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Methods and Rationale for Derivation of a Reference Dose for Methylmercury by the U.S. EPA

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In 2001, the U.S. Environmental Protection Agency derived a reference dose (RfD) for methylmercury, which is a daily intake that is likely to be without appreciable risk of deleterious effects during a lifetime. This derivation used a series of benchmark dose (BMD) analyses provided by a National Research Council (NRC) panel convened to assess the health effects of methylmercury. Analyses were performed for a number of endpoints from three large longitudinal cohort studies of the neuropsychological consequences of *in utero* exposure to methylmercury: the Faroe Islands, Seychelles Islands, and New Zealand studies. Adverse effects were identified in the Faroe Islands and New Zealand studies, but not in the Seychelles Islands. The NRC also performed an integrative analysis of all three studies. The EPA applied a total uncertainty factor (UF) of 10 for intrahuman toxicokinetic and toxicodynamic variability and uncertainty. Dose conversion from cord blood mercury concentrations to maternal methylmercury intake was performed using a one-compartment model. Derivation of potential RfDs from a number of endpoints from the Faroe Islands study converged on 0.1 μg/kg/day, as did the integrative analysis of all three studies. EPA identified several areas for which further information or analyses is needed. Perhaps the most immediately relevant is the ratio of cord:maternal blood mercury concentration, as well as the variability around this ratio. EPA assumed in its dose conversion that the ratio was 1.0; however, available data suggest it is perhaps 1.5–2.0. Verification of a deviation from unity presumably would be translated directly into comparable reduction in the RfD. Other areas that EPA identified as significant areas requiring further attention are cardiovascular consequences of methylmercury exposure and delayed neurotoxicity during aging as a result of previous developmental or adult exposure.

KEY WORDS: Methylmercury; risk assessment; reference dose; Environmental Protection Agency; neuropsychological effects

Awareness of the neurotoxic potential of methylmercury resulted from a mass poisoning episode beginning in the 1950s in residents living near Minamata Bay in Japan. Methylmercury was discharged directly into the bay and was bioaccumulated and bioconcentrated by fish, which were a main dietary component of people in the region. Although both adult and infant or fetal exposure produced signs of methylmercury poisoning, the constellation of effects was different, and effects of developmental exposure were more severe.[1] Consequences of congenital methylmercury poisoning included mental retardation, cerebellar ataxia, seizures, visual abnormalities, and other neurological abnormalities. A

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subsequent episode of methylmercury poisoning occurred in Niigata, Japan, in 1963–1965, involving hundreds of people; the source was a fertilizer factory that released methylmercury into a river that flows into a bay from which fish were caught.[2]

An episode of methylmercury poisoning occurred in Iraq in the 1970s when people consumed methylmercury-treated flour ground from grain intended for planting. Exposure was relatively acute compared to the poisoning episodes in Japan. Highly-exposed children manifested severe sensory impairment, paralysis, hyperactive reflexes, cerebral palsy, and impaired mental development.[3] Other children exposed *in utero* exhibited delayed walking and talking and an increased prevalence of neurological signs.[4] Subsequent epidemiological studies in various areas of the world including the Amazon,[5] Ecuador,[6] French Guiana,[7] Madeira,[8] and Canada (northern Quebec)[9] documented adverse neuropsychological effects associated with developmental exposure to methylmercury, including sensory, motor, and/or cognitive deficits.

The U.S. Environmental Protection Agency (EPA) has, as part of its mandate, a responsibility to perform risk assessments for chemicals present in the environment that may pose a hazard to human health. Risk assessment may be defined as "the characterization of the potential adverse health effects of human exposure to environmental hazards."[10] To accomplish this for noncancer effects, a reference dose (RfD) is derived, defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime." The RfD may be derived from a no adverse effect level (NOAEL) (the highest dose at which no adverse effects are identified), or a low adverse effect level (LOAEL) (the lowest dose at which adverse effects are observed), typically identified in animal studies. It may also be derived from benchmark dose (BMD) analysis, which was used by EPA for derivation of the RfD for methylmercury described herein. In any case, a point of departure (POD) is identified, which is the point on the dose-response curve that marks the starting point for derivation of the RfD. Typically, this POD is divided by one or more uncertainty factors (UFs) in order to account for various extrapolations and perceived insufficiencies in the database.[11]

EPA derived an RfD for methylmercury in 1995 based on the episode of methylmercury poisoning in Iraq. An exposure-effect curve was modeled based on a composite of adverse effects in 81 infants, including onset of walking and talking, soft neurological signs, mental symptoms, and seizures, using a Weibull model. The RfD was 0.1 μg/kg/day of methylmercury, based on estimated intake by the mothers during pregnancy. In 1997, Congress mandated that EPA fund an expert panel under the auspices of the National Research Council (NRC) to determine whether the RfD was scientifically justifiable. The NRC panel concluded that an RfD of 0.1 μg/kg/day was scientifically justifiable based on its analysis.[12] However, NRC recommended that EPA not base its assessment on the Iraqi study, but rather on new large epidemiological studies that had become available since 1995. EPA therefore completed a risk assessment of methylmercury in 2001, using the studies and the analyses suggested by the NRC.

1. STUDIES CONSIDERED IN DERIVATION OF THE RfD

There are three recent epidemiological studies suitable for quantitative analysis that have become available in the peer-reviewed literature since EPA's derivation of an RfD in 1995, and that were used in the extensive quantitative analyses by the NRC.[12] These longitudinal prospective developmental studies were conducted in the Seychelles Islands, the Faroe Islands, and New Zealand. The Seychelles Islands study consisted of 779 mother-infant pairs from a fish-eating population.[13–18] Infants were followed from birth to 5.5 years of age, and assessed at various ages on a number of standardized neuropsychological endpoints. The independent variable was maternal hair mercury concentrations. The Faroe Islands study included about 900 mother-infant pairs; children were tested on a variety of tasks at 7 years of age.[19] The main independent variable was cord blood mercury, although maternal hair mercury was also measured. In the New Zealand study, 38 children of mothers with hair mercury levels during pregnancy greater than 6 ppm were matched with children whose mothers had lower hair mercury concentrations.[20,21] At 6 years of age, a total of 237 children were assessed on a number of neuropsychological endpoints similar to those used in the Seychelles study.[20] Investigators in the Seychelles Islands study reported no evidence of impairment related to *in utero* methylmercury exposure in their main study, whereas the other two studies

Table I. Tests Modeled by NRC, Functions Assessed, and Potential Societal Relevance

Study	Test	Domain/Function Assessed	Societal Relevance
Seychelles	Bender Copying Errors	Visuospatial	Math performance
	McCarthy GCI	Full-Scale IQ	School performance, intelligence
	WJ Applied Problems	Ability to solve problems	Academic skills
	CBCL	Social and adaptive behavior	Antisocial behavior, need for therapeutic services
	Preschool Language Scale	Broad-based language	Learning, intelligence, school performance
	WJ Letter/Word Recognition	Word recognition	Reading ability, school performance
Faroes	Finger Tapping	Motor performance	Motor speed/neuropathy
	CPT Reaction Time	Vigilance, attention, information processing speed	Intelligence, school behavior, and performance
	Bender Copying Errors	Visuospatial	Math performance
	Boston Naming Test	Expressive vocabulary	Reading, school performance
	CVLT: Delayed Recall	Memory	Learning ability, school performance
New Zealand	TOLD Language Development	Broad-based language	Literacy skills, learning, school performance
	WISC-R: PIQ	Performance IQ, e.g., visuospatial, sustained attention, sequential memory	Learning, school performance
	WISC-R: FSIQ	Full-scale IQ, e.g., PIQ + verbal processing, expressive vocabulary	Learning, school performance
	McCarthy Perceptual Performance	Performance IQ, e.g., visuospatial, audition, memory	Learning, school performance
	McCarthy Motor Test	Gross and fine motor skills	Motor system integration

Notes: WJ = Woodcock-Johnson Tests of Achievement; CBCL = Child Behavior Check List; CPT = Continuous Performance Test; CVLT = California Verbal Learning Test; TOLD = Test of Language Development; WISC-R:PIQ = Wechsler Intelligence Scale for Children-Revised Performance IQ; WISC-R:FSIQ = Wechsler Intelligence Scale for Children-Revised Full-Scale IQ.
Source: Reference 26:4–51.

found exposure-related effects on a number of neuropsychological endpoints.

The NRC quantitative analysis included five endpoints from the Faroe Islands study from a total of nine that had been reported as significantly affected by methylmercury exposure[19] (Table I). Similarly, five endpoints negatively associated with methylmercury exposure in the New Zealand study[20] were used in the quantitative analysis by the NRC. The study in the Seychelles Islands did not identify adverse effects of methylmercury exposure on any of the neuropsychological endpoints; the NRC modeled all six of the endpoints assessed in that study. The tests used in the Faroe Islands study were chosen to assess specific functional domains. In contrast, the tests used in the New Zealand and Seychelles Islands studies were apical tests that yield global scores representing the integration of performance over a number of domains. A workgroup convened by NIEHS in 1998 to compare the Faroe and Seychelles Islands studies postulated that differences in the test endpoints assessed might account for the conflicting findings between the two studies.[22] However, the New Zealand study also used apical tests, and the NRC panel concluded that the difference in the results between the Seychelles and Faroe Islands studies was probably not due to differences in assessment methodology.[12] All these endpoints assessed functions that are important for the child's ability to learn, remember, and be successful in an academic setting.

Maternal exposure to methylmercury in the studies was comparable and is unlikely to account for the differential findings in the studies. The maternal hair geometric mean in the Faroe Islands study was 4.3 ppm, with an interquartile range of 2.6–7.7 ppm. The mean maternal hair mercury in the Seychelles Islands study was 6.8 ppm with a standard deviation of 4.5. In the New Zealand study, the mean hair mercury concentration was 8.3 ppm for the 38 mothers in the "high" group, each matched to three "controls" with lower mercury levels. There are other differences between the studies that may account for the

differences in results, which have been discussed extensively elsewhere.[12,22] Among these are children's age at testing, differential genetic susceptibility of the populations, differential pattern of exposure to methylmercury (episodic vs. relatively continuous), and co-exposure to PCBs in the Faroe Islands study (discussed below). It is not possible to determine on the current evidence which if any of these factors is responsible for the different findings in the Seychelles versus Faroe Islands and New Zealand studies.[12,22] NRC[12] points out that the power of the Seychelles study to detect the small effects identified in the Faroe Islands study was only about 50%.

2. DETERMINATION OF THE POINTS OF DEPARTURE FOR THE RfD

EPA chose benchmark dose (BMD) analysis as the most appropriate method of quantifying the dose-effect relationship in these studies, which was the recommendation of the NRC.[12] The *K*-power model was used to model the exposure-effect functions. This model allows for a decreased magnitude of response at lower exposures compared to higher exposures (sublinearity), which would be appropriate if there were a threshold, for example. The *K*-power model was constrained to $K \geq 1$, which precluded a greater apparent response at low exposures relative to higher exposures (supralinearity). $K = 1$ (i.e., a linear dose-effect function) provided the best fit of the *K*-power models for the Faroe Islands data;[23,24] therefore this model was used in the NRC BMD calculations for all three studies.

Benchmark dose analysis requires two additional decisions once an appropriate model has been chosen. When continuous data are used, a point on the curve below which responses are considered "abnormal" must be chosen, termed P_0. A value of $P_0 = 0.05$ was used in the EPA assessment; that is, the cutoff for abnormal response was set at the lowest 5% (5th percentile) of children. Most human characteristics, including children's neurodevelopmental abilities, have an approximately bell-shaped or "normal" distribution. Generally speaking, children who function at or below approximately the 5th percentile would be considered significantly developmentally compromised for the ability that is being measured (e.g., an IQ < 75, or performance on standard scores for more specific abilities such as attention, language, or memory).

The second decision that must be made is the choice of the increase in the proportion of individuals that will be expected to perform in the "abnormal" category in an exposed versus an unexposed population. This is defined as the benchmark response (BMR). A BMR of 0.05 was chosen for this assessment, which would result in a doubling of the number of children with a response at or below the 5th percentile in an unexposed population. Effects were identified on a number of neuropsychological endpoints at approximately the same body burden, which included tests that are predictive of reading and mathematics performance, overall academic performance, and antisocial behavior. The NAS concluded that "[d]eficits of the magnitude reported in the [Faroe and New Zealand] studies are likely to be associated with increases in the number of children who have to struggle to keep up in a normal classroom or who might require remedial classes or special education." [12:325] Such adverse effects are costly for the individual as well as for society as a whole. The choice of a BMR greater than 0.05, which potentially would allow a greater proportion of children to perform in the "abnormal" range, was considered unjustifiable by EPA.

BMDs were calculated for each of the endpoints described above for each of the three studies (Table II). The lower limit on the 95% confidence interval of the BMD (the BMDL) was calculated for each endpoint. These BMDLs served as potential points of departure for the RfD. The BMDLs from the Faroe Islands study were 12–15 ppm in maternal hair, whereas those in the New Zealand study were 4–6 ppm. BMDLs from the Seychelles Islands study were 17–25, about 50% higher than those in the Faroe Islands and 250–300% higher than those from the New Zealand study. It is interesting that the most sensitive measure in the Seychelles Islands study was the Child Behavior Checklist, which is a test of social and adaptive abilities.

It is important to recognize that values derived from a BMD analysis do not represent a threshold, nor are they comparable to a NOAEL or LOAEL as typically derived from animal studies. NOAELs and LOAELs are dependent on the doses chosen for the study. BMD analysis, on the other hand, makes full use of the range of the exposure-effect curve. BMD analysis identifies body burdens that are associated with an identifiable increased risk of a specific adverse effect. Moreover, in the Faroe Islands study at least, there was no evidence for a threshold for adverse effects within the range of maternal exposures in the study, as evidenced by the fact that the $K = 1$ model provided a better fit to the data than *K* values greater than 1. In

Table II. BMD (BMDL) Estimates (ppm MeHg in Maternal Hair) for Endpoints from Three Longitudinal Prospective Studies

Study	Endpoint	BMD[a]	BMDL
Seychelles	Bender Copying Errors	***	25
	Child Behavior Checklist	21	17
	McCarthy General Cognitive	***	23
	Preschool Language Scale	***	23
	WJ Applied Problems	***	22
	WJ Letter/Word Recognition	***	22
Faroe Islands	Finger Tapping	20	12
	CPT Reaction Time	17	10
	Bender Copying Errors	28	15
	Boston Naming Test	15	10
	CVLT: Delayed Recall	27	14
New Zealand	TOLD Language Development	12	6
	WISC-R:PIQ	12	6
	WISC-R:FSIQ	13	6
	McCarthy Perceptual Performance	8	4
	McCarthy Motor Test	13	6

[a]BMDs are calculated from the *K*-power model under the assumption that 5% of the responses will be abnormal in unexposed subjects ($P_0 = 0.05$), assuming a doubling of the excess risk (BMR = 0.05).

Notes: WJ = Woodcock-Johnson Tests of Achievement; CPT = Continuous Performance Test; CVLT = California Verbal Learning Test; TOLD = Test of Language Development; WISC-R:PIQ = Wechsler Intelligence Scale for Children-Revised Performance IQ; WISC-R:FSIQ = Wechsler Intelligence Scale for Children-Revised Full-Scale IQ.

Source: Reference 12:284.

addition, the logarithmic model provided a better fit than the $K = 1$ model for at least some endpoints in the Faroe Islands study.[23,24] In all cases, the logarithmic model yielded BMDLs considerably lower than the $K = 1$ model. EPA used the $K = 1$ model because of concerns about the stability of the logarithmic model at low exposures, as well as questions concerning the biologic plausibility of a supralinear response at low mercury exposures. However, these analyses provide further evidence against a threshold within the range of exposures in the Faroe Islands study.

In addition to methylmercury, the Faroe Islands cohort was also exposed to polychlorinated biphenyls (PCBs). An association was identified between PCB levels in cord tissue and four of the nine measures for which an effect of methylmercury was also identified.[19] Statistical analyses performed by the Faroe investigators suggested that the effects of methylmercury and PCBs were independent of each other.[25] NRC presented additional analyses designed to explore the influence of PCBs (Table III). PCB concentrations were determined on cord tissue from about one-half of the Faroese cohort (about 450 children) and the "adjusted for PCBs" values are based on those children for whom PCB cord tissue levels and cord blood mercury concentrations were available. Analyses were also performed on children in the lowest tertile with respect to PCB levels, which reduced the number of children available for analyses to about 150. No pattern was apparent for the PCB-adjusted analyses compared to the original results, and the observed variability was probably no more than that which would be expected by chance alone. This analysis provides compelling evidence that the effects of methylmercury identified in this study were not the consequence of exposure to PCBs.

In addition to determining the BMDLs for individual endpoints in each of the three studies, NRC also used a hierarchical random effect model to reduce random variation in the estimate for these same endpoints from all three studies.[12:290–294, Table 7-5] Additionally, this analysis was used in calculating BMD and BMDLs for the most sensitive and median endpoints from both the Faroe Islands and New Zealand studies.[12:294,Table7-6] This approach also allowed an integrative analysis based on all three studies.

3. DERIVATION OF THE RfD

To derive the RfD, the level of mercury in cord blood had to be converted to a corresponding intake of methylmercury by the mother. EPA used a one-compartment model for maternal methylmercury kinetics in the assessment.[26] This model provided a reasonable fit to the data,[27] and did not require a series of assumptions necessary for other models.[28] The BMDLs from the endpoints from all three studies modeled by the NRC were converted using a one-compartment model to an ingested dose of methylmercury associated with a corresponding cord blood level (Table IV).

The last step in the derivation of an RfD is the choice of UFs. Since the methylmercury assessment was based on BMD analysis of developmental effects in humans, some of the UFs often employed were irrelevant (e.g., extrapolation from animals to humans, from less-than-chronic to chronic, from LOAEL to NOAEL). The assessment utilized a rich database for developmental neurotoxicity, such that EPA believed that an UF for insufficiency in the database was unwarranted. However, areas where additional

Exposure	Endpoint	Full Cohort	Adjusted for PCBs	Low-PCB Tertile
Maternal hair (ppm)	Finger Tapping	20 (12)	17 (9)	7 (4)
	CPT Reaction Time	18 (10)	27 (11)	13 (5)
	CVLT: Delayed Recall	27 (14)	39 (12)	32 (7)
Cord Blood (ppb)	Finger Tapping	140 (79)	149 (66)	41 (24)
	CPT Reaction Time	72 (46)	83 (49)	53 (28)
	BNT	85 (58)	184 (71)	127 (40)
	CVLT: Delayed Recall	246 (103)	224 (78)	393 (52)

Table III. BMD (BMDL) Estimates from the Faroe Islands Study With and Without Adjustment for PCBs and in the Subset of Children in the Lowest Tertile with Respect to PCB Exposure (Calculated Using the *K*-Power Model)

[a]BMDs are calculated under the assumption that 5% of the responses will be abnormal in unexposed subjects ($P_0 = 0.05$), assuming a doubling of the excess risk (BMR = 0.05).
Notes: CPT = Continuous Performance Test; BNT = Boston Naming Test; CVLT = California Verbal Learning Test.
Source: Reference 12:289, Table 7-4.

information or analyses would be valuable were identified (see below). The only UF factor included in the assessment was a factor of 10 for intrahuman variability.

A composite UF of 10 was used by EPA, $10^{0.5}$ for pharmacokinetic (absorption, elimination, and tissue distribution) variability and uncertainty, and $10^{0.5}$ for pharmacodynamic (biologic response of the relevant organ or tissue) variability and uncertainty. (Half-log units are expressed as 3.0 rather than $10^{0.5}$ by EPA.) For the former, EPA relied on the NRC analyses of variability in the pharmacokinetic factors underlying the conversion from a maternal body burden of methylmercury to an ingested daily dose of methylmercury that corresponded to that concentration.[12:92, Table 3-1] The interindividual variability in ingested dose corresponding to a given maternal body burden from three independent analyses was 1.7–3.0 based on maternal blood, and 1.8–3.3 for maternal hair, using the ratio of the 50th percentile/1st percentile. EPA therefore considered that a factor of three was protective of 99% of the population based on variability of maternal blood mercury half-life.

A quantitative uncertainty analysis was not possible for toxicodynamics. However, the population of the Faroe Islands is descended from Scandinavian stock that settled many generations ago and is extremely homogeneous. The population of the Seychelles Islands is a stable European-African population that may also be more homogeneous than that of the United States. The New Zealand cohort, on the other hand, included individuals from several ethnic groups, and BMDs calculated from this population were lower than those of the Faroe or Seychelles Islands. The relative variability within and between these different populations is unknown, as is the comparability of these populations to that of the United States. A three-fold UF for toxicodynamic variability and uncertainty was applied by EPA.

Support for the choice of an overall UF of 10 is provided by the additional analyses of the Faroese neuropsychological data.[19] Associations remained significant when the observations from offspring of mothers with maternal hair mercury concentrations greater than 10 ppm were excluded from the analyses. This finding indicates that methylmercury-related deficits are present at concentrations below 10 ppm mercury in hair, which corresponds to about 40 ppb in cord blood. The points of departure for the Faroe Islands study were 12–15 ppm in maternal hair, based on the BMD analysis. It may be that effects below 10 ppm are less severe than the magnitude defined by the choices of P_0 and BMR in the BMD analysis. In any event, the fact that the associations were still identified at hair levels below 10 ppm strongly indicates the appropriateness of an UF of at least 10.

The last column of Table IV shows the RfDs for the various endpoints including application of an UF of 10. The calculated RfD values converge on the same point: 0.1 μg/kg/day. Among the endpoints from the Faroe Island study, there are three deviations from 0.1 μg/kg/day: 0.2 μg/kg/day for the CVLT, entire cohort; 0.05 μg/kg/day for CPT, lowest PCB tertile; and 0.05 μg/kg/day for Finger Tapping, lowest PCB tertile. EPA also calculated geometric means from the four endpoints from the Faroe Islands study;[26] RfDs are 0.1 μg/kg/day based on these calculations. For the New Zealand study, smoothed values, both the median value and the results of the McCarthy Perceived

Table IV. BMDLs, Ingested Dose, and RfDs for Various Endpoints from the Faroes Islands, New Zealand, and the NRC Integrative Analysis[a]

Test[b]	BMDL ppb Mercury Cord Blood	Ingested Dose μg/kg/day[c]	RfD μg/kg/day[d]
BNT Faroes			
Whole cohort	58	1.081	0.1
PCB adjusted	71	1.323	0.1
Lowest PCB	40	0.745	0.1
CPT Faroes			
Whole cohort	46	0.857	0.1
PCB adjusted	49	0.913	0.1
Lowest PCB	28	0.522	0.05
CVLT Faroes			
Whole cohort	103	1.920	0.2
PCB adjusted	78	1.454	0.1
Lowest PCB	52	0.969	0.1
Finger Tap Faroes			
Whole cohort	79	1.472	0.1
PCB adjusted	66	1.230	0.1
Lowest PCB	24	0.447	0.05
Geometric mean Faroes			
Whole cohort	68	1.268	0.1
PCB adjusted	65	1.212	0.1
Lowest PCB	34	0.634	0.1
Smoothed values			
BNT Faroes	48	0.895	0.1
CPT Faroes	48	0.895	0.1
CVLT Faroes	60	1.118	0.1
Finger Tap Faroes	52	0.969	0.1
MCCPP New Zealand	28	0.522	0.05
MCMT New Zealand	32	0.596	0.1
Median values			
Faroes	48	0.895	0.1
New Zealand	24	0.447	0.05
Integrative			
All endpoints	32	0.596	0.1

[a]$BMDL_{05}$s from Reference 12:Tables 7-4, 7-5, 7-6. Hair mercury was converted to blood mercury using a 250:1 ratio and an assumption of equivalent maternal and cord levels.
[b]BNT = Boston Naming Test; CPT = Continuous Performance Test; CVLT = California Verbal Learning Test; MCCPP = McCarthy Perceived Performance; MCMT = McCarthy Motor Test.
[c]Calculated using a one-compartment model.
[d]Calculated using an UF of 10.
Source: Reference 26:4–61.

Performance test, yield RfDs of 0.05 μg/kg/day, and the McCarthy Motor Test yields an RfD of 0.1 μg/kg/day. Based on the integrative analysis of all three studies, the RfD would be 0.1 μg/kg/day.

Rather than choose a single measure for the RfD critical endpoint, EPA based the derived RfD on the BMDLs for a number of endpoints from the Faroe Islands study, with supporting analyses from the New Zealand study and the integrative analysis of all three recent large epidemiological studies.

4. OTHER ISSUES NOT ADDRESSED IN THE RfD

In addition to the issues considered to be included in the overall uncertainty factor of 10, EPA recognized additional areas for which data are lacking, and for which there is a reasonable possibility that further information could be important in the RfD determination. A potentially very important issue is the ratio of maternal blood mercury concentrations to the concentrations in fetal cord blood. EPA assumed that the ratio was 1:1 in its dose conversion from cord blood mercury concentrations to methylmercury daily intake by the mother. However, there is evidence that the cord:maternal blood ratio is greater than 1. Review of the literature identified about 20 studies that reported cord and maternal blood mercury concentrations.[26] The composite ratio from the studies indicates that the cord:maternal blood ratio was around 1.7. The calculated ratios are means and do not reflect the full range of variability in the individual mother-fetal pairs. For example, Vahter *et al.*[29] reported the 5th and 95th percentiles of cord:maternal Hg to be 0.88 and 3.1. A deviation from EPA's assumption that cord:maternal blood mercury ratio was 1 would be directly reflected in inaccuracy of the RfD. For example, if the real ratio were in fact 1.7, the RfD should be decreased by a directly comparable amount (to 0.06 μg/kg/day). This issue requires further investigation to determine both the best estimate of central tendency (average) as well as the variability in the ratio for individual mother:infant pairs. The latter calculation would have relevance for the choice of the uncertainty factor: specifically, the factor used for pharmacokinetic variability and uncertainty.

Another area that EPA identified for further analysis was the association between methylmercury exposure and adverse cardiovascular effects. In a study of 1,000 seven-year-old Faroese children, diastolic and systolic blood pressures increased by 13.9 and 14.6 mm Hg, respectively, as the cord-blood mercury increased from 1 to 10 μg/L.[30] A 47% decrease in heart rate variability (an indication of decreased cardiac autonomic control) was also observed. In a seven-year observation period of 1,833 Finnish men, individuals with hair mercury in the highest tertile (2 ppm or higher) had a 2.0 times greater risk of acute

myocardial infarction compared to the rest of the study population.[31] The body burden at which cardiovascular effects are observed in adults should be identified, preferably with quantitative procedures such as BMD analysis.

An additional area of concern for which quantitative data are lacking is the onset or exacerbation of neurological deficits in aging populations previously exposed to methylmercury. "Acts of daily living," which included the abilities to independently eat, bathe, wash, dress, and use the toilet, were evaluated in people diagnosed with Minamata Disease (MD).[32] The prevalence of deficits was relatively greater in persons with MD compared with controls as a function of increasing age. In other words, exposure to methylmercury three decades earlier accelerated the aging process in aged individuals relative to younger ones. Adults who lived in a methylmercury-polluted area near Minamata City in Kumamoto Prefecture in Japan but who were not diagnosed with MD were compared with age-matched adults from a region with low exposure.[33] Complaints that were significantly higher in methylmercury-contaminated areas included a number of neurological signs and symptoms. Animal studies lend support to the conclusion that methylmercury can have delayed effects that are uncovered with age. Neurological and immunological impairment emerged during middle adulthood in mice exposed prenatally to methylmercury.[34] Rats exposed to methylmercury *in utero* and post-natally exhibited a decline in performance in a task that required a substantial motor output at an earlier age than did control rats.[35] Rice and colleagues[36–38] identified accelerated aging of sensory system function in a series of studies in monkeys exposed developmentally to methylmercury. All these observations suggest that either developmental or adult exposure to methylmercury can have adverse long-term sequelae that may not be detected for years or decades following cessation of exposure. However, these effects cannot be quantified in humans based on available data.

5. CONCLUSION

The 2001 EPA RfD for methylmercury is 0.1 μg/kg/day, based on neuropsychological deficits resulting from *in utero* exposure in humans. The RfD is derived from BMD analyses of a number of endpoints from a large longitudinal cohort study in the Faroe Islands, and an integrative analysis of three longitudinal studies. Analyses of a longitudinal study in New Zealand provide supporting evidence that the derived RfD is appropriate. EPA considers that the RfD is for the entire population, not only for women of childbearing age. It is clear that infants and children are more sensitive than adults to methylmercury-induced neurotoxicity. In addition, the potential for cardiovascular effects and delayed neurotoxicity in adults, which are at present insufficiently quantified, suggests that adherence to the RfD by everyone is the most health-protective strategy.

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