

Response to questions posed on 12 November, 1999, by the NAS mercury committee

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This document provides supplementary information in regard to questions asked at the 12 November, 1999, meeting of the NAS Committee on the Toxicological Effects of Methylmercury. This material is provided as background information only for the Committee and is not intended for wider circulation or publication. The contents are as follows:

1. Influential Points
 - (a) Investigation of model stability before inclusion of responses
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1. Influential Points

1.a Investigation of model stability before inclusion of responses

In [1] the mercury effect was estimated in multiple regression after a logarithmic transformation of cord blood concentrations. Here it is illustrated why it is preferable to use the transformed concentrations as opposed to the untransformed concentrations in the regressions.

An observation has the potential to influence the regression result disproportionately if its values on the independent variables are extreme. Such outliers in (multivariate) covariate distributions are identified by calculating the so called Hat Matrix (see for instance [2] page 16). The diagonal elements of this matrix can be interpreted as the distance from covariates of each observation to the vector of covariate means. It would

be desirable if all observations were roughly equally influential, i.e., the hat matrix diagonals are similar.

Graphs 1 and 2 show the Hat Matrix diagonals as a function of the cord blood mercury concentration for the linear and the logarithmic dose-response models, respectively, using the usual set of confounders. These plots clearly favor the logarithmic model. For the linear model a few observations with a very high mercury concentration are seen to be (potentially) very influential. This means that there is a high risk that the estimated mercury effect would change dramatically if one of these observations is excluded. This risk is much lower for the logarithmic model, where the Hat Matrix diagonals are seen to be almost independent of the mercury concentrations.

Based on these graphs alone one could not conclude that the logarithmic model should be preferred to the linear one, since the latter model might explain the variation in the response variable better. However, for most outcomes in the Faroese data, the logarithmic model fits the data better than the linear model does.

1.b Sensitivity of the estimated mercury regression coefficients

This section describes the sensitivity of the estimated mercury regression coefficients in the logarithmic model to exclusion of influential points.

An observation is said to be influential if it has a numerically high *dfbeta*-value. This value is calculated as the difference between the full data (mercury) coefficient and the coefficient estimated after exclusion of the observation at hand divided by the estimated standard deviation of the coefficient ([2] pages 11-13).

Table 1 shows the regression result after exclusion of the 10 most influential observations. The choice of the number 10 is arbitrary, but it corresponds to about 1% of the observations. Overall this exclusion changes the estimated mercury effect relatively little. However, for the Finger Tapping tests all mercury coefficients are attenuated, and none are significant after the exclusion. Conversely, exclusion of influential points for CVLT results generally strengthens the association.

For the Boston Naming Test *graphs 3 and 4* show the association between the *dfbeta*-values and the cord blood mercury concentrations for the linear model and the logarithmic model, respectively. As could be expected from *graph 1* a few high exposure observations are very influential using the linear model. For the logarithmic model the plot is more symmetrical and none of the observations are nearly as influential as the most influential observation using the linear model.

2. Comparison of model fit at low doses

In [3] it was shown that a logarithmic model best fitted the association between a child's score on the Boston Naming Test and the cord blood mercury concentration adjusted for confounders. Here it is investigated whether this is also true looking only at the observations in the low dose range.

On *graph 5* a cutoff dose is applied and the fits of the linear model, the square root model and the logarithmic model (see [3], "Benchmark Calculations") are compared based only on observations with a mercury concentration below the cutoff. *Note that the dose response relations are not re-estimated, the functions being compared are the ones estimated using all observations.* As a measure of fit we have used minus twice the log of the likelihood function. The lower this value is the better is the fit. On the graph the fully drawn curve indicates the difference in fit between the linear model and the logarithmic model while the dotted curve indicates the difference in fit between the square root model and the logarithmic model. Since both curves stay above zero the logarithmic curve gives the best fit at low doses. As was the case for the full data comparison the linear dose response curve gives the worst fit, although the difference from the logarithmic one is not quite significant.

Another approach could be to exclude the high exposure children and only use the low exposure observations in the benchmark calculation. Using this approach the dose response relation should be re-estimated for observations below the cutoff and the model fits compared for the re-estimated relations. However, this approach seems inappropriate since it will yield low BMDLs simply because the estimation uncertainty will increase as a result of the decreasing number of observations used.

3. Benchmark calculations using different logarithmic models

In [3] benchmark doses were calculated using the logarithmic model: $\mu(d) = \beta \cdot \log(d + 1)$, where d is the mercury concentration. The constant of 1 was added to the concentrations to avoid dose-response functions with an infinite slope at zero dose. However, another constant could just as well have been chosen. Here the sensitivity of the benchmark results to the choice of the constant is investigated.

Table 2 gives the BMDLs for the Boston Naming Test calculated for five different values of the constant added to the concentrations before applying the logarithmic transformation. This table also gives minus twice the log of the likelihood function for each model indicating how well the models fit the data. From the table it is seen that the models fit the data almost equally well (although 1 gives a very slightly better fit than the rest), while the BMDLs are quite different. The higher the constant the higher the BMDLs, but all BMDLs are lower than the corresponding BMDLs of the linear model and the square root model reported in [3] and quoted in the table.

4. Scatter plots: Dose-Response association for Boston Naming

Graph 6 shows a scatter plot of the dose-response association adjusted for confounders for the Boston Naming Test, with the mercury concentrations on a logarithmic scale. The three curves are the estimated dose response functions of the linear model (fully drawn curve), the square root model (dotted curve) and the logarithmic model (broken curve). In [3] the models are described in detail, and the plot was shown already at the 12 November meeting.

Graph 7 shows a scatter plot identical to the plot on graph 6 except that the mercury concentrations are now *not* on a logarithmic scale, but on a linear scale.

Graph 8 again shows a scatter plot of the dose response association adjusted for confounders for the Boston Naming Test. Here the mercury concentrations have been transformed using the function: $f(x) = \sqrt{x+1} - 1$. The line is the estimated dose response function of the square root model.

5. Association between methylmercury and organochlorine exposures

The question was asked whether organochlorine compounds other than PCBs could be present in the exposures from marine food and perhaps account for some mercury-associated adverse effects. Our assumption was that PCB was the best overall marker of organochlorine exposures. Calculations have now been performed to determine the association of other organochlorine concentrations with those of PCB and mercury. These calculations were performed for both the original cohort (cohort 1) and for the subsequent Faroese cohort born in 1994 (cohort 2)[4]. More detailed information on organochlorine concentrations is available from the latter.

Cohort 1: Analysis for major PCB congeners and p,p'-DDE was conducted for cord tissue from 436 children clinically examined in 1993. A maternal hair-mercury concentration (HHG) was available from all cases, and a cord blood level (BHG) for 426. Congener 153 was used as the best indicator of the total-PCB concentration, and p,p'-DDE for the DDT group. Because of skewed distributions, all results were log transformed. Organochlorine results below the detection limit (LOD) were set to be 0.05 ng/g. Pearson correlation coefficients were calculated. As overestimation of cord lipid contents may have occurred at low ranges, both fresh-weight and lipid-adjusted concentrations of organochlorines are given (Table 3).

Cohort 2: 132 mother-child pairs had full information on mercury concentrations in cord-blood (BHG) and in maternal hair (HHG) as well as organochlorine concentrations in maternal serum. All results were log transformed. Organochlorine results below the detection limit were assumed to be 5 ng/g lipid. Again, PCB congener 153 (CB-153) was used as being the best representative of the total PCB concentration. p,p'-DDE was the predominant compound detected within the DDT group of compounds (DDE). Likewise, dieldrin was selected as the representative of the -drin compounds (DRIN).

Concentrations of the beta and gamma isomers of hexachlorocyclohexane were added (HCH). Hexachlorobenzene was included as a pollutant from a different set of sources (HCB). All serum concentrations are expressed on a lipid basis. Correlations were calculated as the Pearson coefficients (Table 4).

The overall lipid-based concentrations of CB-153 and DDE are similar for the cohorts, and their associations with mercury levels are also comparable. The conclusion from both cohorts is that PCB correlates better with the mercury concentrations than do any of the other organochlorine compounds. Those compounds that showed a substantial number of samples below the detection limit showed particularly low correlation coefficients, though still somewhat better with respect to PCB than with mercury.

6. Modeling of the association between methylmercury exposure and blood pressure

As described in [5], the mercury concentration in cord blood showed a non-linear association with blood pressure after adjustment for body weight. However, a third-degree polynomial was highly significant ($p < 0.001$) (*Graph 9*). The greatest mercury-associated differences in blood pressures occurred within the low-level exposure range, and the slight increase at the highest exposures was not significant. The same tendency was seen with the maternal hair mercury concentration as the exposure indicator, but the association with the response variables was not as close. This information is provided as a complement to the published paper [5], as the journal in which it appeared discourages the use of p-values.

References

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Tables

Table 1: Regression coefficients and p-values before and after exclusion of the ten most influential observations.

Response	All children		Without Influential	
	coefficient	p-value	coefficient	p-value
Finger tapping				
Preferred hand	-1.0953	0.0492	-0.8965	0.1086
Non preferred hand	-0.3947	0.4595	-0.1902	0.7222
Both hands	-1.6717	0.1359	0.0216	0.9845
HEC				
Error score	0.0341	0.1872	0.0405	0.1269
CPT				
Ln total missed	0.2703	0.0236	0.3483	0.0048
Reaction time	40.2796	0.0002	46.6119	<0.0001
WISC-R				
Digit Spans	-0.2729	0.0491	-0.1636	0.2369
Similarities	-0.0481	0.9015	-0.1099	0.7818
Sqrt. Block Designs	-0.1663	0.1091	-0.2506	0.0170
Bender				
Errors on copying	0.6741	0.1541	0.8853	0.0616
Reproduction	-0.2510	0.1044	-0.2192	0.1646
BNT				
No cues	-1.7703	0.0003	-1.4208	0.0046
With cues	-1.9092	<0.0001	-1.8197	0.0003
CVLT				
Learning	-1.2524	0.1233	-1.6268	0.0479
Short-term repro.	-0.5693	0.0194	-0.7933	0.0016
Long-term repro.	-0.5470	0.0473	-0.8964	0.0015
Recognition	-0.2896	0.1506	-0.2185	0.2469

Table 2: BMDLs using the model: $\mu(d) = \beta \cdot \log(d + a)$, where d is the cord blood mercury concentration and a is a known constant, and $P_0 = 0.05$. Response variable: Boston Naming Test with cues.

a	0.5	1	2	5	10	K -power	Linear	Square Root
$-2 \cdot \log(L)$	7790.43	7790.42	7790.43	7790.50	7790.60	7793.72	7793.72	7790.84
BMR=0.02	0.49	0.93	1.72	3.62	5.88	28.73	26.76	6.74
BMR=0.05	1.65	3.11	5.61	11.11	17.00	61.22	57.50	22.33
BMR=0.10	5.27	9.66	16.73	30.46	42.77	102.22	96.29	53.96

Table 3: Geometric mean, number of analyses below the level of detection (LOD), and correlation coefficients between log transformed values for mercury and organochlorine compounds in the Faroese cohort 1 (N = 436).

	Geometric mean	Number below LOD	Correlations	
			BHG	CB-153
BHG ($\mu\text{g}/\text{l}$)	23.9	0	1.000	0.423
HHG ($\mu\text{g}/\text{g}$)	4.43	0	0.789	0.440
CB-153 (ng/g wet weight) ($\mu\text{g}/\text{g}$ lipid)	0.41	3	0.423	1.000
	0.21		0.346	0.812
DDE (ng/g wet weight) ($\mu\text{g}/\text{g}$ lipid)	1.27	3	0.387	0.888
	0.66		0.316	0.718

Table 4: Geometric mean, number of analyses below the level of detection (LOD), and correlation coefficients between log transformed values for mercury and organochlorine compounds in the Faroese cohort 2 (N = 132).

	Geometric mean	Number below LOD	Correlations	
			BHG	CB-153
BHG ($\mu\text{g}/\text{l}$)	20.4	0	1.000	0.431
HHG ($\mu\text{g}/\text{g}$)	4.08	0	0.774	0.523
CB-153 ($\mu\text{g}/\text{g}$ lipid)	0.26	0	0.431	1.000
DDE ($\mu\text{g}/\text{g}$ lipid)	0.72	0	0.372	0.797
DRIN ($\mu\text{g}/\text{g}$ lipid)	0.007	118	0.294	0.605
HCH ($\mu\text{g}/\text{g}$ lipid)	0.013	53	0.079	0.192
HCB ($\mu\text{g}/\text{g}$ lipid)	0.083	0	0.328	0.850