USE OF BIOMARKERS IN THE BENCHMARK DOSE METHOD

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ABSTRACT

The NRC *Science and Decisions* report discusses methods for assessing noncancer risks using linear extrapolation procedures similar to those used to assess risks posed by exposures to carcinogens. This study was conducted to demonstrate four potential approaches to extrapolating risks below the point of departure, identified using an extension of the Benchmark Dose (BMD) method that allows the development of risk values at doses above the Reference Dose (RfD). The use of an internal measure of dose (blood methylmercury concentration), rather than external concentrations, was also considered in the risk evaluation by incorporating data from the National Health and Nutrition Examination Survey (NHANES). Methylmercury provides an unusual case in which the available BMD/BMDLs (USEPA 2001a) are not only based on human responses, but were also estimated based on biomarkers (i.e., levels in hair and cord blood) measured in individuals as part of the evaluation for potential health effects. Both simple and more complex PBPK models are also available that allow the risk assessor to estimate external exposure rates associated with internal biomarkers. The dose-response conducted here addressed multiple issues raised by the NRC (2009) report including the impact of human variability, sensitive subpopulations, and method of extrapolation above and below the BMD/BMDL and RfD. The results demonstrate that depending on the assumptions used to estimate risks at exposures above the RfD, very different fractions of the population would be expected to have adverse events. This could have significant impacts on decision making in the risk assessment process. This case study exhibits how risk assessments using biomonitoring data for both dose-response and exposure helps to reduce uncertainty in the risk assessment process.

INTRODUCTION

In the *Science and Decisions* (NRC 2009) report, the National Research Council committee made several recommendations related to the practical improvements that USEPA could make in conducting risk assessments. Some recommendations were related to the design of risk assessment and focusing on the planning scoring and problem formulation. Greater attention to design will ensure that the most useful questions are being asked in the context of the risk assessment (NRC 2009).

In applying the results of a dose-response assessment in the screening assessment for potential health effects, it is critical to evaluate not only the potential exposure to the populations of concern, but also the likely fraction of the population that may be exposed to chemical concentrations above an "acceptable" level, such as a Reference Dose (RfD) or Reference Concentration (RfC). When a threshold for effect or nonlinear response at low doses is assumed, it is customary to estimate a point of departure (POD) based on data in the observable range. This can be estimated based on the No-Observed-Adverse-Effect Level (NOAEL) or a Benchmark Dose (BMD). However, instead of extrapolating to a low-dose risk, as is done with cancer endpoints, the POD is divided by "uncertainty factors" to adjust for animal-human differences, human-human differences in susceptibility, and other factors (for example, data gaps or study duration). In attempting to address the potential impact of exposure on a population and the estimated fraction of the population expected to have an adverse event, it may be necessary to extrapolate between the RfD and the POD.

This method is an extension of the benchmark dose (BMD) method that allows the development of risk values at doses above the Reference Dose (RfD) when the existing data are based on human responses. Methylmercury (MM) provides a case in which the available BMD/BMDLs (USEPA 2001a) are not only based on human responses, but were also estimated based on biomarkers (i.e., levels in hair and cord blood) measured in individuals as part of the evaluation for potential health effects. Several BMDs are available for MM for the critical effect in sensitive human populations, so that the usual extrapolation issues of average to sensitive human and experimental animal to human are mollified. Existing literature incorrectly implies that exposures above the RfD are associated with risk to a large population of sensitive humans. This is clearly not correct based on the current understanding of the existing MM data and BMD estimates.

METHODS

The appropriate BMD is chosen in the usual fashion using existing USEPA software and criteria, including p-values, AIC, residuals, BMD to BMDL ratios and visual inspection. The data are modeled to an appropriate point of departure using the usual judgment, and then four different procedures were investigated to extrapolate the potential risk:

- **Approach 1:** A straight line is drawn from both the BMDL and BMD to the RfD, where the RfD is considered to be zero risk
- **Approach 2:** The appropriate BMD model is extrapolated to the RfD and then the risk at the RfD is truncated to zero
- **Approach 3:** The appropriate BMD model is extrapolated to the RfD and this risk is allowed to stand as an upper bound
- **Approach 4:** The appropriate BMD model is extrapolated using a threshold term, where the threshold value is judged to be the RfD, or some higher value

Depending upon the problem formulation, some methods may be more appropriate for screening-level estimates of risk, while others may be more appropriate for more comprehensive estimates. The case study results demonstrate that depending upon the approach applied, the estimated population at risk can vary widely.

Minimum data requirements for the approaches demonstrated:

- **Dose-response data in humans that are the basis for the RfD. (Approaches 2, 3 and 4)**
- **Biomarker information for the chemical of interest**

Dose Response Data in Humans - POD/RfD for Methylmercury

Three longitudinal, developmental studies have been conducted to evaluate the potential neurodevelopmental effects of methylmercury from *in utero* exposure. These studies have been conducted in fish-eating populations from the Seychelles Islands, the Faroe Islands, and New Zealand. The current RfD for methylmercury is based largely on results from a cohort of children evaluated in the Faroe Islands (Grandjean et al. 1997; NRC 2000; USEPA 2001a, b), with selected endpoints from the New Zealand study considered. Benchmark Dose modeling was conducted using results from children administered a battery of neuropsychological tests. Cord blood and maternal hair concentrations of methylmercury were used as the dose metric for BMD modeling.

The current RfD of 0.0001 mg/kg/day reported in the Integrated Risk Information System (IRIS; USEPA 2001a) is based on an integrative analysis of BMDLs estimated from test results from both the Faroe Islands and New Zealand studies. The integrative analysis is consistent with the BMDL recommended by NRC (2000), which is a BMDL of 58 ppb cord blood methylmercury (BMD of 85 ppb) estimated based on the results from the Boston Naming Test in the Faroe Islands cohort. An Uncertainty Factor of 10 was applied for interindividual variability to result in the RfD of 5.8 ppm cord blood (0.1 µg/kg/day, based on the results from a one-compartment model).

Biomonitoring Information

Mercury blood levels from the NHANES survey in 2007-2008 (CDC 2009), were used to estimate levels of organic mercury in the blood of both children (1 to 19 years of age) and women of childbearing age (14 to 45 years of age). Percentiles of the blood organic mercury were calculated using Stata 10 (Release 10, StataCorp LP, College Station, Texas). Organic mercury levels selected for the derivation of the RfD are based on a sensitive subpopulation, children exposed in utero (women of childbearing age provide a surrogate for fetal exposure). Children were also considered, as NHANES provides biomonitoring information. The effect of exposure postpartum remains a potential uncertainty as a contributor to observations in the longitudinal studies.

RESULTS

The results of the NHANES 2007-2008 survey suggested a mean blood concentration of organic mercury of approximately 0.83 ppb. These estimated concentrations of total organic mercury were assumed to be methylmercury for purposes of this case study (Table 1).

TABLE 1: NHANES 2007-2008 Summary Statistics for Blood Organic Mercury (ppb)

	N	Arithmetic Mean	Median	95th	99th	Max
Women, Ages 14-45						
2001-2002*	1757	1.07	0.52	3.92	9.3	22.7
2003-2004	1794	0.9	0.4	3.7	6.6	22.3
2005-2006	1973	0.97	0.52	3.74	7.17	18.2
2007-2008	1535	0.83	0.40	3.34	7.14	10.82
Children, Ages <20						
2001-2002*	1345	0.37	**	1.82	5.32	15.6
2003-2004	3693	0.38	0.1	1.9	4.8	24.6
2005-2006	3862	0.38	0.12	1.87	3.98	11.31
2007-2008	2805	0.33	0.11	1.51	3.63	9.95

* In 2001-2002, blood mercury levels were measured only in children ages 1-5 and women ages 16-49

** Unable to calculate due to relatively high detection limits

For the first method of estimating the potential fraction of population (women of childbearing years and children) expected to have an adverse effect, the risk at the RfD was set to zero and a straight line was drawn from the RfD at zero risk to the BMD and a line to the BMDL at 5% risk, based on the dataset of Boston Naming Test scores for children for the Faroe Islands (Grandjean et al. 1997; Budtz-Jorgensen et al. 1999, 2000). For each of the unique blood mercury levels above the RfD in the NHANES data for both women of childbearing years and children, the risk was determined by interpolating along the straight line between the RfD and the BMD to get a most likely risk level and the line between the RfD and the BMDL to get an upper bound on the risk. This risk value was then multiplied by the population estimate at that blood mercury level (the sum of the weights which represents the US population) to obtain the expected number of cases of adverse events (Table 2).

TABLE 2: Estimated Risks and Number of Adverse Events in Subpopulations of Interest

Approach	RfD (ppb)	Population	Range of organic Mercury Levels	Range of Associated Risks		Estimated number of Adverse Events	
				Most Likely	Upper Bound	Most Likely	Upper Bound
1	5.8	Children (1 to 19 yrs)	6.3 - 9.9	3.2×10^{-4} to 2.6×10^{-3}	4.8×10^{-4} to 3.9×10^{-3}	256	389
	5.8	Women (14 -45 yrs)	6.0 - 10.8	1.3×10^{-4} to 3.2×10^{-3}	1.9×10^{-4} to 4.8×10^{-3}	1276	1936
1	10.5	Children (1 to 19 yrs)	No NHANES Organic Blood Levels above 10.5 ppb				
	10.5	Women (14 -45 yrs)	10.8	5.0×10^{-5}	3.2×10^{-4}	1	9
2	10.5	Women (14 -45 yrs)	10.8	2.5×10^{-5}	3.0×10^{-4}	1	43
3	10.5	Women (14 -45 yrs)	10.8	1.3×10^{-3}	4.3×10^{-3}	37	122
4	10.5	Women (14 -45 yrs)	10.8	Estimated Threshold of 77.8 ppb			

Women of childbearing age with total organic mercury blood concentrations above the RfD were above the 97th percentile in the distribution of blood concentrations (Table 1). The upper bound on the potential number of individuals at risk of having an adverse event was approximately 2000 (Table 2). For children, the total organic mercury blood concentrations above the RfD were above the 99th percentile in the distribution of blood concentrations (Table 1) and the upper bound on the potential number of individuals at risk of having an adverse event was approximately 400 (Table 2).

Shipp et al. (2000) derived a BMD and RfD based on the results of the Seychelles study, which was relied upon for a demonstration of all four approaches (Shipp et al. 2000). A BMD and BMDL were calculated using BENCH_C (ICF 1996) and data digitized from a published scatter plot of residuals of test scores for Visual Recognition (Myers et al 1995). The maternal hair mercury levels were converted to equivalent blood concentrations by dividing by 200 (Budtz-Jorgensen et al. 2004). A k-power model was fit to the data to obtain the BMD (approximately 610 ppb) and BMDL (approximately 105 ppb). An example RfD of 10.5 ppb was calculated as the BMDL divided by an uncertainty factor of 10. The resulting linear extrapolation indicated a very small number of potential adverse events in women of childbearing years, with an upper bound of approximately 10 events (Table 2).

For the second and third methods, Benchmark modeling was used to determine the risk levels from the BMD and the BMDL down to the RfD. The benchmark model which provided the BMD and BMDL was used to determine a maximum likelihood estimate (MLE) and lower bound (LB) on that estimate from a risk of 10% down to a risk for which the estimated LB was equal or below the RfD and then further down to a risk for which the estimated MLE was equal to or below the RfD. This resulted in a table of risks, MLEs and LB with the risks varying from 10% down to 0.1% (Table 3) which represent the shape of the dose-response curve for the MLE and the LB determined by the Benchmark model fit to the data.

For the second method, the risk at the RfD was determined by interpolation for the MLE and the LB along these lines. The lines were shifted so that the risk level at the RfD was 0 by subtracting the risk level at the MLE or LB equal to the RfD from each of the risks. The risk at each of the unique mercury blood levels from NHANES was determined by interpolating between the two MLEs or two LBs in Table 3 which were closest to the NHANES blood level to get a most likely and upper bound on the risk and these risk values were multiplied by the population estimate at that blood mercury level to obtain the expected number of cases of adverse events. The results from the third method were similar to the second, but the dose-response curves were not shifted so that the risk at the RfD was zero, which results in an increase in the most likely and upper bound on risk for each of the NHANES blood levels by the risk associated with the RfD from those used in the second method.

For the final method, a threshold benchmark model (THC; ICF 1997) was fit to the data. Restrictions forced the grouping of the data into 10 approximately equal size groups. The dose level used for each group was assumed to be the mid-point of each group. A polynomial model with a threshold was fit to the data and the threshold predicted by the model (approximately 78 ppb) was larger than any of the blood mercury levels reported in the current NHANES survey (Table 2).

The results demonstrate that regardless of the POD/RfD relied upon or the method of extrapolation between the RfD and the POD that there is not a large fraction of the US population exposed to methylmercury above the RfD. While the different approaches demonstrate some changes in estimates, they are not large due to the small number of women of childbearing age with blood concentrations above the RfD. As shown in Figure 1, this case study does demonstrate that depending on the assumptions used to extrapolate above the RfD, different fractions of the population would be expected to have adverse events. This could have significant impacts on decision making in the risk assessment process.

DISCUSSION

The overall approach applied in this case study to evaluate the potential for adverse events in the general population or even a selected subpopulation has several strengths.

Strengths

- Use of a biomarkers, which are typically closer to the "target tissue" concentration than the use of external exposure concentration
- Ability to evaluate the potential fraction of people exposed above and below the RfD to assess the likelihood of adverse noncancer effects at a specified internal concentration, which may be extended to an exposure level if information are available.
- Ability to estimate potential risk at a specific dose or biomarker concentration above the RfD.

While there are numerous strengths to this approach, there are limitations as well.

Limitations

- Uncertainties for other compounds as to the relationship between biomarker and effects of concern. For methylmercury, there is information on the potential relationship between internal dose metrics, such as hair levels and blood levels, and external intakes, as well as responses. This information is not available for every compound of concern.
- Information characterizing the potential shape of the dose-response curve below the BMD/BMDL. In the case of the Faroe Islands results, which largely make up the basis for the current USEPA (2001a) RfD, these data are not readily available which limits the types of approaches that could be applied. These data are not needed for linear extrapolation (Approach 1), but as demonstrated, the approach and assumptions used can impact the estimate of the potentially affected population.

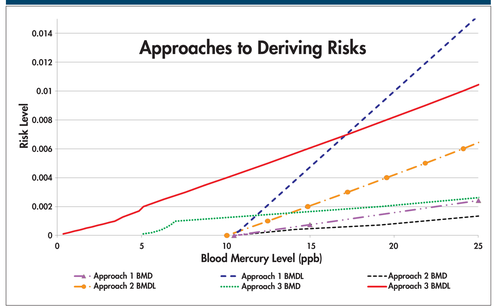
The case study approach demonstrated can address several specific issues raised by the NRC (2009) report. First, intraspecies variability and sensitive populations are usually addressed by the use of an intraspecies uncertainty factor of up to 10. This method can be used to consider measured biomarkers of exposure in sensitive subpopulations or selected populations, such as women of childbearing years, and to evaluate the relationship to the RfD or the BMD/BMDL. Secondly, this case study specifically focuses on background exposure and potential responses. Consideration of the NHANES data focuses on background levels of compounds in the general population. This method can be extended to biomarker information for specific populations as well, if data are available. And finally, the method is easy to apply, as long as the critical data are available. It can also answer questions as part of the problem formulation in a screening level risk assessment to help risk assessors in decision making.

The study authors also investigated the use and incorporation of mode of action (MOA) data for MM to better inform the choice of approach for extrapolating risks below the point of departure. However, at the present time, there is no single hypothesized potential MOA for the neurodevelopmental effects that serve as the basis for the RfD. Currently, the available MOA studies for MM suggest that the observed neurodevelopmental effects likely occur by more than one mechanism (Faustman et al. 2002; Atchison and Ware 1994).

TABLE 3: MLE and LB on Dose associated with Specific Risks Determined from the K-Power Benchmark Model

Risk	MLE (ppb)	LB (ppb)
0.1	610.63	105.80
0.09	566.59	104.04
0.08	520.36	101.98
0.07	471.58	99.51
0.06	419.85	96.48
0.05	364.62	97.23
0.04	305.20	81.38
0.03	240.65	64.17
0.02	169.68	45.24
0.01	90.41	24.11
0.009	81.93	21.85
0.008	73.33	19.55
0.007	64.62	17.23
0.006	55.79	14.88
0.005	46.83	12.49
0.004	37.74	10.06
0.003	28.52	7.60
0.002	19.16	5.11
0.001	7.01	3.42

FIGURE 1: Estimation of Risk Associated with Blood Mercury Level Using Approaches 1, 2, and 3



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