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Uptake and Retention of Mirex by Fish Maintained on Formulated and Natural Diets in Lake Ontario Waters

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Abstract.—Fish with no detectable levels of the contaminant mirex were grown in Lake Ontario waters under conditions simulating commercial aquaculture. Benthic black bullheads (*Ameiurus melas*) were grown in cages placed in a bay of the lake. Pelagic rainbow trout (*Oncorhynchus mykiss*) were grown in terrestrial raceways served with Lake Ontario waters. Contaminant-free fingerlings were reared to a large size on a commercial ration in these systems, which partially isolated them from the contaminant-laden food web and bottom sediments. Black bullheads fed a mirex-spiked, commercially prepared food had mirex concentrations that exceeded the U.S. Food and Drug Administration (FDA) action level of 0.1 μg/g, significantly higher than concentrations in fish receiving the same commercial food without mirex. Ninety percent of fish receiving the unspiked ration had nondetectable levels of mirex (values below 0.002 μg/g). The 10% containing mirex had concentrations 94% below FDA action level. In the rainbow trout study, 97% of the fish had no detectable levels of mirex. This investigation demonstrated that bioaccumulation of the lipophilic contaminant mirex by fish cultured under simulated commercial conditions in Lake Ontario waters was not significant. These findings have implications for commercial aquaculture, regulatory decisions, and health-conscious fish consumers in the Great Lakes Basin.

The Great Lakes constitute a unique North American aquatic resource that has substantial aquaculture potential. However, organic contaminants present in tissues of fish that inhabit the lakes frequently exceed action levels established by the U.S. Food and Drug Administration (FDA), which restricts the sale and consumption of these fish. For example, discovery of the pesticide mirex in Lake Ontario fish (Kaiser 1974) contributed to the State of New York's decision to implement a ban on eating fish from the lake in 1976. The subsequent repeal of the ban and the establishment of the present advisory that restricts or prohibits ingestion of fish caught from Lake Ontario (NYSDEC 1990) has eliminated the commercial and restricted the recreational consumptive use of fish. Similar advisories also exist in the Province of Ontario (Ontario Ministry of the Environment 1988) and in states along the upper Great Lakes (Minnesota Department of Health 1991; Wisconsin Department of Natural Resources 1991). Regulations designed for wild-caught fish are also being applied to cultured fish (Fong and Brooks 1989; National Fisheries Institute Communications 1992).

The presence of contaminants in the waters and fish of the Great Lakes has had a major effect on the development of aquaculture in these lakes. A workshop that explored the potential of aquaculture in the Great Lakes identified the uncertainty about levels of organic toxics in fish cultured in the U.S. waters of Lake Ontario as the number one impediment to development of an aquaculture industry in these waters (Buttner 1988). With increased consumer awareness and concern about the quality of their seafood, the issue of contaminants has been magnified and extends to other waters (e.g., Anonymous 1992a, 1992b; National Fisheries Institute Communications 1992).

Because of organic contaminants in the water and in naturally occurring fish, some regulatory agencies have suggested that aquaculture in the Great Lakes is not desirable or feasible, because the fish produced will contain unsafe levels of contaminants. The assumption is cultured fish will have similar levels of contaminants as wild-caught fish if grown in the same contaminated waters. The basis for this argument is that some investigations have demonstrated that chemical uptake from water, through the gills, is a major route of contamination (e.g., Hamelink and Spacie 1977; Connell 1991). Conversely, other studies have shown that uptake through the food web is the major pathway (e.g., Johnson 1973; Addison 1976; Guiney et al. 1979; Skea et al. 1981; Niimi 1983). Thus, fish cultured in tainted waters could bioaccumulate contaminants, even though they are raised on an uncontaminated commercial feed. Without a demonstration project, regulatory agencies will employ and enforce the most cautious and conservative regulations to protect the con-

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sumer, and prospective culturists are justifiably hesitant to pursue commercial aquaculture if the harvested product may not be marketable.

Fish accumulate organic contaminants, such as mirex, in three ways: (1) absorption from water through their gills or integument; (2) ingestion of contaminated food; and (3) inheritance from parental sources through yolk or oil (e.g., Johnson 1973; Addison 1976; Hamelink and Spacie 1977; Guiney et al. 1979; Skea et al. 1981; Niimi 1983). The three accumulation pathways allow testing of a method for producing clean fish in waters of the Great Lakes. It is possible that fish cultured in these waters, isolated from the contaminant-laden sediments and partially isolated from the natural food web, would not acquire contaminant levels that exceed FDA action levels. Because uptake through inheritance can be controlled by the use of progeny from contaminant-free parents, our study tested the hypothesis that the major uptake route of mirex is via diet rather than through integument and gills. Uptake of mirex from lake water seems unlikely, because it is hydrophobic. Thus the question being tested becomes, Will fish in Lake Ontario with limited access to natural forage and feeding largely on a prepared food ingest enough natural food to elevate mirex levels in edible portions (fillets) of their bodies and exceed FDA guidelines?

Methods

Cage and pen culture.—Between 12 and 14 June 1990, eight cages (1-m^3 volumes, 1-cm plastic mesh; Buttner 1992a, 1992b) were anchored in Braddock Bay (Figure 1). Each cage received 100 or 300 mirex-free fingerling black bullheads (*Ameiurus melas*). On 16 July, four pens were installed. Each pen enclosed a 15-m^2 area of the bay and was made of plastic webbed (1-cm mesh) fencing that extended below the substratum and above the water surface. Each pen received 15–45 black bullhead fingerlings (1–3 fish/m^2). Average weight of black bullhead fingerlings was 28.2 g (range, 22.4–33.5 g). Fingerlings were third- and fourth-generation fish obtained from mirex-free parental fish spawned at the State University of New York College at Brockport.

Fish in four cages were fed once each day (1–3% body weight/d) with Catfish Cage Chow (Ralston Purina 5144; Buttner 1992b). The diet was analyzed and found not to contain mirex. Fish in four other cages received an identical ration spiked with mirex. To prevent cross contamination of spiked and unspiked rations, cages were equipped with feeding rings to retain food and were separated by approximately 1,000 m. Mirex-spiked feed was prepared with an mirex–acetone solution (1 mg/L in July, 2 mg/L in August, and 3 mg/L in September) that was sprayed on the commercial feed and fed to fish between 16 July and 24 September at rates (total for the four cages) of 1.6 mg mirex/d in July, 3.3 mg/d in August and 5.0 mg/d in September. Pen-reared fish fed only on natural food for 93–107 d. Water quality was monitored weekly (Table 1). Cages and pens were harvested on 27 September 1990 and 17–31 October 1990, respectively, and fish were frozen immediately at −4°C.

Raceway culture.—On 18 April 1990, each of six flow-through raceways (1.8 × 0.6 × 0.3 m; 310 L) at the Russell Power Generation Station (Figure 1) received mirex-free fingerling rainbow trout (*Oncorhynchus mykiss*). The Nashau (New Hampshire) strain trout (mean weight, 59 g; range, 23–123 g) were stocked at a rate of 0.32 fingerlings/L. Fingerlings were obtained from the Caledonia (New York) State Fish Hatchery and were fed once daily to satiation with a 40% protein ration (Ralston Purina Trout Chow 5105 and 5106). Because of problems with permit acquisition, mirex was not added to the daily ration as a control. Raceways received unfiltered Lake Ontario water alone or mixed with heated lake water discharged from the coal-fired electricity generating plant. Between mid-July and late August, water temperature frequently exceeded 20°C and trout were noticeably stressed; after mid-August hot water was no longer available due to prophylactic treatments for zebra mussels (*Dreissena polymorpha*). Routine maintenance and monitoring included adjustment of water flow (maintained at ~8–15 L/min), alternate-day scrubbing and treatment with copper sulfate (0.4–0.8 mg/L) or formalin (45–65 mg/L) as a 30–40-min bath to control monogeneric trematodes and *Ichthyophthirus,* daily water temperature measurements, and weekly measurements of dissolved oxygen, pH, alkalinity, and total ammonia-nitrogen (Table 1). All fish were removed on 18 October 1990 and most were immediately frozen. Sixty fish were returned to the raceways (10 per unit) and maintained on pelleted food another 6 months before they were harvested and frozen on 28 May 1991. Between 18 October 1990 and 28 May 1991, fish were fed to satiation three to five times a week and water quality was not monitored.

Wild fish.—Naturally occurring, age-2 or older brown bullheads (*Ameiurus nebulosus*) were seined

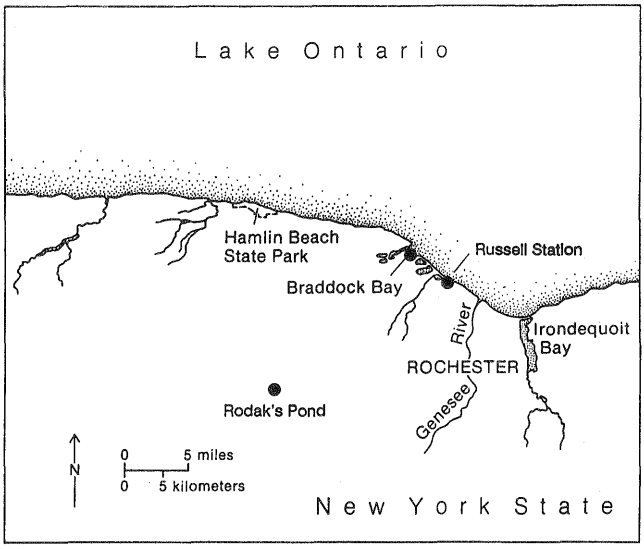

FIGURE 1.—Bullhead or rainbow trout culture and sample sites.

from a nearby inland pond with no known sources of mirex, Rodak's Pond (N = 6; 140–289 g), and electrofished from Braddock Bay (N = 6; 261–611 g) (Figure 1). Rainbow trout (*Oncorhynchus mykiss*) were electrofished from Lake Ontario (N = 5; 989–4,478 g).

Mirex analyses.—Five or six fish from each cage, pen, raceway, and natural habitat were filleted, and the skin was removed from the fillets. The tissue was ground and homogenized in a food processor. Five grams of tissue were mixed with 20 g of anhydrous sodium sulfate and extracted overnight (Soxhlet—200 cycles minimum) with 75 mL of methylene chloride and hexane (20:80 volume : volume). A 30-mL aliquot was evaporated to 1 mL under nitrogen, then cleaned up through a 5-g florisil column (50 mL at 4 mL/min); the eluant was further concentrated under nitrogen. Mirex was determined by electron capture (^{63}Ni) gas chromatography; the Hewlett Packard (HP) 5890A gas chromatograph was equipped with a HP101 25-m × 0.2-m capillary column and HP 3396A integrator. Recovery efficiencies were 96.1% (N = 8). The mirex detection limit was 2 μg/kg (wet weight) for fish samples, and one blank and two replicate analyses were performed for every five fish analyzed. A one-way analysis of variance (ANOVA) was used to compare the means (Minitab statistical package; Ryan et al. 1985).

Results

Mirex levels in fillets from black bullheads maintained on the mirex-spiked ration (cages A–D) were significantly higher than concentrations observed in fish (cages E–H) receiving the uncontaminated ration (ANOVA; $P < 0.001$) and exceeded the FDA action level of 0.1 μg/g (Figure 2). Bullheads fed the mirex-free ration either had nondetectable levels of mirex (below 0.002 μg/g) or levels 94% below the FDA action level (Figure 2). Of 20 black bullheads analyzed from the cages fed the mirex-free ration, 18 (90%) of the fish contained no detectable levels of mirex. Black bull-

TABLE 1.—Methods used to monitor and ranges observed for water quality variables at Braddock Bay (3 June–24 September) and Russell Station (18 April–17 October) fish culture sites in 1990.

Variable	Analytical method	Braddock Bay cage culture	Russell Station flow-through
Temperature (°C)	Thermometer	13.5–28.0	6.2–23.1
Dissolved oxygen (mg/L)	Polarographic meter and Winkler method (APHA et al. 1980)	3.8–10.0	4.8–12.0
pH	Meter	7.6–8.7	7.6–8.8
Alkalinity (mg/L as $CaCO_3$)	Potentiometric (APHA et al. 1980)	104–127	86–104
Water clarity (cm)	Secchi disk	25–69	
Total ammonia-nitrogen (mg/L)	Nesslerization (Hach Co. 1985)		0.01–0.82

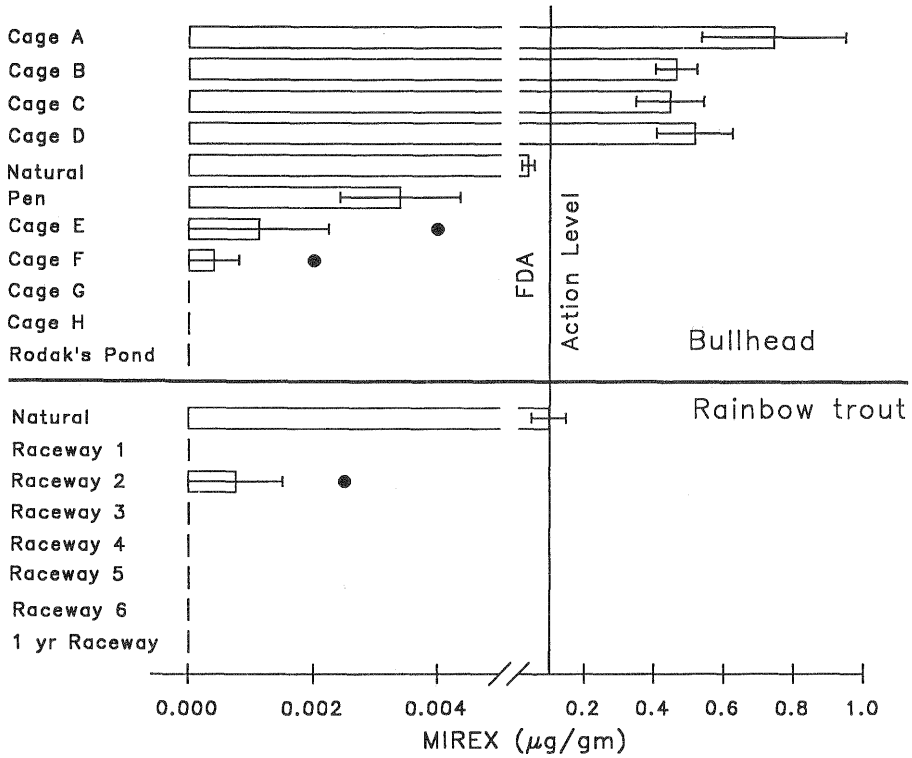

FIGURE 2.—Mirex concentrations in black bullheads cultured on a commercial ration spiked with mirex (cages A–D), the same commercial ration without mirex (cages E–H) and in pens on natural feed; in wild brown bullhead from Rodak's Pond and Lake Ontario (natural); and in wild rainbow trout from Lake Ontario (natural) and rainbow trout cultured on a commercial ration in raceways for 6 months (1–6) and one year (1 yr raceway). Bar graphs represent the mean ± SE. A solid circle indicates the actual mirex concentration in analyzed fish that contained mirex.

heads maintained on the mirex-free ration had mirex levels more similar to those of brown bullheads from Rodak's Pond, with no known source of mirex, than to those of fish naturally occurring in Lake Ontario (Figure 2). Black bullheads maintained in pens on natural forage contained mirex at concentrations averaging 0.004 μg/g. Naturally occurring brown bullheads electrofished from Braddock Bay had an average concentration an order of magnitude higher (0.04 μg/g). Growth of cultured black bullheads analyzed averaged 224%.

Rainbow trout fed a commercial ration in raceways receiving Lake Ontario water grew 261% in a 6-month period to an average weight of 213 g. Of the 30 fish analyzed, only one contained a detectable level of mirex (raceway 2; Figure 2). Even after an additional 6 months' residence time in water from Lake Ontario, six rainbow trout that by then averaged 490 g had no detectable levels of mirex. Wild rainbow trout electrofished from Lake Ontario, heavier than the cultured fish, had mean mirex levels of 0.097 μg/g, which approached the FDA action level (Figure 2).

Discussion

Lake Ontario is generally considered to be contaminated with mirex (Kaiser 1974; Holdrinet et al. 1978). At the cage culture site in Braddock Bay, we measured mean mirex concentrations in sediments of 1.92 μg/kg dry weight and 0.025 μg/g organic carbon. Fish populations naturally occurring in Braddock Bay were contaminated with mirex: skinless fillets of brown bullhead contained 0.04 μg/g ($N = 5$); whole bodies of pumpkinseed (*Lepomis gibbosus*) contained 0.015 μg/g ($N = 8$); and skinless fillets of rainbow trout contained 0.097 μg/g ($N = 5$). In addition, seston that settled in the raceways at nearby Russell Station contained a mean mirex concentration of 0.015 μg/g dry weight. Because Lake Ontario's and Braddock Bay's waters, sediments, seston, and food chain are contaminated with mirex, fish grown in these

waters could be expected to contain mirex in their tissue. However, we have demonstrated in this study that the presence of mirex in food resulted in the greatest concentration of mirex in fish muscle tissue.

Absorption of contaminants from the water through gills and integument, ingestion of contaminated food, and transfer of contaminants from parents via yolk and oil are potential mechanisms by which fish accumulate organic contaminants such as mirex. Standard aquaculture practices control these sources. Contaminant-free, high-quality fingerlings are cultivated and fed a commercially prepared ration. The fish are cultivated in systems like cages and land-based raceways that largely isolate them from the contaminant-laden natural food chain and sediments. By controlling the pathways of uptake in simulated commercial conditions, we have demonstrated the uptake of mirex through the integument and incidental ingestion of natural food that enters culture units is not significant.

These results have implications for aquaculture in the Great Lakes. The underlying assumption of regulators, industry, and consumers has been that any body of water, such as Lake Ontario, that is specifically included in a health advisory for recreational fishing would be a poor choice for aquaculture, because the fish are likely to contain contaminants (Buttner 1988; N. Kim, New York Department of Health, personal communication). Our data indicate that fish with significantly reduced or nondetectable levels of an organic contaminant, like mirex, can be cultured in contaminated waters. Fillets of both the pelagic, coldwater rainbow trout, a fish generally considered to have a high lipid content, and the benthic, coolwater black bullhead contained no detectable levels of mirex when cultured for up to 12 months in Lake Ontario water and fed a mirex-free food. The health advisory and associated restrictions correctly applied to consumption of fish feeding on the natural food web in Lake Ontario fish (and perhaps elsewhere) should not necessarily apply to fish commercially cultured. Future research should determine whether other organic pollutants, especially PCB congeners having dioxin-like biological activity, will accumulate in fish raised on commercially prepared diets.

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References

Addison, R. F. 1976. Organochlorine compounds in aquatic organisms: their distribution, transport and physiological significance. Pages 127–143 *in* A. P. M. Lockwood, editor. Effects of pollutants on aquatic organisms. Cambridge University Press, Cambridge, UK.

Anonymous. 1992a. Is our fish fit to eat? Consumer Reports 57:103–112.

Anonymous. 1992b. What else is in fish? And how did it get there? Consumer Reports 57:112–114.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and waste water, 15th edition. APHA, Washington, D.C.

Buttner, J. K. 1988. The obstacles facing Great Lakes aquaculture. Water Farming Journal 3:6–7.

Buttner, J. K. 1992a. Cage culture of black bullhead. Aquaculture Magazine 18(2):32–46.

Buttner, J. K. 1992b. Cage culture of black bullhead. Aquaculture Magazine 18(3):55–65.

Connell, D. W. 1991. Bioaccumulation of xenobiotic compounds. CRC Press, Boca Raton, Florida.

Fong, W. G., and G. M. Brooks. 1989. Regulation of chemicals for aquaculture use. Food Technology 43(11):88–93.

Guiney, P. D., M. J. Melacon, J. J. Lech, and R. E. Peterson. 1979. Effects of egg and sperm maturation and spawning on the distribution and elimination of a polychlorinated biphenyl in rainbow trout (*Salmo gairdneri*). Toxicology and Applied Pharmacology 47:261–272.

Hach, Co. 1985. Water analysis handbook. Hach Co., Loveland, Colorado.

Hamelink, J. L., and A. Spacie. 1977. Fish and chemicals: the process of accumulation. Annual Review of Pharmacology and Toxicology 17:167–177.

Holdrinet, M. Van Hove, R. Frank, R. L. Thomas, and L. J. Hetling. 1978. Mirex in the sediments of Lake Ontario. Journal of Great Lakes Research 4:69–74.

Johnson, D. W. 1973. Pesticide residues in fish. Pages 181–212 *in* C. A. Edwards, editor. Environmental pollution by pesticides. Plenum, New York.

Kaiser, K. L. E. 1974. Mirex: an unrecognized contaminant of fishes from Lake Ontario. Science (Washington, D.C.) 185:523–525.

Minnesota Department of Health. 1991. Minnesota fish consumption advisory. Minnesota Department of Health, Minneapolis.

National Fisheries Institute Communications. 1992. Seafood safety. National Fisheries Institute, Rosslyn, Virginia.

Niimi, A. J. 1983. Biological and toxicology effects of environmental contaminants in fish and their eggs. Canadian Journal of Fisheries and Aquatic Sciences 40:306–312.

NYSDEC (New York State Department of Environmental Conservation). 1990. New York State 1990–91 fishing regulations guide. NYSDEC, Albany, New York. (Available from Bureau of Environmental Protection, Albany.)

Ontario Ministry of the Environment. 1988. Guide to eating Ontario sport fish. OME, Communications Branch, Toronto.

Ryan, B. F., B. L. Joiner, and T. A. Ryan. 1985. Minitab handbook. Duxbury Press, North Scituate, Massachusetts.

Skea, J. C., H. A. Simoin, E. J. Jacling, and J. Symula. 1981. The pickup and retention of mirex by brook trout fed a contaminated diet. New York State Department of Environmental Conservation, Division of Fish and Wildlife, Rome.

Wisconsin Department of Natural Resources. 1991. Health guide for people who eat sport fish from Wisconsin waters. Wisconsin Department of Natural Resources, Publication IE-019REV, Madison.