Toxicity and Accumulation of Arsenic in Green Sunfish, *Lepomis cyanellus*, Exposed to Arsenate in Water

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Aquatic fauna has long been known to accumulate arsenic. CHAPMAN (1926) found up to 70 ppm arsenic in Portuguese oysters, as much as 119 ppm arsenic in mussels, up to 110 ppm arsenic in lobsters, and a maximum of 10 ppm arsenic in plaice (a marine flat fish). These aquatic fauna had been exposed to 0.14 to 1.0 ppm arsenic in sea water according to CHAPMAN (1926). Largemouth black bass from the southern United States retain up to 40 ppm arsenic (ELLIS et al. 1941). Few laboratory studies, however, have been reported on arsenic toxicity and accumulation in fish; moreover, most of these studies were conducted using such toxic arsenicals as arsenite (GILDERHUS 1966) which, being trivalent, is considered more potent than the pertavalent congener (HARVEY 1970).

The purpose of this study was to determine to what extent green sunfish, an active freshwater fish, was affected by short-term exposures to high concentrations of a less potent arsenical, sodium arsenate. Acute or short-term effects of this arsenical were assessed on the basis of mortality; lethal time for 50% mortality (LT_{50}) and lethal dosage for 50% mortality (LD_{50}) were calculated by straight line graphic interpolation. Arsenic accumulation in whole fish at the time of death was measured using neutron activation analysis (NAA) and these values were compared with arsenic exposure, time before mortality, and fish condition.

Neutron activation analysis is an established, nondestructive method employed by SMALES and PATE (1952a, b, c), LUNDE (1967a, b), SMITH (1961, 1964), and MOLOKHIA and SMITH (1967). After irradiation, 100% of the stable isotope (arsenic-75) undergoes a neutron-gamma nuclear transformation to arsenic-76 which has a 26.5 hr half-life and major gamma ray energies (in MeV) and intensities of 0.560 (45%), 0.646 (6%), and 1.205 (6%) (WEAST 1968). The 0.560 MeV photopeak was used for all net peak area calculations, made according to the ATKINSON et al. (1970) modification of Covell's method (COVELL 1959).

Materials and Methods

Green sunfish were seined from a pond 5 km northeast of Manor, Texas and transported in styrafoam coolers to 50-l aquaria. After 4 days in charcoal-filtered, aerated well water, fish were randomly divided into 4 groups of 25 fish each. Each group was placed in an environmental chamber in 14-l aquaria containing 0, 100, 500, and 1000 ppm arsenic (as sodium arsenate) in aerated, well water at 20°C and constant fluorescent lighting. At regular

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intervals, aquaria were examined for dead specimens, which were classified as such when they did not move after being prodded. Dead fish were removed as soon as possible, weighed, measured (for total length), and frozen in sequential order of death. This experiment was conducted in triplicate and then cumulative % mortality values were calculated.

One-third of the dead specimens were homogenized, placed in 2/5 dram plastic polyvials, dried to stabilization at 60°C, weighed, and analyzed for the arsenic quantity in whole fish using NAA. Known quantities of arsenic (i.e. 1, 10, and 100 µg) were irradiated with fish tissue over 360 KW-hr in the rotary specimen rack of the University of Texas TRICA Mark I Nuclear Reactor at a flux of 2×10^{12} thermal neutrons/cm^2/sec. After a one- to three-day decay period for reduction of sodium-24 activity, each sample was counted for 800 sec on a lithium-drifted germanium detector connected to a Nuclear Data multichannel analyzer system. Arsenic accumulation was compared with arsenic exposure, time lag before death, fish condition, fish wet weight, fish dry weight, and fish total length.

Results and Discussion

Cumulative % mortality (Fig. 1) for all fish in the triplicate experiment increased with increasing exposure concentrations. Lethal times for 50% mortality for 100, 500, and 1000 ppm arsenic were 46, 17, and 12 hr, respectively. This effect was also observed at lower exposure levels: at 30 and 60 ppm arsenic and 20°C, LT_{50} decreased from 527 to 210 hr for green sunfish (SORENSEN 1974). Other metals produced the same effects. An increase in zinc concentration from 5 to 40 ppm decreased survival time in zebrafish from about 8 to 2 hrs (SKIDMORE 1967). An 11-mo exposure of the fathead minnow to a range of 4.4 to 95 µg/l copper reduced survival from 80 to 55% (MOUNT 1968).

When these same data were plotted as cumulative % mortality for 3 size classes (Fig. 2), no excessive variation was apparent in 500 and 1000 ppm arsenic exposure levels. The smallest fish exposed to 100 ppm arsenic, however, died at a slightly more rapid rate than those fish in the other 2 size classes. The LT_{50} values for small, intermediate, and large fish exposed to 100 ppm arsenic are 39, 55, and 73 hr as calculated by straight line graphic interpolation. WODZICH and TAYLOR (1957) simularily found that arsenic trioxide kills younger before older rabbits. LD_{50} values were calculated from arsenic exposure versus cumulative % mortality data (Fig. 3). The LD_{50} values for 12, 18, 24, and 48 hr were 1000, 350, 175, and 150 ppm arsenic, respectively.

Arsenic accumulation increased with the arsenic exposure level (Fig. 4); mean arsenic accumulation was 33.4, 541.2, and 581.6 ppm arsenic for exposure levels of 100, 500, and 1000 ppm arsenic, respectively. About one-third of all specimens in this triplicated experiment were analyzed for arsenic. Such biological variation in metal accumulation was consistant with that found

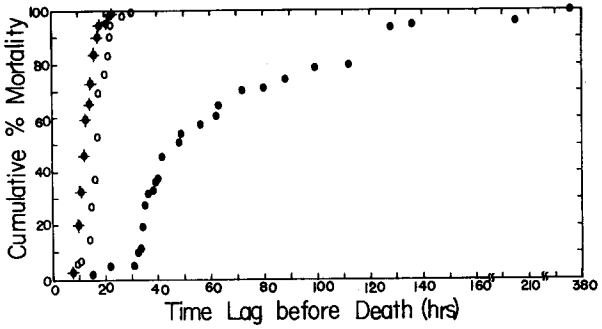

Figure 1. Mortality of green sunfish exposed to 100 (●), 500 (○), and 1000 (◆) ppm arsenic. No mortalities occurred in fish exposed to 0 ppm arsenic.

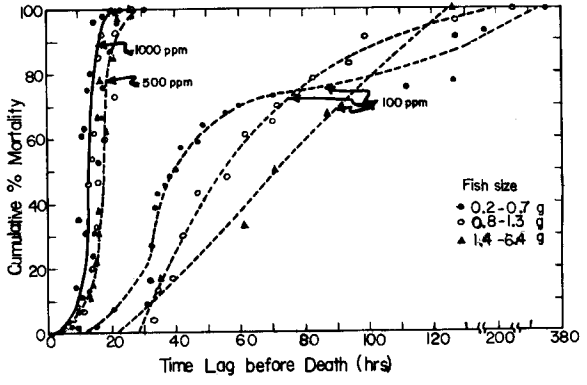

Figure 2. Mortality of specific size classes of green sunfish exposed to 100, 500, and 1000 ppm arsenic.

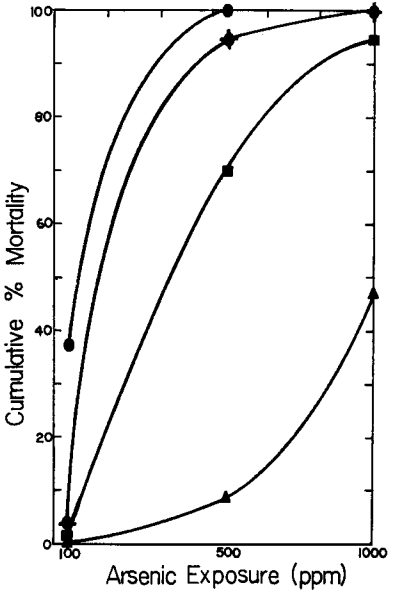

Figure 3. Cumulative % mortality at various arsenic exposure levels for straight line graphic interpolation of LD_{50} values at 12 (▲), 18 (■), 24 (◆), and 48 (●) hrs.

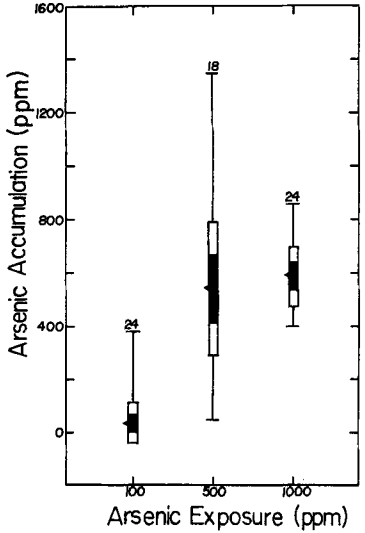

Figure 4. Dice-grams (as modified by HUBBS and HUBBS (1953)) of arsenic accumulation at various exposure levels. The triangle indicates sample mean; the heavy horizontal line, total range; the black bar, 2 estimated standard errors ($2s_{\bar{y}}$) to each side of the mean; and one-half of each black bar plus the white bar at either end, one sample standard deviation on either side of the mean.

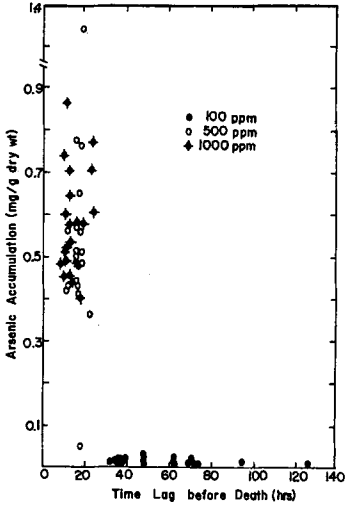

Figure 5. Time before mortality versus whole body arsenic accumulation in fish exposed to 3 concentrations of arsenic.

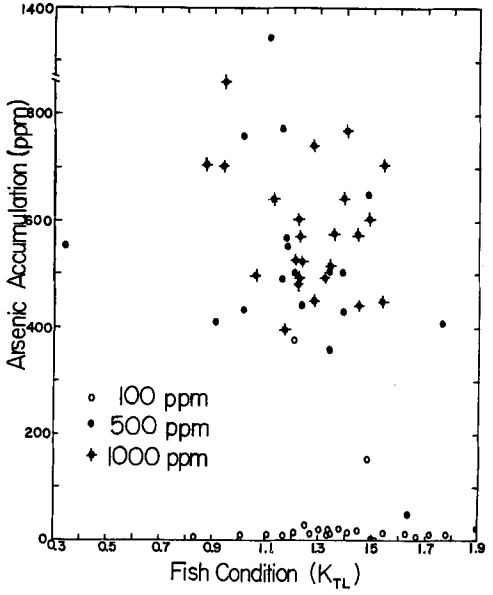

Figure 6. Arsenic accumulation in individual fish versus condition ($K_{TL} = \frac{W \times 10^5}{L^3}$, where W is specimen wet weight in g and L is total length (TL) in mm).

by OPHEL and JUDD (1967) for bullhead, carp, and shad and by LOVETT et al. (1972) for pike, sturgeon, and catfish. Aside from this variability it was apparent that the direct accumulation of arsenic from water did follow the established pattern for direct uptake of other metals such as cadmium (MOUNT and STEPHAN 1967) and zinc (MOUNT 1964).

Time of death was compared to total arsenic accumulation (Fig. 5). Fish exposed to 100 ppm arsenic appear to be more successful in removing arsenic from the body over short periods of time. Arsenic retention in organs of L. cyanellus appeared to be short in duration, decreasing markedly the first week after transferring fish to 0 ppm from 30 or 60 ppm arsenic (SORENSEN 1974). The high and variable arsenic accumulation before mortality of fish exposed to 500 and 1000 ppm arsenic seems to indicate that mortality might be caused by a second mechanism (perhaps mucous-clogging of the gills due to metallic irritation?) at these levels.

No correlation was observed in comparison of arsenic accumulation with fish total length, wet weight, dry weight, and condition (Fig. 6). Literature citings of the relationship between toxicant accumulation and such specimen parameters are rare; however, no correlation was observed between fish weight and cadmium uptake in salmon or drum (LOVETT et al. 1972), between fish weight and zinc accumulation by about 1000 fish of 22 species (MOUNT 1964), or between fish weight and cesium-137 uptake in carp (KEVERN 1966). This lack of a correlation between various specimen parameters and fish weight might be due, in part, to the fact that nonessential elements such as arsenic (LIEBSCHER and SMITH 1968) are merely contaminations of tissue and have no significant function.

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