Bull. Environ. Contam. Toxicol. (1994) 53:211-218

Environmental Contamination and Toxicology

Estimation of Appropriate Background Concentrations for Assessing Mercury Contamination in Fish

G. R. Southworth, M. J. Peterson, S. M. Adams, B. G. Blaylock

Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Building 1505, MS 6036, Oak Ridge, Tennessee 37831-6036, USA

Received: 10 June 1993/Accepted: 14 January 1994

One goal of environmental restoration at contaminated industrial or hazardous waste sites is the prevention of any further release of contaminants. As a consequence of successful remediation, it is hoped that elevated contaminant concentrations in biota will return to levels characteristic of environments uncontaminated by point sources. To evaluate the efficacy of such an environmental cleanup, it is necessary to know what background contaminant concentrations would typify uncontaminated conditions in the systems of interest. In the case of environmental mercury contamination, determining appropriate reference concentrations is complicated by the complex natural biogeochemistry of this element and the wide-scale atmospheric transport of mercury from diffuse sources. Concentrations of mercury in freshwater fish from sites relatively unimpacted by human activities vary widely (Hand and Friedman 1990; Schmitt and Brumbaugh 1990; Phillips et al. 1987; Huckabee et al. 1987; Heit et al. 1989). Much of the variation is associated with major differences in productivity, pH, hardness, and alkalinity (Bjornberg et al. 1988). Thus, a degree of mercury contamination in fish that represents the natural background level in one lake or stream may indicate contamination in another. An accurate estimate of an appropriate background mercury concentration in fish is needed to determine the extent to which industrial mercury discharges produce elevated mercury concentrations in fish in receiving waters, and to determine the concentrations in fish that would represent restoration to uncontaminated status.

Losses of large quantities of mercury in the 1950s at the U.S. Dept. of Energy (DOE) facilities in Oak Ridge, Tennessee resulted in continued chronic contamination of several small streams and the downstream river/reservoir system (Van Winkle et al. 1984) . Mercury concentrations in axial muscle of fish exceed 1 µg/g wet wt. near the source, and decline to much lower concentrations 20 km downstream in Watts Bar Reservoir (Van Winkle et al. 1974; TVA 1985; Kornegay et al. 1991). Although remedial efforts are underway, the facility remains a continuing source of mercury contamination to the downstream waters.

Correspondence to: G. R. Southworth

The primary objective of this study was to measure mercury concentrations in bluegill and redbreast sunfish from streams and reservoirs near Oak Ridge, Tennessee that are presumed to be relatively unimpacted by anthropogenic point sources of mercury in order to determine appropriate background levels in fish to apply in evaluating local contaminated streams and reservoirs. A secondary objective was to explore interspecific differences in mercury bioaccumulation between bluegill and redbreast sunfish and to examine background mercury concentrations in bluegill from stream and reservoir environments.

MATERIALS AND METHODS

Fish were collected by backpack or boat electrofishing from streams and reservoirs in the watersheds of the Clinch and Tennessee Rivers in east Tennessee as part of several regulatory monitoring programs. Under these programs, mercury concentrations in sunfish have been monitored at contaminated sites downstream from DOE facilities in Poplar Creek, East Fork Poplar Creek, Bear Creek, and White Oak Creek (Fig. 1). Two important goals of these programs are assessment and remediation of environmental damage caused by mercury contamination; therefore multiple reference sites were sampled to define background concentrations of mercury in sunfish in environments similar to those that were contaminated. Sunfish were collected from seven streams (Hinds Creek, Paint Rock Creek, Bull Run, Beaver Creek, Brushy Fork, Ballplay Creek, and Little Sewee Creek) that were assumed to be unimpacted or distant from major anthropogenic mercury sources (Fig. 1). Reservoir reference sites were located in Tellico Reservoir, Norris Reservoir, Fort Loudon Reservoir, Melton Hill Reservoir, Watts Bar Reservoir, and Chickamauga Reservoir (Fig. 1). Except for Tellico Reservoir, all waters sampled arise in watersheds rich in limestone and dolomite strata, and are characterized by baseflow alkalinities of approximately 1.5 to 3.5 meq/L and pH 7.5 - 8.5. Tellico Reservoir has water chemistry more consistent with streams draining the Appalachian Highlands, with alkalinity of approximately 0.5 meq/L and pH 7.5 - 8.5.

The predominant sunfish species in small to medium size lowland streams in the Tennessee River drainage of east Tennessee is the redbreast sunfish (*Lepomis auritus*). Bluegills (*L. macrochirus*) are also found in such streams but are much less common. Both species are found in the streams impacted by mercury discharges from the Oak Ridge DOE facilities. Redbreast sunfish predominates in East Fork Poplar Creek and specific reaches of White Oak Creek. Bluegill is more widespread in White Oak Creek, where movement is impeded by weirs, and in lower Poplar Creek. Collections were restricted to fish exceeding 50 g wet wt to minimize the impact of possible covariance between fish-size mercury content and also to measure directly mercury concentrations in the population of fish likely to be taken for food by recreational fishermen. A 1 to 2-g sample of the anterior dorsal portion of the skinless fillet was excised, wrapped in aluminum foil, labeled, and frozen at -20° C until analyzed. Mercury analyses were conducted by the Analytical Chemistry Division at Oak Ridge National Laboratory. Samples were digested in a mixture of nitric acid, perchloric acid, and potassium dichromate, after which the mercury was reduced with

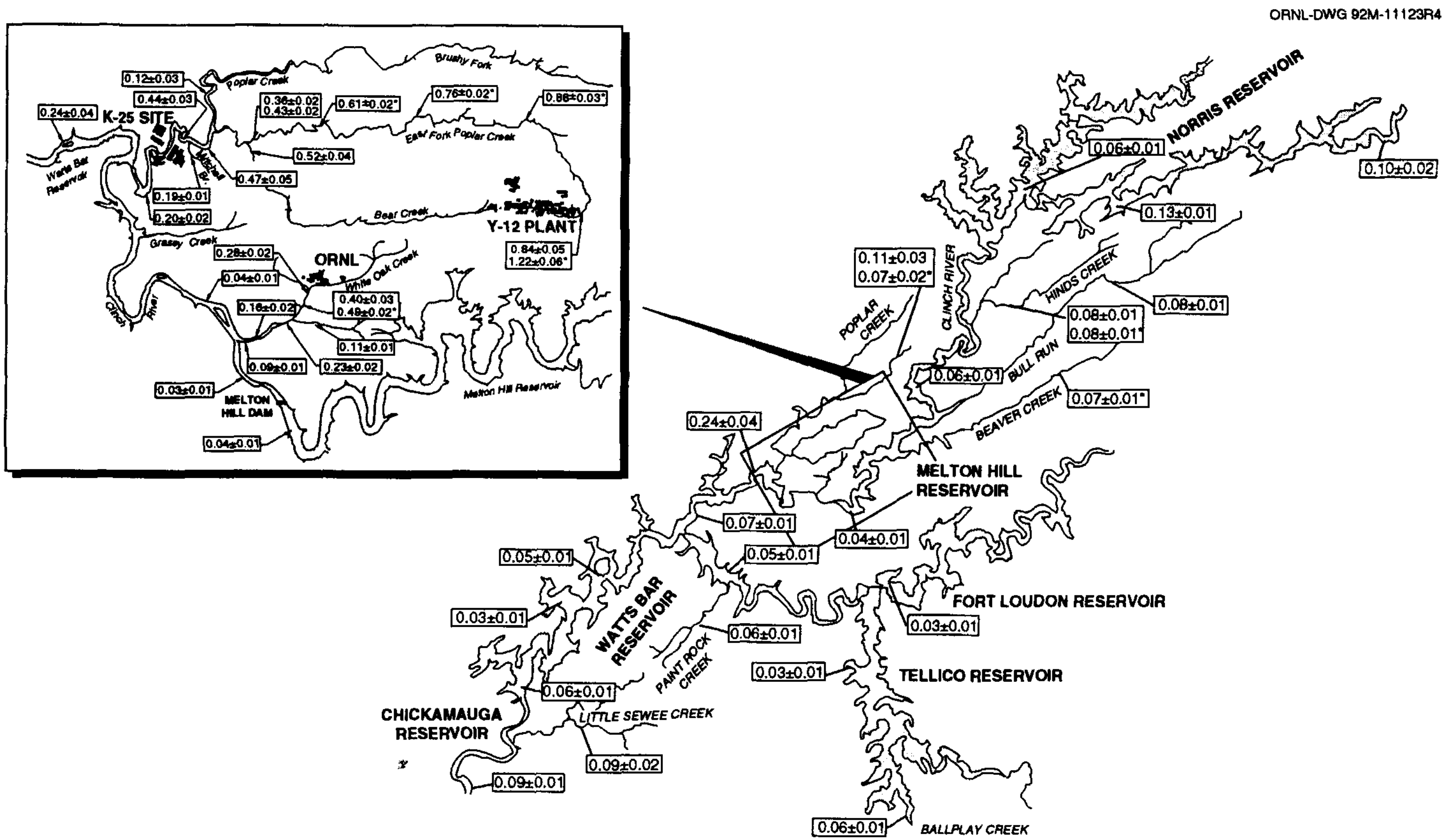

Figure 1. Concentrations of mercury (mean ± SE, μg/g wet wt) in sunfish collected from sites in east Tenn., 1985-1991. Data marked by asterisks are for redbreast sunfish (*Lepomis auritus*), all other data are for bluegill (*L. macrochirus*). Samples per site range from 4 to 123.

stannous chloride and analyzed by cold vapor atomic absorption spectroscopy (Feldman, 1974). Results of analyses of EPA reference fish agreed well with the published value (2.52 µg/g), averaging 2.48 ± 0.13 µg/g (SD, N = 68). Blind duplicate samples from both uncontaminated and contaminated sites had a mean coefficient of variation of 10.0%. Statistical evaluations of data were made with analysis of variance, Tukey's multiple comparison test, linear regression analysis, and t-tests (SAS 1985). Dunnett's test was used for comparisons between fish contaminated sites and reference site collections. The Shapiro-Wilk statistic was used to assess normality, and Levene's test was used to evaluate homogeneity of variances among sites. In general, $\log_e$-transformation of data improved normality and homogeneity of variances when comparisons were made among sites. The Type I error rate α used for all statistical comparisons was 0.05.

RESULTS AND DISCUSSION

Mean mercury concentrations in bluegill and redbreast sunfish collected from the seven reference stream sites varied little, ranging from 0.06 to 0.11 µg/g wet wt, with a median of 0.08 µg/g. The mean concentration (± SE) of mercury in redbreast sunfish (Hinds Creek, Beaver Creek, Bull Run and Brushy Fork) was 0.079 ± 0.005 µg/g wet wt (n = 89), while the average concentration in stream bluegill (Hinds Creek, Brushy Fork, Paint Rock Creek, Ballplay Creek, and Little Sewee Creek) was 0.079 ± 0.004 µg/g (n=94). In reference streams where both species were collected (Hinds Creek and Brushy Fork), mean concentrations of mercury in bluegill and redbreast sunfish did not differ (Table 1). It seems that these species can be used interchangeably for estimating background mercury concentrations in sunfish in streams from this geographic region.

Mean mercury concentrations in sunfish (each species treated separately) from stream sites downstream from the known mercury point sources were significantly higher than the mean concentrations in fish from reference streams (one-tailed Dunnett's test, $\log_e$-transformed data). The one exception was White Oak Creek embayment, a flooded embayment of Watts Bar Reservoir that is more appropriately classified as a lake rather than a stream site.

Bluegills are abundant throughout large reservoirs in the Tennessee River drainage, presenting no problem for collecting adequate numbers at any site. Redbreast sunfish, primarily a stream species, are much less abundant in lake habitats. Therefore, bluegill is the obvious choice of sunfish as a monitoring species in such environments. Mean mercury concentrations varied more in bluegill from lakes in eastern Tennessee than in stream sunfish, ranging from 0.03 to 0.13 µg/g. The highest mercury concentrations in bluegill from reference sites occurred in Norris Reservoir (Fig. 1), an oligotrophic reservoir with little urban or industrial development in its watershed. Mercury concentrations in bluegill from this lake exceeded those at all other reservoir sites, including those downstream from known mercury sources, except for the site in Watts Bar Reservoir nearest the largest mercury source. Statistical comparison (Tukey multiple comparison test) of mean mercury concentrations in bluegill at sites in Norris Reservoir, all other lake reference sites, and all stream reference sites indicated that all differed significantly, averaging 0.110 ± 0.008, 0.039 ± 0.002, and 0.079 ± 0.004 µg/g, respectively.

Table 1. Mean mercury concentrations (µg/g wet wt., number of fish analyzed in parentheses) in redbreast sunfish and bluegills from uncontaminated and contaminated sites

Site	Bluegill	Redbreast sunfish	Means different?[1]
Contaminated			
East Fork Poplar Creek (kilometer 23.4)	0.84 (105)	1.22 (123)	yes
White Oak Creek (kilometer 2.9)	0.40 (19)	0.49 (36)	yes
East Fork Poplar Creek (kilometer 2.1)	0.36 (97)	0.43 (94)	yes
Uncontaminated			
Hinds Creek	0.08 (62)	0.08 (64)	no
Brushy Fork[2]	0.11 (10)	0.07 (12)	no

[1]Significant difference between mean mercury concentrations in bluegill and redbreast sunfish, two-tailed t-test with assumption of unequal variances where appropriate
[2]Bluegill data influenced by two samples (0.26, 0.29 µg/g) with [Hg] well above range typical of reference sites

Mercury concentration in individual fish was correlated with fish weight in Norris Reservoir fish, but not in the other two groups. The collections from Norris Reservoir also contained more very large bluegill than the other collections, raising the concern that the high concentrations were due in part to the presence of larger fish in this collection. However, because the slope from the mercury vs weight regression was shallow, there was actually little change in mercury concentration with fish weight. The mercury concentration predicted in a 70 to 75-g fish from Norris Reservoir (the mean weights of fish in the other two reference collections) by the regression expression was 0.10 µg/g, similar to the mean of 0.11 µg/g actually measured for the Norris sample, which had an average fish weight of 112 g. Results of the comparisons among these water bodies therefore reflected true differences, rather than artifacts related to differing fish sizes among samples.

The higher mercury concentrations in fish from Norris Reservoir may be related to the large seasonal fluctuations in water level in this lake. Each year, large expanses of lake bottom are converted to terrestrial environments as the lake is drawn down over the summer to winter pool levels. These areas are then flooded again in the spring, mimicking in some respects conditions that occur when new reservoirs are created. Elevated mercury levels in fish are a well known consequence of flooding terrestrial environments to create new reservoirs (Abernathy and Cumby 1977; Cox et al. 1979; Bodaly et al. 1984).

Determining appropriate reference concentrations for assessing contamination in sunfish in reservoirs therefore appears to be more difficult for reservoirs than for streams. A reservoir in the same river system, but upstream from known or suspected point sources of mercury, would normally be a logical reference site for assessing mercury contamination in fish from downstream point sources. However, our data indicate that Norris Reservoir (upstream from the mercury source) would not be an appropriate reference site for Watts Bar Reservoir (downstream from the mercury source). Mean mercury concentrations in bluegills from sites between Norris Reservoir and the major mercury source in the Poplar Creek drainage ranged from 0.03 to 0.06 µg/g (Fig. 1). Similar mercury concentrations were found in bluegills at most other sites in Watts Bar Reservoir downstream from the mouth of Poplar Creek. Clearly, if Norris Reservoir, where bluegills averaged 0.11 µg/g, was used as a reference site rather than the other lakes sampled in this study, it would be impossible to determine if small increases in mercury concentration in fish downstream from mercury point sources were the results of contamination.

Statistical comparison (ANOVA, Dunnett's test with $\log_e$-transformed data) of mercury concentrations in bluegills from lake sites downstream from mercury sources with bluegills from lake reference sites (excluding Norris Reservoir) discriminated all sites in the Poplar Creek drainage as contaminated. In Watts Bar Reservoir downstream from Poplar Creek, contamination was clearly evident at the site nearest Poplar Creek, but the only other sites at which mean mercury concentrations significantly exceeded the reference site concentrations were those farthest from the source, near Watts Bar dam and in the downstream tailwater (Chicamaugua Reservoir). The mean mercury concentration in bluegills from White Oak Creek embayment, which was not classified as contaminated based on comparison with bluegills from stream reference sites, was significantly elevated when the comparison was made with lake reference sites. Embayments such as this represent transitional habitats between stream and lake habitats, and it is not clear whether lake or stream sites are most appropriate as references. In Poplar Creek embayment, the site upstream from the mouth of East Fork Poplar Creek is upstream of the mercury source, but there are no barriers to movement of fish between contaminated and uncontaminated reaches of the embayment, which contains streamlike habitat in its upper reaches. The mean mercury concentration in bluegill was higher at this site than at lake reference sites, but not higher the mean concentration in stream reference fish.

Although mean mercury concentrations did not differ between redbreast sunfish and bluegills in uncontaminated streams, the concentrations of mercury differed between the two species in contaminated streams (Table 1). At the three contaminated stream sites where substantial numbers of both species were collected, mean mercury concentrations in redbreast sunfish significantly (two-tailed t-test) exceeded those in bluegill. The ratio of excess mercury (mercury concentration minus reference fish mercury concentration) in redbreast sunfish to that in bluegill ranged from 1.25 to 1.5. These results suggest that the redbreast sunfish is a more sensitive bioindicator of mercury contamination than bluegill. Differences in feeding habits or bioenergetics may explain the differences in mercury bioaccumulation. Whatever the cause, redbreast sunfish may be the better species for assessing mercury contamination in streams due to its higher accumulation potential, more widespread distribution, and greater abundance in such habitats.

Differences were apparent among mean mercury concentrations in sunfish from different sites in this study; however, the overall variation was not great. This similarity in background mercury concentrations in sunfish suggests that, in areas where regional water chemistry is fairly homogeneous and not favorable to facile methylation of environmental mercury, little variability in background mercury concentrations is to be expected. This is in contrast to regions such as north Florida and the upper midwest, where local variability in water chemistry and the presence of low pH, low alkalinity waters in some systems results in large variations in mercury concentrations in fish from different lakes and streams, with many uncontaminated sites yielding fish with higher mercury concentrations than the highly contaminated sites in east Tennessee (Hand and Friedman 1990; Lange et al. 1993).

Although the background mercury concentrations in sunfish were relatively homogeneous in the geographic area studied here, there was enough variability to merit caution in selecting an appropriate reference area. Stream sunfish would not provide an appropriate reference value for evaluating the degree and extent of mercury contamination in a reservoir downstream from known mercury sources. Similarly, lake sunfish could in many instances erroneously indicate contamination in streams if used as a reference. The variation in mercury contamination in bluegill from different lakes within the same drainage system underscores the need for the use of multiple reference sites, and upstream-downstream comparisons in close proximity to the suspected source, in evaluating mercury contamination. The possible misleading interpretation of data from a single reference site was noted by Rada et al 1986. Our results suggest that urbanization and industrial development, such as occur on Fort Loudon Reservoir and to a lesser extent, Melton Hill Reservoir, do not necessarily affect mercury concentrations in fish. Even the large point source input of mercury via Poplar Creek to Watts Bar Reservoir yielded little evidence of contamination in bluegills throughout most of the lake.

The occurrence of significant differences in mercury concentrations between redbreast sunfish and bluegills from contaminated sites indicates the need for caution in combining data from different species, even congeneric species. In some cases interspecific differences could be misleading.

Acknowledgements

Sponsored by Environmental Management Depts. at the Oak Ridge Y-12 and K-25 Plants, and Oak Ridge Natl. Laboratory. These facilities are managed by Martin Marietta Energy Systems, Inc., under contract DE-AC05-840R21400 with the U.S. Dept. of Energy. Portions of this research were conducted on the Oak Ridge National Environmental Research Park. Environmental Sciences Division Publication No. 4181

REFERENCES

Abernathy A R, and Cumbie P M (1977) Mercury accumulation by largemouth bass (*Micropterus salmoides*) in recently impounded reservoirs. Bull Environ Contam Toxicol 17: 595-602

Bodaly R A, Hecky R E, and Fudgé R J P (1984) Increases in fish mercury levels in lakes flooded by the Churchill River Diversion, Northern Manitoba. Can J Fish Aquat Sci 41: 682-691

Bjornberg A, Hakanson L, and Lundbergh K (1988) A theory on the mechanisms regulating the bioavailability of mercury in natural waters. Environ Pollut 49: 53-61

Cox J A, Carnahan J, DiNunzio J, McCoy J, and Meister J (1979) Source of mercury in fish in new impoundments. Bull Environ Contam Toxicol 23: 779-783

Feldman C (1974) Perchloric acid procedure for wet ashing organics for the determination of mercury (and other metals). Anal Chem 46: 1606 -1609

Hand J, and Friedman M (1990) Mercury, largemouth bass, and water quality: a preliminary report. Florida Department of Environmental Regulation, Bureau of Surface Water Management. Tallahassee, Florida

Heit M, Schofield C, Driscoll C, and Hodgkiss S (1989) Trace element concentrations in fish from three Adirondack lakes with different pH values. Water Air and Soil Pollut 44: 9-30

Huckabee J W, Feldman C, Talmi Y (1974) Mercury concentrations in fish from the Great Smoky Mountains National Park. Analytica Chimica Acta 70: 41-47

Kornegay F C, West D C, Evans R A, Goodpasture S T, Tardiff M F, and Wilson A R (1991) Oak Ridge Reservation environmental report for 1990. ES/ESH-18/V1. U.S. Dept. of Energy Y-12 Plant, Oak Ridge National Laboratory, and K-25 Plant, Oak Ridge, Tennessee

Lange T R, Royals H E, and Conner L L. (1993) Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. Trans Am Fish Soc 122:74-84

Phillips G R, Medvick P A, Skaar D R, and Knight D E (1987) Factors affecting the mobilization, transport, and bioavailability of mercury in reservoirs of the Missouri River Basin. U.S Fish and Wildlife, Fish and Wildlife Technical Report U.S. Fish and Wildlife Service, Washington, D.C.

Rada R G, Findley J E, Wiener J G (1986) Environmental fate of mercury discharged into the upper Wisconsin River. Water Air Soil Pollut. 29:57-76

SAS Institute, Inc. (1985) SAS User's Guide: Statistics, Version 5 Edition. SAS Institute, Inc. Cary, North Carolina

Schmitt C J, and Brumbaugh W G (1990) National contaminant biomonitoring program: concentrations of arsenic, cadmium, lead, mercury, selenium, and zinc in U.S. freshwater fish, 1976-1984. Arch Environ Contam Toxicol 19: 731-747

Tennessee Valley Authority (TVA) (1985) Instream contaminant study, task 4: fish sampling and analysis. Report to the U. S. Dept. of Energy, Oak Ridge Operations Office. Tennessee Valley Authority, Office of Natural Resources and Economic Development, Knoxville, Tennessee.

Van Winkle W, Counts R W, Dorsey J G , Elwood J W, Lowe V W, McElhaney R, Schlotzhauer S D, Taylor F G, and Turner R R (1974) Mercury contamination in East Fork Polar Creek and Bear Creek. Oak Ridge National Laboratory, Oak Ridge, Tennessee. ORNL/TM-8894