

Underestimation of human methylmercury toxicity due to exposure misclassification

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Abstract: Epidemiological assessment of human methylmercury toxicity relies on observational data, and the results are therefore susceptible to several potential sources of bias. Exposure misclassification is a particularly important problem. Standard statistical analysis assumes that the exposure is measured without error and produces effect estimates that are biased toward 0. Although adjustment for this problem may be achieved by sensitivity analysis, this approach requires that the degree of imprecision is known. Using results from a prospective study of a Faroese birth cohort (N = 1,022), structural equation analysis showed that the total imprecision of these markers was 30-50% (i.e., much more than the laboratory error). The magnitude of exposure imprecision is perhaps surprising, but the results will at least make it possible to adjust for the resulting bias. This exposure misclassification must be taken into account as an important source of bias toward the null. The exposure misclassification also impacts on calculated benchmark doses by overestimating the true levels. If ignored, this error may result in recommendation of exposure limits that offer less protection than intended.

Key words: Children, Epidemiology, Exposure Limits, Human Health, Neurotoxicity

Introduction

Exposure misclassification is a major obstacle to obtaining accurate dose-response relationships, because the extent to which a true association can be observed depends on the precision with which the key parameters are assessed. Non-differential exposure measurement error results in underestimation of the risk: The larger the imprecision of the exposure parameter, the greater the underestimation. Because a gold standard is rarely available, the degree of misclassification is seldom known for sure, and its magnitude must therefore be assumed. Research studies therefore aim at providing exposure parameters of high validity and the highest possible precision. Exposure biomarkers are usually validated by comparison of different methods, analysis of reference materials and replication studies. The imprecision is then expressed as the coefficient of variation (CV) (i.e., the standard deviation divided by the mean of a large number of analyses of a uniform sample).

The most commonly used methylmercury exposure biomarkers are the mercury concentrations in scalp hair and in blood. Toxic damage during brain development is thought to constitute the critical effect of this toxicant. We have therefore used data from the prospective study of the first Faroese birth cohort to estimate the true extent of imprecision associated with different methylmercury exposure parameters^[1]. Mercury

was measured in cord blood and in 9-cm maternal hair sampled at the time of parturition, and the laboratory imprecision on both chemical analyses was thought to be below 5% (coefficient of variation, CV). The log transformed results showed an approximately linear relationship with an excellent correlation between the two parameters, although the differences between the two were greater than suggested by the laboratory CV. Each mother responded to a dietary questionnaire and indicated the number of whale meat dinners per month during pregnancy, the main source of methylmercury exposure^[1]. The children were examined at age 7 years with regard to nervous system development.

Results and discussion

Measurement error variances in mercury biomarkers were estimated using two different structural equation models. These models view observed variables as manifestations of underlying latent variables and they assume causal relations between the latent variables possibly after accounting for covariates. The first model included only exposure variables^[3]. The true exposure is not known but is observed through the error-prone exposure parameters. According to the measurement part of the model, each exposure parameter Hg_i (after logarithmic transformation) was expressed as a function of the true methylmercury exposure level Hg and a random error:

$$\log(Hg_i) = \alpha_i + \beta_i * \log(Hg) + \varepsilon_i,$$

where α_i is an intercept for exposure parameter i , β_i is the factor loading, and ε_i is an error function. The standard deviations of these error terms are the parameters of main interest here. Because of the log-transformation error standard deviations can be interpreted as CVs in the raw concentrations.

The factor loadings allow observed variables to have different scales. Latent variables are not observed and therefore have no inherent scale. In this case, the true exposure was expressed on the scale the cord blood concentration by fixing the factor loading of this indicator at one ($\beta_{\text{blood}} = 1$). In models where factor loadings vary across indicators, error variances are on different scales and cannot be directly compared. Instead, meaningful comparisons of precisions can be based on the correlation to the true exposure. The exposure model contains too many unknown parameters in relation to the number of observed variables to allow estimation of the error variances. This problem was solved by inclusion of information on the number of pilot whale meat dinners consumed by the mother (Whale). This variable was assumed to affect the true prenatal mercury exposure:

$$\log(Hg) = \mu + \lambda * \log(\text{Whale} + 1) + \varepsilon.$$

A second more sophisticated model, which also includes neurobehavioral test scores, was then developed^[2]. Again, the latent exposure variable is modeled from the two exposure biomarkers and the questionnaire information. Similarly, neurobehavioral test scores were considered manifestations of major nervous system functions. The model further assumes a linear effect of the latent exposure on the latent responses, taking into account the confounders. In the simple model including only exposure

variables, the cord blood concentration showed the strongest correlation to the true exposure (Table 1). The maternal hair concentration correlated significantly weaker than the blood parameter ($p = 0.007$). Thus, the cord-blood mercury concentration is clearly the least imprecise indicator of prenatal mercury exposure.

Table 1. Calculated total error for two exposure biomarkers that reflect the prenatal exposure to methylmercury, as estimated by two different statistical methods^[4]. Each parameter has been transformed to a logarithmic scale.

Indicator	Factor Loading	Error Variance	Coefficient of Variation	Correlation to true exposure
Exposure model				
Blood mercury	1	0.10	0.32	0.93
Hair mercury	0.84	0.19	0.44	0.84
Full model				
Blood mercury	1	0.080	0.28	0.94
Hair mercury	0.81	0.20	0.45	0.82

The results of the expanded structural equation model are quite similar to those based on the simple exposure model (Table 1). However, the cord-blood parameter appears to be even less imprecise than the hair analysis. Thus, inclusion of outcomes and confounders in the exposure calibration changed the parameters only slightly. This is because the latent exposure variable is highly dependent on the three exposure indicators and only weakly associated with the effects and the confounders. The definition of the latent variable is therefore almost unaffected^[4] by incorporation of these variables. The results show calculated CVs that much exceed the known laboratory variations, with the total error being at least 5-fold greater than the laboratory imprecision.

The results of these analyses are in agreement with the observation that the cord-blood mercury concentration is a stronger predictor of cognitive deficits than the maternal hair concentration^[5]. However, the magnitude of estimated total imprecision is far in excess of previous determinations of laboratory variability. The imprecision of each exposure biomarker can be looked upon as a sum of two different types of error: laboratory measurement imprecision and biological or preanalytical variation. The second error component arises due to variability in mercury concentrations in the fetal circulation, individual differences in the distribution of mercury, variations in the hematocrit of the fetal blood, etc. Hair concentrations may depend on hair structure, growth rates, external contamination, and the effects of cosmetic hair treatments.

These findings illustrate that measurement error may be greatly underestimated if judged solely from laboratory quality data. If based on laboratory performance, the precision is therefore confused with the quality of the information. In the present example, the mercury concentration in maternal hair or some other sample is erroneously assumed to represent the dose at the fetal target organ.

Given the magnitude of the total imprecision observed in this study, limited benefit will arise from intensified efforts to improve the quality of the chemical analyses

in the laboratory. The analytical imprecision is already only a small part of the total error. Thus, the preanalytical sources of imprecision deserve increased attention. Regression analysis generally assumes that the exposure variable has been assessed without error. Any non-differential error will bias the results toward the null hypothesis. Additional bias toward the null may occur in the presence of confounders that have a greater precision. On the other hand, if the population examined represents a very wide exposure range, then the bias will be less severe.

Conclusions

Laboratory reproducibility may greatly underestimate the total imprecision of exposure biomarkers. In epidemiological studies, unrecognized random error will result in underestimation of the true effects of the exposure. Biomarkers of prenatal methylmercury exposure were found to be at least 5-fold more imprecise than suggested by laboratory quality data. Structural equation modeling was found to be useful to ascertain the total imprecision. Because serious underestimation of exposure imprecision is likely to be common, the significance of environmental hazards may be underestimated.

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