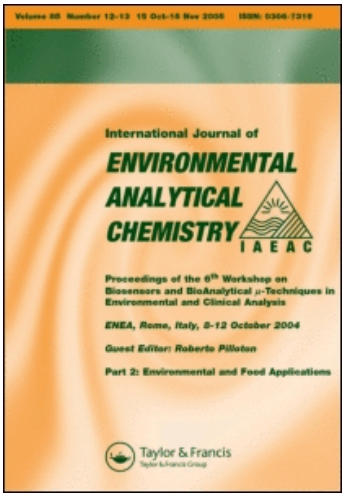

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Comparison of Different Analytical Techniques for the Determination of Organic Mercury

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Comparison of Different Analytical Techniques for the Determination of Organic Mercury

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Methods for the determination of (1) total organic mercury (Hg) using an extraction + neutron activation analysis, (2) the sum of methyl-Hg + phenyl-Hg using $^{131}I^-$-Cl^- exchange and (3) methyl-Hg using two different Westöö modifications, have been compared. Sample materials were 8 falcon livers, 5 pike livers and 2 pike muscles. Although differences were found between the methods, interaction effects caused by either sample inhomogeneity or bad performance of the analytical methods impeded a clear interpretation of the comparison. Total Hg in the samples was determined by neutron activation analysis (NAA) and atomic absorption spectrometry. The accuracy of the total Hg determination using NAA was verified by the analysis of certified reference material.

In addition to the other organic Hg determinations phenyl-Hg was determined separately in some of the samples by an isotope exchange method using $^{203}Hg^{2+}$.

The main conclusion of the study was that there is a demand for reference materials certified for at least total organic Hg and methyl-Hg.

INTRODUCTION

The toxicity of alkylmercuries have been known for decades.[1,2] This knowledge, added to an understanding of the ecological transformation of mercury (Hg) into potentially more hazardous compounds, has motivated increased concern about Hg levels in the environment. Methods for the detection of small amounts of Hg, but

also to identify the compounds containing it are therefore of considerable importance.

Several studies have shown that a large fraction of the Hg assimilated into plant and animal tissues is present as the toxic methyl-Hg (see e.g., Ref. 3). The most widely employed technique for the determination of methyl-Hg in biological material is still based upon the procedure devised by Gage[4] in 1961 and later developed into a gas chromatographic (GC) method by Westöö.[5,6] The need, however, for greater specificity regarding other organomercury compounds and simple and more sensitive methods has led to the development of a variety of different procedures, e.g., the combination of Westöö's GC procedure with atomic absorption spectrometry (AAS)[7] and the combination of the Westöö procedure, without the GC, with AAS[8] or neutron activation analysis (NAA).[9] The latter two combinations are used for the determination of total organic mercury. Other sensitive techniques employ thin-layer chromatography of dithizone extracts combined with AAS,[10] and GC after separation of methyl-Hg with the aid of hydrocyanic acid and cysteine paper.[11] Greater sensitivity together with higher specificity are achieved using GC combined with microwave emission spectrometry[12] or mass spectrometry,[13] and more recently by liquid chromatography with differential-pulse electrochemical detection.[14] Another group of common methods are the selective reduction methods using either $SnCl_2/NaBH_4$ (Ref. 15) or $SnCl_2/CdCl_2$ (Ref. 16), while the use of radioisotope methods like exchange reactions with either $^{203}Hg^{2+}$ (Ref. 17) or $^{131}I^-$ (Ref. 18) seem less abundant.

Contrary to this diversity of methods the occurrence of certified reference materials is very limited. On that basis it seems difficult to evaluate the performance of all these methods unless direct comparisons are made.

Aiming to check our analytical techniques for the determination of organic mercury we therefore compared our methods with methods used on a routine basis by other laboratories. The results of these collaborative tests are reported in this work.

EXPERIMENTAL

The methods used, together with their primary references, are listed

TABLE I

Methods used for the determination of organic mercury.

Method No.	Analyte	Principle	Reference	Remark
1	total Hg	NAA[a]	19	DIC[c]
2	total Hg	AAS[b]	24	
3	total organic Hg	extraction + NAA	9	DIC
4	Σ (methyl-Hg + phenyl-Hg)	$^{131}I^-$-Cl^- exchange	20	DIC
5	methyl-Hg	Westöö modification	22	
6	methyl-Hg	Westöö modification	23	
7	phenyl-Hg	$^{203}Hg^{2+}$-Hg^{2+} exchange	21	DIC

[a]NAA, neutron activation analysis.
[b]AAS, atomic absorption spectrometry.
[c]DIC, Danish Isotope Centre.

in Table I. The methods employed by the Danish Isotope Centre (DIC) were methods No. 1, 3, 4 and 7 shortly described below.

Total Hg was determined by *Method No. 1* using radiochemical NAA.

In *Method No. 3* total organic Hg was determined using a modified Westöö extraction, i.e., extraction of the organic Hg with toluene and back-extraction with a cysteine acetate solution, followed by NAA of the cysteine acetate solution.

Method No. 4 was employed for the determination of the sum of methyl-Hg and phenyl-Hg using the exchange reaction between their chlorides and the radioactive $^{131}I^-$. The exchange reaction, which is a two-phase reaction, employs as its first part a separation of the methyl-Hg and phenyl-Hg from the sample using several extraction and purification steps ending up with a toluene phase (cf. Ref 18). To the toluene phase is added $^{131}I^-$ in an aqueous ascorbic acid solution causing an exchange between the chloride of methyl-Hg-Cl and phenyl-Hg-Cl in the toluene phase and $^{131}I^-$ in the water phase. The exchange reaction has been shown to be selective, fast and quantitative as long as $[Cl^-]/[I^-] \leq 600$; after only 1 min the measured activity in the toluene phase is proportional[18] to the concentration of the sum of methyl-Hg and phenyl-Hg. The

concentration was determined by comparison with standards treated similar to the samples.

Method No. 7 was used for the determination of phenyl-Hg. It is based on the isotope exchange between the Hg of phenyl-Hg-Cl and the radioactive $^{203}Hg^{2+}$. The reaction is carried out by addition of HCl and $^{203}HgCl_4^{2-}$ to the sample, followed by 20 min of standing (exchange reaction going on), isolation of the phenyl-^{203}Hg/Hg-Cl by extraction with toluene and radioactivity measurement of the toluene. As long as inorganic Hg is added in excess the exchange reaction will cause no significant fall in the specific activity of the ^{203}Hg and the activity of the toluene phase will be proportional to the phenyl-Hg concentration. The method has been shown to be very selective also towards methyl-Hg (cf. Ref. 21). The concentration was determined by comparison with similar treated standards.

Also included in Table I are the methods used by the other two laboratories, i.e., *Method No. 2*, total Hg determination using AAS and *Methods No. 5* and *6*, two Westöö sample modifications for methyl-Hg determination (extractions: sample→toluene→cystein acetate solution→benzene—GC with electron capture detection). The first modification used $CuSO_4$ in the first extraction step to displace sulphur-bound Hg.

Sample materials were falcon livers obtained from West Greenlandic falcons and pike liver and muscle samples from Wänern, Sweden. A total of 8 falcon livers, 5 pike livers and 2 pike muscles were analysed.

The samples were homogenized prior to analysis. Due to lack of sample material not all methods were employed on each sample. In each case determinations were carried out in duplicate.

RESULTS AND DISCUSSION

The accuracy of the total Hg determination using *Method No. 1* is documented by the results of analyses of various reference materials certified by the National Bureau of Standards in U.S.A. as shown in Table II.

The relative precision of the methods used by us was generally better than 10%.

TABLE II
Analysis results for NBS[a] standard reference materials using neutron activation analysis.[b]

	Our result ± S.E.[c] (ng/g dry matter)	Certified by NBS (ng/g dry matter)
NBS 1571 Orchard leaves	151 ± 7 (22)	155 ± 15
NBS 1645 River sediment	949 ± 55 (10)	1100 ± 500
NBS 1577 Bovine liver	16.2 ± 0.8 (6)	16 ± 2
NBS 1566 Oyster tissue	49 ± 7 (4)	57 ± 15
NBS 1632a Coal	129 ± 10 (8)	130 ± 30

[a]NBS, National Bureau of Standards (U.S.A.).
[b]Ref. 19.
[c]Numbers in parentheses indicate number of determinations.

Falcons

The results for the falcon livers are shown in Table III. The AAS determination of total Hg tended to give a higher result than the NAA determination at the lower concentrations. No statistical difference, however, could be found between the methods when the logarithmized data were tested by a paired Student *t*-test.

The phenyl-Hg constituted only on one occasion a significant amount of the total Hg content in the falcon livers. The sum of methyl-Hg and phenyl-Hg, determined by the $^{131}I^{-}$-Cl^{-}-exchange, *Method No. 4*, therefore reflects the methyl-Hg concentration. If we make the reasonable assumption that the total organic Hg is mainly methyl-Hg (cf. Ref. 3) the results within the broken line rectangle in Table III show the methyl-Hg content in these specific samples measured by four different methods, i.e., *Methods No. 3*, *4*, *5* and *6*. These results as percentage of the total Hg content measured by NAA, were subjected to a two-sided analysis of variance, using a mixed model with samples as random and methods as fixed factors (cf. Ref. 25).

The results of this analysis revealed a difference between the methods on a 10% significance level. The reason for the low significance was a highly significant interaction effect caused by

TABLE III

Results for falcon livers (mean ± S.E. of two separate determinations).[a]

Method No.	1	2	3	4 Σ (Methyl-Hg + phenyl-Hg)	5	6	7
Analyte:	Total Hg	Total Hg	Total organic Hg		Methyl-Hg	Methyl-Hg	Phenyl-Hg
Sample 1	0.217 ± 0.003 (100)	0.29 ± 0.01 (131)	0.204 ± 0.008 (94)				0.010 ± 0.001 (4.7)
Sample 2	0.602 ± 0.001 (100)	0.60 ± 0.04 (100)	0.574 ± 0.011 (95)			0.52 (86)	
Sample 3	1.05 ± 0.07 (100)	1.22 ± 0.04 (116)	0.90 ± 0.08 (86)			0.92 (88)	0.004 (0.04)
Sample 4	1.24 ± 0.04 (100)	1.26 ± 0.01 (102)	1.12 ± 0.10 (90)				0.004 (0.03)
Sample 5	1.52 ± 0.01 (100)	1.35 ± 0.15 (89)	1.24 ± 0.01 (89)	1.38 ± 0.27 (91)	1.02 ± 0.08 (67)	1.16 ± 0.14 (76)	0.0011 ± 0.0002 (0.007)
Sample 6	2.24 ± 0.04 (100)	2.29 ± 0.08 (102)	1.93 ± 0.05 (86)	1.58 ± 0.18 (71)	1.22 ± 0.23 (54)	1.72 ± 0.06 (77)	0.0005 ± 0.0002 (0.02)
Sample 7	2.59 ± 0.25 (100)	2.41 ± 0.08 (93)	1.87 ± 0.21 (72)	2.71 ± 0.27 (105)	1.07 ± 0.10 (41)	2.07 ± 0.17 (80)	
Sample 8	3.60 ± 0.07 (100)	3.50 ± 0.02 (97)	3.23 ± 0.02 (90)				0.0011 ± 0.0002 (0.03)

[a]Result as percentage of the result of method No. 1 shown in parentheses.

either bad performance of the methods or by sample inhomogeneity. The significance, however, was found to be caused by *Method No. 5*, which generally produced lower results on these samples than *Methods No. 3, 4* and *6*.

Comparing *Methods No. 5* and *6* this finding was rather surprising and unexpected. Both methods are Westöö modifications, the only difference between them being the use of $CuSO_4$ in *Method No. 5* in order to mask any free sulfhydryl groups and displace Hg bound to sulphur.[26] Although this procedure has been proved to be inefficient compared to the use of proteolytic enzymes[27] one would still expect *Method No. 5* to produce results equal to or greater than results produced by *Method No. 6*.

Pikes

The results for the pike livers and muscles are shown in Table IV. Total Hg was determined by NAA (*Method No. 1*). Total organic Hg, the sum of methyl-Hg and phenyl-Hg, and methyl-Hg were determined by *Methods No. 3, 4* and *5* (cf. Table I), respectively. Phenyl-Hg was not determined but is not likely to occur in significant amounts (cf. Ref. 14). For the pike livers only two results were obtained using *Method No. 4*. These results agree with the ones of *Method No. 5*.

The results within the broken-line rectangles in Table IV were subjected to the same two-sided analysis of variance as the results of the falcon livers. The results, however, are very sparse and the following findings should only be considered indicative.

For the pike livers neither the method factor nor the interaction was found to be significant, i.e., no difference was found between *Methods No. 3* and *5*.

For the pike muscles the interaction effect overshadowed any differences between the methods. Again this interaction effect might be caused by sample inhomogeneity or bad method performance. Comparing the results two by two, however, revealed the results of *Method No. 5* in all but one case to be significantly lower than the results of *Methods No. 3* and *4*.

A possible, but not very likely explanation of the differences, could be the presence of a significant amount of phenyl-Hg in the samples, which would be included in the results of *Methods No. 3* and *4*.

TABLE IV

Results for pike livers and muscles (mean ± S.E. of two separate determinations).[a]

Method No.:	1	3	4	5
Analyte :	Total Hg	Total organic Hg	Σ (Methyl-Hg + phenyl-Hg)	Methyl-Hg
Pike liver 1	0.351 ± 0.018 (100)	0.24 ± 0.06 (69)		0.27 ± 0.04 (77)
2	0.440 ± 0.005 (100)	0.16 ± 0.03 (37)		0.26 ± 0.06 (59)
3	0.177 ± 0.010 (100)		0.12 (68)	0.16 ± 0.01 (90)
4	0.481 ± 0.008 (100)	0.23 (48)		0.26 ± 0.04 (54)
5	0.279 ± 0.022 (100)		0.21 (75)	0.24 ± 0.03 (86)
Pike muscle 1	0.596 ± 0.0020 (100)	0.52 ± 0.04[b] (86)	0.66 ± 0.03[b] (111)	0.29 ± 0.04[c] (49)
2	0.686 ± 0.002 (100)	0.63 ± 0.04[b] (91)	0.43 ± 0.11 (63)	0.27 ± 0.04[c] (39)

[a]Results as percentage of the result of method No. 1 shown in parentheses.
[b, c]Difference between results marked a and b at a 5% significance level.

CONCLUSIONS

The purpose of this study was to evaluate the performance of the methods used by our laboratory for the determination of organic Hg by comparison with common and well established routine methods. The comparison with one of these methods showed good agreement, while some discrepancies were found regarding the comparison with the second routine method. As no other value than the total Hg content could be determined with a documented accuracy using certified standard reference materials none of the results could, however, be rejected as being wrong. Dissimilarities might be attributed to sample inhomogeneity or bad performance of any of the methods. On this basis the main conclusion of this work must be

that there is a great need for the development of reference materials certified for at least total organic Hg and methyl-Hg.

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