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Tissue Distribution and Histopathological Effects of Dietary Methylmercury in Benthic Grubby *Myoxocephalus aenaeus*

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There is a need to test deterministic models predicting the behavior and effects of chemicals on aquatic systems (Connelly 1991) by conducting experiments with more than one trophic step at a time. This approach requires the set-up of an experimental food chain in pounds or mesocosm facilities which can be used for dietary uptake studies and assessment of sublethal stress induced by contaminated food. In the course of our current research program at the INRS marine mesocosm facilities (Roy et al. 1991), a model benthic food chain including inter- and sub-tidal species such as the mussel (*Mytilus edulis*), clam (*Mya arenaria*), starfish (*Leptasterias polaris*), polychaete (*Nereis virens*), amphipod (*Gammarus sp.*), gastropod (*Buccinum undatum*), and fishes (*Pleuronectes americanus, Myoxocephalus aenaeus*), is used for testing food uptake models and for the development of sublethal toxicity tests which could be used in the environmental assessment of coastal and estuarine waters. Among these test organisms, the grubby (*M. aenaeus*) is a small coastal fish (12-15 cm) characterized by a broad head. The grubby is tolerant of water temperature and salinity variations and lives on a wide variety of bottom strata at low depths (Scott and Scott 1988). The grubby is carnivorous and consumes a wide variety of molluscs and the young of many species of fish. Because of its size, its estuarine and coastal distribution and its large spectrum of prey, this species was seen as an ideal fish to fit into our experimental food chain model.

This paper reports a preliminary experiment designed to measure distribution of mercury in tissues and to test the response of various histopathological and biochemical stress indicators in grubby exposed to dietary contamination by methylmercury (MeHg) for a 20-day exposure period. MeHg was chosen because it is rapidly bioaccumulated by most living organisms and its toxicity has been studied for decades in numerous aquatic ecosystems.

MATERIALS AND METHODS

Mature grubby were captured in the subtidal waters of the St. Lawrence Estuary

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(Canada) and kept in running seawater under natural conditions of temperature, salinity, and photoperiod. They were fed with smelt pieces. In February, three months before the beginning of the experiment, 10 fish were placed in individual buckets (35.6 cm x 22.9 cm) within a large rectangular tank (335.0 cm x 40.6 cm x 30.5 cm) having a seawater flow rate of 10 L/min. This experimental set-up ensured the observation of feeding behavior and a correct evaluation of the quantity of food ingested by each fish. In May, when water temperature allowed regular daily feeding, the contamination experiment was started. The five control fish were in the five buckets nearest to the water source while the five fish receiving mercury-contaminated food were in downstream buckets in such a manner that dissolved or particulate mercury could not reach control fish. Mean weight and length (ranges given in square brackets) in controls and experimental fish were, respectively, 36.6 $\pm$ 8.9 [30.3-51.9] g, 13.7 $\pm$ 1.2 [12.8-15.7] cm and 42.5 $\pm$ 13.2 [27.6-63.4] g and 14.4 $\pm$ 1.6 [12.8-17.0] cm.

Contaminated food was prepared by dipping a weighed amount of small pieces (0.3 to 0.5 g each) of uncontaminated smelt muscle in a solution of methylmercury (II) chloride to achieve a concentration of 6.0 $\pm$ 1.0 ng/g ww in each piece of food. For 20 days, control and experimental fish received one meal per day consisting of 0.3 g of smelt pieces of non-contaminated or contaminated with methylmercury, respectively. Food was immediatly eaten in most cases (94%) after 20 min; food not eaten was discarded. The mean daily mercury consumption can be estimated to 2.1 $\pm$ 0.7 ng/day. On day 21, fish were not fed and on day 22 they were anaesthetized with MS222 (3-aminobenzoic acid ethyl ester), 0.02% (w/v) buffered to seawater pH (7.8 $\pm$ 0.2). The fish were weighed, measured, and blood sampled by caudal puncture. Some blood aliquots were kept in hematocrit tubes or diluted in Drabkin's solution for further determination of blood hemoglobin concentration; the rest were centrifuged at 7,000 x g for 3 min. Plasma was separated into aliquots for plasma glucose and cortisol determinations. Gill filaments from the second left gill arch and a small piece of liver were excised and put in a 3% (w/v) formaldehyde - 4% (v/v) glutaraldehyde fixative solution. Other organs (remaining gills and liver, stomach, intestine, muscle, gonads, and skin) were dissected out and kept frozen for mercury determination.

Total mercury and inorganic mercury were determined using an adaptation of published method (Rouleau et al. 1992). Briefly, fish samples were acid digested in flat-bottomed flasks at 55°C for 1 hr using a mixture 1:1 of concentrated nitric and sulphuric acids. Total mercury was determined in duplicate using a Fisher HG-3 flameless atomic absorption spectrophotometer. Standard mercury solutions for calibration purposes were prepared from a commercially available 1000 ppm $HgCl_2$ standard solution. The detection limit was 10 ng/g ww and the overall variability of the method was estimated to be $\leq$ 15%. Samples (1-2 g) for the determination of inorganic mercury were digested in 5 ml of 45% (w/v) KOH and 1 ml of 1% (w/v) L-cysteine for 1 hr at 80°C. Samples were successively treated with 8 M urea and 0.5 M $CuSO_4$ and finally acidified with 2 ml of concentrated

hydrochloric acid. This mixture was extracted twice with 20 ml of toluene and the mercury remaining in the aqueous phase was analyzed by flameless AAS as described above. This extraction method ensured a quantitative recovery ($\geq$ 98%) of methylmercury spiked in the sample. Mercury found in the aqueous phase was assumed to be water soluble inorganic mercury and the difference between total mercury and inorganic mercury was assigned to methylmercury.

Blood hemoglobin and plasma glucose were measured, respectively, by colorimetric (Sigma kit No. 525) and enzymatic methods (Sigma kit No. 16 UV). Plasma cortisol was measured by radioimmunoassay (Kallestad, No. 825). Tissues used for histological examination were post-fixed in 1% (w/v) osmium tetroxide, dehydrated in ethanol, and embedded in Epon. When embedded, gill samples were oriented in order to procure sagittal sections. Serial semi-thin sections (0.5 μm) were stained with toluidine blue. Only qualitative observations were made on liver sections. Qualitative and quantitative measurements were made on gill sections by the method of Audet and Wood (1992). Presence or absence of significant differences between control and experimental fish were analyzed by Student's t test for all measured parameters.

RESULTS AND DISCUSSION

Tissue distribution of methylmercury in control and exposed fish (Table 1) indicated the presence of MeHg in all organs analyzed, with a maximum value recorded in the digestive tract of contaminated fish and the minimum observed in gonads in the control.

Liver and muscle tissues of control animals showed relatively high concentrations of methylmercury. As control fish were located upstream from contaminated fish in our flow-through tank, their accidental contamination via seawater or food particles was not possible. The presence of mercury in control animals indicated the ability of grubby to bioconcentrate mercury from their natural coastal habitat along the St. Lawrence Estuary. Low Hg concentrations in the digestive tract, gills, and skin of controls confirmed that seawater pumped from the Estuary near Pointe-au-Pere (Rimouski) was cleaner than brackish waters near Riviere-du-Loup (100 km upstream to Rimouski) where fish were collected. However, the cleansing efficiency of seawater from the laboratory for a long adaptation period (8 months of uncontaminated feeding) was not enough to remove mercury (mainly as MeHg) from liver and muscle. In fish consuming contaminated food, highest mercury concentrations were detected in the digestive tract and liver, indicating a very recent contamination. Lowest levels were found in gonads, where MeHg ranged from 0.183 to 0.667 μg/g ww, with a mean value of 0.315 $\pm$ 0.184 μg/g.

Comparison between the total methylmercury dose ingested over the exposure period and the total body burden indicated that mean assimilation efficiency of MeHg from food was 57% for grubby, assuming the natural background methyl

Hg level observed in control fish was the same as in experimental fish. Published values for absorption efficiency for dietary methylmercury range from ≤ 20% for northern pike, *Esox lucius* (Philips and Gregory 1979), to 70% for rainbow trout, *Salmo gairdneri* (Philips and Buhler 1978), and 86 to 90% for flounder *Platichthys flesus* (Riisgard and Hansen 1990) and plaice *Pleuronectes platessa*, respectively (Pentreath 1976). Assuming a regular accumulation rate for methylmercury over the contamination period as previously observed in rainbow trout and flounder, we estimated the average uptake rates in liver, muscle, and gonads to be 62, 25, and 17 ng/g ww/d, respectively. These values are 8 to 12 times higher than those reported for flounder and are probably related to the higher concentration of MeHg (6.0 ppm compared to 1.15 ppm) used in the present study.

Table 1. Tissue distribution of methylmercury in the grubby, *Myoxocephalus aenaeus*, exposed for 20 days to a methylmercury-contaminated diet (6.0 μg/g ww). Mean ± standard deviation.

Tissue	Control fish (n = 3)	Contaminated fish (n = 5)
Liver	0.244 ± 0.105	1.230 ± 0.396
Digestive tract	0.083 ± 0.043	2.986 ± 0.465
Gonad	0.019 ± 0.010	0.315 ± 0.184
Muscle	0.205 ± 0.173	0.505 ± 0.076
Skin	0.093 ± 0.075	0.572 ± 0.151
Gill	0.083 ± 0.043	0.428 ± 0.110

The determination of inorganic mercury was made to assess the possible enzymatic or bacterial demethylation of Hg in the digestive tract and other tissues of the grubby. Concentrations of inorganic mercury in control and experimental fish were either very low (i.e., ≤ 0.03 μg/g) or below the detection limit. Although some reports mentioned the degradation of methylmercury in the intestines of some fish species (Riisgard and Hansen 1990), we found no evidence for such a process in the grubby. Even in the liver of contaminated fish, the proportion of inorganic mercury never reached ≥ 2% of total Hg measured. Bloom (1992) observed that virtually all (> 95%) mercury detected in wild fish and marine invertebrates was present as methylmercury, while inorganic mercury

was absent in most biological tissues. Our results are in agreement with his study, as control grubbies were MeHg-contaminated but no inorganic mercury (i.e $\leq$ 10 ng/g ww) was detected in any organs or tissue.

The survey of different stress indicators revealed the absence of significant response to dietary methylmercury contamination in grubby (Table 2). Concentrations of all measured blood variables were similar between control and experimental fish.

Table 2. Biochemical stress indicators measured in grubby following feeding with methylmercury-contaminated food and control fish. Mean $\pm$ standard deviation.

Stress indicators	Control fish (n = 5)	Contaminated fish (n = 5)
Hematocrit (%)	17.6 $\pm$ 1.06	20.8 $\pm$ 1.86
Blood hemoglobin (g/100 mL)	3.4 $\pm$ 0.32	3.6 $\pm$ 0.23
MCHC (g/100 mL)	19.1 $\pm$ 1.12	17.7 $\pm$ 1.17
Plasma glucose (mg/100 mL)	50.6 $\pm$ 18.9	74.8 $\pm$ 17.0
Plasma cortisol (μg/100 mL)	0.29 $\pm$ 0.18	0.25 $\pm$ 0.22

Biological data on grubby are scarce but values obtained for variables examined were in the range observed in other marine fish species kept in captivity under similar temperature and salinity conditions (Audet et al. 1993). Biochemical indicators are largely recognized as useful tools for the assessment of fish health (Thomas 1990). They are sensitive to sublethal stressors and to the severity of the stressor. Consumption of diet contaminated by methylmercury in the present study did not induce a stress response.

Livers from both groups of fish exhibited large amounts of lipids in hepatic cells and displayed similarity in histological features. Liver was shown to be the site of a mercury pool in trout tissue (Baatrup et al. 1986), and liver histopathological changes were detected following waterborne mercury contamination (Sinovnic et al. 1980). The recognition of different histopathological changes in liver as stress indicators (Hinton and Lauren 1990) and the type of contaminant loading suggested that liver histology would be an efficient way to diagnose health

impairment in the present study. However, despite the presence of elevated methylmercury contamination in the liver of experimental fish, no histological changes were observed.

Similarly, no qualitative gill damage was observed in experimental fish compared to the controls; localized signs of lamellar epithelium necrosis were observed in two fish of each group. Quantitative measurements did not show any change in terms of number of mucous or chloride cells on both filaments (2 / 200 μm $\pm$ 0.23 (n=9); 3.9 / 200 μm $\pm$ 0.8 (n=9)) and lamellae ((0.2 / 200 μm $\pm$ 0.07 (n=9); 0.6 / 200 μm $\pm$ 0.17 (n=9)), or in terms of interlamellar distance (36.2 μm $\pm$ 2.4 (n=10)) or diffusion distance (6.5 μm $\pm$ 0.3 (n=10)). We examined the histology of the gills to detect the possible presence of water mercury contamination. Gills are continually exposed to pollutants present in water. A vast statistical review (Mallatt 1985) on fish gill structural changes indicated that observed changes mentioned in the literature were largely non-specific and that some particular types of lesions were frequently detected with waterborne heavy metal contamination. Thus in windowpane flounder (*Scophthalmus aquosus*), long-term sublethal mercury exposure induced gill epithelial response especially in terms of number of chloride cells and epithelial thickness (Pereira 1988).

The grubby seems to possess many biological characteristics which are highly suitable for use in mesocosm studies. It is well-adapted to the estuarine environment, inhabits coastal areas, eats a broad range of prey, is easy to stock and maintain healthy in captivity, and is small-sized, which allows a high density loading in mesocosms. However, the grubby is also highly resistant to modifications of its environment. Even though fish were exposed for a period long enough to cause significant contamination, our results clearly showed that dietary mercury contamination was not initially stressful to the fish. This is certainly a strong indication that more sensitive stress indicators have to be developed before measuring detectable adverse effects in the fish population when studying contamination from within the food chain. Further work will be conducted on other biomarkers such as stress proteins and macrophage responses using different contaminants such as organotin compounds and weathered oil residues.

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