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METHYL MERCURY INDUCED CHANGES IN THE SERUM PROTEINS OF BLUEGILLS—*LEPOMIS MACROCHIRUS* (TELEOSTEI)[1]

HIRAN M. DUTTA, S. B. LALL[2] and ALI Z. HAGHIGHI[3], Department of Biological Sciences, Kent State University, Kent, OH 44242

ABSTRACT. There were qualitative and quantitative changes in the serum proteins of bluegills, *Lepomis macrochirus*, exposed to 8.728×10^{-4} ppb (W/V) of methyl mercury (MeHg) for 24, 48, and 72 hr. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of serum proteins revealed significant changes in the qualitative and quantitative profiles at 24 and 48 hr. However, at 72 hr a trend to return to control levels was noted. The data suggest that at the dose tested, MeHg produced repairable lesions in certain tissues or organs of bluegills.

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INTRODUCTION

Methyl mercury (MeHg) can cause a variety of abnormalities in the gills, gut, liver, kidney, or brain of fishes. The intensity of these changes depends on the species, its age, physiological status, concentration of MeHg and exposure time (Choi et al. 1978, Mehra and Choi 1981).

Data are available in the literature on the movement of Hg in aquatic system (Hildebrand et al. 1980), haematological alterations in the blood of fishes exposed to various forms of Hg (Hilmy et al. 1980), mucus secretion (Lock and Van Overbeeke 1981), changes in cellular elements (Harper 1968), developmental anamolies (Weis and Weis 1977, Sharp et al. 1980) and the bioaccumulation and biomagnification of Hg in fish tissues (Waldichuk 1974, Yamazaki 1978). However, there is little information on changes in serum pro-

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[2]Present Address: Associate Professor, Department of Zoology, University of Udaipur, Udaipur, India.

[3]Present Address: Ph. D. Student, Department of Chemistry, Kent State University, Kent, OH 44242.

teins of fishes exposed to MeHg (Lagler et al. 1977, Hilmy et al. 1980).

Estimation of qualitative and quantitative changes in serum proteins as measured by sodium dodecyl sulphate-polyarcylamide gel electrophoresis (SDS-PAGE) can be correlated with toxicological effects of chemicals. The purpose of this study was to measure any changes in serum proteins of bluegills (*Lepomis macrochirus*) exposed to MeHg.

METHODS AND MATERIALS

Adult bluegills (5–8 cm in total length) were collected locally. The bluegills were acclimated to aquaria water (pH 7.25–7.35, temperature 25–27 C) for 3 days, and food was available *ad libitum.*

Concentrations of MeHg ranging from 1.255×10^{-5}, 1.255×10^{-4}, 1.255×10^{-3}, 1.255×10^{-2} and 8.728×10^{-4} ppb (W/V) were tested to determine a sub-lethal dose.

Fish (N = 4–5) were exposed to 8.728×10^{-4} ppb (W/V) MeHg in all glass aquaria. Controls were treated under identical conditions, minus the MeHg. Samples of blood were taken from unanesthetized control and treated fishes at 24, 48 and 72 hr. Blood was removed by cardiac puncture with 1.0 ml sterile plastic syringes. The samples were allowed to stand at room temperature for one hr and then placed in a refrigerator for overnight storage. The following day, the samples were centrifuged at 700 g for 10 min and the serum was removed and frozen for subsequent biochemical and electrophoretic assays.

Sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) was performed essentially according to the method of O'Farrel (1975). Serum samples (30–40μl) were thawed and applied to slab wells. Albumin, oval albumin, β-lactoglobulin, pepsin, trypsinogen and lysosyme were run simultaneously as marker proteins. The gels were stained with Coomassie brilliant blue G-250 for 1–1.15 hr and then destained in 7% acetic acid until the polypeptide bands displayed maximal staining effect and no background staining. Gels were scanned with a densitometer to determine the quantitative profile of peptides. Schematic maps were prepared to display the site and number of bands. Total serum proteins were estimated according to the method of Lowry et al. (1951). All protein samples and SDS-PAGE were run in duplicate.

RESULTS AND DISCUSSION

MeHg at 8.728×10^{-4} ppb (W/V) caused the fish to swim erratically and increased opercular movements. The levels of total serum proteins in controls were 2.65 mg/ml compared to 1.17, 1.778 and 1.40 mg/ml in fish treated with MeHg for 24, 48 and 72 hr respectively (table 1). SDS-PAGE of serum protein hydrolysates showed changes in serum proteins of MeHg treated fish (fig. 1). A total of 28 major plus minor (weakly staining) bands were observed in the serum of controls as opposed to 22, 51, 27, from fish exposed to MeHg for 24, 48, 72 hr. A variety of peptides, showing differential loci, staining intensity, mobility, and molecular weight were seen at 48 hr compared to 24 hr. However, 72-hr samples showed a reduction in polypeptide bands approximating a profile similar to controls. Although the number of bands at 72 hr and control are nearly the same, they differ in their loci and staining intensity indicating quantitatively and qualitatively different peptides. Additional peptides could be added to the serum of MeHg treated fish as a result of breakdown of intracellular haemoglobin, red blood cells or other cellular components. The total protein as measured by Lowry's method decreased in MeHg treated fish, even though there was an increase in the total number of bands. This could have resulted from rapidly hydrolysed low molecular weight proteins which yielded fast-moving peptides that stained weakly and occupied different loci on the gel.

Giblin and Massaro (1974) stated the binding of MeHg to intracellular haemoglobin of rainbow trout can be reversed by extracellular sulfahydryl groups. Nakao et al. (1973) showed intact red blood cells can be penetrated by various mercurial compounds which induce inhibition of both Na^+-K^+ ATPase and Na^+-K^+

TABLE 1

Total serum protein in control and MeHg treated bluegills.

Treatment	Total serum protein (mg/ml)
Control	2.65
MeHg (24 hr)	1.17
(48 hr)	1.78
(72 hr)	1.40

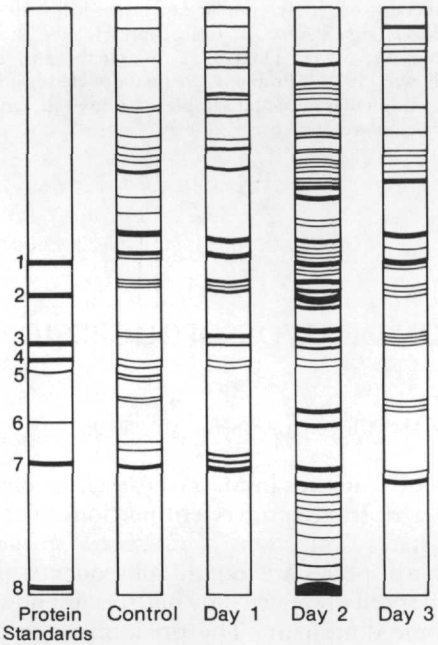

FIGURE 1. Diagrammatic representation of serum polypeptides as resolved by SDS-PAGE from control and MeHg treated bluegills.

insensitive ATPase. Dawson (1975) and Saad et al. (1973) emphasized blood alterations or damage to the haemopoietic organs also may be associated with pathological conditions related to waterborne pollutants. Passow et al. (1961) suggested binding of Hg may be in either "sensitive" or "non-sensitive" areas of subcellular compartments and may influence the outcome of toxicity.

Regardless of the mechanism(s) of cellular damage, our data show that MeHg caused considerable changes in the serum proteins of the bluegills. With further refinements in methodology, serum proteins could be useful indices of contaminant-induced toxicity in fish populations.

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