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Toxicity and Tissue Uptake of Methylmercury Administered Intraperitoneally to Rainbow Trout (*Salmo gairdneri* Richardson)

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The acute toxicity of methylmercury (MeHg) to fish has been examined by administration of MeHg in the water (McKIM et al. 1976); oral injection (MIETTINEN et al. 1970); and intraperitoneal injections (present study). McKIM et al. (1976) found that the 96 h LC50 for yearling brook trout (102 g) was 65 ug Hg/L. MIETTINEN et al. (1970) found that the 30 day LD50 for MMC (methylmercuric chloride) administered orally in three to four portions was 20 - 25 mg MMC/kg of body weight.

An intraperitoneal (IP) injection was employed as the mode of toxicant application in the present study since administration of MMC in food (oral catheterization) or in water generally leads to a great deal of between animal variability in the rates and magnitude of MMC accumulation. These differences in accumulation may be due to differences in the quantity of food ingested, regurgitated, and MMC passed in the feces; and to differences in the intensity of activity and the physical parameters of the MMC test water. The use of an IP injection facilitates the examination of sublethal effects of metabolizable toxicants such as MeHg since one is able to reproduce tissue levels of the toxicants which are similar to those found in some natural populations. This then allows measurement of some parameter to be made at different tissue concentrations of the toxicant.

Different tissues possess varying capacities for the uptake and the elimination of MeHg and show differences in the magnitude of pathological damage. For instance, GIBLIN & MASSARO (1973) state that the brain of rainbow trout was found to accumulate and release MeHg at a slower rate than other tissues probably because the blood brain barrier exerts some control over the passage of MeHg. However, it has been reported that following long exposure, the Hg concentration in the brain may exceed that of the muscle (McKIM et al. 1976, MIETTINEN et al. 1970). This may be a consequence of the high concentration of sulfhydral containing compounds in the nervous system which bind irreversibly with MeHg (MANALIS & COOPER 1975). The magnitude of neuropathological damage has also been shown to be highly correlated with regional differences in the concentration of selenium (EVANS et al. 1977).

Many studies involving the neurotoxicity of MeHg have stressed the importance of the latency of neurological symptoms subsequent to MeHg exposure (EVANS et al. 1977, BERLIN et al. 1973). The

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duration of this latent phase is dependent on the frequency of MeHg administration, size of the dose, and species of the test organism. Most studies of primates cite latencies of 20 - 25 days depending on the behavioral parameter measured.

The objective of the present study was to determine the 15 day LD50 for a single intraperitoneal dose of MMC and secondly to measure the concentration (tissue uptake) of Hg in the brain, eye, and epaxial musculature 30 days following an injection of different sublethal doses of MMC.

MATERIALS AND METHODS

Toxicity

Four flow through, temperature controlled (15 ± 1°C) 20-L aquaria were used. The test fish were obtained from Duggans Trout Farm (Namao, Alta.) and Sam Livingston Fish Hatchery (Calgary, Alta.). The size of the fish ranged from 10 - 20 cm (fork length). Each fish was anesthetized with 100 ppm MS222 (ethyl-m-amino benzoate methanesulfonate) then weighed and tagged. The fish were distributed to their respective tanks by stratified random assignment. They were given a one week acclimation period before injection and fed EWOS pellets ad libitum daily. The methylmercuric chloride (MMC) solution consisted of MMC salt dissolved in a 0.02 M Na_2CO_3 solution and adjusted to a pH of 7.2 with 5% HNO_3 yielding a solution containing 2.57 mg of MMC per mL. The control solution was 0.02 M Na_2CO_3 adjusted to a pH of 7.2 with 5% HNO_3. The MMC was administered weight specifically, intraperitoneally, just anterior to the pelvic fins. The fish in the control group were injected with an array of injection volumes equivalent to those given to the experimental fish.

Three separate experiments were carried out to estimate the 15 day LD50. The protocol of these experiments is illustrated in Table 1a.

The time of death was recorded for each fish and the surviving fish were all sacrificed by placing them in a 1000 mg per L solution of MS222 on the fifteenth day of the experiment. The fish were then frozen and held at -30°C until total mercury analysis was performed.

Whole fish were wet ashed in preparation for total mercury analysis. Each individual was weighed and subsequently placed in 100 mL of concentrated H_2SO_4 and 50 mL of concentrated HNO_3 for 24 h at room temperature. Three 3 mL aliquots of each digested fish were taken for total mercury analysis. Each aliquot was then oxidized by adding 7 mL of 7% $KMnO_4$ and reduced by 0.1 mL of a 1% solution of $SnCl_2$. The analysis for total mercury content in digested samples utilized a flameless atomic absorption spectrophotometer (ARMSTRONG & UTHE 1971, MUNSON, In prep.).

TABLE 1a

Experimental Protocol and Percent Mortality in 15 Days.

Experiment I	Dose	0[1]	10[2]	15	20
	n	6	6	6	6
	% mortality	0	83	100	100
Experiment II	Dose	0	2	5	8
	n	6	6	6	6
	% mortality	0	33	83	67
Experiment III	Dose	0	2	4	6
	n	9	8	8	8
	% mortality	0	0	13	50

[1]controls injected with saline at a similar array of injection volumes to those of the experimentals.

[2]all dose values are in mg of Hg/kg of body weight. Note that MMC was injected, however the values were reported as Hg since 80 percent of MMC is Hg. Therefore if we inject 12.9 mg MMC/kg we have actually injected 10 mg HG/kg.

TABLE 1b

Experimental Protocol for Tissue Uptake Experiments.

Experiment I	Dose	0	0.3[1]	0.6	0.8
	n	6	6	6	6
Experiment II	Dose	0	0.3	0.6	0.8
	n	6	5	5	5

[1]toxic units corresponding to 0, 1.8, 3.6, 4.8 mg Hg/kg (0, 2.3, 4.6, 6.2 mg MMC).

A standard curve was estimated for each set of samples of fish, using a mercuric chloride standard (absorbance versus ug Hg). Chemical blanks were used to test for background concentrations of Hg.

A recovery of 87 - 102% of the total mercury present was determined by recovery experiments using samples containing known amounts of $HgCl_2$ and CH_3HgCl. Accuracy of the analysis was also monitored by measuring the total Hg content of standard orchard leaves, 0.147 ± 0.056 S.D. ug per g (n = 24) standard reference material 1571. The Hg levels obtained were 95% of the values reported (0.155 ± 0.015 ug per g) for these leaves (MUNSON, In prep.).

Tissue Uptake of MeHg

Two experiments were employed to examine the effect of dose on tissue Hg concentration and to determine the difference in Hg uptake between tissues, 30 days after the fish were intraperitoneally injected. The test fish were obtained from Sam Livingston Fish Hatchery and ranged in size from 10 - 20 cm in fork length. All fish for a given experiment were placed together in a 200-L continuous flow through tank and allowed one week acclimation at 15 ± 0.1°C. The fish were fed EWOS pellets ad libitum daily.

The fish were divided into four dose classes: control, 0.3, 0.6, 0.8 toxic units. The dose equivalent to one toxic unit was taken to be the 15 day LD50 which had previously been determined experimentally. The corresponding dose levels were: 0, 1.8, 3.6, 4.8 mg Hg (0, 2.3, 4.6, 6.2 mg MMC) per kg of body weight.

The experimental protocol is given in Table 1b. Each fish was anesthetized, weighed, tagged, injected intraperitoneally and then returned to the holding tank. The injection solutions were identical to those utilized in the toxicity test.

Fish were sacrificed on day 30 and the tissue samples were removed, placed in glass containers and frozen at -30°C. Total mercury analysis of these samples was identical to that for whole fish except the smaller quantities of tissue were digested in 2 mL of concentrated H_2SO_4 and 1 mL of concentrated HNO_3. Total mercury was expressed in ug Hg per g of wet tissue weight.

RESULTS

Toxicity

Figure 1 illustrates the percent mortality 15 days after injection as a function of the dose of methylmercury administered. A regression analysis was performed in the 2 - 10 mg range of the independent variable to minimize error in the prediction of the 15 day LD50 (10 mg was chosen as a maximum dose for the regression since curve inflection occurs at this level of the independent variable). The y-intercept was not significantly different ($P > 0.01$) from the origin, therefore a line was fitted to pass through the origin and the $\bar{X}$, $\bar{Y}$ point for the 2 to 10 mg range. The standard error of this line was estimated to be 4.1 and the regression coefficient (slope) was estimated to be 8.7.

The 15 day LD50 for a single intraperitoneal injection in rainbow trout is 5.7 ± 0.4 mg Hg per kg of body weight, determined by interpolation. The mean uptake (body burden) for this was 5.1 ± 0.6 ug Hg per g of body tissue (Fig. 2) indicating that the fish accumulated approximately 90% of the dose administered.

The fish from toxicity experiments two and three (Table 1a) were used to measure the body burden of Hg (Fig. 2). A one way

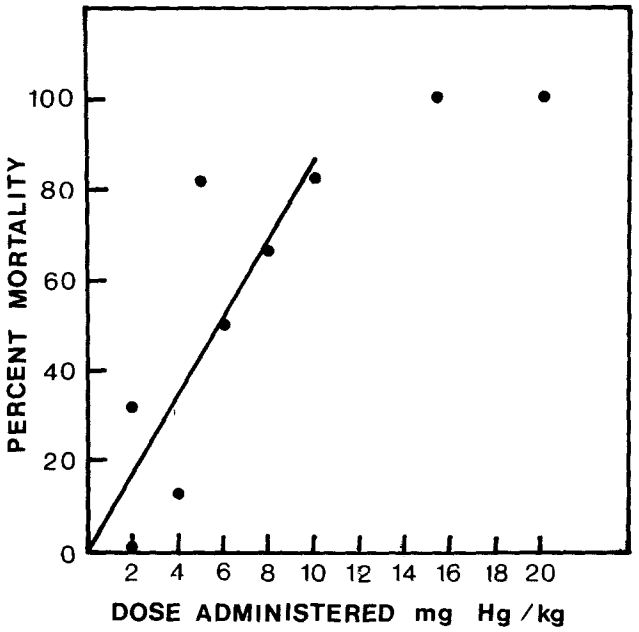

Figure 1. Percent mortality versus the dose administered (mg Hg/kg of body weight). A regression analysis was performed on 2 - 10 mg range of independent variable and the equation is Yi = 0 + 8.7 (Xi).

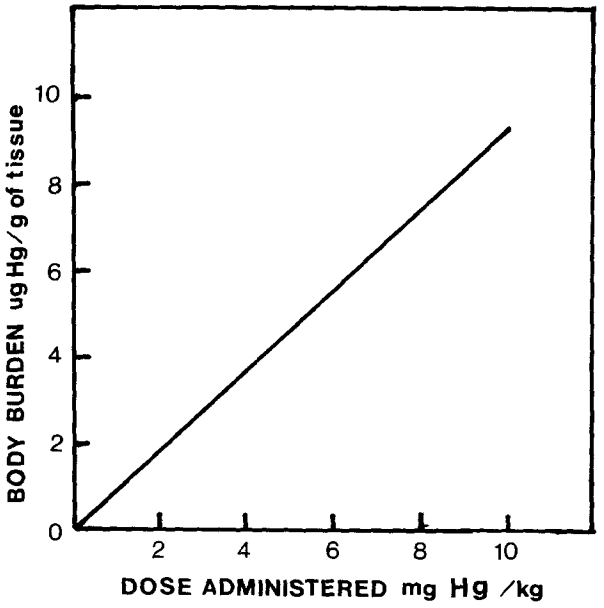

Figure 2. Body burden or the whole fish mercury content (ug Hg/g of tissue versus the dose administered (mg Hg/kg of body weight). The regression equation is Yi = 0 + 0.91 (Xi).

analysis of variance demonstrated a significant difference in the mean uptake between the various dose classes ($P < 0.001$). The y-intercept of the regression equation was not significantly different from origin ($P > 0.01$) and thus a straight line was fitted through 0 and $\bar{X}$, $\bar{Y}$ yielding a regression coefficient of 0.91 and a standard error of 0.11 (Fig. 2).

For the remainder of this paper the 15 day LD50 will be referred to as 1 toxic unit (tu) and any dose less than 1 toxic unit will be considered as a sublethal dose.

Tissue Uptake

A one way analysis of variance indicated a significant difference between the mercury content of the three tissues tested (brain > muscle > eye) (all $P < 0.001$) for each dose level: 0.3, 0.6, 0.8 tu (Fig. 3). The concentration of mercury in each tissue increased significantly ($P < 0.001$) with increasing sublethal dose of methylmercury.

The y-intercept for the regression equations of all three tissues was not significantly different from origin (all $P > 0.01$) and their respective regression coefficients (method previously explained) and standard errors are as follows: 4.76 ± 0.49 (brain); 3.91 ± 0.39 (muscle); 2.03 ± 0.26 (eye).

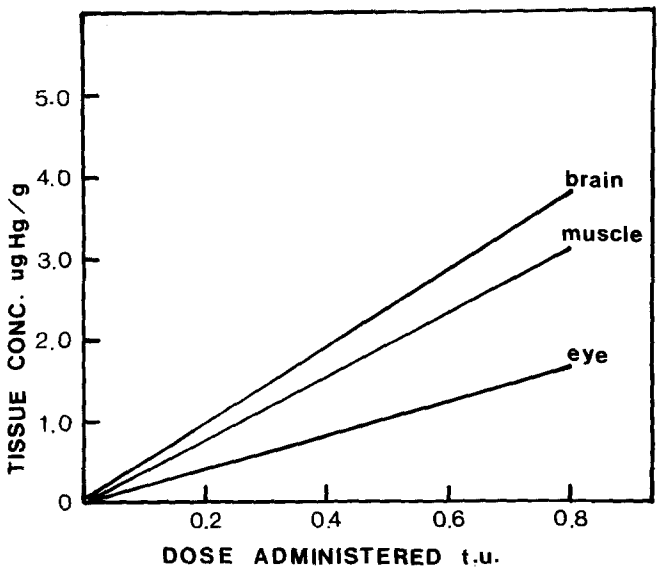

Figure 3. Tissue concentration; brain, muscle, eye; of mercury (ug Hg/g of tissue) versus the dose administered (toxic units: 0.3, 0.6, 0.8). The respective regression equations are: $Yi = 0 + 4.76\ (Xi)$, $Yi = 0 + 3.91\ (Xi)$, $Yi = 0 + 2.3\ (Xi)$.

DISCUSSION

Acute toxicity studies dealing with the intraperitoneal or oral mode of administration of methylmercury have been shown to be influenced by the rate of application of the toxicant. This unfortunately, prevents the establishment of standard methods and therefore reduces the comparability of results.

The LD50 of the present study and that of MIETTINEN et al. (1970) differ unequivocally MIETTINEN et al. (1970) applied 9.6 - 19 mg MeHg per kg body weight in three or four portions with a two day interval, whereas we used a single dose of 2 - 10 mg Hg per kg of body weight. An 8 or 10 mg Hg/kg dose was highly lethal in our experiment whereas this same dose administered in several portions over a period of days resulted in survival beyond the thirtieth day of the test period (MIETTINEN et al. 1970).

It appears that the retention of methylmercury differs with respect to the method of application. Intraperitoneal injections resulted in retention of 90% of the dose administered, on the other hand 53% of the dose orally administered was retained (MIETTINEN et al. 1970). However, once MeHg is in the circulatory system, elimination occurs through both the feces and urine regardless of the mode of administration (MUNSON, in prep.).

The duration of toxicity test is an important consideration when designing acute lethality experiments of this nature. The mode of toxicity of MeHg associated with chronic exposure to MMC appears to be via pathological changes in the nervous system (EVANS et al. 1977, BERLIN et al. 1973, MANALIS & COOPER 1975, BÄCKSTRÖM 1969, MIYAKAWA et al. 1970). Therefore, a reasonably long period of chronic exposure is necessary for the MeHg to accumulate to levels which inflict neuropathological damage. Moreover, this latency between injection and the onset of behavioral aberrations, seems to be dependent on the volume and frequency of dosing as well as species specificity (EVANS et al. 1977).

McKIM et al. (1976) report that the concentration of MMC in any given tissue at any time following exposure is dependent on the concentration of MMC in the water. Our results support this contention indirectly since we were able to demonstrate a difference in the uptake of Hg between the dose categories within each tissue. In fact the relationship showed a significant ($P < 0.001$) degree of linearity for each tissue.

Several laboratory studies have indicated that brain concentrations of mercury exceed epaxial musculature concentrations, however this is by no means a consistent trend for all species of fish. The difference in magnitude of uptake between tissues is illustrated in Figure 3. The high brain levels could be explained by preferential accumulation in nervous tissue or by a slow turnover of MeHg in the brain compared with other tissues such that

after 30 days the other tissues had lost most of their accumulated MeHg but the brain had not. The chemical nature of the nervous system could also influence the mobility of MeHg (MANALIS & COOPER 1975).

The relationship between body concentrations of Hg and dose does not differ from that found for muscle concentration of Hg (slope test $P > 0.05$) and dose in this study. This has also been shown by McKIM et al. (1976) and by MIETTINEN et al. (1970) and they point out that this is obviously so since the muscle tissue represents the largest component of the body.

The small variance in the dose response relationship which was found in the three tissues studied, indicates that intraperitoneal administration maybe particularly suitable for sublethal behavioral or physiological studies dealing with MeHg. In addition, we were able to produce some tissue levels which are near those that are commonly found in areas of mercury contamination.

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