Interference of Fats in the Determination of Mercury Residues in Fish by Atomic Absorption Spectrometry[1]

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The most commonly used methods for determining total mercury content of biological samples are the wet oxidation methods and these methods have been briefly reviewed by Smart (1). In general, these methods consist of digesting the samples with a mixture of oxidizing agents such as HNO_3-$KMnO_4$, HNO_3-H_2SO_4, H_2SO_4-$KMnO_4$, HNO_3-$HClO_4$, HNO_3-H_2O_2, etc. The mercuric ions are then extracted with a chloroform solution of dithizone and estimated by atomic absorption spectrophotometry. While numerous methods are available in the literature for determining mercury in biological samples, very few of them report on the interference of other materials. The object of the study reported here was to compare two methods for determining mercury residues in fish and also to investigate the effect of organic matter, particularly fat, on the determination of mercury by atomic absorption spectrometry.

Methods

Mercury determination by the method of Wobeser *et al.* (2) consisted of digestion of about 0.5 g fish tissue with concentrated H_2SO_4, followed by oxidation of the sample with potassium permanganate. Excess oxidant was destroyed by hydroxylamine hydrochloride and the pH of the solution was adjusted to 1.0 to 1.5. Mercuric ions were then extracted with a chloroform solution of dithizone and determined by atomic absorption spectrometry.

In the modification of this method the digestion, oxidation and the destruction of excess oxidants were carried out as mentioned above. The resulting solution was then extracted twice with 10 ml chloroform and the chloroform extracts were discarded.

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[1]Contribution No. 452

Bulletin of Environmental Contamination & Toxicology, Vol. 7, No. 5, 1972, © by Springer-Verlag New York Inc.

The mercuric ions were then extracted with chloroform solution of dithizone and determined by atomic absorption spectrometry.

In the method of Saha *et al.* (3) a 4 to 5 g sample of fish tissue was digested under reflux with concentrated nitric acid and perchloric acid to destroy all organic matter. The acidity of the digest was adjusted to approximately normal and hydroxylamine hydrochloride added to destroy excess oxidants. The solution was then extracted twice with 10 ml chloroform to remove any organic matter in the digest and the chloroform extracts were discarded. Mercuric ions were then extracted with chloroform solution of dithizone and determined by atomic absorption spectrometry.

The effect of the presence of fat on the determination of mercury by the methods of Wobeser *et al.* (2) and Saha *et al.* (3) was studied in the following way. About 5 or 10 mg of fat (lard, corn oil or cod liver oil) was added to the reagent blanks (without any fish tissue) in both the methods after destruction of the excess oxidant with hydroxylamine hydrochloride, the rest of the procedure followed as usual, and apparent mercury content determined. Mercury content of the reagent blanks (without addition of any fat or fish tissue) in both the methods was determined and the results were corrected accordingly.

All experiments were carried out in duplicate and average values are given in Table 1.

TABLE 1

Mercury content of fish as determined by two methods using atomic absorption spectrometry

	Mercury (ppm)		
Specimen No.	Wobeser *et al.* (2)	Wobeser *et al.* with modification	Saha *et al.* (3)
1	6.56	4.37	4.44
2	2.70	1.65	1.60
3	2.10	0.60	0.53
4	1.60	0.40	0.40
5	1.00	0.81	0.80

Results and Discussion

The method of Wobeser et al. (2) gave consistently higher values for the mercury content of the five fish specimens examined here (Table 1). In some cases these values were four times those obtained by the method of Saha et al. (3). However, removal of organic matter from the digest with chloroform prior to the extraction of mercuric ions with dithizone (modified Wobeser et al. method) gave results similar to those obtained by the method of Saha et al.

Addition of 5 or 10 mg of lard, corn oil or cod liver oil to the reagent blank in the method of Wobeser et al. (2) gave an apparent 'mercury' peak in the atomic absorption spectrometer (Table 2). Irrespective of the nature of the added fat 'apparent mercury' content was 0.27 µg/mg of fat. It is possible that there could be a few mg of fat remaining in the digest in the method of Wobeser et al. (2) which would interfere with the determination of mercury and give high values. Since Wobeser et al. used only 0.5 g of sample for each determination and if only 5 mg of fat remained in the digest it could increase the 'mercury content' of the sample by 2.7 ppm. This perhaps explains the higher values obtained by Wobeser et al. method (Table 1) as compared to that of Saha et al. However, addition of the same amounts of fat to the digest in the method of Saha et al. (3) gave no 'apparent mercury' as such fats were removed with chloroform before extraction of mercuric ions with dithizone.

These results indicate that in the wet oxidation methods of determining mercury in fish tissue any fat remaining in the digests can interfere in the estimation of mercury by atomic absorption spectrometry and give high values. This interference can, however, be easily removed by extraction of the digests with chloroform prior to the recovery of mercuric ions with dithizone.

TABLE 2

Apparent mercury content of reagent blanks in the method of Wobeser et al. (2) after adding fat to the digest*

Fat	Amount added (mg)	Apparent mercury µg	Apparent mercury µg/mg of added fat
Lard	5	1.25	0.25
	10	3.00	0.30
Corn oil	5	1.32	0.26
	10	2.78	0.28
Cod liver oil	5	1.26	0.25
	10	2.91	0.29
			av. 0.27

*Addition of same amounts of fat to the digests in the method of Saha et al. (3) showed no 'apparent mercury'.

Acknowledgement

The authors wish to thank G. Wobeser of the Western College of Veterinary Medicine, University of Saskatchewan, for providing the five fish specimens.

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