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Uncertainties in the Reference Dose for Methylmercury

Michael L. Dourson*, Andrea E. Wullenweber, Kenneth A. Poirier

Toxicology Excellence for Risk Assessment (TERA), 1757 Chase Avenue, Cincinnati, OH 45223, USA

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Abstract

This paper critically examines the National Academy of Sciences and the National Research Council report on the toxicological effects of methyl mercury and the recently published US Environmental Protection Agency Reference Dose (RfD) for Methylmercury. Particular scrutiny is placed on the choice of the critical study and the underlining assumptions utilized in the selection of specific uncertainty factors (UFs) and the rationale for using a less-than-default factor of 10. The UFs that were utilized or considered by other agencies and organizations are also critically examined, explained and compared to one another. Based on these analyses, the authors suggest research that could be performed that would ameliorate the uncertainty of choosing a more precise partial UF or that may even provide completeness of database to allow for selecting of a UF for unity, thus improving the precision of the current published RfD.

Keywords: Risk assessment; Methylmercury; Reference dose; Toxicology; Uncertainty factors

INTRODUCTION

The National Academy of Sciences and the National Research Council (NAS/NRC) recently produced a report entitled, "Toxicological Effects of Methylmercury" (NAS, 2000), in which the NAS evaluated the toxicological effects of methylmercury (methyl Hg) and prepared recommendations on the establishment of a scientifically appropriate reference dose (RfD). The NAS report confirmed the current US Environmental Protection Agency (EPA) RfD for non-cancer effects of methyl Hg at 0.1 μg/kg per day intake. For establishing a scientifically appropriate RfD, the NAS report recommended the use of a benchmark dose (BMD) of 12 ppm in maternal hair from a Faroe Islands study (Weihe et al., 1996) of the effects of prenatal exposure to methyl Hg. The critical effect was judged to be neurobehavioral effects in children; and the use of two uncertainty factors was recommended (one for the toxicokinetic variability in sensitive individuals and one for uncertainty in the choice of critical effect).

NAS convened the Committee on Toxicological Effects of Methylmercury to complete its report. This committee was charged with the following tasks: evaluate the body of evidence leading to EPAs current RfD for methyl Hg, evaluate new data that could affect the adequacy of EPAs methyl Hg RfD for protecting human health, consider exposures in the environment relevant to evaluation of likely human exposures, and identify data gaps and make recommendations for future research. The committee was not charged to calculate an RfD for methyl Hg. Instead, the committee provided scientific guidance to EPA on the development of an RfD (NAS, 2000).

In this paper, we briefly comment on the basis of the deliberations and conclusions of the NAS report, and identify additional research that we believe should be addressed in any future deliberations of a methyl Hg RfD (whether it is by EPA or other groups). The NAS report is much more comprehensive than our brief analysis; and we select and focus on key areas that need additional research to improve the basis for the development of an RfD for methyl Hg.

* Correspondence author. Tel.: +1-513-542-7475/ext. 14; fax: +1-513-542-7487.
E-mail address: dourson@tera.org (M.L. Dourson).

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US EPA METHODS FOR DEVELOPING REFERENCE DOSES (RfD)

To better understand the process NAS used to complete this study, we will briefly summarize EPA's methods for deriving an RfD value. A more complete discussion on the derivation of RfD values can be found in Barnes and Dourson (1988) and Dourson (1994). An introduction to the concept of Margin of Exposure (MOE) is also described in the former paper.

EPA defines an RfD as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (EPA, 2001b). Developing an RfD involves several judgments. These judgments include: (1) the critical effect (i.e. the first adverse effect or its known precursor that occurs as dose rate increases); (2) the most appropriate no-observed-adverse-effect level (NOAEL)[1] or benchmark dose lower limit (BMDL)[2] of this critical effect; and (3) appropriate uncertainty factors (UFs)[3] based on a review of the entire toxicity database.

RfDs are considered accurate but not precise estimates of doses below a toxicity threshold. They are considered accurate because they are based on a review of all toxicity data, and because the use of individual UFs are considered to be somewhat protective. It is also important to note that the use of several UFs together can yield conservative estimates. RfD estimates are not very precise because each uncertainty factor has a range of likely values of approximately 10-fold. Several factors are generally multiplied together to estimate an RfD, thereby increasing variability and decreasing precision. Therefore, deriving a risk estimate will not provide a "precise" number, like a simple mathematical equation, but it will provide a good estimate that is within a certain range and can be considered "accurate".

When available, adequate data from acceptable human studies are often used as a basis of the RfD, because the problem of interspecies extrapolation is avoided and the confidence in the estimate is often greater. In the absence of such data, RfDs are estimated from studies in experimental animals. The primary study evaluated by NAS used human data.

Generally, the presence of a "complete" database indicates that the acquisition of additional toxicity data is unlikely to result in a change to the RfD. Scientists at the EPA typically consider such RfDs to be "high confidence", reflecting the likely stability of the value to additional data. At the other end of the spectrum, EPA considers a single, well-conducted, subchronic mammalian bioassay by the appropriate route as a minimum database for estimating an RfD. However, for such a database the likelihood that additional toxicity data may change the RfD is higher, and the associated confidence in the RfD is lower. Due to the conservatism inherent in the uncertainty factor approach, the acquisition of additional data often results in higher RfDs (i.e. a conclusion that higher exposures are "safe"). This is because while the new data might suggest a lower NOAEL, the need for uncertainty factors is often reduced. The result is an RfD that is slightly higher than before because the reduction in the NOAEL is not as large as the decrease in the uncertainty factor. For example, the RfDs for mirex (EPA, 2001a) and tributyltin oxide (Benson, 2000) were both increased in light of new data received. This may be the case for methyl Hg as well, because the new data from the Faroe and Seychelles Islands have dramatically increased our knowledge of methyl Hg toxicity in children, and have decreased our uncertainty about where the likely threshold for response in sensitive individuals may be.

SUMMARY OF THE NAS (2000) ANALYSIS

NAS evaluated all available information related to the toxic effects of methyl Hg to determine the target organ, critical study, end point of toxicity, and dose on which to base the RfD. NAS critically evaluated information from epidemiological studies of populations in

[1] A NOAEL is an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse, nor precursors to adverse effects (EPA, 2001b).

[2] A BMDL is an exposure level that corresponds to a statistical lower bound on a standard probability of an effect, such as 10% of people affected (NAS, 2000).

[3] An UF is one of several, generally 10-fold factors, used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, i.e. interhuman or intraspecies variability; (2) the uncertainty in extrapolating animal data to humans, i.e. interspecies variability; (3) the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure, i.e. extrapolating from subchronic to chronic exposure; (4) the uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) the uncertainty associated with extrapolation from animal data when the database is incomplete (EPA, 2001b).

a variety of areas, including such locations as Amazon, Ecuador, Faroe Islands, French Guiana, Iraq, Japan, New Zealand, Peru, and Seychelles Island. Based on this evaluation, the NAS focused on the studies from three different geographic areas (Seychelles Island, New Zealand, and Faroe Islands), each of which evaluated neurodevelopmental effects following prenatal exposure to methyl Hg. NAS determined that the Faroe Islands study, which evaluated exposure to methyl Hg, DDT and PCBs from consumption of pilot whales (Weihe et al., 1996), was the most appropriate of the three studies for use as the basis for the RfD. NAS specifically selected this study as the critical study because it showed toxic effects at a low dose, it had the largest number of people tested, and the investigators made great efforts to control confounders. The BMD for the Faroe Islands study was 12 ppm methyl Hg in maternal hair. Based on a comprehensive analysis, the NAS determined the critical effect of methyl Hg to be neurological impairment from in utero exposure.

The NAS reviewed the scientific basis for UFs and determined that a UF of not less than 10 would be appropriate on the basis of variability in human kinetics and uncertainty in the choice of critical effect. Setting this UF at not less than 10 gave room for the uncertainty that neurological impairment may or may not be the correct critical effect, as compared with immunotoxicity or cardiotoxicity. (Concerns about the potential for the latter two effects occurring at low doses have been raised by recent studies, as discussed later in this paper.)

The NAS also conducted an analysis of the methyl Hg MOE, using established guidelines (Barnes and Dourson, 1988). The MOE is the ratio of the critical dose (NOAEL or BMD lower limit) to the estimated population exposure level, such that the smaller the ratio, the greater the cause for concern (NAS, 2000). This analysis is important because it gives risk managers another tool besides the RfD for making public health policy decisions. In a comparison of various BMDs developed by the NAS panel and mean exposures in three populations of interest, the panel found that MOEs varied from as low as 7.5 (New Zealand) to as high as 77 (Seychelles). However, when the upper 95th percentile exposure distributions were considered, the MOEs were uniformly lower (as expected), with values ranging as low as 2 (New Zealand) to as high as 22 (Seychelles). (NAS noted that MOEs below 10 indicate that population exposures may be approaching levels of concern for public health.) To further characterize these risks, the NAS developed an estimate of the number of newborns at risk from mothers who are high consumers of fish. The panel concluded that over 60,000 newborns annually in the US might be at risk for adverse neurodevelopmental effects from in utero exposures to methyl Hg.

METHYLMERCURY RISK VALUES COMPARED

Table 1 shows methyl Hg risk values developed by several organizations: ATSDR, ICF Kaiser and International Toxicity Estimates for Risk (ICF/ITER), and EPA. These risk values vary by a factor of 3–10, depending upon the choice of study used as the basis of the risk value, whether a BMD or a NOAEL is used, and the judgment of uncertainty factors. Variability in risk values can be attributed to differences in scientific judgments, the methods used by the organization, and the use of more recently published data. For example, EPA's previous value was based on data from an epidemiological study on an episodic exposure to methyl Hg in Iraq (Marsh et al., 1987). At the time of the development of this value, neither data from the

Table 1
Methyl Hg risk values compared

Organization[a] name	ATSDR (1999)	ICF/ITER (1998)	EPA (2001a)	NAS (2000)
Risk value[b] (μg/kg per day)	MRL 0.3	Fish RfD 0.3–1	RfD 0.1	RfD nd
Study location	Seychelles Island	Seychelles Island	Iraq	Faroe Islands
Basis[c] (ppm hair)	NOAEL 15	BMD 21	BMD 11	BMD 12
Basis (μg/kg per day)	1.3	0.9–3	1.1	nd
Uncertainty factor	4.5	3	10	10
Critical organ	CNS	CNS	CNS	CNS

[a] ATSDR: Agency for Toxic Substances and Disease Registry; ICF/ITER: ICF Kaiser/International Toxicity Estimates for Risk (ITER); EPA: US Environmental Protection Agency; NAS: National Academy of Sciences.
[b] MRL: minimal risk level; RfD: reference dose; nd: not done.
[c] NOAEL: no observed adverse effect level; BMD: benchmark dose; CNS: central nervous system.

Seychelles Island nor the Faroe Islands were available to serve as the basis of the RfD; once these studies were published, EPA scientists realized that these studies might form a better basis of the RfD.[4]

BRIEF COMMENTS ON THE CONCLUSIONS OF THE NAS REPORT

Overall the NAS report was well researched, referenced, thought-out and written. Potential problems with studies were anticipated (or discovered) and original investigators were contacted to ask for their help and for additional data, where appropriate. The analysis of the toxicokinetic variability in human response was exceedingly well done. The NAS report will serve as an adequate and nearly comprehensive basis of the revision to the existing RfDs (as described in Table 1). However, several issues need to be further addressed before a new RfD can be completed. Some of these issues were discussed by the NAS, but additional effort is needed to resolve them. Other issues were absent and original work is needed. Critical comments on the report are shown below.

Biological Variability (NAS, Chapter 3)

In this chapter, NAS discusses the factors that can affect variability in individual responses to methyl Hg exposure. The NAS made the following conclusions about such factors: sensitivity is related to the age at which exposure occurs; sex differences appear to affect the metabolism, tissue uptake, excretion and toxicity of Hg; and dietary nutrients and supplements may protect against toxicity. The NAS also stated that additional intraindividual factors are noted in response to similar exposures, including nutritional factors and unknown genetic susceptibilities. These conclusions of the NAS panel are well wrought. Furthermore, the NAS panel conducted an excellent analysis on the toxicokinetic analysis conducted by three groups of investigators on the interpretation of the uncertainty factor for within human variability. In its conclusions, the NAS identified that when using a methyl Hg biomarker concentration as the exposure metric in a methyl Hg risk assessment, a toxicokinetic model should be used to estimate the ingested dose that gave rise to the biomarker concentration; and that lack of consideration for interindividual toxicokinetic variability can result in an RfD that is not protective of a substantial portion of the population. This analysis will enable the use of data, rather than defaults, in the eventual determination of the RfD. This option is explored later under comments to the NAS, Chapter 8.

Dose Estimation (NAS, Chapter 4)

Developmental toxicity can occur from either peak concentrations of a toxicant (e.g. ethyl alcohol), or from more continuous exposures reflecting different body burdens. The NAS report investigated whether peak exposures of methyl Hg from pilot whale meat found in the Faroe Islands, and the "spiky" exposures to methyl Hg of the New Zealand study were likely to cause effects that would not occur if the same doses were administered in a more continuous fashion. For the Faroe Islands, NAS used a one-compartment model to conclude that the whale meat consumption resulted in a peak dose that was approximately twice the estimated background concentration. Because the estimated peak is not much higher than the background concentration, the NAS suggested that differences in findings in neurological impairment between the Faroe and Seychelles Islands might not be caused by differences in peak concentration. As implied by the NAS panel, however, the differences in peak concentration should be carefully studied (as explained below). The NAS also suggested a single hair XRF analysis for methyl Hg from the Faroe Islands. This analysis might confirm the NAS estimate that twice background concentrations may be due to peak exposures from pilot whale meat, as suggested by the NAS from the one-compartment PBPK model.

We suggest looking further at dose estimation because methyl Hg exposures in the Faroe and Seychelles Islands studies are very different. In the case of the Seychelles Island, methyl Hg exposure is mainly, if not exclusively, through the consumption of fish on a-several-times-a-week basis. In the Faroe Islands, methyl Hg exposure also occurs from a-several-times-a-week fish consumption, but also, and to a greater extent, in an occasional ingestion of pilot whale meat. The difference in total methyl Hg exposure between these two sources in the Faroe Islands is approximately four-fold, where exposure from whale meat is greater than exposure from fish. However, taking into account the frequency of fish (e.g. twice

[4] Just after this paper was accepted for publication, EPA promulgated its new RfD for Methylmercury based on the Faroe Islands study (EPA, 2001a). We recognize that although the RfD value has not changed, the rationale and UF used to calculate the RfD are different. Nonetheless, the suggested research in this paper is still applicable to use in reducing the uncertainties in deriving the RfD.

Table 2
Comparison of other possible critical effects of methyl Hg

Organ system	Species	NOAEL	LOAEL	Effect	Study
Immune	Rat	None	5 ppb in water of dams (~0.6 μg/kg per day)	Altered mitogen response	Ortega et al. (1997)
Immune	Rat	None	5 ppb in water of dams (~0.6 μg/kg per day)	Increased thymic weight in pups	Wild et al. (1997)
Cardiovascular	Human males	<2 ppm hair	>2 ppm and less than 16 ppm hair (high concentration is ~1.4 μg/kg per day)	2.9-fold adjusted increased risk of cardiovascular death	Salonen et al. (1995)

a week) or whale (e.g. twice a month) ingestion, the bolus dose difference between these sources is approximately 15-fold, again, with higher exposure from whale meat.[5]

Health Effects of Methylmercury (NAS, Chapter 5)

To date, the overriding consensus among scientists worldwide has been that neurological impairment in offspring of mothers consuming contaminated food is the critical effect of methyl Hg toxicity. However, as indicated by NAS, at least one recent study suggests that cardiovascular effects of methyl Hg may occur in adult men at doses that are close to the (maternal) NOAEL or BMD for impaired neurological development in infant humans (Salonen et al., 1995) (Table 2). We suggest that the results of the Salonen et al. study should be compared with the results of other studies of fish eating populations that have found beneficial effects of fish consumption on cardiovascular performance (e.g. Daviglus et al., 1997). A recent report describes many of these studies from the perspective of comparative risk (TERA, 1999), and the American Heart Association has also issued a statement on the benefits of fish as a source of *n*-3-polyunsaturated fatty acids and protein without high saturated fat (AHA, 1996).

[5] These ratios are estimated as follows. From Weihe et al. (1996), page 142, average grams of fish consumed per day in the Faroe Islands is 72; for whale meat this value is 12. Using average total Hg concentration values of 0.07 ppm for fish (almost all of which is methyl Hg) and 3.3 ppm for whale meat (half of which is methyl Hg) also given by Weihe et al. (1996) yields a methyl Hg daily dose estimate of 5 μg from fish and 20 μg from whale meat (~four-fold difference). Further assuming twice weekly fish meals, and twice monthly whale meat meals yields methyl Hg doses of 20 μg per fish meal and 300 μg per whale meat meal (~15-fold difference). See Weihe et al. (1996), Tables 2–4 for the basis of these latter assumptions. Other assumptions based on these tables would also be reasonable.

If an evaluation of the Salonen et al. (1995) study determines it to be adequately conducted and confounders are appropriately addressed, then a BMD for the cardiovascular toxicity of methyl Hg should be determined. Such a BMD can then be compared with the BMD for neurological impairment. If the BMD for cardiovascular effects is below those estimated for neurological impairment in the various studies, then the RfD might be more properly developed from cardiovascular effects, rather than neurological impairment. Uncertainty factors for this critical effect may, or may not be the same as currently suggested (see Table 1). If the BMD for cardiovascular effects is the same or higher than those for neurological impairment, then this would reduce the need for an uncertainty factor for database deficiencies as suggested by the NAS report.

Similarly, the relevance of immunological effects at low dose should be further studied (Table 2). The studies of both Ortega et al. (1997) and Wild et al. (1997) indicate immunological effects in experimental animals at doses that are close to, but slightly below, the NOAEL or BMD for impaired neurological development in infant humans. These immunological effects should be confirmed, their potential adversity should be evaluated, and their relevance to the choice of critical effect in humans should be studied.

Such an analysis would enable the determination of whether immunotoxicity is a more sensitive effect than neurological impairment. If so, then this might change the basis of the RfD. Alternatively, immunotoxicity may not be considered as a critical effect if the effects seen: (1) are considered enhancements to the immune system; (2) are not considered relevant to humans; or (3) represent a NOAEL with a corresponding LOAEL that is higher than the BMD for neurotoxicity. Any of these three possibilities would lessen the need for an uncertainty factor for database deficiencies, as suggested by the NAS report.

Table 3
Multiple average selected chemical exposures in fish studies[a]

Study	Methyl Hg (μg/kg per day)	PCBs (μg/kg per day)	DDT (μg/kg per day)	Total (μg/kg per day
Faroe Islands adults (Weihe et al., 1996)	0.5	3 × 150 RfD × 0.6 LOAEL	2 × 4 RfD × 0.04 LOAEL	~6
Faroe Islands infants (Steuerwald et al., 2000)	–	12 × 600 RfD × 2 LOAEL	7 × 14 RfD	–
Seychelle Islands (ATSDR, 1999)	1.3	Low	–	–

[a] For this comparison we selected the RfD for Aroclor 1254 of 0.02 μg/kg per day and the monkey LOAEL for Aroclor 1254 of 5 μg/kg per day. This is because Fangstrom et al. (2000) state that the most prevalent congeners are 138, 153, and 180. None of these congeners are present in Aroclor 1016. Aroclor 1254 has all three of these most prevalent congeners (TERA, 1998).

Table 4
Table calculations

PCBs (Weihe et al., 1996)
30 μg/g (PCBs in blubber) × 7 g per day (estimated daily blubber consumption)/70 kg (average body weight) = 3 μg/kg per day (PCBs consumed from blubber)
EPAs reference dose (RfD) for Aroclor 1254 = 2E−5 mg/kg per day = 0.00002 mg/kg per day = 0.02 μg/kg per day
3 μg/kg per day (PCBs consumed from blubber)/0.02 μg/kg per day (RfD) = 150-fold greater than RfD (PCBs consumed from blubber is 150 times above RfD)
DDT (Borrell and Aguilar, 1993, as cited in Weihe et al., 1996)
20 μg/g (DDT in blubber) × 7 g per day (estimated daily blubber consumption)/70 kg (average body weight) = 2 μg/kg per day (DDT consumed from blubber)
EPAs reference dose (RfD) for DDT = 5E−4 mg/kg per day = 0.0005 mg/kg per day = 0.5 μg/kg per day
2 μg/kg per day (DDT consumed from blubber)/0.5 μg/kg per day (RfD) = four-fold greater than RfD (DDT consumed from blubber is 4 times above RfD)
PCBs (Steuerwald et al., 2000)
1.52 mg/kg (PCBs in milkfat) × 0.05 (5% milkfat) × 0.64 l per day (average daily milk intake) × 1 kg/l (conversion factor)/4 kg (average infant body weight) = 0.012 mg/kg per day or 12 μg/kg per day (PCBs consumed from milk)
EPAs reference dose (RfD) for Aroclor 1254 = 2E−5 mg/kg per day = 0.00002 mg/kg per day = 0.02 μg/kg per day
12 μg/kg per day (PCBs consumed from milk)/0.02 μg/kg per day (RfD) = 600-fold greater than RfD (PCBs consumed from milk is 600 times above RfD)
DDT and analogs (Steuerwald et al., 2000)
0.87 mg/kg (DDT in milkfat) × 0.05 (5% milkfat) × 0.64 l per day (average daily milk intake) × 1 kg/l (conversion factor)/4 kg (average infant body weight) = 0.007 mg/kg per day or 7 μg/kg per day (DDT consumed from milk)
EPAs reference dose (RfD) for DDT = 5E−4 mg/kg per day = 0.0005 mg/kg per day = 0.5 μg/kg per day
7 μg/kg per day (DDT consumed from milk)/0.5 μg/kg per day (RfD) = 14-fold greater than RfD (DDT consumed from milk is 14 times above RfD)

Comparison of Studies for Use in Risk Assessment (NAS, Chapter 6)

The NAS provides a good analysis of different studies for use in risk assessment, including issues associated with in utero PCB exposure. However, we are not convinced that in utero PCB exposure is the only way to look at the possible PCB toxicity demonstrated in the Faroes. The fact that two very well conducted sets of studies, those of the Faroes and Seychelles, indicate differences in methyl Hg toxicity (one essentially showing toxicity and one not), suggest to us that a complicating factor might exist between them. We suspect that the complicating factor might be the enormous exposures to PCBs in breast milk in the Faroes that is not found in the Seychelles.

For example, we have found that exposures to PCBs in the Faroe Islands are very high, far in excess of the RfD for Aroclors 1016 and 1254 on EPA's Integrated Risk Information System (IRIS) (EPA, 2001a). For example, an average dose of 12 μg/kg per day for infants that can be estimated from the PCB concentration in maternal milkfat is 600 times the Aroclor 1254 RfD (0.02 μg/kg per day) and twice the monkey Aroclor 1254 LOAEL (5 μg/kg per day) for clinical signs and immunotoxicity in infants (see Tables 3 and 4).[6] Exposures to other chemicals are also of note, e.g. DDT

[6] For this comparison we selected the RfD for Aroclor 1254 because Fangstrom et al. (2000) state that the most prevalent congeners are 138, 153, and 180. None of these congeners are present in Aroclor 1016, while Aroclor 1254 has all three of these most prevalent congeners (TERA, 1998).

and analogs. PCB exposures in the Seychelles Islands are extremely low as per NAS.

Several recent studies have described both negative and positive neurological effects from exposure to PCBs either in utero or through breast milk. For example, published work of Jacobson and Jacobson (1996) concluded that in utero, but not breast milk, exposure to PCBs at concentrations slightly higher than those in the general population from consumption of high levels of fish from Lake Michigan can have a long-term impact on infant intellectual function. PCB exposures in this study were lower than that found in the Faroe Islands as measured by PCBs in mothers' milk.

In contrast, the work of Budtz-Jorgensen et al. (1999) indicates that many of the neurological indices in the Faroe Islands are not correlated with in utero PCB exposures. Similar correlations were not done on the basis of breast milk PCB exposures. Since the greater exposure to PCBs to these infants appears to be from breast milk in this study, some uncertainty exists with the authors' conclusions that total PCBs exposures are not associated with neurological indices in the Faroes. Another possibility that may explain the lack of correlation of Budtz-Jorgensen et al. (1999) is that only about one-half of the cohort was analyzed for PCBs in cord tissue.

Steuerwald et al. (2000) specifically looked at PCBs in breast milk and correlation with a neurological optimality score (NOS) at 2 weeks after birth in what appears to be a different group of Faroes infants. They found no correlation with PCB in utero exposures, in agreement with Budtz-Jorgensen et al. (1999), nor with milk PCB exposures, although they did find a correlation with methyl Hg in cord blood.

Huisman et al. (1995) show neurotoxicity from PCB and related chemical exposures through breast-feeding, but not in utero, exposures. This is in contrast to both the work of Jacobson and Jacobson (1996) and Steuerwald et al. (2000) (for different reasons), but is consistent with the Faroe investigators for in utero PCB exposures. These doses in Huisman et al. (1995) were slightly lower than that found in the Faroes.

Patandin et al. (1999) show that in utero, but not breast milk, exposure to PCBs is related to reduced neurological scores on standard tests in children at 42 months of age (this is a continuation of the Huisman et al. (1995) study). The authors also note that the breast-fed infants were more advantaged and that substances in breast milk or other factors associated with breast-feeding may have counteracted the negative influence of PCB exposure on cognitive development.

These somewhat confusing results are echoed in part by similar perinatal PCB exposures and neurological effects in experimental animals (as described below).

In order to postulate that a high post-birth PCB exposure is the principal reason for some of the neurological effects seen in the Faroes, this PCB exposure would need to be correlated with at least some measures of methyl Hg exposure, since these neurological effects were associated with methyl Hg exposure. However, at least for some measures of in utero PCB and methyl Hg exposures, this correlation does not exist, suggesting that the neurological effects are not associated with post-birth PCB exposures. Unfortunately, the PCB dose to infants via breast milk was not estimated in the Faroe studies for the neurotoxicity effects of concern (not any one study can monitor everything, of course). Thus, the correlation between neurotoxicity effects and breast milk PCB dose is not known. Alternatively, the correlation of neurotoxicity with methyl Hg exposures might be enhanced with this large PCB post-birth dose. Such enhancement would be consistent with some of the animal work described below.

Typically, neurological test batteries measure changes in various neurological parameters, which can include IQ, spatial acuity, memory, etc. and are general changes that measure a cause and effect relationship. The childhood cohort in the Faroe Islands was given a battery of neurobehavioral and cognitive tests that included such common measurements as continuous performance test, finger tapping, hand–eye coordination, intelligence testing, visual motor tests, and naming tests. When one has a multiple exposure to chemicals, such as in the Faroe Islands study, one cannot always easily differentiate which of the chemicals individually or in combination are eliciting those changes. Moreover, when exposure to at least one of these multiple chemicals is extraordinarily high, as it is with PCBs (e.g. exposure to breast-fed infants is 600 times the Aroclor 1254 RfD), then it is more difficult to argue that the neurological effects seen are solely due to one chemical such as methyl Hg.

Experimental animal work also supports the neurotoxicity of PCBs at low doses. EPA's current Aroclor 1016 RfD was set to 7E−5 mg/kg per day in 1996 based on a monkey reproductive bioassay. Female monkeys were exposed to 0.007 or 0.028 mg/kg per day Aroclor 1016 for 22 months, ranging from 7 months prior to breeding to offspring weaning at age of 4 months. The critical effect in this study was judged as decreased birth weight in the high dose group. Neurobehavioral tests of infant monkeys were also

conducted. At age of 14 months, the offspring in the high dose group (0.028 mg/kg per day) were significantly impaired in their ability to learn the spatial position discrimination problem, requiring more than 2.5 times as many trials as their age-matched controls. After over-training, the difference in learning the spatial position discrimination was no longer significant. This may suggest a marginal neurobehavioral effect. In contrast, the same dose (0.028 mg/kg per day) also facilitated learning ability on shape discrimination. An uncertainty was also observed in offspring at the age of 4–6 years. At this later time, the two test groups were not significantly different from controls in spatial learning and memory tasks, but the exposed groups did significantly differ from each other. The difference between the two exposed groups was due to a combination of decreased performance at the high dose and facilitated performance at the low dose. Although these data are insufficient for establishing an exposure effect relation due to the lack of difference between exposed and control groups, the investigators suggested that the performance deficit at 0.028 mg/kg per day may have been exposure-related.

Except for the critical study and the supportive reports from the human study, EPA does not summarize additional neurobehavioral tests in its IRIS entry for Aroclor 1016 (no additional data was available at the time of EPA's review and RfD development). However, enough evidence appears to exist on IRIS to say that behavioral effects at the high dose (0.028 mg/kg per day) may also be adverse, and an RfD for neurobehavioral toxicity should also be based on the same NOAEL of 0.007 mg/kg per day as the current RfD for Aroclor 1016. The resulting neurobehavioral effect RfD might also be 7E−5 mg/kg per day.

This LOAEL of 0.028 mg/kg per day, showing possible neurotoxicity in infant monkeys, is about two- to three-fold higher than the dose of 0.012 mg/kg per day PCBs to which we estimate in this report that human infants are being exposed in the Faroe Islands through mother's milk.

The monkey LOAEL used to derive the RfD for Arochlor 1254 is not based on neurotoxic endpoints. Rather, this RfD is based on a LOAEL of clinical signs and immunological deficits in non-human primates. Neurological endpoints were not examined in this particular battery of studies. However, other studies in different species, reported after the promulgation of the Arochlor 1254 RfD, have shown that neurological impairments occur from exposure to PCBs (Roegge et al., 2000; Crofton and Rice, 1999). Crofton and Rice (1999) specifically show developmental ototoxicity in offspring of rats exposed at doses lower than that found in humans of the Faroe Islands as we estimate here. Recent work of Crofton et al. (2000) shows impact on neurological function in experimental animals from postnatal-only Aroclor 1254 exposures (the only maternal dose given was 6 mg/kg on gestation days 6 to postnatal day 21).

Recently, an in vitro study in rat brain tissue (Bemis and Seegal, 1999) demonstrated that PCB and methyl Hg in combination acted synergistically to reduce dopamine. These data suggest that not only may we not be able to differentiate between the effects of PCBs and methyl Hg, but that the end effect may be exacerbated when both are present. Although this is a limited study performed in vitro and may not be applicable to other endpoints, it suggests that these effects need to be considered when examining complex mixture exposures. Additional studies on PCB and methyl Hg interaction are underway in EPA's Neurotoxicity Division of the National Human and Ecological Effects Research Laboratory.

We consider that a very useful and unique finding of the data from the Faroe Islands studies is the mixture of chemicals found. Since many fish in the world are contaminated with similar mixtures, it would be very useful to develop a mixtures RfD. (Some preliminary work has been done on this; see, for example, Poirier et al., 2001.) This could be done specifically for PCBs, DDT and analogs, and methyl Hg on the basis of the Faroe Islands data using established guidelines (EPA, 1986). For an example of the use of mixtures RfD for fish contamination, see the appendix in Dourson and Clark (1990), where a specific mixtures RfD for contaminated fish in the Great Lakes is developed.

Risk Characterization and Public Health Implications (NAS, Chapter 8)

Uncertainty Factors in the Determination of the RfD

Uncertainty factors for each of the risk values listed in Table 1 include an uncertainty factor for variability in the human population. It is now common practice for this uncertainty factor to be divided into roughly equal subareas of toxicodynamics and toxicokinetics: a value of 1/2 $\log_{10}$ each, or about three-fold (Dourson et al., 1996). For variability in human toxicokinetics, each RfD listed in Table 1 includes a toxicokinetic uncertainty factor (the ICF/ITER factor determines the range in the given RfD).

The NAS text does a good analysis of this subfactor, as shown in Tables 5 and 6. As discussed in research

Table 5
Comparison of results of variability in the ingested dose of methyl Hg:hair (from NAS, 2000, Table 1–3, 1 ppm hair)

Study (as cited in NAS, 2000)	50th percentile (μg/kg per day)	Ratio of 50th to 5th percentile	Ratio of 50th to 1st percentile
Stern (1997)	0.03–0.05 (mean = 0.04)	1.8–2.4 (mean = 2.1)	2.3–3.3 (mean = 2.7)
Swartout and Rice (2000)	0.08	2.2	Data not reported
Clewell et al. (1999)	0.08	1.5	1.8
Mean	–	1.9	2.3

needs, future research and development on this particular subfactor would include incorporating the recent guidelines of the International Programme on Chemical Safety (IPCS) (Meek et al., 2000). The IPCS guidelines suggest the choice of best study and kinetic parameter on which this judgment is made. If a choice among these studies could not be made, then averaged values for this uncertainty factor could be 1.8, 1.9, or 2.3, depending on choice of median dose in hair or blood, and whether a 95 or 99% degree of confidence is desired.

The uncertainty factor for variability for human toxicodynamics in the RfDs for ICF/ITER and EPA is judged to be one-fold. The NAS panel also surmises that a one-fold factor would be appropriate. ATSDR, however, judges this factor at a value of 1.5. All of these toxicodynamic factors are reduced from the usual default value of three-fold because the critical effect, neurological impairment, is determined to be in a sensitive subgroup of the human population. Some attempt could be made here to harmonize these values, however, it is unlikely that additional research is warranted.

The uncertainty factor for database deficiencies in the RfDs for ICF/ITER and EPA is judged to be three-fold. The NAS panel also surmises that an approximate three-fold factor would be appropriate. ATSDR, however, judges this factor at a value of 1.5. The reasons for these factors varies as shown below.

- ATSDR: a factor of 1.5 is employed to account for the possibility that the domain-specific tests, as employed extensively in the Faroe Islands but not the Seychelles Island, might be able to detect very subtle neurological effects not tested for in the 66 months Seychelles Island cohort.
- EPA: a factor of 3 is applied for lack of a two-generation reproductive study and lack of data for the effect of exposure duration on sequelae of the developmental neurotoxicity effects and on adult paresthesia.
- ICF/ITER: a factor of 3 is included to address concerns regarding the possibility of chronic sequelae as well as concerns raised by other studies of fish-eating populations.
- NAS: a factor of 3 is considered because of possible low dose sequelae and latent effects, and immunotoxicity and cardiovascular effects.

All of these database deficiency factors are based on deliberations of the scientific uncertainties in the choice of critical effect as discussed by Dourson et al. (1992). The deliberation of such uncertainties does not reflect policy considerations, or policy choices, as suggested by the NAS.

As discussed more fully below, research suggested under the "critical effects" can be used to further reduce the scientific uncertainties associated with the use of this database uncertainty factor. However, a general argument for reducing this uncertainty factor without additional research would be that the types of bioassays available for determination of the RfD have improved dramatically since 1995, the date of EPA's RfD on IRIS where a factor of three-fold was used. Other arguments might include that a two-generation reproductive study in rodents would not likely yield a dose lower than the current BMD, because the human epidemiology studies do not suggest a concern for reproductive effects. Furthermore, chronic exposure at the BMD will not likely evoke neurological damage, since infants appear to be more sensitive individuals

Table 6
Comparison of results of variability in the ingested dose of methyl Hg:blood (from NAS, 2000, Table 1–3, 1 ppm blood)

Study (as cited in NAS, 2000)	50th percentile (μg/kg per day)	Ratio of 50th to 5th percentile	Ratio of 50th to 1st percentile
Stern (1997)	0.01	1.5–2.2 (mean = 1.8)	1.7–3.0 (mean = 2.4)
Swartout and Rice (2000)	0.02	2.1	2.8
Clewell et al. (1999)	0.07	1.4	1.7
Mean	–	1.8	2.3

than adults. For example, the large Seychelles population has been exposed for a lifetime and there are no reported anecdotal observations of people exhibiting adverse neurological effects. The absence of anecdotal mention of neurological effects in this and other studies from Peru, New Zealand, Canada and the Faroes is also a powerful argument to support the use of a database uncertainty factor of 1.

Margin of Exposure Analysis

The NAS also conducted an analysis of the methyl Hg MOE. The NAS panel found that MOEs ranged from as low as 2 ppm to as high as 22 ppm, using the upper 95th percentile exposure distributions. NAS also developed an estimate of over 60,000 newborns annually in the US that might be at risk of neurodevelopmental effects from mothers who are high consumers of fish. The basis of this latter estimate is not entirely clear, however, and needs to be more carefully described. If, for example, this value is based on a comparison of methyl Hg from fish consumption of 100 g per day, and a dose derived from a BMD of 12 ppm of maternal hair divided by a 10-fold uncertainty factor, then the estimate may be misleading. This is because the expected "threshold" for neurodevelopmental effects would be the BMD divided by an uncertainty factor of 2–3 (as per the NAS) to reflect the expected human variability in toxicokinetics, plus division of a further uncertainty factor of 3 or less to reflect database uncertainties specifically due to delayed neurodevelopmental effects. Other database considerations such as potential cardiovascular effects and immunotoxicity in adults do not reflect uncertainties with the sensitive population, and should not be used as part of this calculation. Alternatively, the NAS might indicate that this latter analysis is for neurodevelopmental effects in children and latent effects in adults that include neurological, cardiovascular and immunological endpoints. In the latter case, the effects would be for lifetime exposure, including both in utero exposures and exposures in adults.

SUMMARY AND SOME RESEARCH NEEDS

Determine the Critical Effect for Methylmercury

The determination of the critical effect for methyl Hg is important. Investigating other potential critical effects will either change the basis of the RfD or reduce the need for uncertainty factors. Specifically, the results of the Salonen et al. (1995) study should be compared with studies of other fish eating populations where beneficial effects on cardiovascular performance have been shown to check for possible confounders. A comparison of such studies exists in TERA (1999) in which a comparative dietary risk framework was developed to compare the possible health risks of consuming contaminated fish, while considering the potential health benefits lost by not eating fish. The result of using this framework is a crude quantitative representation of the risks and benefits of consuming contaminated fish. Such analysis would be useful for further evaluating the Salonen et al. (1995) study. If the Salonen et al. (1995) study is appropriate, a BMD for the cardiovascular toxicity of methyl Hg should be determined.

Similarly, the immunological effects of Ortega et al. (1997) and Wild et al. (1997) should be confirmed and their relevance to the choice of critical effect in humans should be studied. Such an analysis would, again, enable the determination of whether immunotoxicity is a more sensitive effect than neurological impairment.

Analyze the Impact of Mixed Chemical Exposures in the Faroe Islands

The Faroe Islands data are from exposures to a mixture of chemicals. The Seychelles Island data are from exposures to primarily one chemical, methyl Hg. These different exposures may allow us to use studies from both areas to derive RfDs for a mixture and a single chemical exposure, respectively. Specifically, the importance of investigating the extraordinarily high PCB exposures (Aroclor 1254 RfD × 600; twice the monkey Aroclor 1254 LOAEL) in the Faroe Islands should not be understated. It may well be that these huge PCB exposures to breast-fed infants in the Faroe Islands are affecting the health of the population independent of any neurological response, or they may be enhancing the toxicity of methyl Hg. Here is a listing of some studies that support and do not support the neurotoxicity of PCBs from breast-feeding.

- Steuerwald et al. (2000) did not find neurological effects in newborns from PCB exposures in breast milk; their exposures were in excess of EPA's RfD for Aroclor 1254 by 600-fold, and in excess of the monkey Aroclors 1254 LOAEL on EPA's IRIS by two-fold. Such high exposures in experimental animals, including infants, are in the range of doses that caused neurotoxicity with some PCBs.

- The NAS reported non-significant regressions of PCB exposures in cord tissue with several neurological effects, but significant associations with other neurological effects in the Faroes study. The NAS depended on cord tissue levels of PCBs that were readily available for regressions, but did not account for excess PCB exposures in breast milk (data were not available).
- Jacobson and Jacobson (1996) show that in utero (but not breast milk) exposure to PCBs cause developmental neurotoxicity similar to methyl Hg in children.
- Huisman et al. (1995) show that breast milk (but not in utero) exposure to PCBs are related to reduced neonatal neurological optimality and higher incidence of hypotonia in newborn infants.
- Patandin et al. (1999) show that in utero, but not breast milk, exposure to PCBs is related to reduced neurological scores on standard tests in children at 42 months of age (this is a continuation of the Huisman et al. (1995) study).
- Bemis and Seegal (1999) show that PCBs and methyl Hg act synergistically for neurotoxicity endpoints.

We would support an analysis by EPA to confirm the positive and negative correlations between neurological impairment and PCBs in cord tissue by Budtz-Jorgensen et al. (1999), but also, and perhaps more importantly determine the correlation on the basis of postnatal PCB dose from breast-feeding. Although this was not analyzed in the original Faroe Islands study, recent indications from Dr. Grandjean and colleagues suggest that this will be done shortly. Such data would be extremely helpful in EPA's analysis.

In addition, further investigations should be conducted on the differences in neurological response from in utero PCB exposures between Faroe Islands (which was negative) and others (which was positive) shown in the work of Jacobson and Jacobson (1996) and Patandin et al. (1999). This is especially important since the PCB exposures in the Faroe Islands were greater than at least one of these investigations, that found in Lake Michigan (as measured by PCBs in mothers' milk).[7] The results of Jacobson and Jacobson (1996) and Patandin et al. (1999), which were published after the development of the Aroclor RfDs, do support the potential for PCB neurotoxicity in humans at low doses, although the former study remains controversial (Schell et al., 2001). The Jacobson and Jacobson (1996) study was also done based on fish consumption, and so the congener mix may be more relevant to that in the Faroe Islands than the mixture in the Aroclor studies that form the basis of EPAs RfDs.

We would also encourage EPA to use the Seychelles Island data as the basis of its methyl Hg RfD. Although fish from the Seychelles are likely to also have additional contaminants, the huge doses of PCBs found in the Faroes will not be one of them. The Faroes in turn would make ideal studies to develop mixture RfDs. Many of our fish are contaminated with both chemicals.

Develop a Mixtures RfD

A mixtures RfD for PCBs, DDT and breakdown products, and methyl Hg from data found in the Faroe Islands could be done on the basis of established guidelines (EPA, 1986). Some preliminary work has already been done in this area (Poirier et al., 2001). Fish throughout the world are usually contaminated with multiple chemicals, and the Faroe Islands data enable us to approach a mixtures RfD with some confidence. Once this RfD is established, one may then be able to develop a method to scale this mixture's RfD to fish with other proportions of these same chemicals. As risk assessors, we need to identify how we can best use the all of our epidemiology data.

Evaluate Continuous versus Peak Exposures in Methyl Hg Exposure

Small changes in continuous dose appear to make a contribution to methyl Hg risk assessment. Methyl Hg bolus dose differences of approximately 15-fold in the Faroe Islands between fish and pilot whale consumption, and by implication bolus dose differences between the methyl Hg exposure in the Faroe Islands and the Seychelles Island should be afforded the same level of analysis. Specifically, we agree with the implication of the NAS panel that the approximately two-fold differences in peak concentration over background due to peak exposures in the Faroe Islands should be carefully studied. We strongly urge that this conclusion be confirmed by a multiple compartment PBPK analysis. Some work has already been done in this area (Gentry et al., 2001).

In addition, the NAS panel recommended that a continuous single-strand XRF analysis of hair be conducted from both the Seychelles Island and Faroe

[7] Specifically, the highest PCB exposure in Jacobson and Jacobson (1996) looks to be ~1.25 μg/g of fat (see Fig. 1 on page 787). In contrast, the highest exposure in the Steuerwald et al. (2000) appears to be ~18.5 μg/g of fat (Table 1, page 601). Mean values can also be found in these same tables.

Islands studies. Such an analysis would enable a better comparison of the data between these areas, and might allow the development of RfDs that reflect both dose and frequency of exposure.

Uncertainty Factors in the Determination of an RfD

Current EPA RfD guidelines (Dourson, 1994) can be enhanced with new approaches to uncertainty factors and data unique to methyl Hg. For example, new research in the area of human variability in toxicokinetics has been incorporated into the recent guidelines of the International Programme on Chemical Safety (Meek et al., 2000) for compound-specific adjustment factors (formerly referred to as data-derived uncertainty factors). This guidance is now undergoing an international review, including review by scientists with ATSDR, EPA and FDA. In brief, the excellent analysis of the NAS (2000) panel, as shown in Tables 5 and 6, may be considered directly as the factor for human variability in toxicokinetics, in lieu of a default value of either 2 or 3 as per the NAS panel. Averaged values for this uncertainty factor could be 1.8, 1.9, or 2.3, depending on choice of median dose in hair or blood, and whether a 95 or 99% degree of confidence is desired. Alternatively, and perhaps preferred, one study could be selected as the basis of this uncertainty factor and the results used directly.

Research suggested under the "critical effects" discussion above can be used to address scientific uncertainties associated with the use of this database uncertainty factor and further reduce its need. For example, if a BMD for cardiovascular effects is similar or higher than the BMD for neurological impairment, then the former effect is not likely to be a critical effect, and the need for the database uncertainty factor is correspondingly reduced. Likewise, the immunotoxicity effects listed in Table 2 should be further studied as to whether they are considered adverse, adaptive, compensatory or beneficial. If they are adverse, then their relevance to humans needs to be judged. If both adverse and relevant, then they would likely be used as the critical effect and the RfD for methyl Hg can then be revised accordingly. The need for the database uncertainty factor is then correspondingly reduced.

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