

SUPPLEMENTAL MATERIALS

Evaluation of the Cardiovascular Effects of Methylmercury Exposures: Current Evidence Supports Development of Dose-Response Function for Regulatory Benefits Analysis

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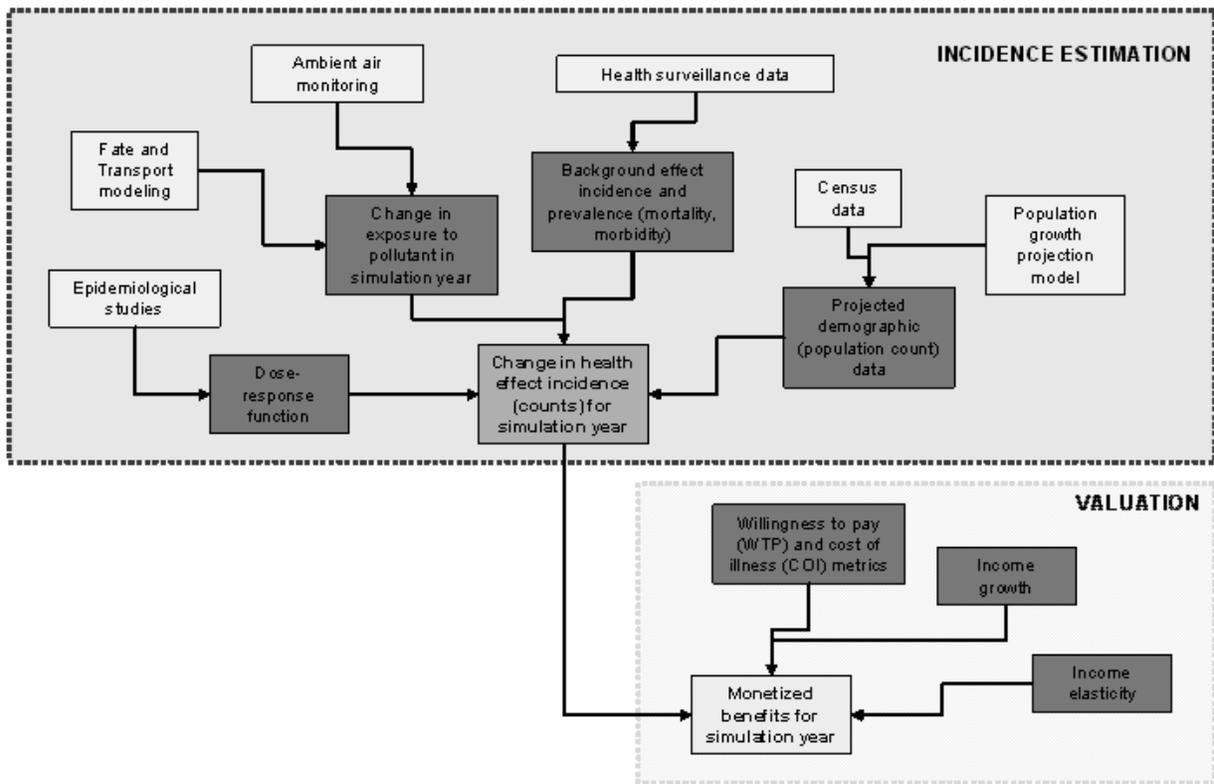
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Adapted from figure created by Neal Fann, U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards, personal communication, January 12, 2010.

**Supplemental Material, Figure 1:
EPA FRAMEWORK FOR QUANTIFYING AND MONETIZING BENEFITS**

US EPA WORKSHOP ON THE CARDIOVASCULAR TOXICITY OF METHYL MERCURY:

CHARGE QUESTIONS

Biologic Plausibility

1. Considering both positive (or supporting) data, negative data, and data showing no effect, what epidemiologic, animal bioassay or in vitro studies should be considered when evaluating the plausibility that the epidemiologic studies reporting an association between methyl mercury and cardiovascular effects reflect a causal association?
2. What are the strengths and weaknesses of the identified studies? Based on this evaluation can the identified studies be categorized (e.g., high or low quality)?
3. What scientifically-defensible approaches are available to evaluate the different lines of evidence?
4. Considering these various sources of data, what is the likelihood that there is a causal relationship between human methyl mercury exposures and increased risk of cardiovascular effects? Please consider the different cardiovascular effects that have been associated with methyl mercury in the epidemiology literature.

Methyl mercury Toxicokinetics

5. Several prominent studies report associations between human toenail mercury concentrations and cardiovascular effects. Previously, EPA evaluated risk of decreased childhood IQ due to maternal methyl mercury exposures during pregnancy based on blood or hair methyl mercury concentrations. Are the toxicokinetics of methyl mercury accumulation in toenails

adequately understood to consider such studies in an analysis of biological plausibility of cardiovascular effects?

6. Are the toxicokinetics of methyl mercury accumulation in toenails adequately understood to include such studies in an analysis of dose-response for cardiovascular effects that would relate dietary methyl mercury intake rates to increased risk of cardiovascular events? Are there adequate scientific data to describe the relationship between toenail and blood or hair methyl mercury concentrations?

Dose-Response Modeling

7. Which study or studies should be considered in the development of dose response function/s for cardiovascular impacts?
8. What statistical techniques should be used in modeling a dose-response function?
9. Should separate dose-response functions be developed for children and adults? Are the end points the same?
10. How should potential effects of polyunsaturated fatty acids, selenium or other cardioprotective compounds in fish be treated in the development of dose-response functions?

Implementation Issues

11. How should EPA address uncertainty and variability in an assessment of cardiovascular impacts of methyl mercury including uncertainties due to the paucity of data on women, small number of studies, limited number of U.S. studies, and other important input factors?

ADDITIONAL INFORMATION ON GENERALIZED ADDITIVE MODELS

Generalized Additive Models (GAMs) combine parametric terms and smooth nonlinear functions into a single regression model. These models can accommodate both nonlinear dose-response (D-R) relationships and flexible control for confounding. Therefore, GAMs could be used to evaluate the shape of the D-R function across the existing epidemiological studies of methylmercury (MeHg) and risk of myocardial infarction (MI). In addition, GAMs could be used to reanalyze the epidemiological data to incorporate non-linear adjustments for negative confounding by n-3 polyunsaturated acids (PUFAs) in order to reduce the possibility of an artificially attenuated D-R coefficient. Several recent environmental epidemiology studies have used GAMs to assess the D-R relationship between MeHg exposure and cardiovascular outcomes (Grandjean et al. 2004; Guallar et al. 2002; Thurston et al. 2007).

Different implementations of GAMs exist, including those based on local smoothing (LOESS), parametric splines (Dominici et al. 2002), and penalized splines (Eilers and Marx 1996; Ruppert et al. 2003; Wood 2006). Each of these approaches depends on a tuning parameter that determines the degree of smoothness for a given function, which is typically estimated from the data. Spline models fit a different polynomial function in different ranges of the exposure. A penalized spline form fits separate polynomials in different ranges (usually deciles) of the exposure data. It allows for flexibility in the shape of the curve, while penalizing overfitting by constraining the change in coefficients between different deciles, and reducing sensitivity to the choice of the boundary points (called knots) that separate exposure into different regions.

Several years ago, Dominici et al. (2002) noted that the implementation of LOESS in the most popular program for fitting GAMs at the time, Splus, yielded incorrect results, which

caused much controversy over the appropriate use of GAMs in environmental epidemiology settings. However, recent advances in both GAM theory and software have resolved these issues (Ruppert et al. 2003; Wood 2006).

ANALYSIS COMPARING EXPOSURE LEVELS ACROSS RECOMMENDED EPIDEMIOLOGICAL STUDIES TO THE DISTRIBUTION OF EXPOSURES IN THE US POPULATION

As discussed in the main body of the manuscript, we recommend the use of two epidemiological studies of methyl mercury's (MeHg's) effect on the risk of myocardial infarction (MI) for use in benefits assessment, the European Community Multicenter Study on Antioxidants, Myocardial Infarction and Breast Cancer (Guallar et al. 2002; the "EURAMIC study") and the Kuopio Ischaemic Heart Disease Risk Factor Study (Virtanen et al. 2005; the "KIHD study"). In order to increase statistical power and widen the range of exposure levels to which the dose-response function (D-R) derived from these studies could be applied, it may be beneficial to pool across the results of these two studies.

In order to assess whether it would be reasonable to pool the results of these two studies, we compared the hair-mercury (Hg) levels of the study participants in the two studies. The KIHD study reports a mean hair-Hg level of 1.9 $\mu\text{g/g}$ (Virtanen et al. 2005). The EURAMIC study measured Hg in toenails, rather than hair. Therefore, we converted the toenail-Hg levels in that study to hair-Hg using a steady-state ratio of 2.44 $\mu\text{g Hg/g hair per } \mu\text{g Hg/g toenail}$ reported in Ohno et al. 2007. We selected a ratio from this study because the different biomarker samples were collected simultaneously, the study subjects were free from occupational Hg exposures and dietary questionnaires showed that fish intake accounted for most of the Hg biomarker concentrations in the study population. The resulting overall mean hair-Hg concentration among cases in the EURAMIC study that we calculated is 0.66 $\mu\text{g/g}$. The hair-Hg means for each individual study center that we calculated range from 0.34 to 1.66 $\mu\text{g/g}$. The hair-Hg levels observed across the two studies cover a range of values across the distribution of MeHg

exposures and therefore, we believe pooling the results of these studies for benefits assessments is reasonable.

We also assessed whether the hair-Hg levels of the study populations were comparable to the distribution of exposures in the US population to determine whether a pooled D-R function could be applied directly in a benefits assessment, or whether some adjustment would be necessary. We utilized hair-Hg measurements in two studies as the basis for our assumptions about the distribution of hair-Hg in the US, one including women of childbearing age in the US from the 1990-2000 National Health and Nutrition Examination Survey (NHANES) (McDowell et al. 2004) and one including women of childbearing age from 12 US states (Knobeloch et al. 2005).

The KIHD hair-Hg study mean of 1.9 $\mu\text{g/g}$ is slightly larger than the 95th percentile of hair-Hg in women of childbearing age in the US (1.73 $\mu\text{g/g}$ in McDowell et al. 2005 (see Table 2) and 1.58 $\mu\text{g/g}$ in Knobeloch et al. 2005 (see Table 5)). However, since men consume somewhat larger portion sizes than women, this corresponding percentile for men is likely to be somewhat less (Stern 2005).

The EURAMIC study overall hair-Hg mean of 0.66 $\mu\text{g/g}$, calculated above, falls between the 75th and 90th percentile of women of childbearing years in the US according to McDowell et al. 2004 and between the 75th and 95th percentile relying on the distributions reported in Knobeloch et al. (2005). In addition, the lowest hair-Hg level measured at an individual EURAMIC study center (0.34 $\mu\text{g/g}$ in Zeist, the Netherlands) falls between the 50th and 75th percentiles of hair-Hg in US women of childbearing age (0.19 and 0.42 $\mu\text{g/g}$ in McDowell et al. 2004 and 0.29 and 0.59 $\mu\text{g/g}$ in Knobeloch et al. 2005). The highest hair-Hg in an individual EURAMIC study center (1.66 $\mu\text{g/g}$ in Málaga, Spain) falls between the 90th and 95th percentile in

the McDowell study (1.11 and 1.73 $\mu\text{g/g}$) and is above the 95th percentile in the Knobeloch study (1.58 $\mu\text{g/g}$).

In conclusion, it appears as though the hair-Hg levels of study participants in the two studies we recommend as the basis for deriving a pooled D-R function for MeHg and MI are at the upper end of the distribution of hair-Hg in the US population. Therefore, consideration could be given to adjusting the D-R function derived from these studies before applying it in a benefits assessment or restricting its application only to those with higher end exposures (i.e., assume that there is a threshold for MeHg toxicity). However, there is some evidence supporting a linear D-R function (e.g., the Generalized Additive Model analysis in the EURAMIC study). If the relationship is in fact linear, it would be appropriate to apply a single slope across all levels of MeHg exposure.

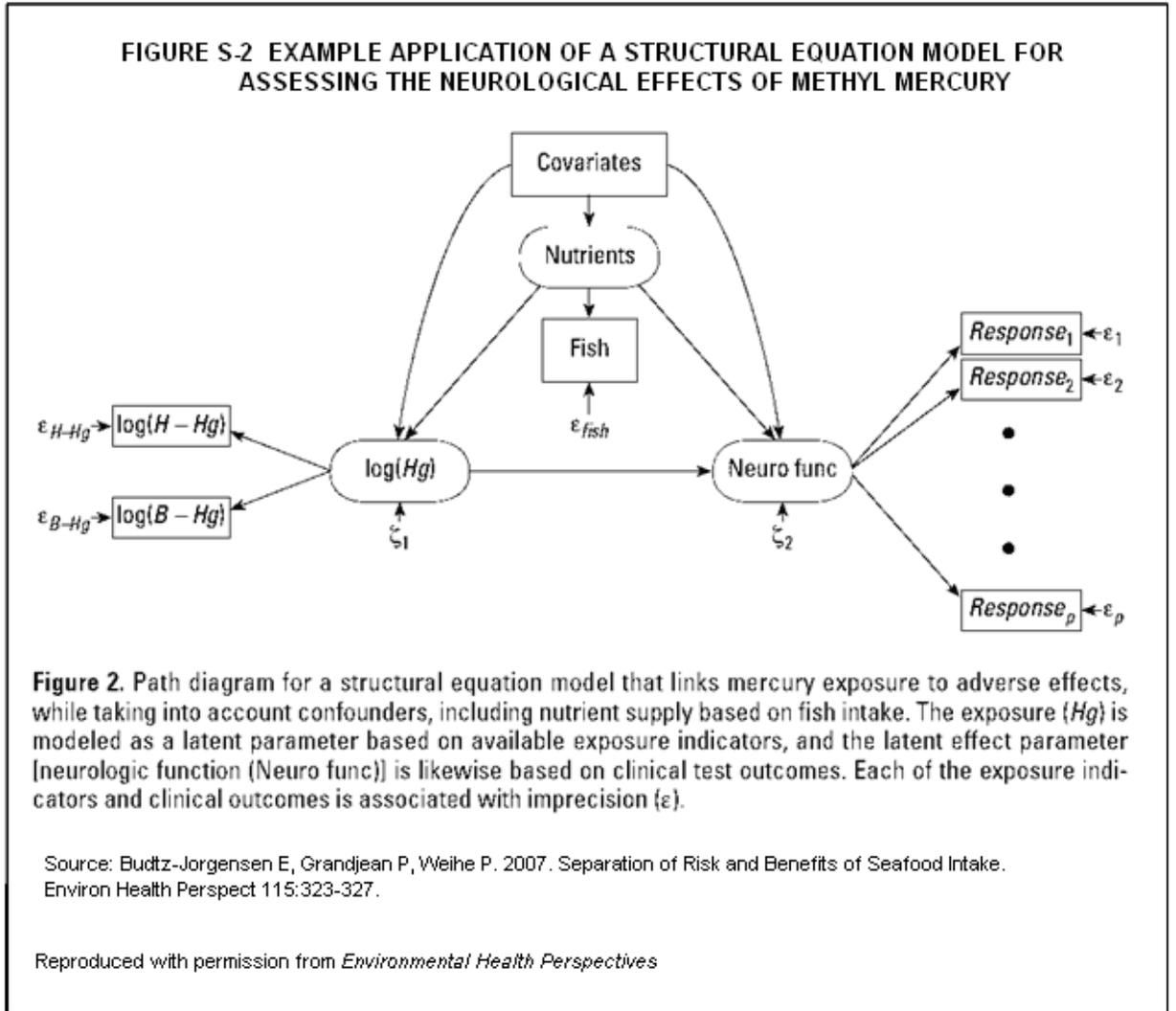
ADDITIONAL INFORMATION ON STRUCTURAL EQUATION MODELS

We recommend that investigators in future studies of methylmercury (MeHg) and cardiovascular effects consider applying Structural Equation Models (SEMs) to better address the limitations associated with the available biomarker measures and the multiple dimensions associated with cardiovascular health endpoints. The models have been used extensively in social science (Bollen 1989), and more recently, in the analyses of the health effects of lead (Chuang 2001; Sanchez et al. 2006) and MeHg (Budtz-Jorgensen 2003a, 2003b, 2007; Choi et al. 2009).

SEMs can effectively reduce the dimension of both exposure and response by assuming that each set of surrogate variables jointly reflect a relatively small number of unobservable, or latent, variables of interest. This approach has several advantages: it avoids multiple testing problems because the SEM typically contains fewer latent variables than observed health and exposure surrogates; it can yield more powerful tests of association by pooling evidence of a health effect across both outcomes and exposures; and it adjusts out any measurement error associated with individual exposure surrogates, which typically biases effect estimates downward (Budtz-Jorgensen et al. 2003b). SEMs are able to reduce measurement error because only the variation in a given surrogate that is common across all surrogates for a particular latent variable is captured.

Supplemental Material Figure 2 shows an example of an SEM structure used to assess neurobehavioral effects of MeHg. Budtz-Jorgensen and colleagues applied SEMs to estimate associations between exposure to MeHg, measured through several different exposure surrogates (i.e., cord blood-Hg concentrations, maternal hair-Hg concentrations, maternal whale meat consumption and toenail-Hg concentrations), and multiple neuropsychological test results

thought to jointly reflect neurobehavioral development (Budtz-Jorgensen et al. 2003a, 2003b; Choi 2009).



**Supplemental Material, Figure 2:
EXAMPLE APPLICATION OF A STRUCTURAL EQUATION MODEL FOR
ASSESSING THE NEUROLOGICAL EFFECTS OF METHYL MERCURY**

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