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MEMORANDUM

Release

To: Melody Roelke

From: Clint Moore *CM*

Date: 26 October 1989

Subject: Preliminary analyses of mercury loads in Florida panthers

I present to you a summary of some quick analyses of the mercury concentration data. For all of the analyses, I log-transformed (natural log, base e) the data. Multiplicative effects on the untransformed scale become additive (or linear) on the transformed scale. For example, concentration values 80, 8, and 0.8 differ from each other by a multiplicative factor of 10. On the log scale, their values become 4.382, 2.079, and -0.223, respectively. These values differ by approximately an additive factor of 2.303. For data that vary as widely as these, this is a reasonable transformation to rely upon. Not only is log-transforming done for intuitive reasons, it is also helpful for satisfying certain statistical assumptions. Without the transformation, these data are be distributed in a skewed manner with a long tail to the right. As the untransformed values increase in size, so does their variability.

Comparison of labs

The first result I report is that of the comparison of liver analyses done at the Patuxent and GFC labs. Table 1 presents the ratio of the untransformed GFC liver value to that of the Patuxent value, and the logarithm of this ratio, for each sample. Notice that the log of the ratio is exactly equivalent to taking the difference of the transformed lab values. An untransformed value ratio of 1.0 corresponds to equivalent measures at the labs. On the log scale, equivalence corresponds to a value of zero. For log-ratio values less than zero, the GFC lab reported a lower value than the Patuxent lab. For values greater than zero, the converse is true. The mean of these log-ratios were tested for equivalence to zero by a one-sample t-test. Actually, because I looked at difference of transformed values, this would more properly be called a paired t-test. To get an idea of the magnitude of the "between-lab" variability, I compared it to the total variability of the data, without regard to labs or pairings.

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For the 11 samples, the mean lab difference of transformed values was 0.5432 (i.e., GFC values 1.722 times as great as Patuxent values, on the untransformed scale) with a standard error of 0.3634. This difference was not significant at the 0.05 level ($t = 1.495$, $P = 0.166$). The 95% confidence interval for this difference is (-0.2663, 1.353). On the untransformed scale, this interval corresponds to (0.7662, 3.868). The pooled variance estimate for the data without regard to labs or pairings was 16.62. The between-lab variability was only 8.7% of this value, indicating that relative to overall variability in these bioassay values, the variability due to labs is fairly small.

Notice the 2 extremely high log-ratio values for samples 13 and 20 in Table 1. These values correspond to relatively low Hg concentrations in these liver samples. For bioassay values this low, the 2 labs had more trouble in agreeing on a value, and the GFC lab reported relatively much higher values than did Patuxent. When these 2 values were excluded from the analysis, the mean difference dropped to 0.006476 (1.006 untransformed) with a standard error of 0.05956. This tiny difference was not significantly greater than zero ($t = 0.109$, $P = 0.916$). The 95% confidence interval, on the original scale, shrank to (0.8773, 1.155). The between-lab variability, expressed as a percentage of total variability, dropped to 0.53%. For Hg concentration values greater than about 1.0, the labs appear to be in good agreement.

Relationship between liver and hair bioassays

Six pairs of Patuxent liver and hair samples were available for analysis. Transformed liver Hg concentration was fit to transformed hair Hg concentration via linear regression. Predicted values of liver Hg concentration for living animals, given values of hair Hg concentration, were estimated and prediction limits calculated.

The regression model was just significant at the 0.05 level ($t = 2.77$, $P = 0.051$, $r = 0.8105$, $s.e.(b) = 0.3172$). The estimated regression equation is

$$\ln y = 0.1750 + 0.8778 \ln x,$$

or on the untransformed scale,

$$y = 1.191 x^{0.8778}.$$

Figure 1 displays the regression model. The vertical lines drawn from the points to the regression line get smaller as hair Hg concentration increases. This causes some concern not so much in the estimation of the model parameters, but in estimation of the variability of the parameters. The precision in the model undoubtedly suffers because of the high variability observed in liver Hg concentration at low values of hair Hg concentration. As a result, the confidence we can place in the prediction of future values is diminished.

The asterisks on the regression line in Figure 1 are predicted liver Hg concentrations for 5 cats. The 95% prediction interval is displayed as a pair

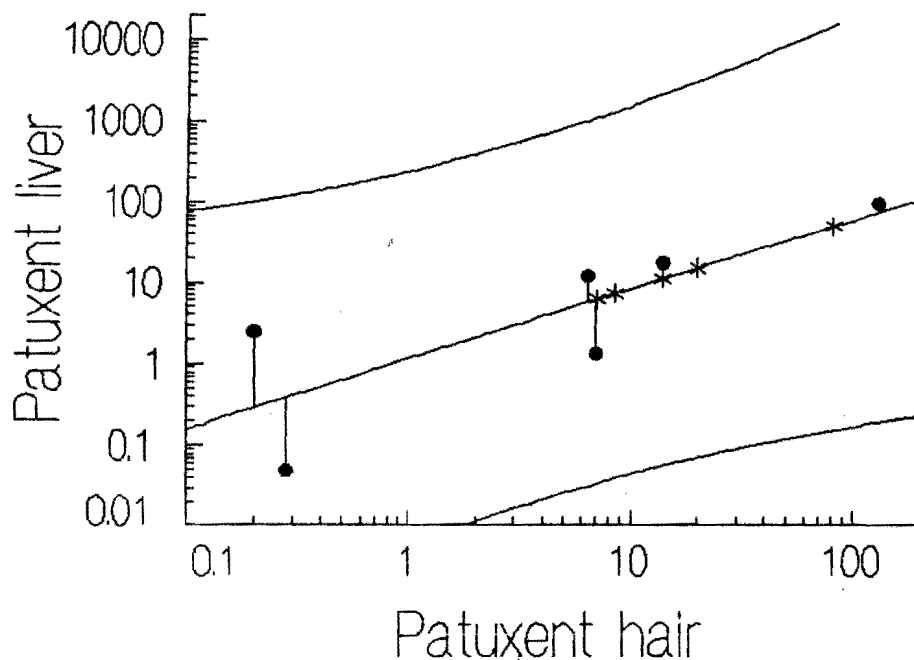


Figure 1. Estimated regression of $\log(\text{liver Hg})$ on $\log(\text{hair Hg})$, with prediction limits.

of concave lines. For predicting a *mean* for several cats with a particular hair Hg value, these lines would be much closer to the regression line. In Table 2, I have provided predicted values and 95% prediction intervals for liver Hg concentrations for the 5 cats. All values are on the original scale.

Age and region comparisons of liver samples

All samples could be classified into young and old age categories, and east and west Florida region categories. Three liver values were known for east, young cats, and 4 more were predicted from hair samples (mean = 2.891, s.d. = 1.059). Four values were known for west, young cats, and no more were predicted from hair samples (mean = -1.190, s.d. = 2.110). Three values were known for west, old cats, and 1 more was predicted from a hair sample (mean = 2.713, s.d. = 0.4481). There were no old cats in the eastern region.

The difficulty in making comparisons of means calculated with the aid of estimated data is that there should be a penalty built in for the use of these data. This penalty results in a loss of power of the analysis, and a popular way of implementing this penalty is to reduce the number of degrees of freedom in the error term by the number of data values estimated. For example, in a t-test with a sample size of 25, you would normally need to look up the t-value with 24 degrees of freedom, 2.064 in this case for a 0.05 error rate. If 5 of the values were estimated however, you should look for the t-value with 19 degrees of freedom, 2.093. Since 2.093 is larger than 2.064, you need to find a larger mean in the latter case than in the first case in order to declare the mean significant.

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The eastern region mean was significantly greater than the western region mean in young cats ($t = -3.685$, 5 df, $P = 0.014$, liver Hg concentrations average 59 times greater in east than in west young cats). Among western region cats, the young age mean was significantly smaller than the old age mean ($t = 2.835$, 5 df, $P = 0.036$, liver Hg concentrations average 50 times greater in old than in young western region cats). For this latter test, there was an indication that age group variances were unequal, a violation of test assumptions. Even when corrected for this, the difference is still fairly noticeable ($t = 2.835$, 3.27 effective df, $P = 0.060$).

I was curious to see what effect ignoring estimated values would have on the results above. Very little, as it turns out. Even with the reduced sample sizes, the differences are still strong. For young cats, the eastern region mean was significantly greater than that for the western region ($t = -3.198$, 5 df, $P = 0.024$, liver Hg concentrations 85 times as great in east cats as in west cats), and for western region cats, the old age mean was significantly greater than that for young cats ($t = 3.035$, 5 df, $P = 0.029$, liver Hg concentrations 48 times as great in old as in young cats).

Conclusions

Liver Hg values did not appear to differ appreciably between the 2 labs when the Hg concentration exceeded 1.0. At lower values, GFC lab values were many times greater than those obtained by Patuxent.

Liver Hg values and hair Hg values were positively related. However, for relatively small values of hair Hg, variability in liver Hg values increased.

Assuming that the western region young cats do not constitute an unusual sample, there is evidence that eastern region young cats carry greater liver Hg burdens than do their western counterparts. Additionally, older western region cats have on average higher concentrations of liver Hg than do younger cats in that region.

cc: Jim Brady
WLD 9-3-4

Table 1. GFC/Patuxent liver Hg value ratios and log transformations.

Sample	Ratio	Transformed
— 27	86.4 / 110.0	-0.2415
04	11.2 / 7.8	0.3618
07	16.3 / 20.0	-0.2046
C08	21.0 / 19.0	0.1001
13	1.0 / 0.049	3.0159
— 15	11.95 / 13.0	-0.0842
20	0.91 / 0.05	2.9014
25	2.5 / 2.5	0.0
A64	1.5 / 1.4	0.0690
A59	21.1 / 20.0	0.0535
A20	12.05 / 12.0	0.0042

Table 2. Values of liver Hg concentration, predicted from hair Hg.

Sample	Predicted value	95% prediction interval
— 21	12.081	(0.0581, 2511)
— 14	7.7959	(0.0403, 1509)
— 16	57.008	(0.1634, 19887)
— 22	6.6565	(0.0350, 1266)
W08	16.522	(0.0739, 3693)