USGS

WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: DEVELOPMENT OF A NOVEL *IN VITRO* SCREENING ASSAY FOR ANALYSIS OF BIOAVAILABLE METAL

Duration: August 1, 1997 - July 31, 1998

Federal funds requested: $9,000

Non-federal funds: pledged: $29,606

Principal investigator:

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Congressional district: Sixth

STATEMENT OF THE CRITICAL REGIONAL WATER PROBLEM:

The contamination of aquatic systems by metals is widespread and has been extensively investigated. As a result, ambient water quality criteria (AWQC) have been defined and sediment quality. criteria are being established for many metals (U.S. EPA, 198 6; Ankley *et el.,* 1996). There is an ongoing change in the way metals are regulated. Criteria based on total metal concentrations are being replaced by limits derived from dissolved metal concentrations (Davies. 1996). The latter are operationally defined as the fraction of metals that pass through a 0.45Ftm filter and are thought to better represent the bioavailable fraction (APHA, 1985). Although the dissolved fraction generally is the most bioavailable. there are problems associated with their u se (Birge *et. al,* 1996). One important issue is the uncertainty associated with their quantification. Current filtration techniques may be unreliable and it is not clear if this issue can be rectified (Bergman *et al.,* 1997; Birge and Black, 1996; Davies, 1996). The situation gets even more complicated by the fact that bioavailable metal is not always toxic. For example, silverthiosulfate is bioavailable but 15,000 times less toxic than silver nitrate ($AgNO_3$) to freshwater fish (Ho gstrand *et al.,* 1996a; Wood *et al.,* 1996). Such problems stress the need for more reliable methods that can gauge not only bioavailable metal, but also intracellular reactivity (bioreactivity) of metals. This proposal describes the developme nt and characterization of a novel *in vitro* metal sensor (biosensor) which can be used as a superior method to reliably and precisely measure metal bioavailability and bioreactivity.

Water Quality and Aquatic and Environmental Protection are two of the five areas in which research and technology transfer have been prioritized for the Southeastern and Island region. Research of stated importance within the water quality area include s "development and improvement of monitoring techniques," which is the very objective of

the proposed work. The biosensor will be constructed for use on stream and lake waters from Kentucky to selectively monitor metals that are likely to pose threat to f ish populations. Such activities have been identified as central to the Aquatic and Environmental Protection research priority for the Southeast and Island region. It should be noted, however, that the metal biosensor could have regulatory impact that exc eeds regional needs.

RESULTS, BENEFITS, AND INFORMATION

The immediate results and benefits from the proposed research will be the presence of a unique tool to determine the presence of bioavailable and bioreactive silver (Ag), cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn) in natural waters. Whi le complicated procedures, such as analysis of dissolved metal, can at best estimate metal bioavailability, the metal biosensor will provide an actual readout of the bioavailability in a specific water. No currently existing technique can offer this advan tage. In addition, as this is a cell culture technique it would be less time and space consuming, and more cost-effective than current analytical and whole animal approaches. Recently, it has been suggested that the existing AWQC approach should be phased out by mechanistic-based chemical models that employ affinity constants for metals binding to the gill surface (Bergman *et al.*, 1997). This method is based on the principle that gills are the primary target of most acute metal-mediated toxicity (J anes and Playle, 1995; Bergman, 1997). Thus, the metal biosensor, which should respond to bioreactive metals entering across the apical gill surface, would directly complement gill binding models that only gauge surface binding of metals. In this and othe r respects, the biosensor shows promise to develop into a useful regulatory tool for metals.