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Chronic Dietary Toxicity of Methylmercury in the Zebra Finch, *Poephila guttata*

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Mercury (Hg) contamination of the environment through anthropogenic activity continues to be significant (Evans, 1986; Meger, 1986; Bjorklund et al., 1984), and has resulted in the accumulation of elevated levels of Hg in invertebrates, fish and wildlife in certain Hg contaminated habitats (Barr, 1986; Gardner et al., 1978; Vermeer et al., 1973; Wren, 1986). In addition, the availability of methylmercury (MeHg), a highly toxic and readily absorbable form of Hg, to the food chain is enhanced at low pH (Wood, 1985), and this has resulted in higher concentrations of Hg in various biota which inhabit environments sensitive to acid precipitation (Stokes et al., 1983; Suns et al., 1980; Wiener, 1983; Wren et al., 1983).

The chronic dietary toxicity of MeHg has been investigated in a number of bird species including mallard ducks (Anas platyrhynchos) (Heinz, 1974, 1976; Pass et al., 1975), black ducks (Anas rubripes) (Finley and Stendell, 1978), pheasants (Phasianus colchicus) (Fimreite, 1971; Borg, 1969), quail (Coturnix coturnix) (Stoewsand et al., 1974), and chickens (Gallus gallus) (March et al., 1983; Tejning, 1967). Except for the subchronic feeding study of Finley et al. (1979), the effects of MeHg on small passerines have not been studied. The present report describes the tissue accumulation and toxicity of MeHg in zebra finches (Poephila guttata) in response to chronic dietary exposure.

MATERIALS AND METHODS

Adult male/female pairs of aviary-bred zebra finches were randomly assigned to one of four dietary groups of 8 pr/group. Group 1 (Control) received a commercially available finch mash (Ralph Moore Ltd., Norwich, Ontario) and distilled, deionized water ad lib. The Hg concentration of the Control diet was <0.02 ug/g dry wt. Groups 2, 3, and 4 received the same diet supplemented with MeHg at a level of 1.0, 2.5, or 5.0 ug

Hg/g dry wt. Daily lots of mash were prepared by diluting appropriate volumes of a 1000 ppm Hg stock solution (CH_3HgCl dissolved in 70% ethanol and stored at -20° C.) with H_2O and mixing thoroughly with the dry mash in a wt.:vol. ratio of 2:1. The four Groups were maintained on their respective diets for 76 days during which time they were monitored daily for the possible development of behavioral abnormalities. All birds were sacrificed on day 77 by chloroform inhalation, and liver, kidney, and brain were removed immediately, and frozen until required.

Hg analysis was performed by the Ontario Research Foundation, Mississauga, Ontario. Total Hg was determined by cold vapor atomic absorption spectrophotometry (Hatch and Ott, 1968) after complete oxidative digestion of tissue samples in $H_2SO_4/HNO_3/KMnO_4$. Reagent blanks contained <0.009 ug Hg, and the recovery of Hg from spiked samples was typically >95%.

All Hg concentrations in the following Discussion are total Hg, ug/g wet wt. if referring to a tissue, and ug/g dry wt. if referring to a dietary Hg concentration, unless otherwise stated.

RESULTS AND DISCUSSION

Kidney, liver, and brain tissue from control finches contained <0.04, <0.02, and <0.01 ug Hg/g respectively. Accumulation of Hg in the three tissues was dose-dependent and linear over the range of dietary Hg used. Figure 1 shows the relationship between the dietary Hg level and the resulting organ levels. For all three tissues, the coefficient of correlation (r) was >0.9. The relative rates of accumulation of Hg were kidney ~ liver > brain. No statistically significant differences were observed between males and females with regard to terminal Hg concentration in any of the tissues analysed. In all three Hg-supplemented dietary Groups, liver and kidney accumulated Hg to approximately 30-fold, and brain 13-fold, the corresponding dietary Hg level when a dry wt.:dry wt. comparison was made. The estimated daily exposure to ingested Hg for the three Hg-supplemented Groups is given in Table 1.

None of the finches in Groups 1-3 developed signs of MeHg intoxication over the course of the experiment, nor was there any mortality in these Groups. In Group 4, however, behavioral signs typical of MeHg intoxication developed in certain individuals, the earliest indication being observed around day 40.

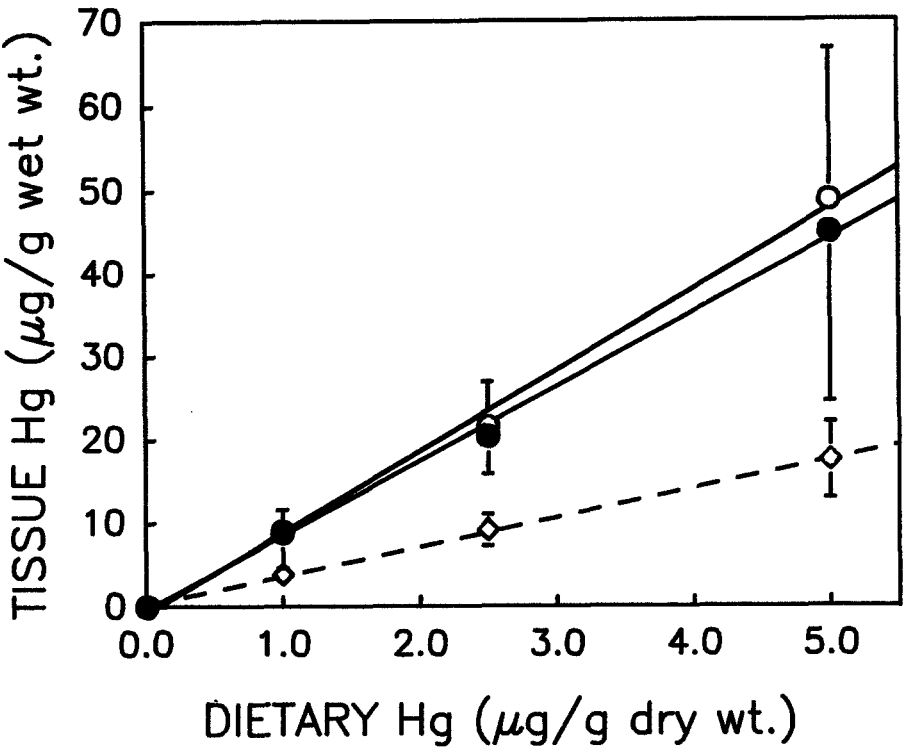

Figure 1: Best-fit linear regression lines for the accumulation of Hg in kidney (—○—) liver (—●—) and brain (--◇--) of zebra finches 77 days after beginning to consume <0.02, 1.0, 2.5, or 5.0 ug/g dietary Hg.

Table 1. Estimated intake of dietary Hg by zebra finches

	Daily Exposure		Cumulative Exposure	
	ug/g body wt.	ug/bird	ug/g body wt.	ug/bird
Grp 1	<0.007	<0.1	<0.53	<7.6
Grp 2	0.35	4.9	26.6	372
Grp 3	0.88	12.3	66.9	935
Grp 4	1.75	24.5	133	1862

Affected birds became lethargic, their feathers were fluffed, and they had difficulty balancing on their perches. When induced to fly, they fluttered weakly and had difficulty landing properly. The earliest death was on day 68, and by the end of the experiment (day 77), 25% (4) of Group 4 birds had died, and of the remainder, 40% (5) showed signs of neurological impairment.

I attempted to distinguish among dead, symptomatic, and asymptomatic birds in Group 4 on the basis of their tissue-Hg concentrations (Table 2). Generally, neurological signs did not develop until Hg concentrations of 15 ug/g or greater had accumulated in the brain, and at least 30-40 ug/g in liver and kidney. Finches dying of MeHg poisoning subsequent to developing neurological impairment did not show any further elevation of brain-Hg, but liver- and kidney-Hg concentrations tended to be higher (>50 ug/g) than in neurologically affected finches which had not died by the end of the experimental period. Asymptomatic birds of Group 4 typically had liver- and kidney-Hg concentrations <40 ug/g and brain-Hg <15 ug/g. Brain-Hg levels of finches in Group 3, none of which developed signs of MeHg intoxication, were 7-11 ug/g.

Table 2. Hg Residues (ug/g wet wt.) in dead, neurologically impaired, and asymptomatic birds of group 4 (mean±SD)

	Dead (n=4)	Impaired (n=5)	Asymptomatic (n=7)
Liver	73±16	43±13	30.5±6.1
Kidney	65±13	55±18	35.5±9.6
Brain	20±5	20±4	14.1±3.1

It may be suggested from the above results that a critical concentration of Hg must be attained in the zebra finch brain before neurological impairment can be induced, but that once such levels have accumulated, further increases are not necessary for death to occur. The results of other studies, in which termination of MeHg exposure at the onset of neurological signs did not prevent later mortality (Hill and Soares, 1987; Fimreite and Karstad, 1971), support this conclusion. However, the possible involvement of the liver and kidney should also be considered. In the present study, hepatic Hg concentrations were the most useful measure for discriminating among dead, neurologically impaired, and asymptomatic birds in Group 4 (Table 2).

A dietary level of 5 ug/g Hg as MeHg was sufficient to cause a significant degree of neurological impairment and death in zebra finches. However, mallard or black ducks chronically fed diets containing 3.0 ug/g (Heinz, 1976; Finley and Stendell, 1978), 3.3 ug/g (Gardiner, 1972), 2.8 ug/g (Pass et al., 1975), or 5.0 ug/g (Bhatnagar et al., 1982) Hg as MeHg experienced no mortality nor did they develop neurological dysfunction. Similarly, adult pheasants and chickens were able to tolerate 9-10 ug/g Hg in the diet without

developing symptoms of MeHg poisoning (Fimreite, 1971; Tejning, 1967). A major reason for the high sensitivity of zebra finches to the relatively low concentrations of MeHg used in the present investigation is probably their high metabolic rate, and the large amount of food which they consequently consume relative to their body wt. I have calculated that an average zebra finch weighing about 14 g consumes 300 - 400 g food/kg body wt./day. In contrast, Fimreite (1971) estimated that pheasants weighing about 1.2 kg consumed only 50-60 g food/kg body wt./day. Therefore, the dietary Hg concentration required for pheasants to achieve a daily dose of Hg equivalent to that of zebra finches would be 5-8-fold that contained in the corresponding finch diet. A similar relationship likely holds for other larger birds, such as ducks, as well. Thus, 15 ug/g Hg in feed was required to induce weight loss and paralysis in mallards (Bhatnagar et al., 1982).

In ducks, brain- Hg concentrations <10 ug/g, and/or liver and kidney levels <50 ug/g were not sufficient to produce mortality or obvious neurological dysfunction (Bhatnagar et al., 1982; Finley and Stendell, 1978; Heinz, 1976; Pass et al., 1975). However, paralysis in mallards (Bhatnagar et al., 1982), and a high incidence of mortality in quail (Stoewsand et al., 1974) were observed at brain-Hg concentrations of 18-25 ug/g, a level similar to that observed in the present study in finches which developed signs of MeHg intoxication. In a comparative study, Gardiner (1972) fed MeHg at a dietary concentration of 33 ug Hg/g feed to pheasants, Rouen ducks, and broiler-type chickens, and noted an 80-90% mortality in the ducks and pheasants over the 35 day experimental period, but only a 7.5% mortality in the chickens. It is perhaps significant that liver-Hg concentrations in the ducks and pheasants were 50-70 ug/g, but <50 ug/g in the chickens. In the present study, liver-Hg <50 ug/g was not associated with mortality in zebra finches (Table 2). In the only other study of dietary MeHg toxicity in passerines, Finley et al. (1979) fed a diet containing 40 ug/g MeHg to a variety of species and observed the time required to reach 30% mortality. The most sensitive species, the grackle (Quiscalus quiscula), did so within 6 days. Brain-Hg levels (20-22 ug/g) were similar to those of finches which died of MeHg intoxication in the present study (Table 2).

The results reported herein, when compared with data from numerous previous studies, support the contention that tissue-Hg concentrations which are associated with neurological impairment and/or death in birds are frequently similar despite differences in species, body size, the Hg content of the food consumed, or the time required for the birds to become affected. It should

be noted, however, that certain factors are able to greatly modify the toxicity of MeHg. Chief among these factors is dietary selenium (Se). Stoewsand et al. (1974) observed that 5 ug/g Se in the diet afforded complete protection from the toxic effects of 40 ug/g dietary Hg (as MeHg) in quail even though tissue-Hg levels were highly elevated. A knowledge of tissue-Se concentrations, along with tissue-Hg levels, is thus important if any meaningful judgement is to be made regarding the possible health effects in birds based on the concentrations of Hg accumulated in target organs.

Reproductive effects in birds due to MeHg exposure occur at considerably lower dietary Hg concentrations than those required to produce overt signs of MeHg intoxication. Pheasants and ducks experienced significant decreases in hatchability of eggs, and an increased incidence of embryonic and hatchling mortality when consuming a diet containing 2-3 ug/g Hg, even though the adult birds exhibited no signs of MeHg intoxication and had not accumulated high tissue concentrations of Hg (e.g. liver-Hg = 2-12 ug/g) (Fimreite, 1971; Heinz, 1976). Brain-Hg concentrations as low as 3-7 ug/g can be lethal to newly hatched ducklings (Finley and Stendell, 1978; Heinz and Locke, 1976). Levels at least 4-times these values are required to produce the same response in adults of a variety of species (Finley et al., 1979; Stoewsand et al., 1974; present study). Adult mallards with brain-Hg levels 3-10 ug/g developed no brain lesions and did not exhibit behavioral signs of MeHg intoxication (Bhatnagar et al., 1982; Pass et al., 1975). All one-day-old chickens consuming 7.8 ug/g dietary Hg died within 3 weeks after accumulating brain-Hg concentrations of only about 8.5 ug/g (Soares et al., 1973), a finding which stands in stark contrast to the response of adult chickens which demonstrated a low mortality (7.5%) when exposed to dietary MeHg even at concentrations as high as 33 ug/g (Gardiner, 1972). These examples serve to illustrate that avian embryos and hatchlings are far more sensitive to the toxic effects of MeHg than are adult birds. The fact that significant reproductive impairment can occur in birds at dietary Hg concentrations which are only about 1/5 those required to produce neurological impairment in adults of the same species raises the possibility that reproductive effects in small birds, as exemplified by the zebra finches in the present study, may occur at dietary Hg levels of 1 ug/g or less. Such possibilities have yet to be explored.

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