OCS Study
MMS 97-0021

Effects of Barium and Divalent Cations Associated with Oil Production Wastes on Developing Marine Organisms

Final Technical Summary

Final Study Report

U.S. Department of the Interior
Minerals Management Service
Pacific OCS Region

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Authors

Gary N. Cherr
Teresa W-M. Fan
Principal Investigators

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Southern California Educational Initiative
Marine Science Institute
University of California
Santa Barbara, CA 93106

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REPORT TITLE: Effects of Barium and Divalent Cations Associated with Oil Production Wastes on Developing Marine Organisms- Final Report.

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PROJECT MANAGERS: [1]G.N. Cherr and [2]T. W.-M. Fan

AFFILIATION: [1]Bodega Marine Laboratory and [2]Dept. Land, Air & Water Resources

ADDRESS: [1]P.O. Box 247, Bodega Bay, CA 94923 and [2]University of California at Davis, Davis, CA 95616

PRINCIPAL INVESTIGATORS: G.N. Cherr

KEY WORDS: Santa Barbara Channel; produced water; chronic toxicity; embryo development; marine organisms; barium; divalent cations; biomarkers; contaminant effects; toxic mechanisms

BACKGROUND: Barium is known to be one of the most abundant inorganic constituents in produced waters, and is a major component of drilling muds and fluids (Neff, 1987; Neff, 1993). Barium can also be found at high concentrations in sediments surrounding oil platforms. However, it has been assumed that the high concentrations of barium (up to 2000 mg/L) in oil production wastes are not of environmental concern in the marine environment since sea water contains high concentrations of sulfate, and thus free barium ions (which are bioavailable) would associate with sulfate too rapidly to be of ecological significance (Neff, 1993).

Recently, barium has been shown to be the predominant divalent cation present in the fraction of produced water which perturbs embryonic development (larval shell development) in molluscs (Higashi *et al*., 1992). In addition, studies have indicated that barium incorporation occurs in shells and tissues of adult mussels which were outplanted near a produced water discharge site; in these mussels, increased barium concentrations in the animals correlated with decreased mussel performance (Cherr *et al*., 1993). Furthermore, both produced water, and its barium-containing fraction, appear to exhibit cellular effects through their possible perturbation of microtubule function (Cherr *et al*., 1993; Garman *et al*., 1994). Finally, low concentrations of barium (~200 ppb) have been found to inhibit normal embryonic development in mussels and sea urchins (Cherr *et al*., 1993).

OBJECTIVES: 1. Barium and the barium-containing produced water fraction perturbs ciliary function in developing embryos. We tested this using mussel and sea urchin embryos exposed to either barium (as barium acetate), the divalent cation fraction of produced water, strontium (as a negative control), or control sea water. Ciliary beating of trochophore, veliger, and prism stages was analyzed through videorecordings.

2. Barium and the barium-containing produced water fraction perturb veliger shell development by incorporation into the shell matrix. We tested this by exposing mussel embryos to the different treatments described above, and assessing the normality of shell development using polarization microscopy and also attempted direct analyses of barium/calcium ratios in the larval shells.

3. Barium and the barium-containing produced water fraction directly affect intracellular calcium homeostasis in developing embryos. We tested this by attempting to directly measuring the heavy metal concentration in sea urchin and mussel embryos using a new fluorescent indicator for metals, including barium. We also attempted to quantitate intracellular divalent cation concentration in embryos using specific fluorescent indicators for calcium and magnesium.

4. Barium induces chronic reproductive effects in adult sea urchins in a similar manner as that observed for sea urchins exposed to produced water in the field. Since adult sea urchins have been shown to be reproductively impaired when chronically exposed to produced water in the field (Krause, 1994), we determined if chronic exposure of sea urchins, exposed to environmentally relevant barium concentrations, showed similar effects.

5. Barium is bioavailable to marine organisms at a specific concentration range. It has been argued that barium is not bioavailable at toxic concentrations in sea water due to the high level of sulfate, and that it precipitates upon dilution of produced water with sea water (Neff, 1993). We investigated the bioavailability of barium in sea water in relation to bioeffects in marine embryos.

DESCRIPTION: Previous reports indicated that barium (Ba) and/or strontium (Sr) was primarily responsible for the toxicity of a Southern California produced water to developing marine embryos. In order to further investigate toxicity of Ba and Sr in sea water, mussel embryos *(Mytilus californianus)* were subjected to static exposures of barium acetate and strontium chloride from fertilization through veliger formation. Only Ba exhibited bioactivity at environmentally relevant levels. Adverse effects occurred between 200-900 µg/L (ppb); higher concentrations were associated with decreased toxicity and apparent precipitation of Ba salts from sea water. Nominal Ba exposure concentrations between 100 and 900 µg/L yielded measured concentrations of 100-550 µg/L soluble Ba when analyzed by inductively coupled argon plasma emission spectroscopy. Adverse developmental effects included abnormal shell calcification and embryo morphology. Exposure of embryos to Ba in stage-specific experiments revealed that developmental stages were differentially affected, though they exhibited similar abnormalities. Gastrulae were the most sensitive, while blastula and trochophore larvae were less so. Adverse effects in embryos exposed during the gastrula stage were not reversible despite washing and return to clean sea water. These findings are the first to demonstrate that low concentrations of soluble Ba in sea water can be toxic, and are of potential concern in the marine environment.

The assessment of physiologic changes associated with exposure of both whole embryos and an isolated cell type to these low levels of soluble barium. Through the use of cell permeable fluorescent probes and fluorescence microscopy, we investigated changes in several parameters including ion levels and distribution, intracellular pH, and cellular membrane potential associated with low level barium exposure and the gross effects previously described. Changes were observed in Ba-treated embryos as compared to controls, but these could only be done qualitatively due to interference by Ba directly. No effect on ciliary activity was observed in Ba-exposed veliger and -blastual embryos of mussels and sea urchins, respectively. The metal ion probe for Ba turned out to be non-responsive in our hands, and was later recalled by the manufacturer. As such, without a specific probe for Ba, intracellular ion investigations were put on hold. Nevertheless, preliminary investigations provide additional information which may eventually be used in understanding of how soluble, low level barium in sea water acts as a reproductive and developmental toxicant in some species of marine invertebrates.

Chronic exposure (3-5 months) of adult sea urchins to low levels of Ba (as low as 250 ppb) resulted in a significant decrease in gamete function as determined by lowered fertilization rates in sperm and eggs from exposed animals, with the greatest effect being observed in eggs, although sperm were also affected in some animals. No adverse effect on gamete production was observed for Ba exposure. These results are similar to those obtained by Krause (1994) for sea urchins exposed to produced water in the field. Embryos from exposed parent animals also exhibited abnormal/delayed development. Higher development rates in embryos, as compared to fertilization assessments, indicate that embryos have impaired elevation of the fertilization envelope. This effect may be osmotic in that normal elevation of the envelope is dependent on calcium binding proteins released by the egg at fertilization; it is likely that Ba replaces calcium and blocks normal hydration.

We have shown that barium exposure impacts normal skeleton (spicules) formation in sea urchin larvae. In order to specifically address this, we isolated primary mesenchyme cells, which are responsible for secreting and organizing the internal skeleton, and cultured them in the presence of Ba *in vitro*. It is clear that Ba does not prevent cell aggregation and fusion, but does inhibit normal spicule formation in the isolated cells, and is a good biomarker of Ba effects.

Mussels outplanted at Carpinteria for three months were sampled for their new-growth shell material by the project of Osenberg *et al*. Ca was analyzed in addition to Ba in order to normalize to the carbonate shell mass, since shell may also contain non-carbonate material. The results of Ba/Ca were completed for both *Mytilus californianus* and *Mytilus edulis* .

M. californianus, in some outplant sessions, showed a clear decreasing trend with increasing distance from the outfall (Oct '90, Jan '91, May '91, Aug '91, Dec '91), but at the other times did not show a trend at all. Note that, when sampled, site 1000m east of the outfall was comparable to 1000m west. Although there is no immediately obviously explanation to the results, the data is undergoing statistical tests by Osenberg *et al*., particularly in relation to Carpinteria discharge rates, prevailing current and environmental conditions, and biological parameters. This analysis may explain the trends observed in the Ba/Ca data.

M. edulis, for the two outplant sessions, did not show a Ba/Ca trend in May '91, which was a time that *M. californianus* exhibited a strong trend, suggesting that *M. edulis* may resist the incorporation of Ba into their shells. Again, Osenberg *et al*. is in the process of analyzing this data in relation to other factors.

Although subject to the data analysis by Osenberg *et al*., these data support the hypothesis that, in the field, Ba is accumulated by *M. californianus* and recorded in their newly-grown shell material. Some of these trends are striking considering the high sulfate in seawater and dilution factors in the open coastal ocean. Once again, it is assumed that only bioavailable Ba would be incorporated into shells, and supports our findings that Ba is in fact bioavailable at certain concentrations in sea water.

STUDY PRODUCTS:

Spangenberg, J.M. and G.N. Cherr. 1996. Developmental effects of barium in a marine bivalve (*Mytilus californianus*). *Environmental Toxicology and Chemistry*, **15(10)**:1769-1774.

Spangenberg, J.M. 1997. *Reproductive and developmental effects of Barium (Ba) exposure in two marine invertebrates: the California mussel (Mytilus californianus) and the white sea urchin (Lytechinus anamesus)*. Ph.D. Dissertation. University of California, Davis.

Spangenberg, J.M. and G.N. Cherr. Chronic barium exposure perturbs fertilization and development if *Lytechinus anamesus* eggs and embryos. In preparation.

FINAL STUDY REPORT

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APPENDIX 1:

DEVELOPMENTAL EFFECTS OF BARIUM EXPOSURE IN A MARINE BIVALVE (*MYTILUS CALIFORNIANUS*)

J.V. Spangenberg and G.N. Cherr, *Environmental Toxicology and Chemistry* **15**:1769-1774

APPENDIX 2:

EFFECTS OF CHRONIC BARIUM EXPOSURE (3–5 MONTHS) ON SEA URCHIN GAMETE PERFORMANCE IN CLEAN SEA WATER

APPENDIX 3:

BARIUM/CALCIUM RATIOS IN SHELLS OF *MYTILUS CALIFORNIANUS* AT INCREASING DISTANCE FROM THE OUTFALL

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.