenario	Description
#1	Embryos (stage 3-10) were exposed 3-5 hours after initiation of spawning, and exposure continued for approximately 48-hours post hatch.
#2	Embryos were allowed to develop under normal husbandry conditions, and were exposed as eleutheroembryos for 48-hours beginning day of hatch.

2.3 Dosing levels

Preliminary studies employing independent experimental replicates were conducted to assess reasonable ranges for dosing levels. Dosing levels of radium-226 used in the preliminary studies ranged from 6 – 60,000 pCi/L and were based on maximum effluent concentrations of 60 pCi/L to surface water and 600 pCi/L to sewerage, as identified in the United States Nuclear Regulatory Commission's website (www.nrc.gov). Dosing levels of barium chloride used in the preliminary studies ranged from 1 to 1,000 parts-per-million (ppm) and incorporated levels above and below the EPA MCL for barium in drinking water of 2 ppm. Based on the results of the initial range-finding studies, two treatment levels were selected for each alkaline earth metal. 600 and 60,000 pCi/L were selected as dosing levels for radium-226, and 1 and 100 ppm were selected as dosing levels for barium chloride. DI and ERM controls were analyzed concurrently with the experimental treatments for each alkaline earth metal.

2.4 Endpoints

Two endpoints, mortality and time to hatch, were concurrently assessed to characterize toxicity. These endpoints were based on observations made during preliminary studies. Mortality was defined by loss of heartbeat and absence of blood cell circulation, and time to hatch was defined as complete escapement from the chorion, measured in units of days post fertilization. Data was recorded on a master data sheet that identified well location. Dead embryos or eleutheroembryos were removed daily from chamber with disposable pipette. Endpoints were assessed once daily throughout development.

2.5 Embryo collection

Orange red (OR) medaka embryos and eleutheroembryos were used in this study. All care and maintenance procedures followed those approved by the Duke University Institutional Animal Care and Use Committee (protocol registry # A117-07-04). Embryos were collected from the colony maintained at the Duke Forest Aquatic Research Facility. Brood fish used for egg production were 4-12 months old, on a 16 and 8 hour light/dark cycle, maintained at a water temperature of 25ºC and pH of 7-7.5 and fed Otohime B1 (Otohime Beta, Nisshin Feed Co. Ltd., Tokyo) with live brine shrimp nauplii (*Artemia sp*.) as supplements. Eggs were collected 3-5 hours after initiation of spawning and examined under a dissecting microscope. Abnormal or dead individuals were discarded, and viable embryos were subsequently examined to determine embryonic stage of development as described by Iwamatsu (1994). Embryos between stages 3 and 10 of development were used in each assay. A subset of viable embryos were set aside and allowed to develop concurrently under normal husbandry conditions for use in 48-hour eleutheroembryo exposure assays (Exposure Scenario #2).

2.6 Embryonic assessment

Glass coated flat bottom 96-well plates (SUN-Sri, Duluth, GA) were used as experimental exposure vessels. Individual embryos (stages 3-10, Iwamatsu, 1994) were loaded into each well of the microplate using a disposable pipette. Radium and barium solutions were prepared by dissolving radium standard or barium chloride in DI water or ERM. Solutions were carefully changed at 48-hour intervals until 48-hours post hatch. Solution pH was maintained at approximately 7.5 using sodium hydroxide. Plates were stored in an incubation chamber with temperature maintained at 26.5ºC with a 16/8 hour light/dark cycle. Each embryonic treatment level consisted of 32 embryos for radium exposure and 48 embryos for barium exposure. 48-hour eleutheroembryo exposures (Exposure Scenario #2) consisted of 24 newly hatched eleutheroembryos per treatment level for both radium and barium exposures. Randomization was insured throughout the experiment by embryo and plate selection achieving a completely randomized design.

2.7 Data analysis

Data analysis was performed using GraphPad Prism ® Version 4.03 software. Based on the binomial nature of the endpoints assessed (hatched vs. not hatched & dead vs. alive), results were expressed as proportions (% hatched and % alive) and uncertainty was quantified using 95% confidence intervals. Prism computed 95% confidence intervals of proportions using the Clopper and Pearson method based on sample size and variability (Motulsky 2003).

3. Results

3.1 Radium

In Exposure Scenario #1, no statistically significant differences in time to hatch or pre-hatch mortality were observed relative to controls (Figure 1). Similarly, no significant differences in post-hatch (eleutheroembryo) mortality were observed (Figure 2).

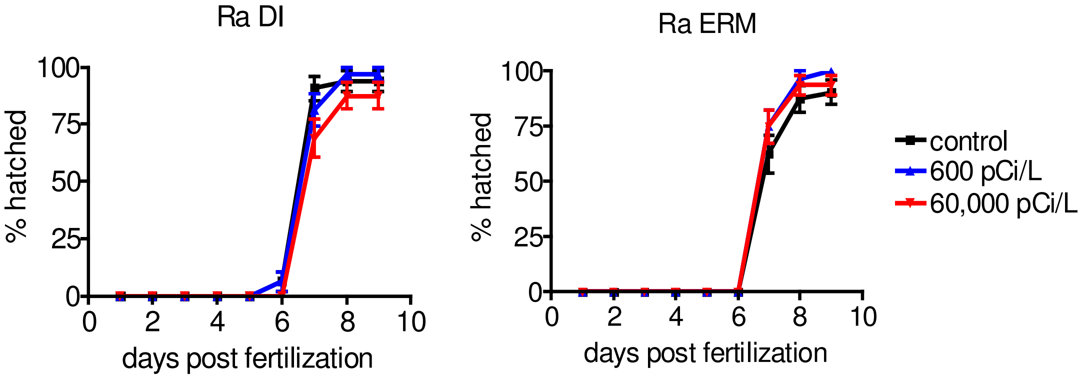

Figure 1. Hatching profiles for medaka embryos exposed to radium under Exposure Scenario #1. No significant differences in time to hatch, hatching rate, or pre-hatch mortality were observed.

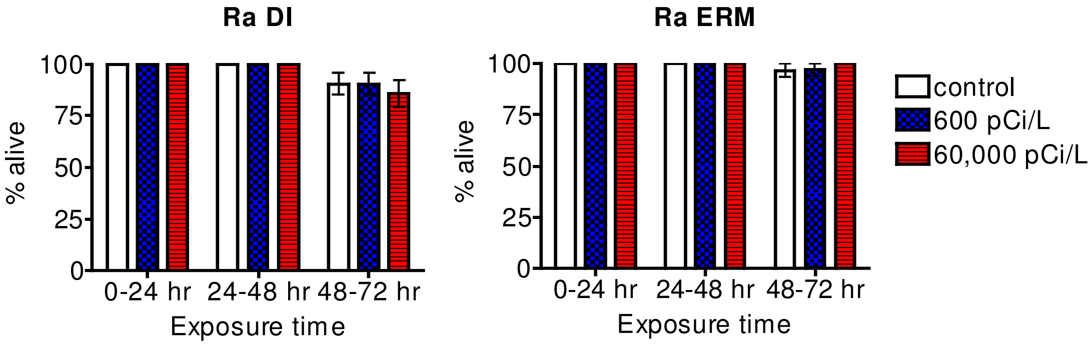

Figure 2. Post-hatch survival in eleutheroembryos exposed for 48-72 hours under Exposure Scenario #1. Exposure time (post-hatch) is given as a range because plates were observed daily, so time of hatch had a 24-hour uncertainty. No statistically significant differences in post-hatch mortality were observed.

In Exposure Scenario #2, no significant differences in post-hatch (eleutheroembryo) mortality were observed relative to controls following exposure to radium (Figure 3).

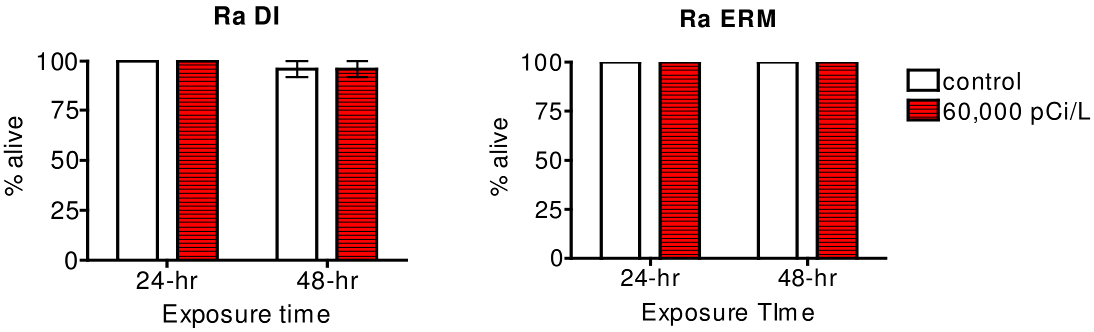

Figure 3. Post-hatch survival in eleutheroembryos exposed to radium for 48 hours under Exposure Scenario #2. Like in Exposure Scenario #1, no statistically significant differences in post-hatch mortality were observed.

3.2 Barium

In Exposure Scenario #1, a statistically significant increase in time to hatch was observed at the highest exposure level (100 ppm) relative to control in both DI water and ERM (Figure 4). No significant differences in pre-hatch mortality were observed at any exposure level except for embryos exposed to 1 ppm barium in DI water. These embryo cultures were contaminated by a fungus, which was deemed as causing the observed mortality. Instructions for care and use of medaka recommended use of slightly saline media for embryonic development and warned of fungal-induced attrition (Kirchen & West 1975). As previously explained, however, DI water was required by constraints of the research design. Embryo cultures that suffered fungal contamination were not incorporated into data analysis.

Exposure post-hatch (as eleutheroembryos) resulted in a significant increase in mortality at the highest exposure level (100 ppm) relative to the control (Figure 5).

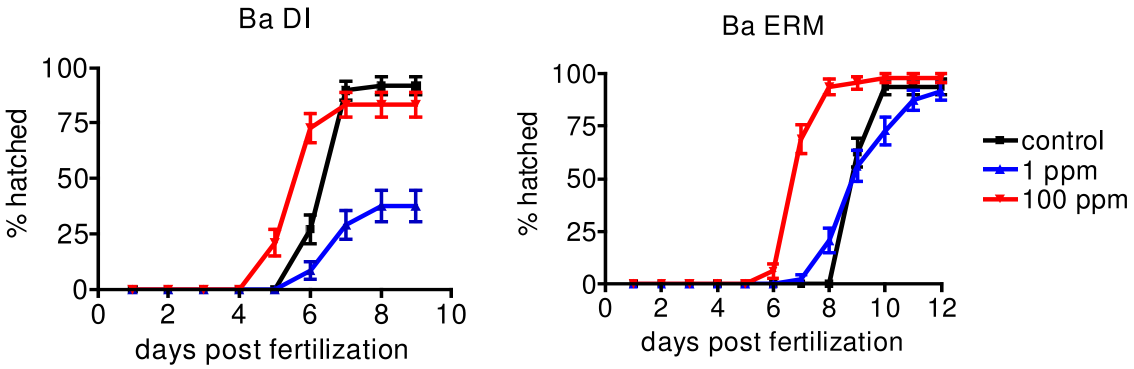

Figure 4. Hatching profiles for medaka embryos exposed to barium under Exposure Scenario #1. Embryos exposed to 100 ppm barium hatched earlier than controls. No significant differences in hatching rate or pre-hatch mortality were observed, except in the case of exposure in DI at 1 ppm; this embryo culture, however, suffered fungal contamination which was deemed as causing the observed mortality.

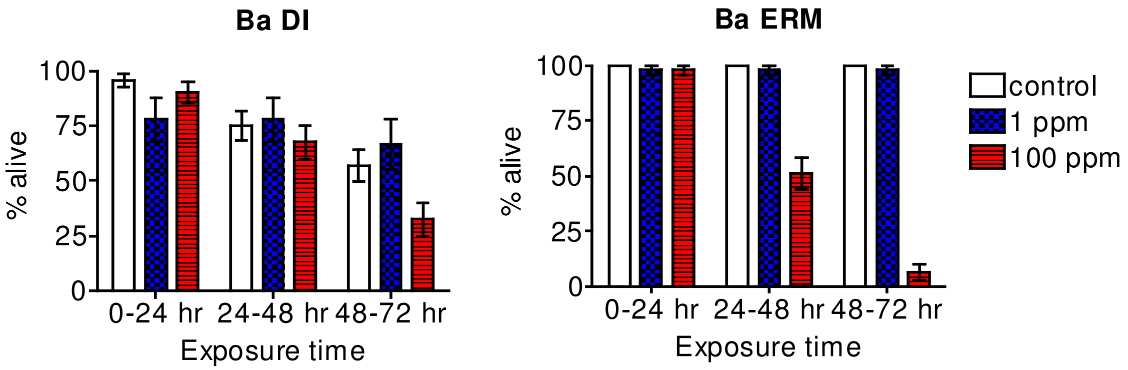

Figure 5. Post-hatch survival in eleutheroembryos exposed to barium for 48-72 hours under Exposure Scenario #1. Exposure time (post-hatch) is given as a range because plates were observed daily, so time of hatch had a 24-hour uncertainty. Eleutheroembryos exposed to 100 ppm barium showed increased mortality relative to controls. Additionally, eleutheroembryos exposed in ERM showed increased mortality relative to DI water.

In Exposure Scenario #2, significant mortality was observed at the highest dosing level (100ppm) relative to controls (Figure 6).

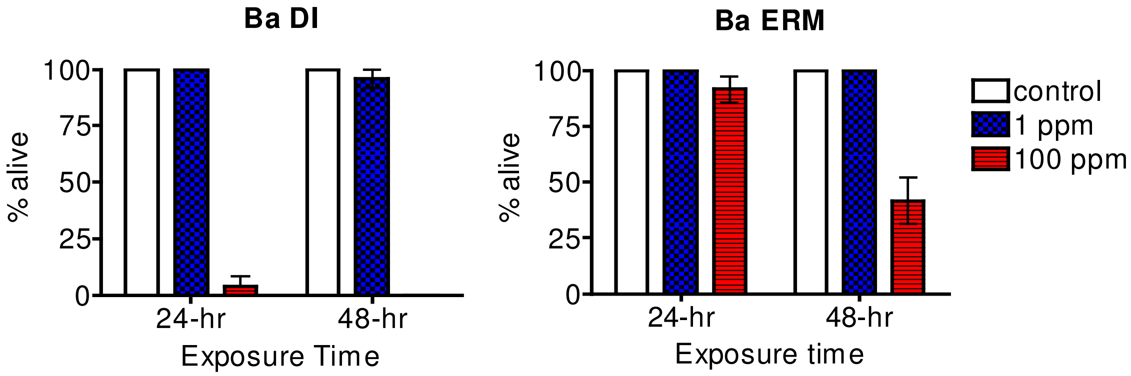

Figure 6. Post-hatch survival in eleutheroembryos exposed to barium for 48 hours under Exposure Scenario #2. Like in Exposure Scenario #1, eleutheroembryos exposed to 100 ppm barium for 48-hours showed increased mortality relative to controls. However, in this case, eleutheroembryos exposed in DI water showed increased mortality relative to ERM.

Eleutheroembryos exposed to barium were also observed as having reduced motility in some cases. No quantitative metrics to measure effects of exposure on motility, however, were assessed as part of this study.

4. Discussion

4.1 Radium

Results failed to demonstrate evidence of embryotoxicity from exposure to radium at levels up to 60,000 pCi/L. A lack of observed embryotoxicity, however, does not definitively demonstrate that exposure to radium, its decay products, and radiation produced as part of the decay process at levels at or below 60,000 pCi/L are not toxic, as other endpoints not assessed in this screening level bioassay may be more sensitive indicators of toxicity. In addition, effects from exposure during development may manifest themselves at later life stages, and these were not assessed. Similarly, later-life stages of the medaka fish may be more sensitive to radium exposure.

4.2 Barium

Results of the bioassay indicated that exposure to barium (in the form of barium chloride) at 100 ppm resulted in earlier time to hatch and increased mortality post hatch. Results of exposure to 1 ppm barium failed to demonstrate evidence of embryotoxicity.

While there was a clear lack of data in the literature regarding toxicity to fish (especially fish embryos) from exposure to barium, the little data that was found suggested that previous toxicological studies of barium on fish focused primarily on later life stages and failed to demonstrate toxicity at levels at or higher than those used in this study (Pan Pesticides Database). These results suggest that developing embryos and eleutheroembryos may be particularly sensitive life stages to barium exposure. Future research efforts on toxicity of barium, therefore, should 1) focus on developing life stages of fish; 2) determine LC_{50} values for 48-hour eleutheroembryo exposure to barium chloride; and 3) incorporate additional endpoints that may be more sensitive indicators of embryotoxicity such as post-hatch motility and inner-eye distance, or other metrics for growth and development.

4.3 Protective role of the chorion

The observation of a lack of significant pre-hatch mortality combined with an increase in mortality post-hatch following exposure to 100 ppm barium suggests that the chorion may play a protective role with regard to metal exposure. This conclusion is supported by a number of studies that have demonstrated that cationic metals, including Ca^{2+},

Cu^{2+}, Mn^{2+}, and Zn^{2+}, preferentially accumulate in the chorion of medaka embryos (Michibata 1981; Nakagawa and Ishio 1989) or salmonoid embryos (Beattie and Pascoe 1978; Woodworth and Pascoe 1982; Peterson et al. 1983) compared to the interior of the embryo (embryonic body and yolk sac). Wedemeyer (1968) hypothesized that such behavior is due to preferential binding of cationic metals to anionic sites on the chorion. No studies assessing the distribution of barium or radium in fish embryos following exposure were found in the literature. More information is therefore needed to support such a conclusion. The use of autoradiography may be the method of choice for detailed analysis of metal distribution in exposed embryos.

4.4 Water chemistry

Results of the bioassay suggest that water chemistry affects the magnitude of post-hatch mortality, although results were ambiguous. In Exposure scenario #1, post-hatch (eleutheroembryo) mortality was unexpectedly greater in ERM than DI water, whereas in Exposure Scenario #2, mortality was greater in DI water than ERM as expected. One possible explanation of the observed difference is that exposure to barium in DI water during embryonic development may allow for physiological adaptation of the developing embryo prior to hatching. Beattie and Pascoe (1978), for example, reported that hatching trout eleutheroembryos exposed to cadmium during embryonic development survived longer in cadmium than eleutheroembryos not exposed during embryonic development, suggesting that pretreatment of embryos with cadmium served some protective function. Physiological adaptation may not have occurred in medaka embryos exposed to 100 ppm barium in ERM as a result of the decreased bioavailability of barium in ERM due to reaction with anions in the water and precipitation out of solution. Further research efforts are needed, however, to support such a conclusion.

4.5 Exposure Routes

In this study, medaka embryos and eleutheroembryos were exposed to radium and barium dissolved in water and uptake was via diffusion across the gill lamellae or dermal absorption. Other routes of exposure, however, may be of greater concern in the environment. Hesslein & Slavicek (1984), in a study assessing geochemical pathways and biological uptake of radium in Canadian lakes, reported that 90% of radium-226 added to the lakes initially sorbed to the sediments. Clulow et al. (1998), in a similar study of radium-226 in water, sediments, and fish from a Canadian watershed containing uranium mining and milling operations, found higher levels of radium-226 in bones of whitefish (a bottom feeding fish) compared to lake trout (a pelagic fish). They proposed that bottom-feeding fish may ingest radionuclide-rich sediment particles along with food or may be exposed to higher levels via their diet. Similarly, Pyle & Clulow (1998), referencing Whicker & Schultz (1982) report that most environmental transport of thorium is through physical processes whereby bottom-feeding organisms ingest thorium through inadvertent consumption of sediments while foraging.

4.6 Mechanisms of toxicity

Mechanisms of toxicity from exposure to barium and radium in developing medaka embryos have not been established in the literature, and assessment of potential mechanisms was beyond the scope of this analysis. However, several mechanisms of toxicity have been proposed in humans and other experimental animal models. In addition, barium and radium may have similar modes of action to other metals, in which mechanisms of toxicity have been established. Available data, summarized below, may serve to generate hypotheses of mechanisms of toxicity from exposure to radium or barium.

Ion regulation in fish via transport of ions across the gill epithelium is essential for fish health. McDonald et al. (1989) reported that trace metals can be expected to have at least some impact upon branchial ion regulation in fish. Specific mechanisms of action, however, are likely to be different for each metal, with some (Cu, Al and H^+) being primarily disruptive to Na^+ and Cl^- balance and others (Cd, Zn, and Mn) interfering with Ca^{2+} balance. It is likely that radium and barium may also inhibit branchial ion regulation in fish.

Radium and barium also share features of calcium as determined physiologically. Calcium is an essential nutrient used by vertebrates for the growth and maintenance of bones (Pyle & Clulow 1998; Whicker & Schultz 1982). Once taken up, radium and barium readily deposit in bone tissue. Radium taken up and stored in bone may undergo radioactive decay resulting in damage to bone or surrounding tissue due to the high linear energy transfer of α-particles emitted as part of the decay process (Pyle & Clulow 1998). Barium, while not radioactive, may also affect health of fishes due to its ability to replace calcium in bone.

Koch et al (2003), in a study referenced in the ATSDR toxicological profile for barium (2007), reported that barium acts as a competitive potassium (K) channel antagonist, blocking the passive efflux of intracellular K. This results in a shift in K from extracellular to intracellular compartments. The net result is a decrease in K concentration in blood plasma and a reduced resting membrane potential, making muscle fibers electrically unexcitable and causing paralysis. Such activity may explain the observed decrease in motility in eleutheroembryos exposed to barium. Future research efforts should explore such behavior.

5. Conclusion

Chemical filtration of groundwater to increase potable water supply and soften water for improved domestic use may come with an environmental price. Residual waters enriched in certain metals, including radium and barium, generated during the filtration process could pose an ecological risk to fish populations if disposed of in surface waters. Freshwater environments, in which radium and barium are likely to be more bioavailable, may be particularly at risk. Other sources of these metals to surface waters may also pose an ecological risk to fish populations. Results of the bioassay suggest that young of the year fish are sensitive to exposure to high levels of alkaline earth metals, with consequences on overall health of fish populations.

Efforts to further characterize embryotoxicity in fishes from exposure to these alkaline earth metals are needed. These efforts should include determination of LC_{50} values for 48-hour exposure of barium to eleutheroembryos and incorporate additional endpoints that may be more sensitive indicators of embryotoxicity, such as post-hatch motility, inner-eye distance, or other metrics for growth and development. While the screening level bioassay failed to demonstrate evidence of embryotoxicity from exposure to radium at levels up to 60,000 pCi/L, monitoring of fishes exposed as embryos is ongoing, and effects of exposure during development may manifest themselves at later life stages. Additionally, exposure routes not applicable to this bioassay, such as intake of radium (or barium) by benthic fishes via diet or incidental ingestion and subsequent bioaccumulation, may be of concern. Future research is needed to characterize ecological risks from these exposure routes.

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