Bull. Environ. Contam. Toxicol. (2005) 74:1078–1083

DOI: 10.1007/s00128-005-0691-2

Bulletin of Environmental Contamination and Toxicology

Trace Metals in Wyoming Fish

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Received: 17 December 2004/Accepted: 11 March 2005

The Wyoming Game and Fish Department collected game fish during the 2000 and 2001 field seasons to survey the state's fisheries for Hg and Se contamination. Twenty-eight lakes and reservoirs throughout Wyoming were sampled, in which 96 fish composites were collected representing 11 species. This type of screening survey aids in identifying possible lakes/reservoirs of concern and consequently in directing future sampling efforts.

MATERIALS AND METHODS

A total of 28 lakes/reservoirs were selected for sampling based on two key criteria. First, sampled waters had a fish population dominated by a U.S. Environmental Protection Agency (EPA) recommended target species. Secondly, sampled waters received moderate to heavy sport fisheries use (USEPA, 1995). Sampling took place between May and October of 2000 and during May and June of 2001. Fish species included in the study: black crappie *(Pomoxis nigromaculatus)*, brown trout *(Salmo trutta)*, channel catfish *(Ictalurus punctatus)*, kokanee *(Oncorhynchus nerka)*, lake trout *(Salvelinus namaycush)*, rainbow trout *(Oncorhynchus mykiss)*, smallmouth bass *(Micropterus dolomieu)*, Snake River cutthroat trout *(Oncorhynchus clarki ssp)*, walleye *(Sander vitreus)*, yellow perch *(Perca flavescens)*, and Yellowstone cutthroat trout *(Oncorhynchus clarki bouvieri)*. Following the initial analysis for Hg and Se, the remaining amount of sample was analyzed for As, V, Cr, Mn, Fe, Ni, Co, Cu, Zn, Mo, Cd, Ba, Pb, and Tl.

Two to three fish of varying age groups were collected using gills nets and purse seines from each site. In a case where 3 fish of similar site, size class, and species were not available, 2 fish composites or a single fish sample was used. An EPA protocol was followed while collecting samples, and all samples were frozen in their original containers until prepared for analysis (USEPA, 1995).

Fish were allowed to thaw at room temperature until filleted into right "A" and left "B" fillets with a stainless steel knife and Teflon cutting board. Fillets were then refrozen in separate bags with fillets from 2 other fish of similar age, collection site and species. Each composite of fillets was treated as a single

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Table 1. Lakes and reservoirs sampled during the 2000 to 2001 field seasons.

Lakes and Reservoirs Sampled 2000-2001		
Alcova Reservoir	Gelatt Lake	Muddy Guard #1
Alsop Lake	Glendo Reservoir	MW Reservoir
Bighorn Reservoir	Goldeneye Reservoir	Pathfinder Reservoir
Buffalo Bill Reservoir	Grayrocks Reservoir	Saratoga Lake
Buffalo Wetlands	Half-Moon Lake	Seminoe Reservoir
Diamond Lake	Hawk Springs Reservoir	Sulphur Creek Reservoir
East Allen Lake	Healy Reservoir	Twin Buttes Reservoir
East Iron Creek Reservoir	Jackson Lake	Viva Naughton Reservoir
Flaming Gorge Reservoir	Lake Hattie	
Fremont Reservoir	Meeboer Lake	

sample for purposes of digestion and analysis. Utensils were rinsed with an aqueous solution of 2% (v/v) HNO_3 (J.T. Baker, Instra-analyzed) prior to filleting each fish.

The "A" fillets were used for the analysis of Se, Fe, Ni, Cu, Zn, V, Cr, Mn, Co, As, Mo, Cd, Ba, Tl, and Pb. Fillets were removed from the -70°C freezer and placed directly onto plastic racks in a Virtis Freeze Mobile 6 lyophilizer (Virtis, Gardner, NY) and subjected to vacuum until dry. Each dried, composite fillet was then ground and homogenized at room temperature in a stainless steel/Rulon blender (Ivan Sorval, Norwalk, CT) and returned to the freezer in the original plastic bag. All non-disposable labware (e.g. homogenization vessels and freezer racks) was washed with 5% (v/v) trace element grade HNO_3 (Baker Instra-analyzed) between samples.

Samples were digested in groups of 12 (9 samples and 3 quality controls). Approximately 0.5 g of sample was weighed into a Teflon microwave bomb and 3 ml each of concentrated HNO_3 and 30% (v/v) H_2O_2 added. After setting for approximately 5 minutes, each bomb was sealed and progressively heated to 180°C in a microwave oven (MDS 2000, CEM Corp.). Each digestor run included two reference materials (DORM-2, NRCC, Ottawa, ONT, Canada and NIST 1577b, NIST, Gathersburg, MD) and a duplicate fish sample (laboratory fortified matrix) spiked with approximately 50% of the anticipated analyte concentration. After 10 minutes at 180°C, samples were cooled and quantitatively transferred to 15 ml polypropylene tubes (Elkay #2086) and q.s. to 10 ml with 18 Mohm deionized water. A reagent blank was created by microwaving a 1:1 mixture of HNO_3 and H_2O_2 according to the same protocol. All reagents used were prepared with deionized water (18 Mohm) prepared with a Millique (Millipore, Bedford, MA) system.

As and Se were analyzed by the method of standard additions (Elan ver. 2.3.1, PE Sciex, Norwalk CT) using an Elan 6100 ICP-MS (PE Sciex, Norwalk, CT). For Se, 1 ml of fish digest was combined with 1 ml of internal standard (200 µL/L Ge, prepared from 10 mg/L Ge, SPEX Certiprep, Metuchen, NJ), 1 ml of 2% (v/v)

HNO_3 and 2 ml of deionized water in a 15 ml tube. Standard additions were prepared from the first sample in each run by adding 1 ml of 0.05, 0.5, 2.5, or 5 μL/L Se (prepared from 1000 mg/L Se, SPEX Certiprep, Metuchen, NJ) to each of 4, 1 ml aliquots of digest. One ml of internal standard and 2 ml of water were then added to each standard addition standard. The reference blank was prepared from 1 ml of reagent blank combined similarly with internal standard, 2% (v/v) HNO_3 and water.

The method of standard additions was also used in the analysis of As, however the method differed slightly from that used in the analysis of Se. One ml of fish digest was combined with 4 ml of internal standard (200 μL/L Ge, prepared from 10 mg/L stock, SPEX Certiprep, Metuchen, NJ). The reference blank contained 1 ml of reagent blank and 4 ml of internal standard. Standard additions were prepared by adding 4 ml of 0.0008, 0.08, and 0.8M 0.40 μL/L As (prepared from 100 mg/L As, SPEX Certiprep, Metuchen, NJ) to 1 of 3, 1 ml aliquots of digest. The method detection limit for As varied between 0.2-0.4 mg/kg depending upon the sample.

The remaining trace metals in this study, excluding Hg, were diluted as follows. One ml of fish digest was combined with 1 ml of internal standard (200 μL/L Ge, Sc, Y, In, Tb, and Bi, prepared from 10 mg/L stock, SPEX Certiprep, Metuchen, NJ), and 4 ml of deionized water in a 15 ml tube. A matrix blank and a set of matrix matched analytical standards were used in building the external calibration curve.

The "B" fillets were used for Hg analysis. Samples were thawed to refrigerator temperature, manually chopped into approximately 15 cm chunks, and homogenized in a food processor (Food Chopper, White-Westinghouse, Mt. Prospect, IL). Samples were again refrozen in the original plastic bag until analyzed. All lab ware was cleaned between uses with 5% (v/v) HNO_3, with the exception of microwave bombs which were soaked overnight in 5% (v/v) BrCl.

Approximately 2 g of fresh, homogenized fish was placed in a microwave bomb with 10 ml concentrated HNO_3 and heated according to the same protocol used for Se. Each run of 9 samples was accompanied by a "high" Hg reference (DORM-2, NRCC, Ottawa, ONT, Canada), a "low" Hg reference (NIST 2976, NIST, Gathersburg, MD) and a duplicate fish sample (laboratory fortified matrix) spiked with approximately 50% of the anticipated sample Hg concentration. After cooling, each sample was quantitatively transferred to a 50 ml polypropylene tube (Elkay, Tyco Healthcare, Mansfield, MA) and diluted to 50 ml with deionized water.

The entire 50 ml was then transferred quantitatively to a glass flask with 2 ml concentrated H_2SO_4 and, after the addition of 15 ml of 5% (v/v) aqueous $KMnO_4$ and 4 ml of 5% (v/v) $K_2S_2O_4$, heated at 95°C for 120 minutes in a water bath.

Appropriate Hg standards (prepared from ICP-080 Hg 1000 mg/ml, Ultra Scientific, N. Kingstown, RI) were included with each water bath run. After heating, 3 ml hydroxylamine was added and the digest allowed to cool and de-gas for 1 hour. The cooled digest and standards were analyzed by cold vapor on a Perkin Elmer cold vapor mercury analyzer (PE FIMS 100, AAnalyst 300) according to EPA 245.1. Results for all metals are reported on a wet weight basis.

Noncarcinogen screening values were used to evaluate resulting metal concentrations. The EPA defines these values as target analyte concentrations, in fish tissue, used as standards against which similar tissue collected from the environment can be compared (USEPA, 1995). Se, Hg, As, and Cd concentrations were compared to the noncarcinogen screening values reported by the EPA (USEPA, 2000). Noncarcinogen screening values for the remaining metals, excluding Fe and Pb, were calculated using the following the equation (USEPA, 2000):

$$SV_n = (RfD \bullet BW)/CR$$

where

SV_n = Screening value for a noncarcinogen (mg/kg)
RfD = Oral reference dose (mg/kg/d)
BW = Mean body weight of the general population (70 kg)
CR = Mean daily consumption rate of fish from fresh waters (0.0175 kg/d)

The most current RfD values were obtained from the EPA Integrated Risk Information System (IRIS, 2004) and Risk Assessment Information System (RAIS, 2004). Since no RfD value for Fe could be obtained, the upper level for dietary intake (45mg/70kg/day) was substituted into the noncarcinogen screening value formula (Food and Nutrition Board, Institute of Medicine, 2000). No screening value was calculated for Pb based on the indication that it is a nonthreshold toxicant (RAIS, 2004).

RESULTS AND DISCUSSION

Nine of the 96 samples exceeded the screening value for Hg 0.40 mg/kg with a detection limit of 0.005 mg/kg. Six of these fish were collected from Bighorn Reservoir located in northwestern Wyoming. The group was comprised of 4 samples each composed of 3 walleye fillets ranging in average length from 14.1 to 19.4 in. Two samples were channel catfish. Each sample was a composite of 3 fillets with average lengths of 14.3 and 23.2 in. The remaining fish exceeding the Hg screening value included 1 walleye from Pathfinder Reservoir, which was also composed of 3 fillets with an average length of 16.8 in. Lastly, 1 walleye composed of 3 fillets with an average length of 14.4 in. and 1 brown trout composed of 3 fillets with an average length of 16.5 in. from Seminoe Reservoir exceeded the Hg screening value.

Walleye seemed to accumulate more of the metal as compared to other species. This can probably be accounted for by sampling location and trophic level. Since walleye are located in the top of the aquatic food web in Wyoming waters, the

higher levels of Hg can be attributed to bioaccumulation through the food chain. Also the majority of the samples exceeding the screening value were from Bighorn Reservoir, which indicates that further sampling should be done to more thoroughly document Hg concentrations. It would also be beneficial to collect additional samples from Pathfinder and Seminoe Reservoirs, as well as from other walleye populations from around the state to better access Hg levels.

The only sample to exceed the screening value for As (1.2 mg/kg) came from Twin Buttes Reservoir in southeastern Wyoming. The sample was a composite of 2 brown trout with an average length of 17 in. The remaining samples contained only a few As concentrations above the method detection limit (0.2-0.4 mg/kg), however these concentrations where well below the noncarcinogen screening value.

The only As concentration exceeding the noncarcinogen screening value of 1.2 mg/kg was found in Twin Buttes Reservoir, a Laramie Plains lake. All other sample As concentrations throughout the state were below this screening value. Given the carcinogen and noncarcinogen properties of As, additional sampling at this location should be conducted to access whether there is a potential problem in this waterbody.

Fish from the Laramie Plains Lakes had slightly elevated Se concentrations. However, none of the Se concentrations recorded exceeded the screening value (20 mg/kg) for recreational fishermen (USEPA, 2000). East Allen Lake recorded the highest concentration (12.73 mg/kg) in a composite of 2 Snake River cutthroat trout with an average length of 13.2 in. This lake also recorded the highest average of Se in fillets with 12.5 mg/kg, which included three samples. Twin Buttes Reservoir recorded the second highest average level of Se in fish fillets (6.3 mg/kg) of the Laramie Plains Lakes.

The increased concentrations of Se in the Laramie Plains lakes, as compared to the other lakes throughout Wyoming, are indicative of the naturally seleniferous geology. While all Se concentrations were below the screening value, continued sampling should be conducted. Not only would additional sampling lead to a better understanding of Se levels in these lakes, but also it is important to continually monitor waters where metal concentrations could influence management practices.

Metals such as Fe, Cu, and Zn were above the method detection limit of 0.05 mg/kg, but remained well below their screening values of 2571, 160, 1200 mg/kg, respectively. Remaining metals in the study had concentrations at or below method detection limits. Metals such as V, Cr, Mn, Ni, Co, Mo, Cd, Ba, Pb, and Tl were all at or below the method detection limit of 0.05 mg/kg, or in the case of V, a method detection limit of 0.1 mg/kg.

This survey was meant to serve as a preliminary screening of Hg and Se levels in

Wyoming and so is not statistically powerful. The small sample sizes per location and species did not allow for a detailed statistical comparison of the results. However, the results can be used for guiding future surveys aimed at identifying metal concentrations in edible portions of Wyoming game fish.

Acknowledgments. This project was made possible by a Wyoming Game and Fish Department grant, as well as Wyoming State Veterinary Lab funds.

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