PROCEEDINGS OF

NIMD FORUM 2003

－The study of fetal methylmercury exposure and children development－

Date: November 20,2003

Venue: Niigata Learning Center for Humans and the Environment

Niigata Prefecture, Japan

Organized by National Institute for Minamata Disease

Contents

Preface

I appreciate for all of you who have prepared to open the conference in Niigata Learning Center for Humans and the Environment. Niigata Prefecture is a second outbreak place of Minamata disease in Japan. Recently, it is a serious problem that the low-level exposure of methylmercury on the human health from a global points of view.

As for the problems of mercury pollution in the recent period, there are artificial environmental mercury pollutions not only from the local mercury pollution of the gold mine and mercury mine, but also big amount of consumption of fossil fuels from thermal power plants, or natural environmental mercury pollutions from the explosion of volcanoes. Therefore the mercury pollution is increasing in every environment in the world. Inorganic mercury is flown into the rivers, lakes and oceans and the inorganic mercury will be changed to methylmercury in the natural circumstances. The methylmercury is accumulating in human bodies and animals by the food chain.

It is well known that methylmercury attacks the nervous system. We have had a tragic experience of severe cases of methylmercury poisoning (Minamata disease) in Minamata and Niigata in Japan. The National Institute for Minamata Disease was established in October of 1978 in Minamata City, Kumamoto Prefecture with the purpose of conducting comprehensive medical research to improve medical treatment for victims of Minamata disease while giving balanced consideration to its deep historical background and social importance.

Concerning up-to-date topics, it is important to study the effects of methylmercury to the fetus, which is the most vulnerable to the toxic agent. We are starting to study the dose-response relation between the hair mercury levels of the mothers and umbilical cords, and estimate the developmental effects of mercury to the children through rather rate in Japan. Today Dr. Satoh and Dr. Nakai, Tohoku University, Graduate School of Medicine of Medical and School of Medicine, and Dr. Murata, Akita University School of Medicine will report Japanese studies of the topics.

We invited Dr. Gary J Myers and Dr. Philip W Davidson, University of Rochester Medical Center, USA in the NIMD Forum last year (2002), and discussed the child development in Seychelles with them. We are very happy to have Dr. Philippe Grandjean, Department of Environmental Medicine, Institute of Public Health, and Dr. Pal Weihe, Research Associate Professor from Denmark, and have an opportunity to discuss the important topics. Fish and shellfish are very important source of protein to the human body. However it is important to study the methylmercury effects of the fetus of which exposed on.

Today we will have two Japanese speakers of Dr. Mineshi Sakamoto, National Institute for Minamata Disease and Dr. Akiyoshi Kakita, Brain Research Institute of Niigata University. Additionally, Dr. Loi VD, National Center for Science and Technology of Vietnam will report on the mercury pollution in Vietnam due to gold mine activity.
I hope we will have fruitful meeting in the forum.

Nov. 20, 2003

Komyo Eto, M.D.
Director General
National Institute for Minamata Disease

Welcome Address

I'd like to thank Dr. Eto for giving me an opportunity of the welcome address in front of all the participants. On behalf of Ministry of the Environment, I deeply appreciate all of you for your participation to the NIMD Forum 2003. Especially, I have the great pleasure of welcoming Dr. Phillippe Grandjean and Dr. Pal Weihe from Denmark.

There has been a growing concern about low-dose exposure to methylmercury in Japan.

Life-style and diet of Japanese people have changed from the previous decade, but we are still mass consumers of fish in the world. According to National Nutrition Survey conducted by Ministry of Health, Labour and Welfare, the average of the fish consumption per day is about one hundred grams. Therefore, it is our great interest to explore the low-dose effects of methylmercury to child development.

In June, 2003, Japanese Ministry of Health, Labour and Welfare announced a caution for pregnant women with regards to several fish species, based on the data of National Nutrition Survey, mercury concentrations of fish arrived in Japanese markets and international policies toward fish intake. Although this announcement was made to provide information on health risk posed by fish intake in a large quantity and only aimed at pregnant women, the fish mentioned in the caution had disappeared in markets for a time, giving a great impact on our society.

The cohort study on the low-dose exposure effect of methylmercury on child development in Japan has just started since last year. It is highly expected that the members of this study, as of the Tohoku study, Dr. Satoh, Dr. Murata, Dr. Sakamoto and their colleagues, could reveal low-dose effects of methylmercury and the total effect of fish intake to child development in Japan.

Today's topic, the study of fetal exposure to methylmercury and child development, is extremely appropriate. I hope this Forum would promote the design of the Tohoku study. I'd like to close my words by giving thanks to Mr. Tsukada, the director of Niigata Learning Center for Humans and the Environment for his kind corporation, and the excellent staffers of NIMD for setting this Forum.

Kazuko Kamiya, M.D.
Director
Special Environmental Diseases Office
Ministry of the Environment

Editorial Board

Proceedings

Affiliation

1. Philippe Grandjean ····· (Denmark)
   Environmental Medicine, Odense University

2. Pál Weihe ····· (Denmark)
   The Faroese Hospital System

3. Vu Duc Loi ····· (Vietnam)
   National Center for Science and Technology of Vietnam

4. Kimiyoshi Arimura ····· (Japan)
   Faculty of Medicine, Kagoshima University

5. Komyo Eto ····· (Japan)
   National Institute for Minamata Disease

6. Noriyuki Hachiya ····· (Japan)
   National Institute for Minamata Disease

7. Fusahiro Ikuta ····· (Japan)
   Niigata Neurosurgical Hospital and Brain Research Center

8. Akiyoshi Kakita ····· (Japan)
   Brain Research Institute, Niigata University

9. Kazuko Kamiya ····· (Japan)
   Ministry of the Environment

10. Katsuyuki Murata ····· (Japan)
    Akita University School of Medicine

11. Kunihiko Nakai ····· (Japan)
    Tohoku University Graduate School of Medicine

12. Mineshi Sakamoto ····· (Japan)
    National Institute for Minamata Disease

13. Kiyomi Sakata ····· (Japan)
    Wakayama Medical University

14. Hiroshi Satoh ····· (Japan)
    Tohoku University Graduate School of Medicine

15. Hitoshi Takahashi ····· (Japan)
    Brain Research Institute, Niigata University

Impact of Scientific Uncertainty on Risk Assessment for Methylmercury in Seafood

Philippe Grandjean

Department of Environmental Medicine, University of Southern Denmark, 5000 Odense, Denmark; and Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA

Introduction

Methylmercury is a ubiquitous contaminant of seafood and freshwater fish (UNEP, 2002). Human exposures to this toxicant have increased over time, due to anthropogenic mercury pollution, and because modern fishing technology allows catching species of large, predatory fish that accumulate methylmercury. The most dramatic reminder of the neurotoxic potential of methylmercury was caused by serious water pollution from a local factory in Minamata, Japan (UNEP, 2002). Food contamination by this substance has now become an important public health issue worldwide.

Recent risk assessments of methylmercury have been published by national and international bodies, i.e., the (U.S.) National Research Council (NRC, 2000), the U.S. Environmental Protection Agency (U.S.EPA, 2001), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003). These assessments suggest that the largest prospective studies of developmental neurotoxicity have reached "different" conclusions. While one study seems to disagree with two others, the risk assessments have only partially considered the impact of scientific uncertainty on the study outcomes. Also, the reports have used only so-called default factors to take into account the uncertainties.

The different interpretations of the epidemiologic evidence are reflected in a four-fold difference between the exposure limits used by the (U.S.) Food and Drug Administration and the U.S. Environmental Protection Agency. Because the agencies have access to the same scientific publications on human health risks due to mercury exposure, the question may be asked: is interpretation of the epidemiologic evidence necessarily controversial?

The answer must first consider the nature of epidemiologic studies. Like other scientific inquiries, they will always render tentative knowledge. While no scientific process can provide absolute proof, observational studies, in particular, will lead to conclusions that are likely to be refined as the depth of understanding improves. Given evidence that can never be final, a truly scientific method of decision-making does not

exist.

Preventive action must therefore be based on all relevant documentation, but as in the case of medical diagnosis, decisions must recognize the uncertainty of the data as well as the potential costs and consequences of the interventions being considered. For example, control of mercury-related air pollution may be very costly and must be balanced in the long term against the benefits associated with decreased contamination of fish. In addition, fish species that accumulate mercury contain essential nutrients; the benefits of avoiding eating contaminated fish as a short-term solution must therefore be balanced against possible nutritional disadvantages. Furthermore, government agencies may be bound by specific mandates and past decisions, some of which may be difficult to change. Yet, while these issues are important considerations in the decision-making process, they should not be confused with a critical assessment of the scientific evidence.

Is the Mercury Evidence Contradictory?

Two major prospective cohort studies on the health effects of prenatal methylmercury exposure have been published, each conducted in a population with a high intake of seafood. One study was conducted in the Seychelles Islands in the Indian Ocean (Myers et al., 2003), and the other in the Faroe Islands in the North Atlantic (Grandjean et al., 1997). A smaller prospective study from New Zealand reached results similar to those obtained in the Faroes, as did several cross-sectional studies (UNEP, 2002).

While no association between deficits and maternal hair-mercury concentrations was evident in developmental tests in children up to 8 years of age in the Seychelles (Myers et al., 2003), clear associations with cord blood mercury levels were seen on neuropsychological tests administered to 7 year-old Faroese children (Grandjean et al., 1997). These findings are robust in the full Faroes data set in analyses controlled for age, sex and confounders, and they persist after exclusion of high-exposure subjects.

However, despite the apparent differences between these two studies of mercury-exposed populations, they may not necessarily be in disagreement. In fact, the confidence intervals for the two studies overlap, and the Seychelles findings are therefore not significantly different from the Faroes results (Keiding et al., 2003). Further, some differences would be anticipated, because the two studies used different methods for assessment of exposures and outcomes (Table 1), and due to different epidemiological settings.

Table 1 Main differences between two of the prospective studies of methylmercury-exposed children

Attribute	Faroes	Seychelles
Source of exposure	Whale, fish and shellfish	Fish
Exposure assessment	Cord blood and maternal hair	Maternal hair
Concomitant exposures	PCBs (whale blubber)	Pesticide use in tropics
Language	Faroese (and Danish)	Creole (English and French)
Socioeconomic setting	Industrialized Scandinavian	Middle-income developing
Family-setting	Traditional	Mainly matriarchal
Neuropsychological tests	Domain-related	Omnibus and domain-related
Clinical examiners	Clinical specialists	Nurse/student
Supporting examinations	Neurophysiological tests	Not possible

When visiting the Seychelles during the most recent examinations in 1999, Dr. Pal Weihe and I noted many differences between the two populations. Residents of the Seychelles live in a tropical climate and have easy access to fruits and vegetables. While important sources of vitamins, their nutritional value also depends on possible pesticide residues. The family structure tends to be matriarchal, with almost 50% of all households being headed by a female (MISD, 2003). About one-half of the children are born out of wedlock; about 25% of the children have no known father. Accordingly, children examined in the Seychelles study were accompanied by a ‘care-giver’, often a relative, with whom the child was living (Myers et al, 2003).

The Faroese live in the northern temperate zone, and their lifestyle is entirely Western. Most food items, other than seafood and lamb, are imported from Denmark. Many Faroese are exposed to polychlorinated biphenyls (PCBs) from eating whale blubber, which is also thought to cause developmental neurotoxicity (Dietrich, 1999). At the same time, alcohol use among women is low. The Faroese family pattern is rather stable, with almost all children living within a traditional family structure.

Responses to Uncertainty

To resolve the confusion that has resulted from one mercury study being perceived as "positive" while the other is seen as "negative," several federal agencies first held a

workshop in late 1998, at which about 30 invited experts spent three days listening to presentations and discussing the evidence (NIEHS, 1998). Almost by default, the primary effort focused on questioning the reported associations between mercury exposure and adverse effects in the Faroes. Still, the meeting concluded that the findings of the Faroes study could not be explained away.

Within the time frame of this meeting, less effort was spent on exploring the reasons why some epidemiologic efforts had failed, at least up to that point, to document adverse effects associated with mercury in seafood. Only recently have efforts attempted to document how uncertainties may bias epidemiological findings toward the null hypothesis.

In the subsequent discussions, several issues emerged as crucial considerations for risk assessment, i.e., the use of benchmark calculations, the validity of exposure biomarkers, variations of the mercury hair-to-blood ratio, and the choice of uncertainty factors when calculating an exposure limit. Each if these issues will be dealt with below in the light of current insight into the impact of uncertainties.

Biomarker Validity

In observational studies, where the exposure is not a matter of design, the validity of the exposure assessment depends on the degree to which the exposure parameters reflect the "true" exposure. As the JECFA summary concludes, "based on a consideration of numerous publications, the Committee confirmed the validity of these biomarkers for both short-term (blood) and longer-term (hair) intake of methylmercury" (JECFA, 2003). This inference agrees with our own (Grandjean et al. 2002), but exposure biomarkers should, at the same time, be considered only proxy variables, which are always imprecise to some extent. This issue is important, because exposure misclassification is likely to be nondifferential and will therefore cause underestimation of the true effect of the exposure.

Until recently, the degree of imprecision has been assumed to be reflected by laboratory imprecisions, although these low levels of imprecision (usually about 5% or less) could not explain why associations between mercury concentrations in hair and blood often show wide scattering. Although frequently used for feasibility reasons, the maternal hair mercury concentration is likely to be a rather imprecise measure, particularly in regard to fetal exposure. Among sources of variability are hair type, hair color, external contamination, and leaching due to permanent hair treatments (Grandjean et al., 2002). Recent studies have now documented that the coefficient of variation for the

hair-mercury imprecision is over 50%, i.e., twice the level found for the blood concentration (Budtz-Jørgensen et al., 2004a).

Although these imprecision levels relate to our own studies, where hair-mercury concentrations have been determined both at the University of Rochester and in Denmark, it is likely that similar uncertainties apply to other studies. The overall effect of such non-differential imprecision is that the regression coefficients decrease, the *P*-values increase, and adjustment for confounders with better precision cause additional bias toward the null hypothesis (Budtz-Jørgensen et al., 2003b).

The Hair-to-Blood Ratio

When calculating an exposure level from the hair mercury concentration, an average hair-to-blood ratio of 250 is generally used (U.S.EPA, 2001). This ratio is in accordance with recent evidence on Caucasian and Oriental hair (Grandjean et al., 2002), but is known to vary considerably between individuals. We have also found that it depends on the concentration level (Budtz-Jørgensen et al., 2004a). JECFA (2003) decided to include a factor of 2 to allow for this interindividual variability. Our most recent data are in general agreement with this conclusion, because the 95th percentile differs from the median by a factor between 2 and 3. However, the hair-to-blood association is not constant. For example, in 7 year-old Caucasian children (with finer hair than adults), the ratio is about 370 (e.g., 50% higher than adults) (Budtz-Jørgensen et al., 2004a).

In international comparisons, three main types of hair structure are recognized (i.e., African, Caucasian, and Oriental), but good data for calibration with blood concentrations exists only for the latter two hair types. Thus, for the African population in the Seychelles, translation of hair-mercury results to blood concentrations and intake levels must currently be based on data mainly from Caucasian populations. In this case, an additional uncertainty factor may be appropriate.

Concerns about Confounding

At the NIEHS (1998) workshop, three major reasons were noted as to why a mercury effect might have been overestimated in the Faroese study: (a) association of mercury intake with exposure to other neurotoxic pollutant(s); (b) other types of residual confounding; and (c) inadequate adjustment for multiple comparisons. A main concern

was whether concomitant exposure to organochlorine compounds, especially PCBs, might explain the reported associations. Detailed analyses failed to show any important impact of PCB exposure on the neurotoxicity outcomes (Budtz-Jørgensen et al., 1999; Grandjean et al., 2002). Inclusion of PCB exposure in a structural equation model attenuated the mercury effect somewhat; mercury remained statistically significant, but PCB was far from significant (Budtz-Jørgensen et al., 2002). Although residual confounding of some unknown type can never be completely ruled out, PCB exposure, at least, does not seem to explain the mercury-associated dysfunctions.

Standard multiple regression methods are often used for controlling for confounding effects. However, in situations were the exposure is measured with some degree of imprecision, this approach may result in biased estimates: Inclusion of a covariate, which is associated with the exposure but without any explanatory power in regard to the effect, will increase the underestimation of the effect of the exposure of interest (Budtz-Jørgensen et al. 2003b). At the same time, the estimated effect of the covariate is biased to reflect an impact on the outcome. Without taking the imprecision into regard, multiple regression analysis will not lead to any deeper understanding of the underlying structure of the data and may add uncertainty (and increased *P*-values) to the estimate of the mercury effect.

In discussing the generalizability of the mercury studies, the suggestion was made (Myers et al., 2003) that methylmercury-associated effects, as demonstrated in the Faroes, are relevant only to whale-eating populations. In other words, the concern was raised as to whether the Faroes findings can be generalized to other populations exposed through consumption of fish. The same point of view concerning generalizability could equally well question whether mercury neurotoxicity is negligible only in a population like that of the Seychelles. Because the studies are observational, no single study is likely to provide a proof of causation (or the lack thereof). An evaluation of the overall data base should take into account the specific circumstances, as well as strengths and weaknesses of each study. In the light of the New Zealand study and several cross-sectional studies (UNEP, 2002), the main question seems to be why significant effects were not documented in the Seychelles.

Multiple comparison issues may complicate the overall assessment of the findings in a battery of neuropsychological tests, and may also make it difficult to choose a test that would appear to be the most sensitive parameter. Standard approaches to adjustments for multiple comparisons may be inappropriate because the outcomes are not independent. A useful way of resolving this concern is to apply a structural equation model that incorporates all exposure parameters, confounders, and outcome variables at the same

time (Budtz-Jørgensen et al., 2002). Our structural equation analyses show that the combined regression coefficients and *P*-values for the cognitive function outcomes were very similar to those obtained from the seemingly most sensitive test (i.e., the Boston Naming Test).

The validity of the findings is also supported by the agreement with neurophysiological findings of mercury-associated delays on evoked potentials in the brain (Grandjean et al., 1997). Also, exclusion of subjects with variable exposures during gestation tended to increase the associations between the mercury exposure and the deficits (Grandjean et al., 2003).

Benchmark Dose Calculations

In calculating exposure limits from epidemiological data, regulatory authorities have increasingly relied upon the use of benchmark dose estimates (Budtz-Jørgensen et al., 2001). According to usual default settings, an exposure at the benchmark dose (BMD) results in an increased frequency of a pathological outcome from 5% to 10%. The benchmark dose level (BMDL) is then the point of departure that represents the lower 95% confidence limit of the BMD.

Despite the statistical definition of the BMDL, JECFA (2003) concluded that the BMDL represents an exposure that is "without appreciable adverse effects in the offspring". This interpretation may be true under some circumstances, but in large epidemiological studies, where the confidence interval is relatively narrow, the BMDL will be closer to the BMD. For example, the results from the Faroes show that exclusion of the subjects with a maternal hair-mercury concentration above 10 :g/g (a cut-off level lower than the BMDL used by JECFA) barely altered the regression coefficients and the *P*-values (Grandjean et al., 1997). The BMDL is therefore not a no-adverse-effect level (NOAEL), but rather a lowest-observed-effect level (LOEL). This consideration is important, because it is likely to affect the choice of uncertainty factors, especially in regard to brain function, where even small decrements may be of substantial social and economic impact.

JECFA (2003) used BMDLs based on the maternal hair-mercury concentration. In contrast to the NRC (2000), JECFA decided to exclude the New Zealand study and therefore arrived at a higher overall average BMDL. For the Faroes study, the BMDL chosen by JECFA was 12 :g/g maternal hair (i.e., an average for the linear dose-response curve for several different functions and not the most sensitive brain function, as

preferred by NRC).

The problem of choosing the most sensitive function may be resolved by using a structural equation model for deriving integrated BMD and BMDL values (Budtz-Jørgensen et al., 2003b; Budtz-Jørgensen et al., 2004b). This calculation includes all exposure information, confounders, and cognitive outcomes, and also takes into regard effects of measurement uncertainty. Using this advanced statistical approach, the overall BMDL is calculated at 6 µg/g maternal hair (or 43 µg/L cord blood). Thus, by incorporating the complete data set in the assessment, the resulting hair-based BMDL is only half the size of the BMDL chosen by JECFA (2003).

This finding is in agreement with the general finding that measurement uncertainty (in the exposure or the response) leads to overestimation of the benchmark results (Budtz-Jørgensen et al. 2003a; Budtz-Jørgensen et al., 2004b). Thus, although the above calculations are based on the Faroes study only, it is likely that such refinements of the BMDL calculations using data from other studies would result in a similar, if not greater, decrease in the BMDL results.

Uncertainty Factors

In calculating an exposure limit from a BMDL, an uncertainty factor is usually applied to take into account sources of variation in individual susceptibility as well as insufficiencies in the data base, e.g., concerning effects on target organs other than the developing nervous system. The NRC (2000) and U.S. EPA (2001) chose a total uncertainty factor of 10. However, JECFA (2003) concluded that "the two study samples represent diverse populations", and that "no uncertainty factor is needed to account for variation in vulnerability among subgroups". This decision is also based on the assumption that the most sensitive effects are the average neurobehavioral outcomes in the two studies, on which the overall average BMDL was based. However, JECFA had included results from a study that did not identify statistically significant decrements, thus hardly representing a vulnerable population.

JECFA included only an uncertainty factor of 3.2 to account for the total human inter-individual variability for dose reconstruction (converting maternal blood concentration to a steady-state dietary intake). This decision is in accordance with default calculations, but omits the consideration of toxicodynamic sources of variation as well as insufficiencies in the data base. In conjunction with JECFA's uncertainty factor of 2 for conversion of hair-mercury concentrations to intake levels, the total uncertainty factor

used was 6.4 (Table 2).

Table 2 Calculated exposure limits for methylmercury

	NRC (2000)	JECFA (2003)
Number of studies	One (three)	Two
Exposure biomarker	Cord blood (hair)	Hair
BMDL selected	58 µg/L cord blood	14 µg/g hair
Uncertainty factor	10	3.2 and 2
Exposure limit	0.1 µg/kg per day	1.5 µg/kg per week

The choice of uncertainty factors explains only in part the difference in the recommended exposure limits. Another decision is which studies to include. Most important perhaps, the adjusted BMDL (see above) will result in lower exposure limits than those arrived at in the risk assessments carried out so far.

Public Health Relevance

Once the scientific data have been considered, the health relevance of the findings needs to be determined. Accordingly, scientific findings should be expressed in terms that would facilitate an evaluation of their public health significance to the extent possible. Important societal issues may be raised – for example, are small deficits of any concern if they fall within the normal variation of performance seen in subjects thought not to be exposed to neurotoxicants? For example, the authors of a *Science* commentary proposed that subtle decrements in neuropsychological test performance of children exposed to mercury through fish consumption would be of questionable relevance in the light of the benefits of eating fish (Egeland and Middaugh, 1997).

The Faroes study showed that each doubling in prenatal mercury exposure corresponded to a delay of one or two months in mental development at age 7 years (Grandjean et al., 1997). Because rapid development occurs at that age, such delays may be important. Also, even small shifts in a measure of central tendency may be associated with large changes in the tails of the distribution. Such developmental delays are likely to be permanent, at least in part, but the long-term implications are unknown. The experience with lead neurotoxicity suggests that such effects are likely to remain and that they may even become more apparent with time.

In addition, the mercury effects may well have been underestimated even in the Faroes study. Neurobehavioral tests differ in their psychometric properties, and the sensitivity may be adversely affected by translation into a new language. Many factors other than mercury may influence test performance. In the three prospective studies, methylmercury exposure originated from seafood, and essential nutrients in fish could have potentially counteracted some of the adverse effects. Such potential interaction does not render methylmercury toxicity unimportant, but suggests that the degree to which this contaminant undermines the benefits of essential nutrients deserves attention.

Similar concerns have been raised in regard to other discussions involving developmental toxicity; perhaps most notably in connection with childhood lead exposure. The potential for overestimation of a toxic effect was raised without paying equal attention to the risk of underestimation. In regard to lead neurotoxicity at low doses, Needleman and Bellinger (2001) discussed the methodological solecisms that have clouded such a judgment. For example, if a *P*-value was above 0.05, that was taken as indication of no lead effect. However, considerations of statistical power and results of meta-analyses would have been more informative. Also, while the existence of residual confounding can never be fully excluded, there is little reason to invoke "phantom" covariates to explain away an association that is biologically plausible.

In this regard, epidemiologists have often debated their relationship to the development of public health policies; in particular, the thorny issue of balancing between being an advocate for particular policies and being an ivory tower scientist (Krieger, 1999). Although this debate is likely to continue, societal concerns can never overrule the need to consider the epidemiologic evidence on its merits alone. Likewise, even weak epidemiological evidence may contain important messages on serious health hazards that should not be overlooked.

Conclusions

The calculation of an exposure limit for methylmercury recently carried out by national and international bodies differs in a number of respects. The selection of studies is one issue where the evaluations differ. None of the reports have taken into account the impact of measurement imprecision, and benchmark results used by the committees are therefore biased toward higher values. The NRC and the U.S. EPA used a larger total uncertainty factor that, at least in part, compensated for this problem. JECFA, on the other hand, decided to use an uncertainty factor only for toxicokinetic differences (supplemented by a

factor for variability in the hair-to-blood conversion). JECFA's decision was based on the questionable inference that the average BMDL would protect the fetus against all adverse neurobehavioral effects, and that effects on other organ systems would not be relevant (even though new information support the notion that methylmercury may cause an increased risk of cardiovascular death). If a BMDL adjusted for exposure misclassification was used, the calculated exposure limit would be lower than any of the ones proposed by the expert committees.

Some scientific uncertainties are bound to remain, although new prospective cohort studies on methylmercury neurotoxicity are starting to provide new evidence, e.g., from ongoing research in Japan. However, the documentation is not going to expand substantially or otherwise provide much clearer guidance for regulatory agencies. It should also be recognized that the question as to whether to base decisions either on proof of harm or on precaution cannot be settled from epidemiological evidence.

The experience with lead research has amply illustrated that apparent disagreement is likely to occur between studies carried out by different methods in different settings. We therefore should not anticipate full coherence among all available evidence. Accordingly, decisions on preventive efforts should be justified by the scientific database at large, taking into account its various uncertainties and inconsistencies.

The potential costs and other societal consequences of policy decisions – including decisions to do nothing - also deserve fair consideration. However, these issues should be addressed in parallel to and separate from the discussion of toxicological and epidemiological concerns. Otherwise, the erroneous impression will be generated that disagreements on preventive measures are solely due to uncertainties in epidemiologic evidence.

Acknowledgments

This paper is based on the international collaboration on birth cohorts in the Faroe Islands and on my inspiring discussions with my closest colleagues, especially Drs Katsuyuki Murata, Pál Weihe and Esben Budtz-Jørgensen.

References

Budtz-Jørgensen E, Keiding N, Grandjean P, White RF, Weihe P (1999). Methylmercury neurotoxicity independent of PCB exposure [letter]. *Environ Health Perspect* **107**, A236-237.

Budtz-Jørgensen E, Keiding N. Grandjean P (2001). Benchmark dose calculation from epidemiological data. *Biometrics* **57**, 698-706.

Budtz-Jørgensen E, Keiding N, Grandjean P, Weihe P (2002). Estimation of health effects of prenatal methylmercury exposure using structural equation models. *Environ Health* **1**, 2.

Budtz-Jørgensen, E., Keiding, N., Grandjean, P (2003a). Application of structural equation models for evaluating epidemiological data and for calculation of the benchmark dose. *Proceedings of the ISI International Conference on Environmental Statistics and Health*, Santiago de Compostela, pp. 183-194.

Budtz-Jørgensen E, Keiding N, Grandjean P, Weihe P, White RF (2003b). Consequences of exposure measurement error for confounder identification in environmental epidemiology. *Stat Med* **22**, 3089-3100.

Budtz-Jørgensen E, Grandjean P, Jørgensen PJ, Weihe P, Keiding N (2004a). Association between mercury concentrations in blood and hair in methylmercury-exposed subjects at different ages. *Environ Res* (in press).

Budtz-Jørgensen E, Keiding N, Grandjean P (2004b). Effects of exposure imprecision on estimation of the benchmark dose (submitted for publication).

Dietrich KN (1999). Environmental chemicals and child development [editorial]. *J Pediatr* **134**, 7-9.

Egeland GM, Middaugh JP (1997). Balancing fish consumption benefits with mercury exposure. *Science* **278**, 1904-1905.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Murata K Sørensen N, Dahl D, Yokoyama K, Jørgensen PJ (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* **19**, 417-428.

Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, Debes F, Murata K, Simonsen H, Ellefsen P, Budtz-Jørgensen E, Keiding N, White RF (2001). Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. *Neurotoxicol Teratol* **23**, 305-317.

Grandjean P, Jørgensen PJ, Weihe P (2002). Validity of mercury exposure biomarkers. In: Wilson SH, Suk WA, Eds. *Biomarkers of Environmentally Associated Disease*. Boca Raton, FL, CRC Press/Lewis Publishers, pp. 235-247.

Grandjean P, White RF, Weihe P, Jørgensen PJ (2003). Neurotoxic risk caused by stable and variable exposure to methylmercury from seafood. *Ambul Pediatr* **3**, 18-23.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). *Sixty-first meeting, Rome, 10-19 June 2003. Summary and conclusions.*
URL: ftp://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf (accessed, 19 December, 2003)

Keiding N, Budtz-Jørgensen E, Grandjean P (2003). Prenatal methylmercury exposure in the Seychelles [letter]. *Lancet* **362**, 664-665.

Krieger N (1999). Questioning epidemiology: objectivity, advocacy, and socially responsible science. *Am J Public Health* **89**, 1151-1153.

Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari E, Sloane-Reeves J, Wilding GE, Kost J, Huang LS, Clarkson TW (2003). Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. *Lancet* **361**, 1686-1692.

MISD (Management & Information Systems Division) web site, Statistics & Database Administration Section (SDAS) of the Ministry of Information Technology & Communication of the Republic of Seychelles. URL: http://www.misd.gov.sc/sdas/ (accessed, 19 December, 2003)

Needleman HL, Bellinger D (2001). Studies of lead exposure and the developing central nervous system: a reply to Kaufman. *Arch Clin Neuropsychol* **16**: 359-374.

NIEHS. Workshop organized by Committee on Environmental and Natural Resources (CENR), Office of Science and Technology Policy (OSTP), The White House: Scientific Issues Relevant to Assessment of Health Effects from Exposure to Methylmercury, November 18-20, 1998 [accessed, December 19, 2003]. Available from:URL:http://ntp-server.niehs.nih.gov/main_pages/PUBS/MethMercWkshpRpt.html (accessed, 19 December, 2003).

NRC (National Research Council), 2000. Toxicological Effects of Methylmercury. National Academy Press, Washington.

UNEP (United Nations Environment Programme), 2002. *Global Mercury Assessment.* Geneva.

U.S. EPA (Environmental Protection Agency), Office of Science and Technology, Office of Water, 2001. *Water Quality Criterion for the Protection of Human Health: Methylmercury, Final.* EPA-823-R-01-001. Washington.
URL: http://www.epa.gov/waterscience/criteria/methylmercury/document.html (accessed, 19 December, 2003).

Faroe Islands Cohort Study

Pál Weihe[1,2], Philippe Grandjean[1]

[1]*University of Southern Denmark, Institute of Public Health, Department of Environmental Medicine, Winsloewsparken 17, 5000 Odense C*

[2]*The Faroese Hospital System, Department of Occupational and Public Health, Sigmundargøta 5, FO-110 Tórshavn.*

Toxicological evidence suggests that humans are much more vulnerable to adverse effects from exposures to pollutants that occur during development, i.e., prenatally or in early childhood. However, the adverse effects may not be immediately apparent and often are expressed fully only when physiological functions have matured. Accordingly, research in environmental epidemiology now emphasizes prospective research, in this case based on birth cohorts. Given the advantages of conducting such research in the Faroe Islands, we have therefore generated three birth cohorts.

Cohort 1: A cohort of 1022 singleton births was assembled in the Faroe Islands during a 21-month period of 1986-1987. The range of mercury concentrations in cord blood and maternal hair was about 1000-fold. Frequent whale meat dinners during pregnancy and, to a much lesser degree, frequent consumption of fish, and increased parity or age were associated with high mercury concentrations in cord blood and maternal hair. Mercury in cord blood correlated moderately with blood-selenium. Lead in cord blood was low (median, 82 nmol/l), particularly when the mothers had frequently had fish for dinner and abstained from smoking. Because the effects of fetal childhood exposure to methylmercury are persistent, detailed examination of children with prenatal exposure to this neurotoxicant would be appropriate at school age. At this time, they have developed sufficiently to perform a wide variety of neurobehavioral tests, and they are capable of cooperating for most functional tasks. The first detailed examination took place at age 7 years, i.e., just before school entry, between early April and late June in 1993 and, for the youngest children of the cohort, at the same time in 1994. A total of 917 of the surviving children (90.3%) completed the examinations. Most of the children were examined at the National Hospital in Tórshavn, the capital of the Faroe Islands. To facilitate travel for the families, examinations also took place at the two smaller hospitals in the Faroes in 1993, and the following year in both Odense and Copenhagen, Denmark (for families who had moved). Four children were examined during the morning and four during the afternoon at five examination stations, with each

station taking up to 60 minutes. Past medical history, current health status and social factors were recorded on a self-administered form by the parent accompanying the child (usually the mother). The physical examination included a functional neurological examination with emphasis on motor coordination and perceptual-motor performance. Visual acuity was determined by Snellen's board and contrast sensitivity by the Functional Acuity Contrast Test. Otoscopy and tympanometry were supplemented by audiometry. Main emphasis was placed on detailed neurophysiological and neuropsychological tests that had been selected on the basis of a range of considerations. Tests were chosen to include tasks that would be affected by the neuropathological abnormalities described in congenital methylmercury poisoning and the functional deficits seen in children with early-life exposure to neurotoxicants. The tests also had to be acceptable to the children and their parents, viz. painless, not too time-consuming, and appropriate for 7-year-old Faroese children who had not yet begun school. Tests that were likely to provide a high statistical sensitivity, i.e., with a wide range of scores possible without floor or ceiling effects, and acceptable test-retest reliability, were preferred. In addition, test versions standardized in Scandinavian countries were favored. The second examinations have just been completed at age 14 years. Again, the participation rate was very high, almost 90%. The overall approach was very similar to the one previously applied, though the clinical tests were adjusted to be appropriate for the teenage participants. The examinations were carried out by a team of health service professionals who had no access to information on individual exposure levels.

Cohort 2: The findings from Cohort 1 suggested that exposure assessment should encompass several lipophilic pollutants in addition to methylmercury. As a follow-up, Cohort 2 was therefore established during a 12-month period in 1994-1995 and included 182 singleton term births from consecutive births at the National Hospital in Tórshavn, Faroe Islands. Maternal residence was required in the central and northwestern region of the primary catchment area, i.e., away from the capital area of Tórshavn. About one-third of the Faroese population resides in this area, where the mercury exposure was expected to vary the most. A total 64% of all births were included, incomplete sampling being mainly due to logistic problems in the busy ward. In addition, four children were excluded because they were born before the 36th week of gestation, and two children because they had congenital neurological disease; none of the children had a birth weight below 2500 g. The overall participation rate was slightly below the one obtained in Cohort 1, but the average birth weight was almost the same in the two cohorts and similar to the Faroese average. Relevant obstetric data were obtained by standardized procedures and supplemented by a brief nutrition questionnaire. These children were

first examined by the Neurological Optimality Score at age two weeks (adjusted for gestational age), and then again at 7 months of age. Subsequent examinations were at age 18 months and then at 12-month intervals up to age 66 months. At 42 months, a comprehensive medical examination with the Neurological Optimality Score was included. For comparison with Cohort 1, detailed neurobehavioral tests were carried out at age 7 years. The complete profile of
neurobehavioral development is currently being being analyzed.

Cohort 3: New insight into health risks caused by environmental pollutants and changing exposure patterns in the Faroes lead to the formation of Cohort 3 from consecutive births in Tórshavn between November, 1997 and February, 2000. Because of dietary recommendations from the Faroese health authorities, methylmercury exposures had now decreased thus allowing better characterization of possible effects of PCBs and other lipophilic contaminants. Cohort 3 consists of 650 children. Inclusion criteria required appropriate biological specimens for exposure biomarker determination and a valid examination by the pediatrician at two weeks of age. The children included represent approximately 60% of all pregnancies. In regard to parity, maternal age, smoking and alcohol consumption (very limited), Cohort 3 is quite similar to the two previously generated cohorts. Serum was again collected from the mother at the last antenatal examination (34th week of pregnancy). Other samples collected from the mother-child pairs include cord blood and serum, maternal hair at parturition, and milk on days 3-5 (before mother and child were released) and at two weeks. Nutritional habits were recorded by questionnaire (number of whale meat dinners per month during pregnancy and before pregnancy; number of fish dinners per week; ingestion of blubber with whale meat or fish). A subgroup of Cohort children is being examined with regard to immunological parameters, but the first comprehensive medical examination will take place at the age of 5 years.

Main conclusions from Faroese cohorts:

In the Faroes study, methylmercury appeared a much stronger neurotoxicant than did PCB, but a weak tendency of PCB neurotoxicity was seen in children who at the same time had a high prenatal exposure to methylmercury (Grandjean et al, 2001). Also, in the second Faroese cohort, a decreased neurological optimality scores was seen at increased methylmercury exposures, while PCB did not have an independent effect (Steuerwald et al. 2000). As discussed in a report from the U.S.National Academy of Sciences (2001), these findings - in conjunction with results from New Zealand, Madeira, and Brazil - therefore indicate that methylmercury is a neurotoxicant at levels

exceeded in several regions of the arctic.

We have found that PCB may interfere with essential fatty acid metabolism (Grandjean & Weihe, 2003), possibly via inhibition of desaturations, and this effect should be considered as a possible toxicological mechanism for PCBs. Perhaps it may also relate to our finding that seafood contaminant exposure is associated with decreased postnatal growth (Grandjean et al., 2003). This effect may be due to both methylmercury and PCBs tranferred via human milk, but intrauterine exposure also seems to affect the programming of postnatal growth. Both substances are known from experimental studies to caused such effects. These findings again illustrate that seafood contaminants should be looked at conjointly.

References

Grandjean Philippe, Weihe P, Burse V W, Needham L L, Storr-Hansen E, Heinzow B, Debes F, Murata K, Simonsen H, Ellefsen P, Budtz-Jørgensen E, Keiding N, White R F: Neurobehavioral deficits associated with PCB 7-year-old children prenatally exposed to seafood neurotoxicants. Neurohehavioral and Teratology 23 (2001) 305-317.

Grandjean P, Budtz-Jorgensen E, Steuerwald U, Heinzow B, Needham LL, Jorgensen PJ, Weihe P. Attenuated growth of breast-fed children exposed to increased concentrations of methylmercury and polychlorinated biphenyls. FASEB J. 2003 Apr;17(6):699-701. Epub 2003 Feb 05.

Grandjean P, Weihe P. Arachidonic acid status during pregnancy is associated with polychlorinated biphenyl exposure. Am J Clin Nutr. 2003 Mar;77(3):715-9.

Steuerwald U, Weihe P, Jørgensen P, Bjerve K, Brock J, Heinzow B, Budtz-Jørgensen E, Grandjean P: Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J Pediatr 2000; 136: 599 – 605.