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Sample Weight and Digestion Temperature as Critical Factors in Mercury Determination in Fish

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The concern about mercury (Hg) pollution of the marine environment started with the well publicized case of Minimata (Japan) where in the 1950s several persons died or became seriously ill after consuming fish or shellfish containing high levels of methylmercury. It is now accepted that Hg contaminated seafoods constitute a hazard to human health (Skerfving, 1972). To safeguard humans, accurate determination of Hg in marine biota is, therefore, very important. Two steps are involved in the determination of total Hg in biological materials: (a) decomposition of organic matrix (sample preparation), and (b) determination of Hg in aliquot samples. Although the procedures for determining Hg using the cold vapor technique are well established, sample preparation procedures have not been standardized (Sadiq and Zaidi 1983). In general, samples of marine biota have been prepared by digesting different weights at different temperatures, by using mixtures of different chemicals and of varying quantities, and by digesting for variable durations. Digestion temperature, composition and quantity of the oxidizing acids, and the duration of digestion have been evaluated by Sadiq and Zaidi (1983). The objectives of the present paper were to evaluate the effects of sample weights and digestion temperatures on Hg determination in fish.

MATERIALS AND METHODS

Two commercially important fish species [grouper, Arabic name hamour, and emperor Arabic name shaeri] from the Arabian Gulf were used in this study. Effects of the digestion temperatures and the weights of sample for digestion on Hg determination were investigated.

To investigate the effects of temperature on digestion, two g of homogenized fish tissue were placed in 250 mL BOD bottles or 100 mL digestion tubes. BOD bottles were used for digestion at 24, 60 and 80 ^{o}C, while digestion tubes were used at 100, 120 and 140 ^{o}C. Eight mL conc H_2SO_4 and four mL conc HNO_3 were added to each sample. The 24 ^{o}C digestion was carried out for 24 h. The other samples were heated for three h at the temperature being investigated. After initial step, the samples were allowed to cool and sufficient $KMnO_4$ (10 g/100 mL) was added to each sample to ensure that color persisted during the next heating step. The samples were then heated at 80 ^{o}C for 30 minutes. After cooling, any remaining $KMnO_4$ was reduced using a slight excess (usually 5 mL)

* Send reprint requests to the above address.

of hydroxylamine hydrochloride (12 g/100 mL). Mercury in the aliquots was determined by cold vapor technique using Milton-Roy mercury analyzer and stannous chloride as reductant. Each treatment was replicated 5 times.

The effects of sample weight on the digestion were investigated using one, two and five gram samples. Digestions were performed at 24, 60 and 100 ^{o}C in 250 mL BOD and at 120 ^{o}C in 100 mL glass digestion tubes. Seven mL conc H_2SO_4 and 3.5 ml conc HNO_3 were added to each sample. The remainder of the procedure was as described above.

To investigate recoveries of Hg, 10 additional replicates, with exactly same amount of tissue, were prepared under each treatment. Five of these were spiked with 0.3 mg Hg kg^{-1} tissue and the remainder five were kept as control. These samples were processed along with others under same experimental conditions. Mean concentration of Hg in the control and spiked samples were determined and recovery of spiked Hg was calculated from this information. The precision of sample preparation procedure for each treatment was determined by preparing 10 samples in the same way and determining Hg. The relative standard deviation of these data were found to be below 10 percent. The Instrumental precision was determined by analyzing 15 times same sample and computing standard deviation of these data. The relative standard deviation of instrumental was found to be less than 3 percent. Several Standard Reference Materials (SRM) were obtained from the United States Bureau of Standard (USNBS). The quantities of these SRM's were not enough to include in all treatments. Therefore, 1.0 g of all SRM's were digested at 120 ^{o}C for three h and mercury was determined as descibed above. The results of these determinations are summarized in Table 1.

Table 1 Determination of Hg (mg kg^{-1}) in SRM's.

Standard Reference Material	This Study	USNBS
River sediment, SRM 1645	1.03 0.1	1.1 0.5
Esturine sediment, SRM 1646	0.088 0.021	0.063 0.012
Bovine liver, SRM 1577a	<0.01	0.004 0.002
Oyster tissue, SRM 1566	0.054 0.008	0.057 0.015
Mercury in water, SRM 1641b	1.56 0.05	1.52 0.04
Fish sample MA-M-2/TM*	0.87-0.95	0.85-1.06

* Sample obtained from IAEA, Monaco as part of interlaboratory calibration study.

RESULTS AND DISCUSSION

Grouper and emperor tissue samples were digested at room temperature, 60, 80, 100, 120, and 140^{o}C and total Hg concentrations were determined in these digestates. The mean concentration data are presented in Figure 1. Both in emperor and grouper, concentrations of total Hg increased gradually with an increase in the sample digestion temperature. Maximum concentrations of Hg were found in samples digested at 100 and 120 ^{o}C and were statistically similar ($P < 0.05$). Comparatively lower Hg concentrations were found in samples

digested at 24, 60 and 80 °C and were probably due to incomplete or partial digestion of organic matter. Concentrations of total Hg were lowest in samples digested at 140 °C. This loss was presumed due to volatilization of Hg. These results challenge the common view that digestion of fish tissue above 60 °C results in volatilization losses. In fact, Hg volatilization was noticed only in samples digested at 140 °C.

To further check on volatilization, recoveries of the spiked Hg in samples digested under similar conditions were determined. These data are plotted in Figure 2. Recoveries of spiked Hg followed a trend similar to that of the total Hg concentrations (Figure 1). Recoveries were found the lowest at 24 °C, increased to maxima at 100 and 120 °C and decreased again at 140 °C. It is conceivable that oxidation of fish tissue was enhanced with an increase in temperature, thus increasing Hg recoveries. Poor recoveries of spiked Hg at low temperatures were a surprise. These observations suggest that inorganic-spiked Hg was bound to the partially oxidized organic matter in such a way that it was not reducible. This hypothesis needs to be tested further.

Volatilization of Hg was suspected in the samples digested at 140 °C whereas poor recoveries were observed in the samples digested at temperatures below 100 °C. It is, therefore, concluded that sample digestion should be carried out between 100 and 120 °C. Some investigators have digested fish samples above 120 °C and reported good Hg recoveries (Hornung et al. 1984; Luten et al. 1987). They probably had used pressure digestion bombs which known to reduce Hg volatilization. Many investigators determined Hg in samples below 60 °C (Arnson et al. 1976; Ashraf and Jaffar 1989; Braune 1987; Uthe et al. 1970; Walting et al. 1981; Zook et al. 1976). Excellent recoveries were reported by Ashraf and Jaffar (1989), Braune (1987), Uthe et al. (1970), and Zook et al. (1976). We could not reproduce these recoveries, especially of Ashraf and Jaffar (1989) and Braune (1987). The results of this paper suggest that these studies had probably underestimated Hg concentrations in fish.

From the foregoing, it could be concluded that partially oxidized organic matter plays an important role in Hg determination in fish. This was further investigated by taking one, two, and five g of fish edible tissue of emperor and grouper and digesting in the same amount of acid. The results of these determinations (mean of five replicates) are plotted in Figure 3. Two conclusions can be drawn from these data. Firstly, for a given sample weight, increasing digestion temperature from room temperature (24 °C) to 120 °C enhanced Hg recovery in both fish species. This is similar to the results given in Figure 1. Secondly, at a given digestion temperature, Hg concentrations decreased with an increase in sample weight. This effect was more pronounced at lower temperatures than at higher temperatures. The results of this study demonstrate that the sample weight used for total Hg determination was critical. Higher sample weight could result in appreciably underestimation of Hg concentrations in fish.

To further verify the above trend, effects of sample weight on the recoveries of spiked inorganic Hg were investigated. Results are given in Figure 4. Except for room temperature, recoveries of spiked Hg in one g samples were close to 90% in all treatments, although a slight enhancement can be observed with an increase in digestion temperatures. Significant ($P < 0.05$) decrease in the recoveries was found in samples where weight was two or five g, especially at low temperature. This situation improved when the samples were digested at 100 or 120 °C. Recoveries of spiked Hg in five g tissue were always below 80 %. Some

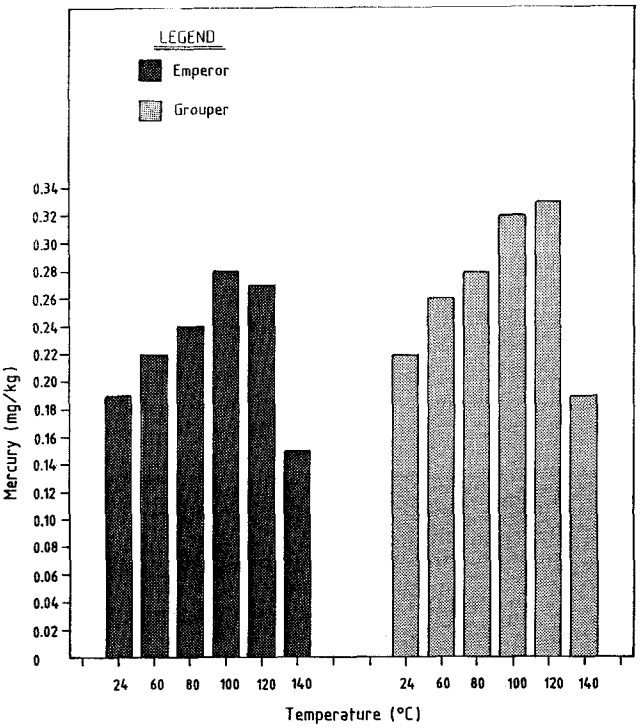

Figure 1 Effect of digestion temperature on mercury determination.

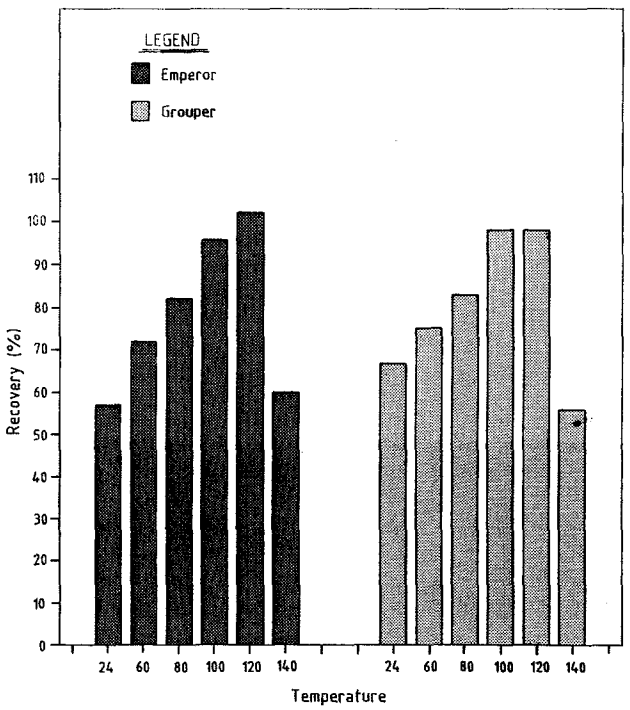

Figure 2 Effect of temperature on recoveries of mercury.

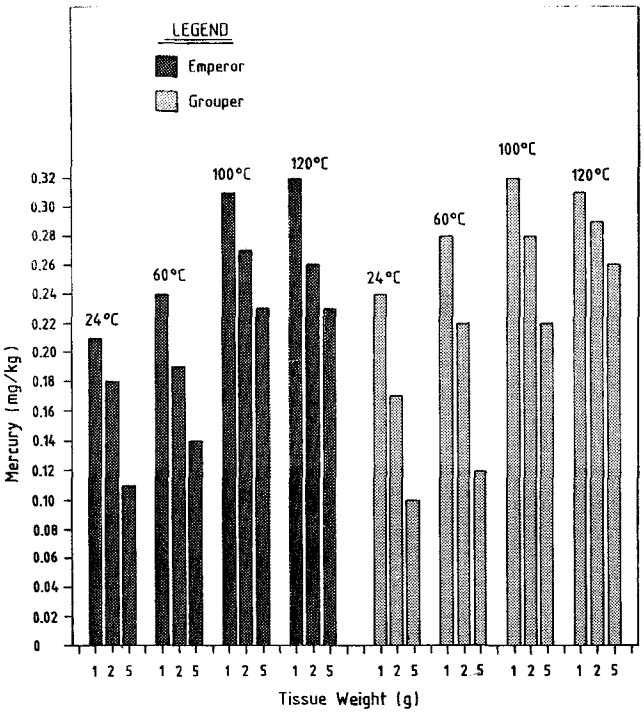

Figure 3 Effect of sample weight on mercury determination.

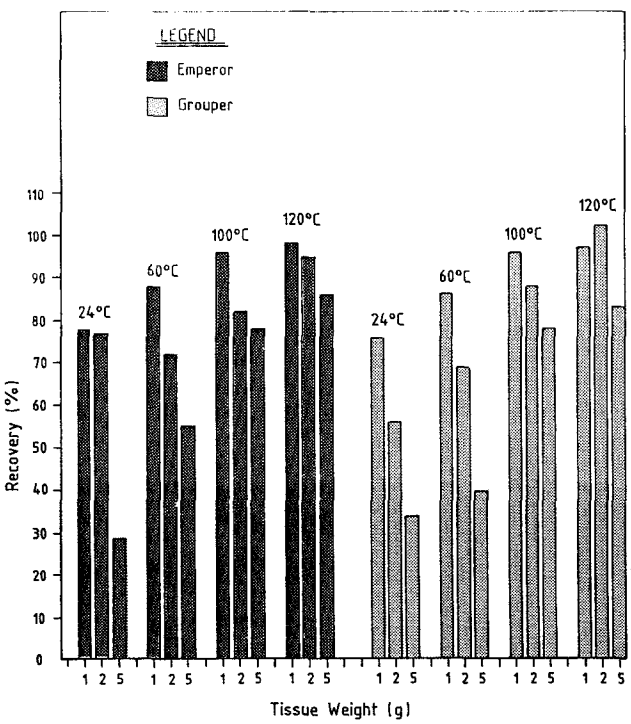

Figure 4 Effect of sample weight on recoveries of mercury.

investigators reported excellent recoveries, (Uthe et al. 1970, Zook et al. 1976) in samples digested below 60°C with sample weight less than 0.5 g. The results of this study support these data.

Some data on Hg determination in fish are given in Table 2. In summary, the amount of fish sample taken varied between 0.1 and 50 g, digestion temperatures from room temperature to 180 °C, and digestion time from 0.5 h to overnight (Table 2). Our results showed that digestion temperature and sample weight could be critical factors and that inter-comparison of the published data could not be achieved in a meaningful way. Because of the difficulties in the digestion procedure, it is suspected that many of these investigators might have under-estimated Hg hazards. From the foregoing, it could be emphasized that wide variations in the conditions of Hg determinations should be minimized and a standard procedure should be proposed and strictly followed.

Table 2 Mercury determination in fish.

Sample Wt(g)	Recovery %	Heating Time/ Temperature	Reference
10.0	97-106	---	Rivers et al. (1972)
1.0	---	3 h/150 °C	Luten et al. (1987)*
1.0	96-97	---	Rincon et al. (1987)
0.1-0.5	100-106	50-60 °C	Uthe et al. (1981)
5.0	91-96	Reflux	Mann & Holland (1977)
2.0	92-102	3 h/95 °C	Sadiq and Zaidi (1983)
0.3	99.8	overnight/ 55 °C	Zook et al. (1976)
2.5	80-90	3-3.5 h	Kureishy et al. (1979)
0.5	---	1 h/55 °C	Aronson et al. (1976)
2.0-3.0	good	4 h/55 °C	Watling et al. (1981)
50.0	>90	overnight/ room temperature	Ashraf & Jaffar (1989)
3.0-5.0	98-105	63-65 °C	Braune (1987)
1.0	---	overnight/ room temperature	Bull et al. (1981)
1.5-2.0	94-102	0.5 h/100 °C	Duve et al. (1981)
0.3-0.5	103	3 h/140 °C	Hornung et al. (1984)
5.0	80-100	---	Harakeh et al. (1985)
2	---	2 h/200 °C	Martincic et al. (1987)*
0.5	---	2 h/160 °C	Ferrara et al. (1987)*

--- No information available
* Used pressure vessel for digestion

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