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Contaminant Levels in Rainbow Trout, *Oncorhynchus mykiss*, and their Diets from Missouri Coldwater Hatcheries

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Abstract Organochlorine and metal contaminants often occur in commercial fish diets and can accumulate in fish to levels of concern for human consumption. Contaminant levels were investigated in diet and rainbow trout fillets from Missouri coldwater hatcheries used in "put and take" fisheries. The average fillet:diet ratio was <0.1 for lead and cadmium, 0.4–0.6 for organochlorine compounds, and about 0.8 for mercury. Trout fillet concentrations for all contaminants were low (<50 ng/g) and below Missouri's fish consumption advisory trigger levels.

Keywords Trout fillets · Diet · Hatcheries · Contaminants

Recent studies indicate that diets used in salmonid hatcheries often contain chemical contaminants which can accumulate in fish tissues to a level where human consumption may need to be restricted (Hites et al. 2004; Carline et al. 2004). Contaminants including organochlorine insecticides (OCIs), polychlorinated biphenyls (PCBs), lead, cadmium, and mercury have been found in commercial fish diets (Maule 2007).

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The Missouri Department of Conservation (MDC) operates five coldwater hatcheries that produce rainbow trout for stocking at trout parks as well as a variety of other public "put and take" fisheries. The objective of this study was to determine if rainbow trout reared at MDC trout hatcheries have contaminant levels considered safe for human consumption and to examine the relationship between the contaminant levels in the diet to that in fish tissue.

Materials and Methods

Ten chemicals were included in this investigation (Table 1) and all but one (cadmium) has Missouri concentration criteria for evaluating the need for fish consumption advisories in Missouri (Missouri Department of Health and Senior Services, personal communication). These contaminants were selected because they are routinely found in fish diets, are persistent and can be toxic.

Five MDC coldwater rainbow trout hatcheries were included in this study: Maramec Spring, Bennett Spring, Montauk, Roaring River and Shepherd of the Hills. The specific diet used during the study period was extruded Sterling Silver Cup™ Fish Feed (Nelson & Sons Inc., Murray, UT) trout production diet (floating) ranging in size from 3.5 to 4.5 mm. The diet typically contains 40%–42% protein, 10%–12% fat, 1%–3% fiber, 9%–12% ash and <10% moisture.

Diet samples (about 200 g) were collected in 300 mL cleaned glass jars between May 10 and June 28, 2005. Rainbow trout in the size range of about 250–300 mm (10–12 inches) and about one-year-old were collected at each hatchery using a net. For each hatchery, three composite samples containing fillets from eight individual fish per composite were collected (24 fish from each hatchery).

Table 1 Missouri fish consumption thresholds for chemical contaminants investigated in this study

Contaminant group	Chemical name	Missouri levels of concern (fillets)[a]	
		One meal per week	Do not consume
Organochlorine insecticide	Lindane	NA	>300 ng/g
	Heptachlor	NA	>300 ng/g
	Dieldrin	NA	>300 ng/g
	Endrin	NA	>300 ng/g
	Chlordane (sum of four isomers)	12 ng/g	>110 ng/g
	DDT (sum of DDE, DDD, and DDT)	NA	>5,000 ng/g
Halogenated industrial organics	Total polychlorinated biphenyls (PCBs)	100 ng/g	>750 ng/g
Industrial metals	Mercury[b]	120 ng/g	>1,000 ng/g
	Lead	NA	>300 ng/g
	Cadmium	NA	NA

[a] Level in fish above which the warning is enacted; NA, not available

[b] Consumption warnings apply to sensitive populations which includes women of childbearing age, nursing, or pregnant women and children under the age of 13 years

Fish were collected December 14 or 15th, 2005. Each fish was measured (total length), immobilized (head trauma), scaled, and a fillet removed from each side (skin-on fillet). Fillets were rinsed in distilled water and wrapped in aluminum foil.

Fish fillets were analyzed for the organic compounds of concern using trace organic analysis methods that include extracting and purifying the residues with size exclusion chromatography/silica gel fractionation followed by quantification with high resolution capillary gas chromatography with electron capture detection (Hinck et al. 2006). The following OCIs were measured and reported: hexachlorobenzene (HCB), lindane, heptachlor, dieldrin, chlordane (as the sum of the *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor isomers), DDT (as the sum of *o*,*p*′-DDE, *o*,*p*′-DDD, *o*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, *p*,*p*′-DDT isomers). A total concentration of polychlorinated biphenyls (PCBs) was measured by summing the individual concentrations of 142 congeners of this complex chemical mixture (These 142 congeners comprised >95% of the PCB mass in the fish samples). Spike recoveries ranged from 72% to 114%. The method detection limits were 0.1 ng/g for all organic compounds except as follows: chlordane (0.4 ng/g), DDT (0.6 ng/g), heptachlor (0.2 ng/g) and total PCBs (11 ng/g).

In preparation for subsequent determination of metals and mercury, fillet samples were homogenized and lyophilized to a coarse powder, whereas diet samples were only lyophilized. Percent moisture was determined as part of the lyophilization process. An aliquot of each dried sample was digested in a microwave digestion system. Final acid matrix of the digestates was 6% nitric acid. The metals, cadmium (Cd) and lead (Pb), were determined using a PE/SCIEX Elan 6000 ICP-MS equipped with a CETAC ADX-500 autodiluter (Hinck et al. 2006). Scandium, rhodium, and bismuth served as internal standards. Mercury was determined by thermal combustion gold amalgamation atomic absorption spectroscopy, where the fillet tissue or diet was introduced directly into a Milestone DMA-80 analyzer (Hinck et al. 2006). Spike recoveries ranged from 75% to 120%. The method detection limits for metals were 2.9 ng/g for cadmium, 12.0 ng/g for lead, and 3.0 ng/g for mercury. All analytical results discussed in this report are presented as ng/g wet weight (part-per-billion) unless otherwise indicated.

Means and standard deviations were calculated for contaminant levels in trout fillets using the three replicate composite samples as the error term. If an individual replicate value was less than the detection limit, then it was not used in calculating the mean. The ratio of contaminant concentration in the fillet tissue to the concentration in the diet was calculated as an index of accumulation. The ratio was calculated for each hatchery and for each contaminant by dividing the mean fillet concentration by the diet concentration (both in ng/g). If the mean fillet concentration for a particular hatchery was below the detection limit then the ratio was calculated using the detection limit. Based on the variability in the diet samples, ratios were not calculated for diet values less than twice the detection limit.

Results and Discussion

Percent lipid in the diet ranged from 11.3% to 13.6% which was close to the 10%–12% range typical for this diet (Table 2). Concentrations of the organic contaminants in the diets were low (less than 50 ng/g). For metals, mercury was less than 50 ng/g, however, lead and cadmium were considerably higher.

Mean fish length varied among hatcheries from 285.8 to 337.4 mm (11.3 to 13.3 in.) and mean weight ranged from 273.4 to 455.4 g (0.6 to 1.0 lb) (Table 3). Lipid content of the fillet tissue ranged from 3.6% to 4.6% of wet weight. All contaminant levels in fillet tissue (means) were below 50 ng/g wet weight (Table 3). The levels observed for all contaminants were considerably below the levels of potential concern for human consumption identified in Table 1.

The Pennsylvania Fish and Boat Commission were among the first to investigate contaminants (PCBs) in trout from state hatcheries used for “put and take” fisheries. In their 1998–1999 investigations, levels of PCBs in trout

Table 2 Concentration of contaminants in diet used in Missouri trout parks during the study period

Contaminant	Concentration in trout diet (ng/g wet weight)				
	Bennett Spring	Maramec Spring	Montauk	Roaring River	Shepherd of the Hills
Lipid (%)	12.5	11.3	13.6	12.9	11.9
Cadmium	147.5	95.2	133.8	95.6	104.6
Lead	393.4	247.5	391.7	230.0	323.2
Mercury	12.4	27.1	23.0	32.3	21.9
DDT	4.1	10.1	2.4	20.0	9.9
Dieldrin	0.5	1.5	0.2	0.7	1.7
Lindane	<0.1	0.1	<0.1	0.2	<0.1
Chlordane	1.3	3.0	<0.4	4.2	3.0
Heptachlor	<0.2	0.2	<0.2	<0.2	0.2
HCB	0.2	0.4	0.2	0.9	0.4
Total PCBs	<11.0	27.6	<11.0	29.7	29.5

fillet tissue occurred in a range (60–200 ng/g) associated with a recommended fish consumption limit of one meal/week in accordance with the Great Lakes Protocol (GLP) (Carline et al. 2004). In 2007, Pennsylvania has a general one meal/week advisory on all sportfish which includes trout.

Commercial diets used to rear the Pennsylvania trout in the 1998–1999 timeframe were in the range of 20–130 ng/g (Carline et al. 2004). Other researchers have reported PCB concentrations in commercial salmonid diets. Hites et al. (2004) found median values for total PCBs in US commercial fish diets of 15 versus 60 ng/g in diets from Europe. Jacobs et al. (2002) found a mean level of PCBs from eight Scottish trout diets of about 105 ng/g. Serder et al. (2006) in a survey of diets in 10 trout hatcheries in the state of Washington found total PCBs ranging from 8.2 to 34.8 ng/g. In the current study, total PCB levels ranged from below detection limits (i.e., 11–29.7 ng/g). The North America data suggests a trend toward lower total PCBs in commercial fish diets in recent years.

Accumulation of contaminants was measured using a ratio of the fillet concentration to the concentration in the diet for each contaminant (Fig. 1). The metals, lead and cadmium, were high in the diet compared to the other contaminants. Maule et al. (2007) also noted cadmium and lead to be at considerably higher levels compared to mercury and organochlorine compounds. Even though these contaminants were relatively high in the diet, low levels were observed in fish tissue, resulting in ratios of <0.1. The relatively low accumulation from diet to fillet tissue was expected given that gastrointestinal absorption in animals is typically less than 15% for lead and cadmium and excretion can be fairly rapid (Goyer 1996). Similar low assimilation efficiencies for these metals in aquatic organisms were observed by Hendriks and Heikens (2001).

PCBs and OCIs had fillet:diet accumulation ratios of about 0.4–0.6. Some researchers have reported a similar range of accumulation from diet into fish for PCBs and several other organochlorine insecticides (Gobas et al. 1993; Fisk et al. 1998), whereas others have reported somewhat higher ranges (Niimi and Oliver 1983; Carline et al. 2004). As an example, Carline et al. (2004) investigated the levels of PCBs in trout fillets after 180 days of exposure to diets ranging from about 69 to 280 ng/g. They found that the 180-day exposure period was sufficient for the tissue levels to stabilize with the diet. The lowest dietary concentration of 69 ng/g PCBs yielded an average trout fillet concentration of 49 ng/g and a fillet:diet ratio of 0.71. Bioaccumulation of PCBs and other organochlorine

Table 3 Mean concentration of contaminants in rainbow trout fillets from Missouri trout parks, 2005

Variables	Mean (standard deviation)[a]				
	Bennett Spring	Maramec Spring	Montauk	Roaring River	Shepherd of the Hills
Length (mm)	295.9 (2.1)	285.8 (6.3)	337.4 (9.3)	289.2 (5.7)	291.3 (1.8)
Weight (g)	277.1 (13.1)	299.0 (9.5)	455.4 (124.4)	273.4 (12.1)	302.4 (8.6)
Lipid (%)	3.6 (0.2)	4.6 (0.3)	4.2 (0.5)	4.4 (0.3)	4.6 (0.2)
Cadmium	<2.9	<2.9	<2.9	<2.9	<2.9
Lead	<12.0	<12.0	<12.0	<12.0	<12.0
Mercury	18.3 (2.3)	20.5 (0.3)	20.1 (1.8)	14.9 (1.9)	15.9 (0.5)
Chlordane	1.2 (0.2)	<0.1	0.7 (0.1)[b]	1.1 (0.1)	1.0 (–)
DDT	3.5 (0.5)	3.6 (–)	4.3 (–)	2.9 (–)	3.8 (0.4)
Dieldrin	0.4 (0.1)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.4 (0.1)
Lindane	<0.1	<0.1	<0.1	<0.1	<0.1
Heptachlor	<0.2	<0.2	<0.2	<0.2	<0.2
HCB	<0.1	<0.1	0.1 (0.1)[b]	0.1 (0.1)[b]	0.3 (0.1)
Total PCBs	<11	<11	16.3 (1.8)	16.1 (3.8)	19.9 (7.0)

[a] All contaminants are in ng/g wet weight. Dashed lines for standard deviation indicate n = 1

[b] Indicates n = 2, n = 3 for all other means

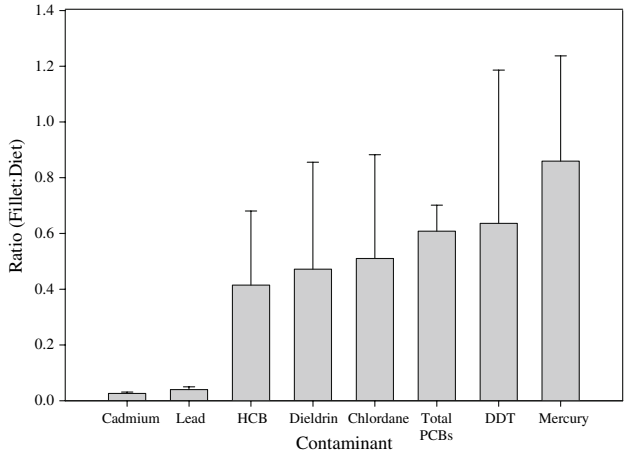

Fig. 1 Mean and standard deviation for the ratio of contaminant concentration in trout fillet to concentration in diet from Missouri coldwater hatcheries

compounds from diet are known to vary with species, size, tissue type, and type of diet (Fisk et al. 1998). Overall, the assimilation values for the organochlorine compounds in this study were within the broad range of values measured in previous studies.

Mercury had the highest fillet:diet accumulation ratio with an average of 0.83. Similar accumulation ratios of 0.67–0.87 were reported by Phillips and Buhler (1978) for methylmercury, the organic form of mercury. Wang and Wong (2003) have shown fish accumulation ratio for diets containing inorganic mercury to be low (0.1–0.27) compared to diets containing methylmercury (0.56–0.95). The high accumulation ratio observed in our study suggests that the dietary mercury was in the organic form. Methylmercury is the predominant form of mercury found in fish fillet tissues from contaminant monitoring programs (Kanan et al. 1998; US EPA 2001).

The European Union (2002) has developed a list of the maximum allowable levels for fish feed (Table 4). The concentrations in the current study were well below the maximum levels specified. A maximum acceptable level for total PCBs was not specified by the European Union. Rather, a maximum level was derived for the dioxin-like PCB congeners. At present in the US, most toxicity thresholds for fish consumption advisories are expressed in total PCBs rather than dioxin-like PCBs. Therefore, it would be useful to consider a total PCB threshold, even though the latter has a better accounting of toxicity potential. Assuming a maximum accumulation ratio of 0.6 from diet to fillet tissue and the GLP meal/week trigger concentration of 60 ng/g, a maximum diet concentration of 100 ng/g is calculated (i.e., 60 ng/g divided by 0.6). The US FDA (2006) has specified a maximum allowable PCB concentration for finished feeds of 200 ng/g. However, it is likely that the GLP advisory trigger for fillet tissue would

Table 4 Comparison of European Union's fish diet contaminant criteria to Missouri trout diet results

Contaminant	Concentration in diet (ng/g wet weight)	
	Finished fish diet concentration criteria from European Union (2002)	Mean concentrations from current study
Cadmium	2,000	115.3
Lead	10,000	317.2
Mercury	100	23.3
DDT	50	9.3
Dieldrin	10	0.9
Lindane	200	0.2
Chlordane	20	2.9
Heptachlor	10	0.2
HCB	10	0.4
Total PCBs	NS[a]	28.9

[a] NS, not specified. Only dioxin-like PCBs reported

be exceeded at this level of total PCBs in fish feed (Carline et al. 2004). Finished fish diets with total PCB concentrations at or below 100 ng/g (wet weight) would be a more appropriate threshold to reduce the likelihood of triggering a fish consumption advisory using the GLP or Missouri advisory limits. If other more sensitive advisory triggers were used, such as EPA's cancer endpoint calculation, then the threshold would be lower (Carline et al. 2004).

The contaminants investigated in this study were not measured in the hatchery water. Therefore, the relative contribution from water, diet and other sources cannot be estimated. In some cases, sources other than diet, such as caulk or paint, can be major contributors of contaminants to fish (Meadows et al. 1998). Results from this study assume no other significant sources of contaminants. Since the levels observed in trout fillets were low overall, this is likely a reasonable assumption. If high levels of contaminants are observed in trout fillets and diet levels are within the allowable levels discussed here, then other sources of contaminants should be investigated.

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References

Carline RF, Barry PM, Ketola HG (2004) Dietary uptake of polychlorinated biphenyls (PCBs) by rainbow trout. North Am J Aquac 66:91–99

European Union (2002) On undesirable substances in animal feed. Directive 2002/32/EC of the European Parliament and of the Council 7 May 2002

Fisk AT, Norstrom RJ, Cymbalisty DD, Muir DCG (1998) Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water coefficient. Environ Toxicol Chem 17:951–961

Gobas F, McCorquodale JR, Haffner GD (1993) Intestinal absorption and biomagnification of organochlorines. Environ Toxicol Chem 12:567–576

Goyer RA (1996) Toxic effects of metals. In: Klaassen CD (ed) Casarett and Doull's toxicology: the basic science of poisons. McGraw-Hill Publishing Co., New York, pp 669–705

Hendriks AJ, Heikens A (2001) The power of size. 2. Rate constants and equilibrium rations for accumulation of inorganic substances related to species weight. Environ Toxicol Chem 20:1421–1437

Hites RA, Foran JA, Carpenter DO, Coreen-Hamilton M, Knuth BA, Schwager SJ (2004) Global assessment of organic contaminants in farmed salmon. Science 303:226–229

Hinck JE, Schmitt CJ, Echols KR, May TW, Orazio CE, Tillitt DE (2006) Environmental contaminants in fish and their associated risk to piscivorous wildlife in the Yukon River Basin, Alaska. Arch Environ Contam Toxicol 51:661–672

Jacobs MN, Covaci A, Schepens P (2002) Investigation of selected persistent organic pollutants in farmed atlantic salmon (*Salmo salar*), salmon aquaculture feed and fish oil components of the feed. Environ Sci Technol 36:2797–2805

Kanan KR, Smith Jr G, Lee RF, Windom HL, Heitmuller PT, Macauley JM, Summers JK (1998) Distribution of total mercury and methyl mercury in water, sediment and fish from south Florida estuaries. Arch Environ Contam Toxicol 34:109–118

Maule AG, Gannam AL, Davis JW (2007) Chemical contaminants in fish feeds used in federal salmonid hatcheries in the USA. Chemosphere 67:1308–1315

Meadows JC, Echols KR, Huckins JN, Borsuk FA, Carline RF, Tillitt DE (1998) Estimation of uptake rate constants for PCB congeners accumulated by semipermeable membrane devices and brown trout (*Salmo trutta*). Environ Sci Technol 32:1847–1852

Niimi AJ, Oliver BG (1983) Biological half-lifes of polychlorinated biphenyls (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gardnerii*). Can J Fish Aquat Sci 40:1388–1394

Phillips GR, Buhler DR (1978) The relative contributions of methylmercury from food of water to rainbow trout (*Salmo gairdnerii*) in a controlled laboratory experiment. Trans Am Fish Soc 107:853–861

Serder D, Kinney K, Mandjikov M, Montgomery D (2006) Persistent organic pollutants in feed and rainbow trout from selected trout hatcheries. Washington State Department of Ecology. Report # 06-03-017

US EPA (2001) Mercury update: impact on fish advisories. US Environmental Protection Agency, EPA Fact Sheet, EPA-823-F-01-011

US FDA (2006) Tolerances of unavoidable poisonous or deleterious substances. Code of Federal Regulations, 21CFR109.30

Wang W, Wong RSK (2003) Bioaccumulation kinetics and exposure pathways of inorganic mercury and methylmercury in a marine fish, the sweetlips *Plectoryinchus gibbosus*. Mar Ecol Prog Ser 261:257–268