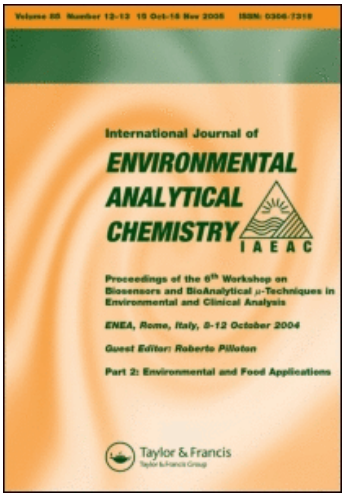

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Statistical Comparison of the Results from Six Analytical Chemistry Laboratories of the Mercury Content of Muscle Tissue of Two Species of Sharks

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Statistical tests were carried out on the results of chemical analysis for total mercury concentrations of replicate samples of muscle tissue of school shark *Galeorhinus australis* (Macleay) and gummy shark *Mustelus antarcticus* Guenther from six independent analytical laboratories. These tests showed that one laboratory produced results 9% below the overall average of all results, another 1% below average while the other four were all 5% above average. Moreover, one laboratory had significantly lower scatter of results than the others, and the percentage scatter (standard error expressed as a percentage of the mean) in two of the laboratories tended to diminish as the magnitude of the results increased. Correction for what were concluded to be wild points indicated that the scatter for all laboratories was below 14%.

KEY WORDS: Statistical laboratory comparison, mercury content.

INTRODUCTION

In 1972 an investigation[1] revealed that total mercury levels in the muscle tissues of sharks ranged from 0.01 to 2.7 parts per million (ppm) for school shark *Galeorhinus australis* (Macleay) and from 0.07 to 3.0 ppm for gummy shark *Mustelus antarcticus* Guenther.

The analytical program involved six laboratories chosen because of their experience, particularly in atomic absorption spectroscopy. Because of the urgency of the investigation it was not possible to standardise analytical techniques and an experimental design was adopted which enabled the results from laboratories to be compared on the basis of two separate criteria, firstly whether there was a tendency for any laboratory to produce consistently higher or lower values than the others and secondly whether the laboratories differed in the degree of scatter (or dispersion) of their results. An account of these comparison tests is presented in this paper.

MATERIALS AND METHODS

Sixty shark carcasses were used in this investigation, one from each of 60 locality-species-sex-length categories and the samples were prepared as described in Walker.[1]

For chemical analysis the vertebral column, skin and exposed surfaces were removed with a scalpel from each thawed sample, which was then macerated in a Hobart 84142 food cutter for ten minutes to produce a fine homogenous paste. Aliquots (approximately 40 g each) of the paste were placed in six separate radiation-sterilsed 60 ml screw-capped polythene jars and stored at −15°C until required.

Replicate samples were submitted to each laboratory for analysis. Four laboratories reported a single result for each sample, Laboratory 5 provided a mean of duplicate analyses and Laboratory 1 reported separate results of duplicate analyses.

Chemical analysis

All the laboratories determined total mercury by cold vapour atomic absorption.[2-4] Five laboratories used a Varian Techtron Atomic Absorption Spectrophotometer (Model A.A.5) and an open-ended mercury kit; Laboratory 4 used a Beckman mercury vapour meter.

The methods used by each laboratory to prepare the samples for atomic absorption spectroscopy are given below.

Laboratory 1

Concentrated nitric acid (3 ml) was added to 1 g of the sample in a 6 × 1″ test tube having a 24/29 ground glass joint, and the mixture was heated under reflux for 10 min, cooled and treated with potassium permanganate (1 g). The mixture was heated under reflux for a further 10 min, cooled, treated with excess 30% hydrogen peroxide added down the condenser, and again boiled for 10 min. At each stage care was taken to ensure that the condensate did not reach the condenser. The mixture was cooled, transferred to a 25 ml measuring cylinder, and treated with a slight excess of 6% potassium permanganate solution. Finally 20% (w/v) hydroxylammonium chloride solution was added to the mixture until the pink colour disappeared.

Laboratory 2

Concentrated sulphuric acid (5 ml) was added to 0.5 g of the sample in a 100 ml conical flask which was then covered with a watch glass. After 1 h in a water bath at 70°C, the flask was cooled and 6% potassium permanganate solution (50 ml) was added. The mixture was again heated at 70°C for 2 h, cooled in ice

water and treated with 20% (w/v) hydroxylammonium chloride solution (8 ml).

Laboratory 3

Concentrated nitric acid (10 ml) and concentrated sulphuric acid (5 ml) were added to 2 g of the sample in an apparatus similar to that described for mercury analysis in the official methods of analysis of the AOAC.[5] The mixture was heated over a flame for 1 h until digestion was complete and copious sulphur trioxide fumes appeared. When necessary additional nitric acid was added dropwise.

Laboratory 4

Concentrated nitric acid (5 ml) and concentrated sulphuric acid (10 ml) were added to 2 g of the sample in a 250 ml Erlenmeyer flask, which was then stoppered, and set aside overnight. The stopper was replaced by a funnel, the mixture was heated on a boiling water bath for 2 h, cooled in an ice bath, and diluted with water (20 ml). The solution was cooled again, treated with 10% (w/v) hydroxylammonium chloride solution, and made up to 50 ml in a graduated flask.

Laboratory 5

Concentrated nitric acid (5 ml) and concentrated sulphuric acid (10 ml) were added to 10 g of the sample in a 250 ml conical flask, and the mixture was heated on a water bath at 70°C for 2 h. The cooled mixture was diluted to approximately 50 ml with water, and filtered through No. 1 filter paper which was then washed at least 4 times. The combined filtrates were made up to 100 ml with distilled water.

Laboratory 6

Concentrated sulphuric acid (5 ml) was added to 0.4 g of the sample in a 100 ml test tube, and the mixture was heated in a boiling water bath for 15 min, cooled in an ice bath and treated carefully with 6% potassium permanganate solution (15 ml). The mixture was heated in a boiling water bath for a further 15 min, and treated with 6% potassium permanganate (12 ml).

The test tube was placed in a heating block and the contents were brought just to the boil, cooled and treated with 20% (w/v) hydroxylammonium chloride (4 ml).

Data analysis

The group of results for each replicated sample, one from each of five laboratories and duplicates from Laboratory 1, were ordered according to

their means from lowest to highest and arranged into six sets of 10. This organisation of the data provided 10 replications of the six mercury level groups (completely randomised design), split for seven laboratory measurements.

To determine whether the results from the laboratories were consistently higher or lower than the means of the other laboratories combined, and to determine the degree of dispersion of the results, two separate statistical analyses were carried out; these are referred to as the test for bias and test for scatter respectively.

The errors in measuring mercury content were expected to be proportional to the mercury level; to ensure these errors were homogeneous the data were transformed by taking natural logarithms.

The parameter used in the test for bias was x_{ijk} where

$$x_{ijk} = \ln (h_{ijk})$$

and the subscripts i, j and k of the mercury level h denote laboratory, size group and sample within that size group respectively. The test adopted was an analysis of variance of the nested or "split-plot" design (see Cochran and Cox)[6] where the mercury level groups represent the "main-plot treatments" and the laboratories the "sub-plot treatments".

The parameter used in the test for scatter was Δ_{ijk} where

$$\Delta_{ijk} = x_{ijk} - \bar{x}_{.jk}.$$

Δ_{ijk} represents the departure of the transformed mercury level of a laboratory from the mean of the transformed levels of all laboratories.

For each laboratory the influence of mercury level group on the variances of Δ_{ijk} was tested by using Bartlett's test[7] to examine the homogeneity of variances.

Estimates of the average scatter s_i of results from each laboratory were made by the following procedure. The variances of the departure values were averaged over the size groups according to the equation

$$v_i = \overline{\text{var}\,(\Delta_{ijk})} = 1/6 \sum_{j=1}^{6} 1/9 \sum_{k=1}^{10} (\Delta_{ijk} - \Delta_{ij.})^2 .$$

It was necessary to modify these values to obtain unbiased estimates of s_i since the scatter from other laboratories affects the average mercury reading and hence the departure values from that average reading. The modification can be shown to be given by the relation

$$s_i^2 = (42 v_i - \sum_{l=1}^{7} v_l)/30 .$$

By adopting a rule developed by Satterthwaite,[8] the appropriate number of degrees of freedom assigned to this estimate was calculated.

RESULTS

The results of the analysis of variance for testing bias are given in Table I. This shows that the effect of laboratory was highly significant. From Table II it can be seen that the results from laboratories fall into three groups: Laboratories 1(a) and 1(b) are 9 % below average, Laboratory 6 is 1 % below average and the remaining laboratories 5 % above average. The absence of a significant

TABLE I

Analysis of variance of the transformed mercury values x_{ijk}

Source of variation	Degrees of freedom	Mean square
Main-plots		
Mercury level group	5	42.6146[a]
Residual	54	0.2035
Sub-plots		
Laboratory	6	0.2622[a]
Mercury level group x laboratory	30	0.0283
Residual	324	0.0198

[a] 1 % significance level.

TABLE II

Differences between laboratories

Laboratory $x_{i..}$		L.S.D.[a]	$x_{i..} - \bar{x}_{...}$	Bias expressed as percentage of $\bar{x}_{...}$
1(a)	−0.792		−0.087	92
1(b)	−0.800		−0.095	91
2	−0.654	5 % 0.050	+0.051	105
3	−0.660	1 % 0.065	+0.045	105
4	−0.658		+0.047	105
5	−0.653		+0.052	105
6	−0.720		−0.015	99
Mean	−0.705	–	0	100

[a] Least significant difference for the $\bar{x}_{i..}$ values.

TABLE III

Variance of Δ_{ijk} values for each laboratory within each mercury level group

Mercury level group	Magnitude range[a] (ppm)	Variance of Δ_{ijk} values within each laboratory							
		1(a)	1(b)	2	3	4	5	6	Mean
1	0.11–0.22	0.0047	0.0092	0.0362	0.0178	0.0068	0.0166	0.0265	0.0168
2	0.22–0.31	0.0152	0.0048	0.0114	0.0101	0.0108	0.0126	0.0449	0.0157
3	0.32–0.51	0.0042	0.0047	0.0123	0.0105	0.0027	0.1364	0.0254	0.0280
4	0.51–0.73	0.0056	0.0046	0.0060	0.0055	0.0070	0.0073	0.0029	0.0056
5	0.74–1.16	0.0654	0.0441	0.0098	0.0125	0.0062	0.0511	0.0059	0.0278
6	1.21–2.56	0.0053	0.0078	0.0027	0.0155	0.0017	0.0163	0.0060	0.0079
	Mean ($=v_i$)	0.0168	0.0125	0.0131	0.0120	0.0059	0.0400	0.0168	0.0170

[a]This is the range from the lowest to the highest mean levels within each mercury level group.

TABLE IV

Test of homogeneity of variance of Δ_{ijk} values for each laboratory using Bartlett's test

Laboratory number	χ_5^2	Explanation for variability of variance of Δ_{ijk} values given in Table III
1(a)	31.2[a]	Wild result in size group 5 ($\chi_4^2 = 5.6$ if mercury level group 5 is omitted).
1(b)	20.9[a]	Wild result in size group 5 ($\chi_4^2 = 2.0$ if mercury level group 5 is omitted).
2	15.8[a]	Trend towards greater accuracy for the higher mercury level groups.
3	3.3	Homogenous over all mercury level groups.
4	8.9	Homogenous over all mercury level groups.
5	27.4[a]	Wild result in size group 3 ($\chi_4^2 = 10.0$[b] if mercury level group 3 is omitted).
6	22.4[a]	Trend towards greater accuracy for the higher mercury level groups.

[a]1% Significance level.

[b]5% Significance level.

interaction effect between laboratory and mercury level group indicates that these differences applied consistently through the mercury level groups.

Results of the test for scatter are given in Tables III and IV; the variances of the Δ_{ijk} values for each laboratory within each mercury group are given in Table III and the results of Bartlett's test are given in Table IV for each laboratory.

Four of the laboratories showed no significant trends in the relationship between the variances of Δ_{ijk} and mercury level group. For Laboratories 2 and

TABLE V

Comparison of scatter of results between laboratories

Laboratory number	Estimated variance (s_i^2)	Approximate degrees of freedom	Standard error	Standard error (%)
1(a)	0.0196	39	0.14	15
1(b)	0.0136	34	0.12	13
2	0.0143	34	0.12	13
3	0.0128	33	0.11	12
4	0.0042	15	0.06	7
5	0.0521	49	0.23	26
6	0.0220	41	0.15	16

6, however, there was a highly significant trend for decreasing variance and hence increasing accuracy with increase in mercury level. In both cases of increasing accuracy, the standard errors for the lower mercury level groups are 2–3 times those for the highest group. However, although the percentage errors are lowest in the highest mercury level groups for the two laboratories, absolute errors remain highest within the highest mercury level groups.

The estimates of s_i^2 and associated approximate degrees of freedom for each laboratory are given in Table V. Applying Bartlett's test of homogeneity of variance to these s_i^2 values gave χ_6^2 equal to 47.2. This is significant at the 1 % significance level and indicates that differences exist in the degree of scatter of the results reported by the laboratories. Using a two tailed F-test it was shown that the variance of Laboratory 4 is significantly lower with a standard error half that of the other laboratories, while the variance of Laboratory 5 is significantly higher; there were no significant differences between the other four laboratories. The higher variance of Laboratory 5 was probably due to one wild result in mercury level group 3. Scatter of results expressed as standard error and conversion of these values to approximate percentages for each laboratory are provided in Table V.

DISCUSSION

The statistical analyses showed that there was good agreement between the results produced by the different laboratories.

However, the test for bias indicated significant differences between the laboratories. Four laboratories were consistent while Laboratories 1 and 6 produced readings that were generally lower throughout all the mercury level groups.

The test for scatter indicated that Laboratory 4 produced results with lower scatter and that Laboratory 5 produced results with considerably higher

scatter than the other laboratories which had scatter of about 14%. For Laboratories 2 and 6 the percentage scatter tended to decrease as the mercury levels increased.

By combining these results it can be predicted with 95% confidence that for a sample containing 0.50 ppm of mercury, which is the current legal limit in Victoria, the measured level would lie within the range 0.38 to 0.66 if measured by an unbiased laboratory with 14% scatter. If the sample was sent to one of the laboratories at random, the range would be 0.36 to 0.68.

Except for the use of the Beckman mercury vapour meter by Laboratory 4, the only obvious difference in the analytical techniques which might explain the observed results was the method of sample digestion.

This step in the analytical procedure for mercury has been a major source of error in the past. Even now there is a compromise between mild oxidation which may contribute interfering materials and the destructive oxidation which may cause variable losses of mercury.

Four of the laboratories digested the samples for one or more hours; Laboratories 1 and 6, the two laboratories providing significantly lower results, heated the digestion mixtures for shorter periods. Apart from Laboratory 4, those were the only laboratories to heat the mixture in a water bath at 100°C; the remaining three laboratories heated the mixtures in water baths at 70°C.

Not all the laboratories carried out similar recovery estimates. The analytical methods therefore cannot be appraised in terms of recoveries other than to mention that the average percentage recoveries using methyl mercuric iodide reported by Laboratories 2, 3 and 4 were 98, 97 and 95 respectively. This only provides a check of processing losses; it has no relevance to whether all the organically combined mercury in the tissue was converted to a form suitable for measurement. Laboratory 4 had carried out a series of digestion variations before this investigation and had selected a technique yielding consistent values.

No "preanalysed" sample was available at the time of this investigation for other interlaboratory comparison.

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