Proceedings of
NIMD Forum 2006

Recent Topics of Fetal Methylmercury Exposure and Its Effects

20 February 2006

Conference Hall, Minamata Disease Archives

National Institute for Minamata Disease

Minamata City, Kumamoto, Japan

Preface

This Forum will be held every year in order to discuss plans for future research subjects in our institute with researchers from foreign countries.

Recently, global mercury pollution has been widely recognized. Two large cohort studies, in the Faroes and Seychelles, on the effects of methylmercury on fetuses, have continued since about twenty years. In Japan, similar studies were started several years ago by the study group of Tohoku University.

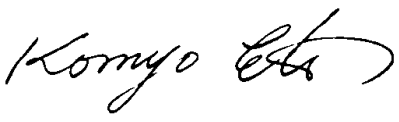
Today we have a chance to discuss theses issues together with the study group of Faroes and Tohoku University. I have invited two the members of World Health Organization (WHO), Dr. Antero T. Aitio and Dr. Tim Meredith, to attend this meeting. It is very important to inform the results of recent cohort studies on the effects of low-level exposure of methylmercury on the fetuses to the members of WHO.

As you know, Minamata City experienced the first instance of methylmercury poisoning caused by man-made environment pollution. A company in Minamata provided economic development but caused environmental pollution. The accident has destroyed not only the environment, but has taken its toll in human life. On May 1, 2006, we commemorate the fiftieth year since the first discovery of patients suffering from Minamata disease. Today victims continue to suffer from the consequences of the disease.

It has been 27 years since NIMD was established in October, 1978. Our institute was designated as a Collaborating Center for Studies on the Health Effects of Organic Mercury by the Regional Office for the Western Pacific of WHO in 1986. We are conducting ongoing studies on the effects of methylmercury and inorganic mercury.

I think this joint meeting of the Faroes study group led by Dr. Grandjean et al., the Japanese study group of Dr. Satoh et al., and Drs. Aitio and Meredith of WHO with the members of NIMD, is very significant. I believe that this forum is a valuable opportunity for good communication among these groups. Finally, I would like to ask all of you here to continue your wonderful environmental protection research efforts in order to leave a comfortable global environment to coming generations.

February 20, 2006



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

Organizing Committee

Mineshi Sakamoto, Ph.D.

Director, Department of International Affairs and Environmental Sciences, National Institute for Minamata Disease

Masaaki Nakamura, M.D., Ph.D.

Head, Clinical Medicine Section, Department of Clinical Medicine, National Institute for Minamata Disease

Masatake Fujimura, Ph.D.

Head, Physiology Section, Department of Basic Medical Sciences, National Institute for Minamata Disease

Kiyoshi Baba

Head, International Affairs and Information Section, Department of International Affairs and Environmental Sciences, National Institute for Minamata Disease

Shuji Tanaka

Officer, Finance and Accounting Section, Administration Division, National Institute for Minamata Disease

Shun'ichi Honda, Ph.D.

Researcher, Survey Section, Department of Epidemiology, National Institute for Minamata Disease

Programme

				Pages
Opening Session				
9:00	-	9:15	**Opening Address** Dr. Komyo Eto, Director General, National Institute for Minamata Disease	
			Welcome Remarks Dr. Tatsuya Aoki, Director, Special Environmental Disease Office, Ministry of the Environment	**1**
Morning Session **Moderators: Dr. Hiroshi Satoh (Tohoku University)** **Dr. Tim Meredith (WHO)**				
9:15	-	9:40	**Dietary advisories and public information** Dr. Pal Weihe (Faroese Hospital System)	**2**
9:40	-	10:05	**Choosing the age for assessment of neurotoxicity** Dr. Frodi Debes (University of Southern Denmark)	**12**
10:05	-	10:30	**Joint analysis of the two Faroese cohorts assessing the effect of prenatal mercury exposure in 7 year old children** Dr. Esben Budtz-Jorgensen (University of Copenhagen)	**31**
10:30	-	10:50	Break	
10:50	-	11:15	**Selenium as a potential protective factor against mercury developmental neurotoxicity** Dr. Anna Choi (Harvard School of Public Health)	**36**
11:15	-	11:40	**Lesson from an animal experiment of combined exposure to methylmercury and PCBs on neurobehavioral influences in mice** Dr. Norio Sugawara (Tohoku University)	**41**
11:40	-	12:05	**Effects of "spike" (short-term peak) exposure to methylmercury on mercury accumulation and neuronal degeneration in the brain** Dr. Shun'ichi Honda (National Institute for Minamata Disease)	**54**
12:05	-	13:05	Lunch	

Welcome Remarks

Dr. Tatsuya Aoki

Director, Specific Disease Control Office, Environmental Health Department, Ministry of the Environment

Substituted by:

Dr. Munehito Machida

Deputy Director, Specific Disease Control Office, Environmental Health Department, Ministry of the Environment

It's my great pleasure to be at NIMD Forum today, and have an opportunity to give a remark.

I appreciate Dr. Komyo Eto, the director of NIMD and his excellent staff for setting MIMD Forum 2006 in Minamata.

On behalf of Ministry of the Environment, I would like to thank all of you, especially Professor Grandjean, Dr. Aitio, Dr. Meredith, for your participation to this Forum.

Today's subject, recent topics of fetal methylmercury exposure and its effects, is now one of the greatest concerns among the field of environmental health all around the world after being issued the advisory on fish consumption for women and children by EPA and FDA in 2001. Japanese researchers have started a cohort study in northern part of Japan since 2002 with corporation of NIMD staff, and I appreciate they are giving report on progress from this morning. In addition, we are happy to have a chance to deepen the understanding of the achievement in cohort study in Faroese islands though this forum.

Hope this Forum would bring us meaningful discussion and promote the understanding of scientific assessment on the cohort studies for fetal methylmercury exposure.

Thank you so much for your attention.

Dietary Advisories and Public Information

Pál Weihe and Philippe Grandjean

Department of Occupational Medicine and Public Health, Faroese Hospital System, F0-110 Torshavn, The Faroe Islands and Institute of Public Health, University of Southern Denmark, DK-5000 Odense, Denmark.

Abstract

Faroe Islanders have consumed pilot whales for hundreds of years. However, the pilot whale meat has been found to be contaminated with methylmercury and the blubber with POPs, including PCB and DDE. Since 1985, investigations have been carrying out studies in the Faroe Islands to establish the exposure levels in pregnant women and the potential adverse effects of the pollutants on the fetus. Current evidence from the Faroes indicates that prenatal exposure to methylmercury and, to a lesser degree, PCBs may impair fetal and childhood development. In August 1998, the Faroese health authorities advised women to reduce their intake of pilot whale meat and blubber to protect the fetus against adverse effects from these food contaminants.

A dietary survey was done two years after the advisory. All together, we obtained 409 24-h recall interviews and a total of 732 food diary recordings. The results from the dietary survey showed a very significant reduction in whale meat and blubber intake, and blood analysis showed a corresponding reduction in the mercury exposure. However, the PCB levels were still high and must be considered to be a potential health problem in the Faroese community. According to the dietary survey, the daily intake of both whale meat and whale blubber has been reduced up to one order of magnitude. However, the concentration of organochlorines has not declined to the same extent as the mercury, indicating that organochlorines can have other significant sources (e.g. seabirds). The longer half-life of some organochlorines compared to methylmercury may be another explanation of this observation.

In the Faroese diet the pilot whale is the main source of mercury and POP´s. The concentrations of mercury in the most common fish species consumed in the Faroes, i.e. cod and haddock, are low, around 0,05 ppm, compared to the concentrations in pilot whale meat, 2 - 3 ppm. Accordingly the public health authorities in the Faroe Islands have not advised people to reduce their intake of fish.

Settlement Structure

The Faroe Islands are situated in the North Atlantic 430 km south-east of Iceland, 600 km west of Norway and a good 300 km north-west of Scotland, in the same time zone as Britain. The distance to Copenhagen, the capital of Denmark, with which the Faroe Islands have constitutional links, is about 1,300 km.

Of the 18 islands, 17 are inhabited. The total area is 1,399 sq.km, and the largest island is Streymoy (373.5 sq.km) with the capital, Tórshavn. The distance north-south is 113 km and east-west 75 km. The land averages 300 m above sea level. The highest point is 882 m.

The climate is oceanic: humid, changeable and windy. In Tórshavn the temperature in the coldest month averages 30 C and in the warmest 110 C. The shortest day is 5 hours and the longest 19 1/2 hours.

There are no woods, but plenty of grassland. A mere 6% of the land is under cultivation while the rest is reserved for the grazing of 70,000 sheep and some cattle and horses. Birds are plentiful, especially sea birds, but other animal life is sparse. In the seas around the Faroe Islands the meeting of the warm Gulf Stream and cold northern currents rich in nutritional matter, results in rather stable ocean temperatures, between 50 and 100 C, favoring fish and animal life.

The population on 31 December 2005 was 48.371 after a tenfold increase in the preceding two centuries. The birth rate is high by European standards, 1.5% in 2000. With a mortality rate of 0.7% the natural increase in population is 0.8%. A modest net emigration of earlier decades was replaced by a certain net immigration in the 1970´ies and 1980´ies. The early nineties saw a massive emigration, the population 1990-95 declining by 7.2%. Since 1996, however, there has been an increasing net immigration.

The pattern of settlement is characterized by a large number of densely populated communities differing greatly in size. There are about 100 villages and towns, of which the largest is Tórshavn with its 19,000 inhabitants including the suburbs. Second largest is Klaksvík with 4,600 inhabitants.

Since 1948 the Faroe Islands have had a special autonomy status within the Kingdom of Denmark, viz home rule, seeking a balance between, on the one hand, the national uniqueness of the Faroe Islands and, on the other, maintenance of the union with Denmark.

The Faroese Home Rule authorities have the legal and administrative competence in those areas, which have been taken over as special Faroese affairs. The Faroe Islands have their own flag, bank notes and stamps and also a special passport.

The economy of the Faroe Islands is overwhelmingly dependent upon fisheries. Fishing, fish farming and fish processing account for a quarter of the gross factor income and almost 100% of exports. Other industries are to a great extent suppliers to the fishing industry. The remaining industries are, like the public sector, highly dependent on proceeds derived from the fishing industry. The Faroese economy is vulnerable to fluctuations in the size of the fish catch, fish prices, exchange rates and the prices of vital import products, including oil.

Lifestyle

The Faroese society is similar to the fishing communities of the western coast of Denmark and Norway. Wages are generally lower than in Denmark, and so are unemployment benefits. In the Faroe Islands, notably fishermen are used to having an income, which fluctuates in keeping with fish catches and prices. There is also a strong tradition for labor mobility, both geographically and between occupations. In the early nineties with high unemployment many Faroese went abroad to work, notably on foreign fishing vessels and in construction, but many of them are slowly drifting back again into the Faroese labor market. Due to freight costs, small units, and high indirect taxation, prices are generally higher in the Faroes, apart from books which are free of VAT, and agricultural produce, which can be bought at world market prices due to the status of the Faroe Islands outside of the Common Agricultural Policy of the EU. Nevertheless most people in the Faroese towns and villages live in fairly good and spacious, brightly painted, centrally heated houses, with most of the gadgets or conveniences considered necessary to day, including TV, video and home computer, and with a car in the garage.

The numbers of smokers in the adult population is declining. According to a survey in 2005 27 % reported daily smoking. In 2003 the same figure was 38 %. With regard to alcohol consumption, the statistics is based on sales figures. These figures indicate that the consumption in the Faroes is in the lower end of the Nordic range. The annual average alcoholic beverages in liters of pure alcohol per capita aged 15 years and older is around 7 in the Faroes and 12 in Denmark. Accordingly, the number of treatment periods/discharges from hospital for alcoholic liver diseases is low in the Faroes.

The diet is dominated by marine food, e.g. cod and haddock. Seabirds are consumed in the season, especially fulmar, puffin and guillemot. However, all types of Scandinavian food items are available in the supermarkets. A part of the traditional diet consists of pilot whale meat and blubber. The pilot whale is a small whale found in large schools in the North Atlantic, the

Mediterranean and a closely related species in the Pacific Ocean. The Faroese pilot whale catch is a traditional, communal, non-commercial hunt aimed at meeting the community's need for whale meat and blubber.

The pilot whale catch proceeds as follows: A school of pilot whales, being observed near the coast, is driven into a fjord and beached, preferably on a flat stretch. Only a limited number of beaches are approved for whaling. The whales are killed by a stab in the neck with a special knife. If the beaching is unsuccessful the whale is fastened by a special hook so that it can be killed in shallow water. It all looks quite dramatic, but each animal is dealt with so fast that the pain is brief. The authorities distribute the meat and blubber according to traditional rules, the main rule being equal shares for all the inhabitants of the district. No export takes place.

The pilot whale is not a threatened species. As a “small cetacean” the whale is not covered by the regulations of the International Whaling Commission (IWC). Working jointly with the IWC, ICES (the International Council for the Exploration of the Sea) and NAMMCO (North Atlantic Marine Mammal Co-operation Organization), - Faroese and international scientists keep a close watch on the size of the whale population. The most recent scientific estimate is that there are approximately 780,000 animals in the North East Atlantic. The annual catch fluctuates with oceanic conditions, but the long term average catch of approximately 1,000 animals, corresponding to only a small fraction of the annual natural rate of increase, is sustainable.

Sometimes the Faroese authorities receive protests, occasionally concluding in threats of a trade boycott, which are often based on exaggerated or misleading descriptions of the whale catch. It is the position of the Faroese authorities that a sustainable harvest of pilot whales occurring in the waters around the Faroe Islands is a legitimate activity. In fact it is one of the most ecological methods of producing meat at 620 degrees Northern latitude, and only one of several examples of sensible traditional utilization of local resources, still practiced in the Faroes. Endeavours to deprive the Faroese people of their right to harvest this resource is seen to be in violation of the UN Covenants of 1966 on Human Rights. Should the scientists so recommend, the authorities will be prepared to limit the catches. Up to now, catches are only limited in order to avoid waste. Whenever the need for whale meat and blubber is considered to be met, a whaling ban is imposed in the district concerned.

The Faroese authorities have acknowledged animal welfare issues regarding the methods of catching the pilot whale. In connection with updating of the age-old rules laid down for the pilot whale hunt, the authorities have strengthened the animal welfare element, such as banning the harpoon and the whale spear and significantly reducing the use of hooks from boats. A veterinarian has been charged with the task of monitoring pilot whale hunts and implementing

further improvements in killing methods. Furthermore the hunt is monitored through an international observer scheme. The Faroese do not believe that the pilot whale meat on their plate represents more animal suffering caused by humans than a similar amount of imported meat and are therefore not prepared to accept the demands for a ban on a catch which makes a larger contribution to the meat production of the islands than all the sheep and cattle put together and which covers about one quarter of the total meat consumption.

Public Health

The disease pattern in the Faroe Islands is primarily known from mortality statistics, statistics on notifiable diseases, and population surveys. Life expectancy at birth is high compared with other Nordic countries both among men (76,6 years) and women (81.3 years). Perinatal mortality is low (2,3 per 1,000 live births for 2003) and so is infant mortality (1,4 per 1000 live births for 2003).

The causes of death show the typical pattern of western industrialised countries, with about 40 % caused by cardiovascular diseases and 25 % cancer. Mortality from acute infections diseases, suicides and accidents is unimportant.

The occurrence of hepatitis B and C and HIV infections is very low. In 2000 no new cases were recorded. The number of diagnosed cases of tuberculosis is low.

A major fall in the incidence of the traditionally sexually transmitted diseases, gonorrhea and syphilis, has been seen over the last two decades. In recent years there were no notified cases of syphilis.

In 1995, the Danish Act concerning central administration of the health care was introduced at the Faroe Islands. The Danish Act concerning the medical officers etc. also applies on the Faroe Islands. The Faroe Islands Act concerning health care came into force in 1996, and according to that Act the Faroe Islands' home rule sets out rules concerning tasks, benefits and administration.

The hospital structure and its organization, specialist fields and their organization as well as the primary health service and its organization largely follow Danish principles. The same applies to nursing homes, home nurses and home help as well as dental treatment.

There is a central hospital in capital and two smaller hospitals in the northern and southern parts of the islands. Complicated cases are treated in Denmark. Social legislation is generally based on the Danish model.

Contaminants in Faroese subsistence food

In the Faroes we have known for a long time that pilot whale meat contains large concentrations of mercury. Relatively large quantities of organic compounds with a slow rate of decomposition such as PCBs, DDE, etc. have also been found. We know now that, in general, marine mammals in the arctic regions are contaminated with these substances; much above levels seen in terrestrial species. The pilot whales, (Globicephala melas), which the Faroese have been eating for centuries, migrate all over the oceans and feed on species from the food webs that have now been contaminated with a variety of industrial chemicals.

Part of the mercury originates from natural release from the earth's crust, but human activities are an important cause as well. Substance groups such as PCBs and pesticides are, however, caused solely by human activities. The substances are carried by rivers, air and currents from coastal waters into the oceans, where they are concentrated in the food chains and may finally end up in the bodies of those far-away Faroese who eat pilot whale meat and blubber.

At the end of the 1970s, we knew that the mercury level in some Faroese people exceeded the international recommendations, which were mainly based on experience from poisoning disasters in Japan and Iraq. As it was not considered absolutely certain that the mercury concentrations found in the Faroes would be harmful if consumed over a long period of time in conjunction with essential nutrients which might be protective, the Faroese Hospital System made an agreement with Odense University to examine the effect of marine pollution on children's development in the Faroes.

Under the presumption that the fetus is particularly sensitive to the effects of mercury, blood and tissue samples were collected from the umbilical cord as well as the mother's hair in connection with more than 1000 deliveries. The analyses showed that about 15% had mercury levels higher than the limit, which was considered safe according to WHO. The children were subsequently monitored until the age of 7 years, at which time they were examined thoroughly by a team of researchers from Japan, the United States, Denmark and the Faroes. The results show that there is a connection between the amount of mercury found in the fetus and the child's memory, attention, language and other mental functions at the age of 7. The results point to the fact that what was previously thought to be a harmless exposure to mercury during pregnancy in reality has a negative effect on the children's development.

Mercury concentrations in Faroese diet.

According to a dietary survey performed in the general population in 1981-82 the daily average intake of pilot whale meat was 12 g per person, 7 g of blubber and 68 g of marine fish, of which cod (Gadus morhus) and haddock (Gadus aeglefinus) is the dominating,(Vestergaard and

Zachariassen, 1987) – table 1. Data on mercury concentrations in pilot whales have been available since the mid 1970ies. Overall the concentrations in pilot whale meat have been constant during the passed three decades, with an average about 2 ppm, min. 0,4 and max 3,3 ppm (Dam M, 2005). The mercury concentrations in the cod and the haddock have been very low compared with pilot whale meat. According to table 2 around 0,02 ppm in recent years and for haddock even lower (< 0,01ppm) (Dam 2005).

Table 1 Faroese diet in general populating (N =637), 1981 – 82

Food item	Daily average consumption per person in gram
Milk products	390
Meat from terrestrial animals	68
Marine fish	72
Vegetables	224
Bread	215
Meat from pilot whales	12
Blubber from pilot whales	7

Table 2 Mercury in cod – wet weight in µg/g

Year	Number of samples	Mercury concentration
1977/78	557	0,03
1994	25	0,01
1997	44	0,03
2000	49	0,02
2001	25	0,02

The first dietary advice issued be the health authorities of the Faroe Islands on pilot whale consumption, was given in 1977. The message was to warn against eating whale liver due to high mercury concentrations which had been found in concentrations up to 270 ppm. The general advice was to abstain from whale liver, and only eat whale meat once a week. Ten years later the advice was tightened so as to suggest that whale dinners were limited to once or twice a month.

After the results of the study of 1023 children prenatally exposed in 1986/87 were published in 1997 the health authorities of the Faroes August 1998 recommended even more restrictions on pilot whale consumption. Based on the demonstrated effects of mercury exposure and on a general assessment of PCB's, the following diet recommendation were issued:

"Meat: The mercury content of pilot whale meat is high and is one of our main mercury sources. Therefore we recommend that adults eat no more than one to two meals a month. Women who plan to become pregnant within three months, pregnant women, and nursing women should abstain from eating pilot whale meat.

Blubber: High PCB's contents in blubber lead us to recommend that adults at the maximum eat pilot whale blubber once to twice a month. However, the best way to protect foetuses against the potential harmful effects of PCB's, is if girls and women do not eat blubber until they have given birth to their children.

Organs: Pilot whale liver and kidneys should not be eaten at all.

The above recommendations are considered the most advisable for the present. When new information is acquired, this diet recommendation will be revised accordingly.

Compared to the diet recommendation of 1989 this recommendation holds further restrictions for women of childbearing age."

Influence of the dietary recommendations.

To examine the effects of the new recommendations, a questionnaire was sent out in 1999 to all women residing in the Faroe Islands, aged 26 to 30 years. They were asked about their dietary habits. Those who wanted to could send a sample of hair to be analyzed for mercury. One year later all women were contacted again with the offer to send a new hair sample for analyses. 45.7% of questionnaires could be used for statistical analyses. 370 single hair samples were available taken either after the first or after the second letter. 146 women provided two hair samples. The geometric mean methylmercury in the first samples was 2.57 µg/g (arithmetical mean 3.54 µg/g with a standard deviation of 2.90 µg/g). In the second sample the results were 1.83 µg/g (2.56 µg/g ± 2.22 µg/g). The difference between the two concentrations is highly significant (Man-Withney U-test: $p<0.001$; CI95 0.52 – 1.44) (see section 6.3 in this report).

In order to make a more exact description of the effects of the diet recommendations a dietary survey was performed among pregnant women in third trimester. To cover the daily variations we used 24 hour recall (24 h recall), as well as a food diary (FD), where all food consumed during one day at a time was reported. To adjust for seasonal variations the women answered a food frequency questionnaire (FFQ) where what is considered Faroese food was listed and we asked about consumption during the past 12 months. All together there were obtained 409 24h recall interviews from the 148 women (116 did all three interviews, 29 two interviews and 3 women only one interview). The main reason for not doing all three interviews was delivery and scheduling difficulties. In total 732 FD recordings were collected (in average five FD´s per woman. The reasons for not doing all six were mainly delivery and lack of motivation. Altogether this totals 1,141 recordings. The results concerning main food items are shown in table 3. The dietary intake of pilot whale meat and blubber is remarkably low compared with the last dietary survey in 1981-82, where the average consumption of pilot whale meat among adult men and women was 12 grams per day and of blubber 7 grams. This reduction is most likely to be a result

of the recommendations to pregnant women to avoid contaminated seafood, such as pilot whale meat and blubber.

Table 3 Faroese diet 2000-2001. Pregnant women, n =148

Food item	Daily average consumption per person in gram
Milk products	517
Meat from terestrial animals	155
Marine fish	38
Vegetables	272
Bread	323
Meat from pilot whales	1,4
Blubber from pilot whales	0,6

According to table 4 a significant reduction in the mercury concentrations in umbilical cord blood occurred in the cohort established after the dietary recommendations – cohort 3. An other confirmation of this trend is the fact that mercury in whole blood from 126 women in 34. week of pregnancy showed an median of 1,4 µg/l, with only 2,4% exceeding the 5,8 µg/l limit (corresponding to the US-EPA Ref. dose).

Table 4 Total mercury in umbilical cord blood in Faroese cohorts

Cohort	Year	N	Geom. mean	Min.	Max.
I	1986-87	894	22,9	0,9	351
II	1994-95	163	20,9	1,9	102
III	1998-00	603	12,3	1,6	193

Conclusion

Whale meat is the dominating mercury source in the diet of the Faroese population. Marine fish species most commonly consumed, such as cod and haddock, are low in mercury. Dietary advisories and public information have focused on reduction of the consumption of pilot whale meat and blubber. Pregnant women have followed these advisories and consequently their mercury burden has decreased significantly.

Acknowledgement:

This paper is in partly from Árni Olafsson: “The Faroe Islands, A brief introduction”,published by the Faroese Government Office, Copenhagen, 1999, and supplemented by Pál Weihe, MD, Depart. of Occupational and Public Health, The Faroese Hospital System, Sigmundargøta 5, P.O. Box 14, 110 Tórshavn, Faroe Islands. The part on contaminants in Faroese subsistence food is mainly based on informations from Maria Dam, Dr. Scient, Food and Environmental Agency, Falkavegur 6, Fo-100 Tórshavn, Faroe Islands.

References

1. Vestergaard T, Zachariassen P. 1987. Føðslukanning 1981–82. Frøðskaparrit 33:5–18.

2. Dam M. Mercury in the Faroe Islands – a review of available data. Fróðskaparrit 52. bók 2004: 85-133.

Choosing the Age for Assessment of Neurotoxicity

Fróði Debes
Psy. D.

Department of Occupational Medicine and Public Health, Faroese Hospital System, Sigmundargøta 5, 100 Tórshavn, Faroe Islands; and Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark

Abstract

Psychometric tests of various mental abilities are widely used to assess possible effects of environmental neurotoxicants. The capability of neuropsychological tests to tap reliable information about cognitive functions in childhood may depend on conditions like the time of emergence of certain intellectual faculties in maturation and development, as well as the ability of the child to establish a stable working cooperation and performance in the test situation for it to demonstrate the best of its ability. Thus, the predictive validity of psychometric tests is known to increase with increasing age in childhood.

The same environment which exposes the fetus and the young child to neurotoxicants may simultaneously provide healthy nutrients during the whole period of the child's development, which could counteract the adverse effects from toxic substances. In addition a host of events and incidences, adverse and beneficial, occurring during development may attenuate the association between cause and effect measured over a long time interval.

Functional deficits or disease outcomes may become apparent only after a substantial latency period, at which time retrospective exposure assessment becomes necessary to reveal possible causal factors. Further, the final effect of neurotoxicant exposures on the development of the individual and his or her attainments in life may be further ameliorated by biological plasticity and mechanisms of compensation as well as the society's level of support, quality of health services and educational remediation. Thus, several factors may influence the time-dependency of manifestations, e.g., of neurotoxicity.

The Faroese birth cohorts have been neuropsychologically tested at various ages in order to try to determine the permanence of the effects associated with prenatal exposure to environmental pollutants via the maternal seafood diet. The timing of these examinations was chosen based in part on experience from developmental lead neurotoxicity. The repeated examinations permit an analysis of the strength of association of some selected neuropsychological tests with measures of neurotoxic exposure as well as with important socioeconomic background variables. Do the effects of exposure show up most clearly at a certain age? This is the question we will try to answer.

Psychometric tests of various mental abilities are widely used to assess possible effects of environmental neurotoxicants on the central nervous system.

The capability of neuropsychological tests to tap reliable information about cognitive functions in childhood may depend on several conditions.

Stability

When we wish to compare test results or the strength of effect from external variables on test results at various ages, the stability and generalizability of the tests has to be considered.

Stability of tests of mental ability is jointly:

1) a monotonically decreasing function of the lenght of time interval between the first and second administration, and
2) an increasing function of the child's age at the first administration
3) Stability cannot become greater than the combination of the reliabilities of the two administrations

An illustration of these principles can be seen in table 1, showing the intercorrelations of Stanford Binet IQs at various ages. Thus the correlation between the global score for the full S-B battery of tests between age 7 and 14 years is .75. For several of the single tests the figure may be lower. This adds to the error of the estimates that we are able to give.

Table 1 Intercorrelations (decimals omitted) of Stanford–Binet IQs at various ages in a group of eighty children.[1]

Age (years)	2½	3	3½	4	4½	5	5½	6	7	8	9	10	11	12	14	15	P.C. I[2]
2½																	.77
3	93																.81
3½	85	82															.83
4	86	86	86														.88
4½	76	78	83	88													.91
5	80	78	83	88	90												.90
5½	68	71	75	80	85	86											.89
6	68	70	77	83	83	87	88										.91
7	66	70	73	78	82	84	84	90									.92
8	59	65	69	74	82	80	78	83	87								.93
9	58	66	66	75	81	79	81	83	88	91							.93
10	59	67	66	72	78	74	77	80	87	91	92						.93
11	57	65	63	71	78	74	78	80	83	90	71	94					.92
12	55	62	62	68	75	70	74	77	77	89	89	92	93				.90
14	49	57	59	65	73	65	68	72	75	83	84	86	86	90			.86
15	48	55	58	62	70	63	65	73	77	84	84	86	86	90	88		.86
17	36	43	47	49	56	49	54	62	62	65	67	69	67	74	71	89	.71

[1]Correlations courtesy of Dr. Robert McCall and the Fels Research Institute.
[2]P.C. I is the first principal component derived from this correlation matrix (see text).

Causes of score instability

Numerous causes of score instability can be listed. Some are formal and technical, while other stem from the daily routines of executing the tests in a field setting, maintaining optimal conditions, and standardized procedures to a more or less degree. While some of these factors may seem to be on the verge of the banal, they are nevertheless very important for achieving reliable results. It is worth to keep in mind that the reliability of tests may often be less in clinical practice and in research field settings, than the figures obtained in test manuals with trained testers under well controlled experimental conditions.

1) Measurment error
    a) Reliability, internal, test-retest, attentiveness, willingness, rapport, motivation, fatigue, emotional state, health, frustration tolerence, self conficence, level of aspiration, anxiety, reaction to failure, failure in instruction and administration, etc.
2) Practice effects
    a) Familiarity w. test taking (most in younger children, from 3 up to 10 IQ points or more).
    b) Practice-tests may improve reliability and stability.
    c) Negligable beyond third grade.
3) Environmental changes
    a) Drastic changes like moving from orphanage to good adoptive home, marked changes in familiy cirumstances like separation, trauma or loosing parents that may affect interests and opportunities for intellecual development
4) Emotional problems
    a) Serious long standing problems and stresses, nervous breakdowns, or recovery from.
    b) Fluctuations from 20 to 40 IQ points are not uncommon.
    c) Inconsistencies like missing easy items, or passing very difficult items. Great discrepancies between subtest scores
5) Remediation of physical deficencies
    a) Poor eysight, hearing loss, endocrine (thyroid) gland dysfunction.
    b) Dramatic improvement.
6) Remediation of scholastic deficiencies
    a) Reading skill, specfic disabilities.
    b) “Late bloomer” (rare, and most in achievement).
7) Individual differencies in rate of maturation
    a) Intrinsic individual differencies in rate of development.
    b) Apparent in physical and mental growth.
    c) Spurts and lags at different periods. Partly genetically determined (closer coincidence in identical than fraternal twins), but mostly a reflection of environmental influences.
    d) The constant aspect of mental growth rate is more genetically determined.
8) Changes in factor composition

a) Change of test items at increasing ages. The same test does not measure exactly the same admixture of abilities at every age level.
b) Reduces age to age correlations.
c) Beyond age 2 most variance in S-B IQ. is attributable to a general factor rising from 60 to 90 pct. by age 10.
d) Different abilities vary in stability from age to age. Higher g-loaded tests are more stable.

9) Scale artifacts

Factors contributing to variance

A child's performance on psychometric or neuropsychological tests is dependent on vast field of different factors and influences each of which may vary over time and contribute to instability of measurement (se figure 1).

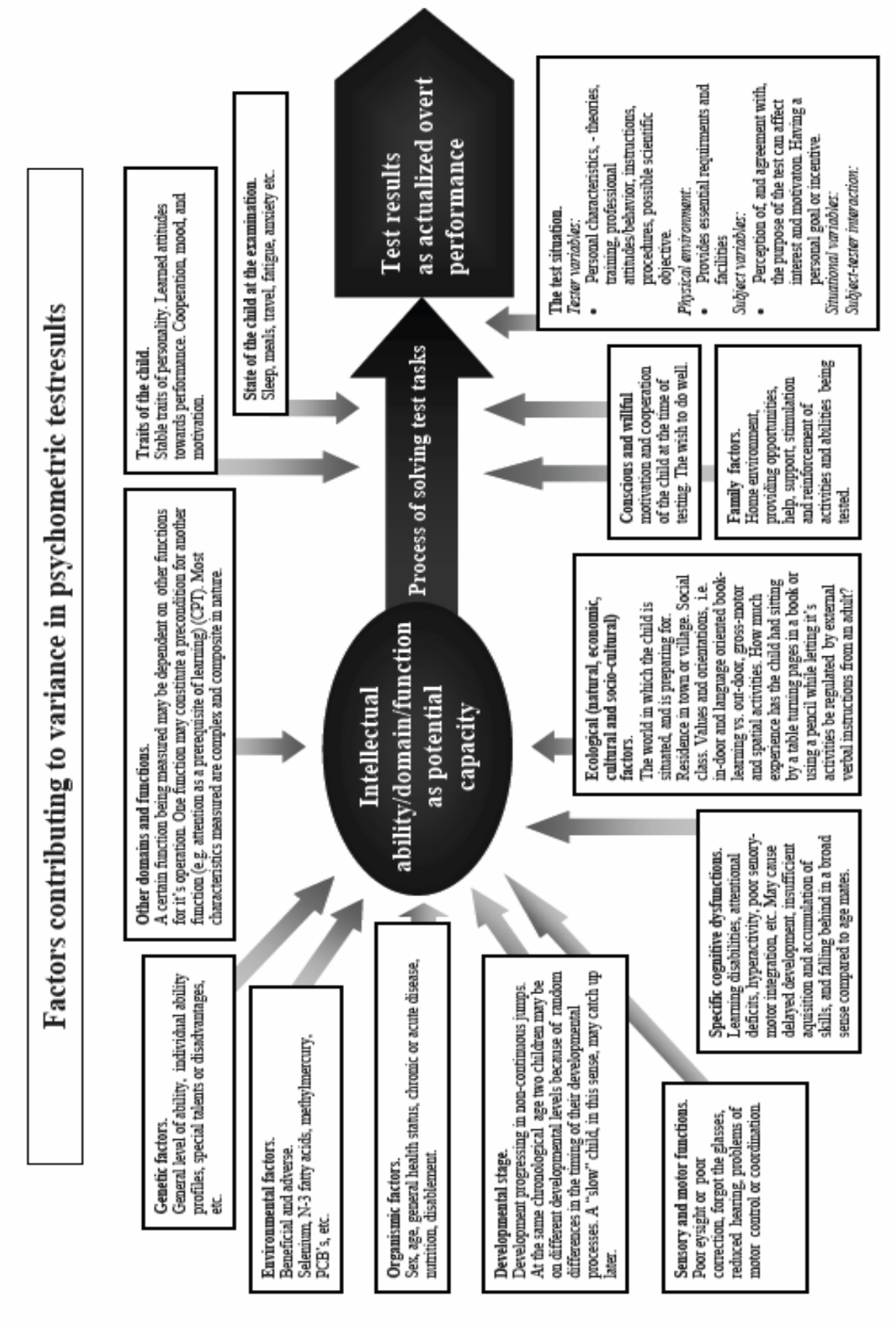
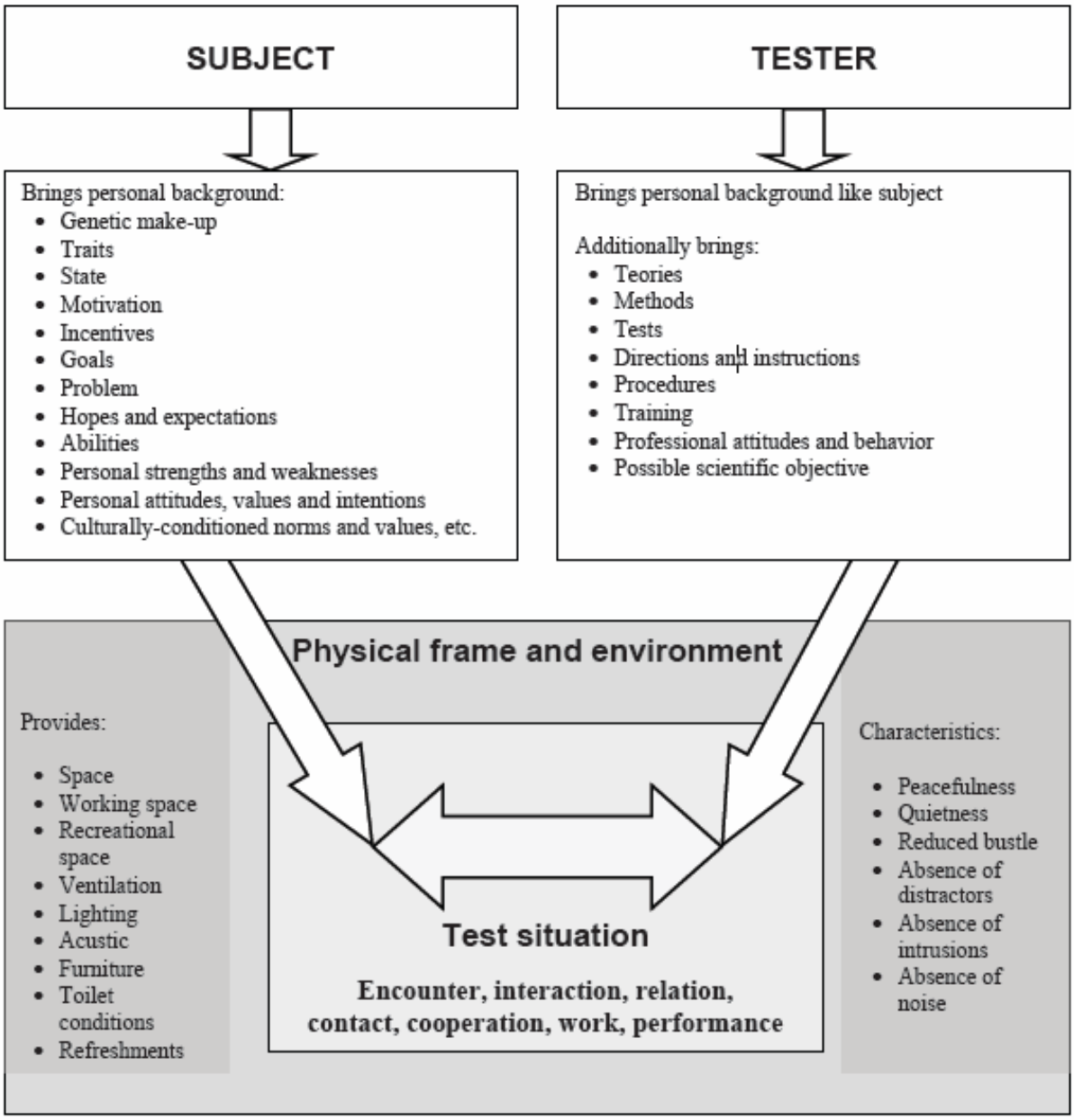


Figure 1. Factors contributing to variance in tests

The test situation

Other causes underlying test stability or instability of tests scores relate to the test situation itself. The test situation can be viewed as a **human encounter** taking place in a **physical environment** where both the subject and the tester bring their **personal background**, and where the tester in addition brings his **professional training** to the scene (se figure 2). The nature of these conditions and interaction of these factors (optimal/non-optimal) all contribute to valid, reliable and stable test results. The development of the ability of the child to establish a stable working cooperation and performance in the test situation is of particular importance.

Figure 2



The time of emergence of certain intellectual faculties in maturation.

Theories of child development have stressed different aspects of the process of intellectual development, some emphasizing the genetic and **biological determination** in the progressive emergence and unfolding of new abilities, while others emphasize the importance of **environmental opportunity, richness and quality of learning experience**.

In the first view development cannot be "speeded up", but has to run it's predetermined course, while in the other environmental or educational programs might be initiated in attempts to enhance the basal level of intellectual competence.

Most theories combine these aspects in various ways, often conceiving ability as largely biologically determined and achievement as additionally dependent on learning experience.

Theories also have varied in the extent that they present development either as an **even, constant and uniform progression** of steadily growing intellectual capabilities, or as a process advancing in **discontinuous leaps** from one qualitatively different **stage** to the next, building up levels of increasing complexity, abstraction, differentiation and cognitive integration. Another view again presents the notion that a **variously timed development of many cognitive microprocessies** may on the surface appear as a progression through broad stages intellectual development.

A classic example of a theory of intellectual development is the well known theory of the Swiss psychologist Jean Piaget. Piaget described the intellectual development of the child as a largely biologically controlled progression through successive stages ranging among others from the sensory-motor stage in the infant, to the preoperational and the concrete operational stages of the young child to the eventual formal logical stage of mental operations achieved in late childhood. On each stage experience and learning is being assimilated and consolidated in a period of time, until the acquired knowledge is eventually reorganized on higher level of perception, understanding and reasoning. Intellectual development is thus seen as an alternation between relatively quiet periods of **assimilation** of experience interrupted by qualitative changes of **accommodation** with a jump to a higher level of intellectual functioning.

It is evident that young children cannot be tested for performance on tasks that require complex and abstract thinking, only revealed by older children and adult. Also sensory and motor functions, which are the only testable functions at a young age, predict later intellectual function rather poorly. Since performance on simpler functions may not predict performance on higher and more complex cognitive functions, characteristic of adult functioning, very well, **researchers therefore has to wait for the emergence in development of advanced functions in order to obtain more**

precise information on how the functioning of the older child or adult may become affected by neurotoxicants.

Also the **timing** at which the individual child enters the transition from one stage to the next may be crucial for a child's ability to solve certain types of tasks.

Differentiation of abilities

Researchers of human intelligence have been interested in the structure of mental abilities as it is demonstrated in inter-individual differences in large populations. After 100 years of scrutiny by various methods of exploratory and confirmatory factor analysis, and in recent years with structural equation modelling, researchers are today in broad agreement that **abilities, viewed in this manner, are hierarchically organized**. All tests of complex mental abilities from diverse areas of functioning show moderate positive correlations with each other. Correlation matrices of such tests show a so called "positive manifold". Abilities in different functional domains do therefore not vary independently and at random between individuals. A hierarchical model allows for a general ability factor at the apex, saturating all kinds of specific mental abilities, and possibly indicating a biological efficiency factor in the nervous system. At intermediate levels such models postulate a limited number of broad ability factors, while a greater number of narrow and specific abilities are postulated at the basic stratum, indicating the lowest level of generality. The theoretical and empirical strength of such models lies in the fact that they allow for both general and specific dimensions of abilities.

An example of a hierarchical model is shown in figure 3. At the basis are a greater number of narrow abilities, tested with specific psychometric tests. The actual tests have some variance in common, thereby defining a number of broad first order group factors. These again share some variance, thereby defining a smaller number of super ordinate second order factors, which again define one common third order general ability factor.

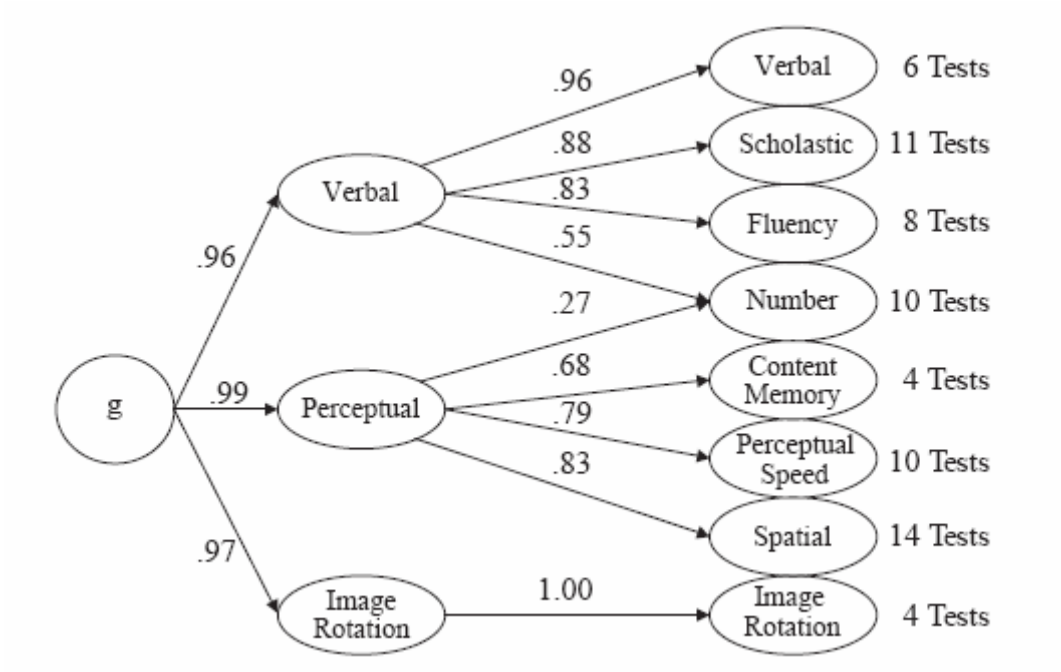


Figure 3. From W. Johnson, T.J. Bouchard (2005). The structure of human intelligence. A SEM model based on previous factor analytic theories, and fitted on a sample of 436 people taking 42 mental ability tests.

From a developmental perspective the hierarchical structure of abilities undergoes a differentiation during maturation in childhood (conversely in old age a dedifferentiation is taking place). When testing children at various ages it may therefore not be exactly the same structure which is being tested. How various predictors may influence specific domains and levels of ability in such a model and perhaps its differentiation during development is another interesting research question. The timing of adverse influence hitting the yet undifferentiated system in young children may be crucial for the effects in later development. This also raises the question of the effect of development and timing of compensatory mechanisms. Lastly there may be a variation in heritability of general and specific abilities.

Genetic and environmental factors

1) Behavioural geneticists have demonstrated that the heritability of the general ability factor is the highest compared to the more specific factors
   a) This implies that the partitioning of the total variance of a test into it's genetic and non-genetic variance is differentially proportioned for various tests
   b) Certain types of environmental influences may therefore be of greater importance for the performance on certain tests than on others
2) Also the relative role of genetic and certain types of environmental influences change during development
   a) Shared familial factors (resulting in between family variance) decrease

b) Genetic factors increase

c) This leaves non-shared familial factors (creating within family variance) as the most important environmental influences by late teen age (se figure 4)

3) While prenatal environmental neurotoxicants may be hypothesized to leave a lasting imprint on the brain, the size of their effect, relative to certain other effects, may be changing during development

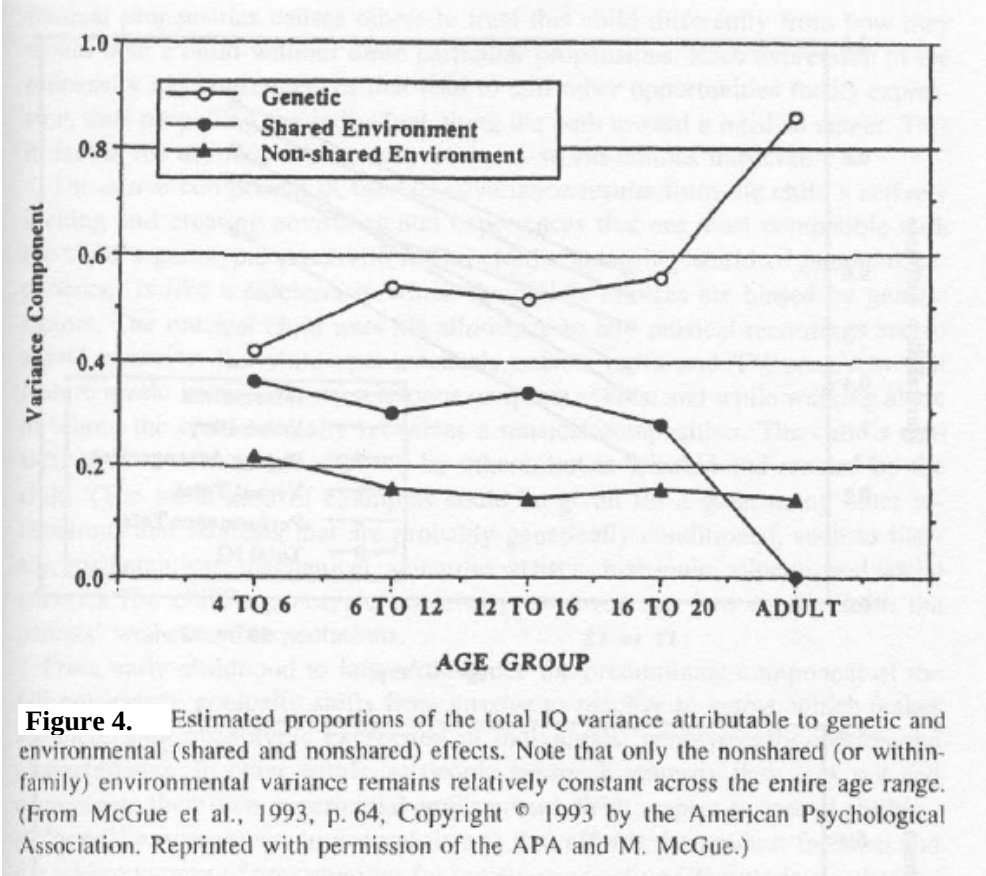
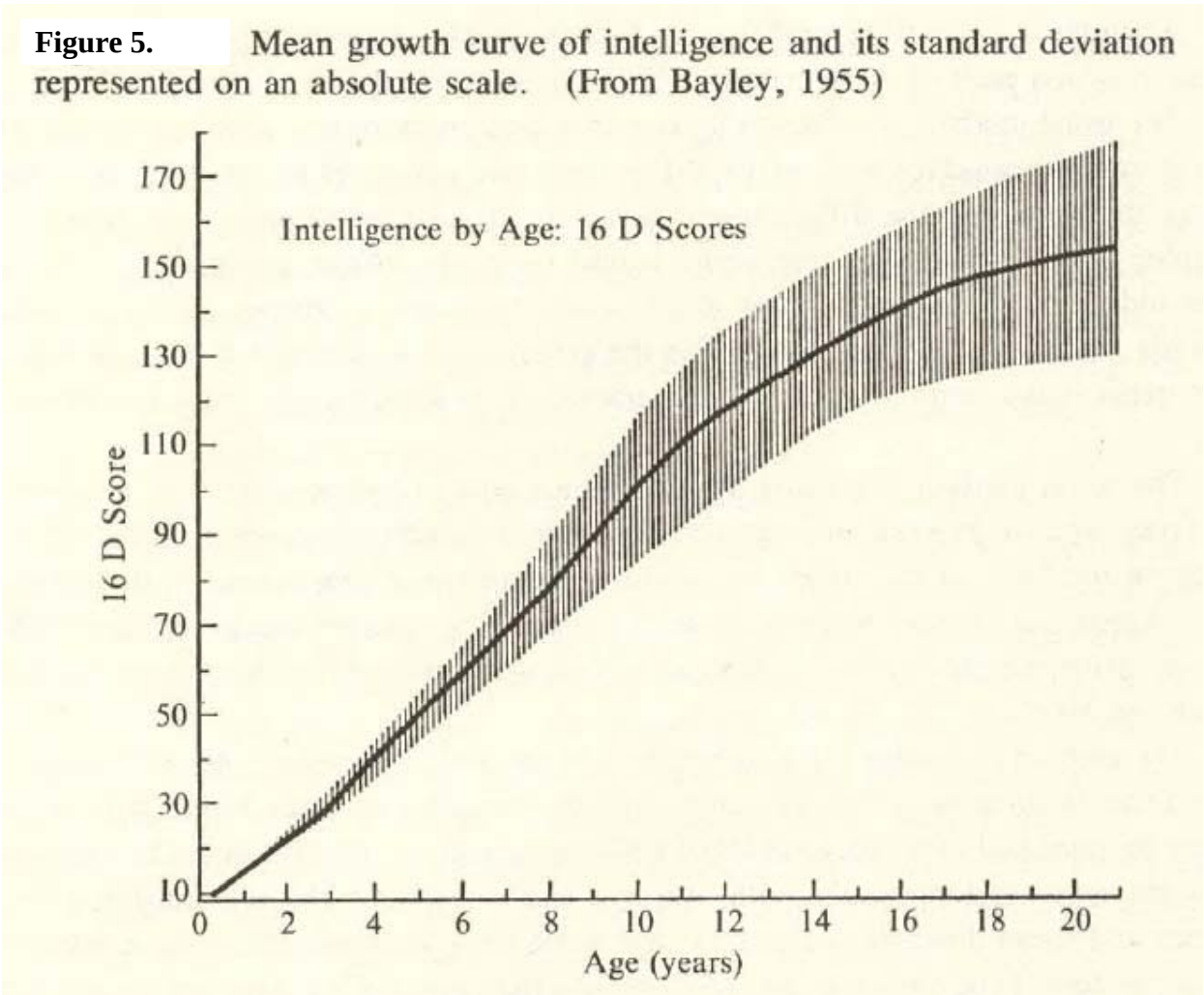


Figure 4. Estimated proportions of the total IQ variance attributable to genetic and environmental (shared and nonshared) effects. Note that only the nonshared (or within-family) environmental variance remains relatively constant across the entire age range. (From McGue et al., 1993, p. 64, Copyright © 1993 by the American Psychological Association. Reprinted with permission of the APA and M. McGue.)

The quantitative nature of mental growth

1) In attempts to construct growth curves of intelligence correllation matrices show simplex features (decreasing correlations at increasing age intevals)
   a) Such a model involves variance in only a single factor that remains constant (maintains constant rank order i each individual across time)
   b) plus random variation (sources are uncorrelated with the constant factor, with each other, or from age to age)
2) The simplex model explains empirical findings as:
   a) Increasing variability among persons as age increases
   b) Greater variability and less age-to-age stability of scores among persons in the upper half of the distribution than in the lower half
   c) Very low correlation between gains in one year and gains in another
   d) Gradual increasing correlations between parents' and children's IQ from infancy to maturity (assuming that the constant factor represents genotypic potential)

Figure 5. Mean growth curve of intelligence and its standard deviation represented on an absolute scale. (From Bayley, 1955)



The constancy of the growth curve is thought to represent mainly gentoypic potential for consolidation of experience.

Positive environmental effects (the Flynn effect)

Positive environmental effects on IQ have been noted over the last century, which may be helpful in understanding certain mechanisms of compensation for other adverse environmental effects, and which may also affect the time stability of test results across populations studied only a decade apart. The strength of the effect may be different in different countries and regions depending on the current living conditions of these regions, thereby adding to the uncertainty of comparison of results across regions.

1) A secular increase in IQ has been observed
    a) Several studies in different countries have shown an intelligence gain during the XX century
    b) The size of the gain is considerable (equivalent to 1 S.D over the last half of the XX century) with greater rates of gain in visuo-spatial than verbal abilities

Two hypotheses have been formulated to explain the so called Flynn effect

2) The cognitive stimulation hypothesis
    a) Predicts an equal gain over the entire continuum of the intelligence distribution.
3) The nutritional and health hypothesis
    a) Predicts a greater gain in the lower and medium halves of the IQ distribution (benefitting the most deprived) caused by improved health, decreased rate of infant disease, and better nutrition

In a study by R. Colom et al. 2005 (se figure 6), gains in IQ are concentrated in the bottom and medium half of the distribution, and gains decrease gradually from the bottom to the top half of the distribution. These findings are consistent with the nutritional hypothesis.

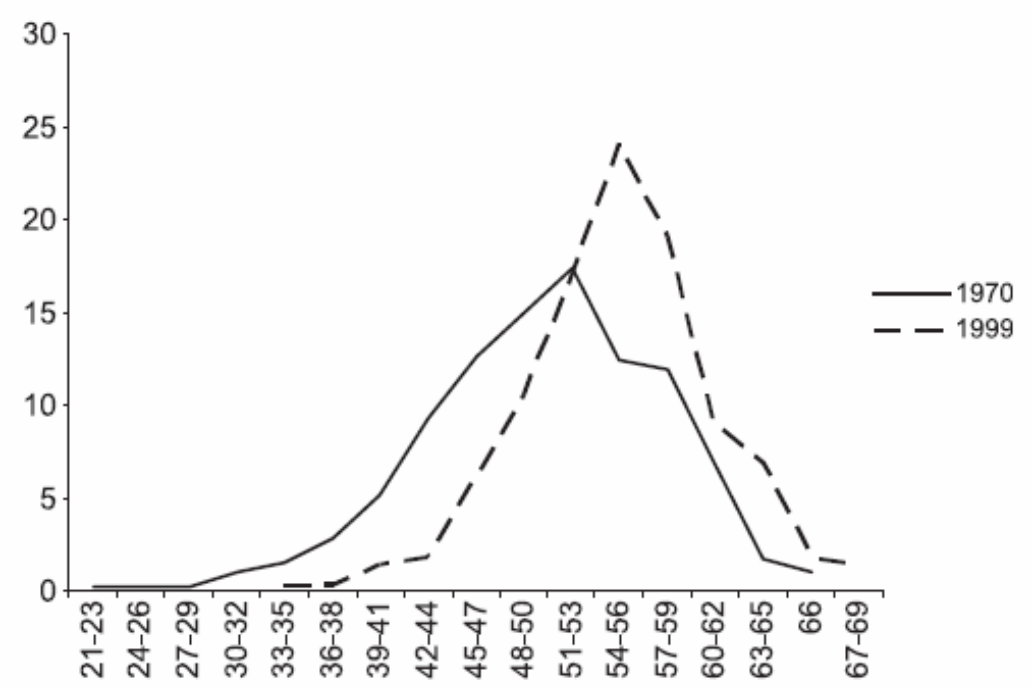


Figure 6. Distribution for the samples tested in 1970 and 1999.

In the Faroes low education and low Raven test score in the mothers is associated with higher levels of mercury in the children. Assuming that the secular increase in intelligence is also taking place in the Faroes, the lower and medium half of the IQ distribution may be simultanously subjected to both adverse and beneficial effects. The secular rise in IQ may be indicative of a "reserve" which may be of importance in ameliorating adverse neurotoxic effects in absolute terms. Also it draws a clearer picture by ascribing particular importance to specific environmental factors in contrast to a theory of diffuse random factors. It seems likely that an even clearer picture of beneficial factors will provide effective means of intervention for at risk individuals in the future. Adverse effects operating most strongly at the lower end of the ability distribution will amount to an increment in the proportion of children falling under critical limits for successful functioning. Beneficial effects operating most strongly at the same end of the ability continuum will have an opposite and much needed effect of raising the level above ctirical thresholds for effective functioning.

Mechanisms of compensation

Several mechanisms of compensation for adverse effects on the brain and the functioneing of the individual may be listed

1) Biological
    a) The ability of the nervous system to compensate for damage and reorganize functional systems of the brain is well known in neuropsychology
    b) The plasticity and reduncant capacity in the brain is greatest in the young child before 2

2) Developmental, psychological
   a) Some children may aquire increasing strategies of compensation, as development progresses and more advanced functions are developed.
3) Environmental
   a) Saigal, et al. 2006 found remarkable successful attainment on markers of transition from childhood to young adulthood (educational attainment, getting a job, living independently) in persons born preterm and with extremely low birth weight (< 1000 gr.) in Canada
   b) Contrary to the authors' hypotheses and much of the literature, they found that a significant majority of the extreme low birth weight participants made a fairly successful transition from adolescence to adulthood
   c) Explanatory factors have been speculated to be:
      i) From advantaged homogeneous population
      ii) Regional intensive care was well-established
      iii) All participants had access to universal health care through provincial-based insurance systems
      iv) Children with disabilities were integrated into regular schools and were provided teaching assistants
      v) Home and workplace accommodations were made by the Canadian society for persons with disabilities
   d) Person-environment interaction
      i) A person's functioning and disability is conceived as a dynamic interaction between health conditions and contextual factors, which include both personal and environmental factors.
      ii) Environmental factors that interact with functioning and disability include the facilitating or hindering effect of features of the physical, social, and attitudinal world
4) The Faroese population shares quite similar socio-demographic characteristics with the Canadian study population, and although adverse effect deriving from neurotoxicants may not be absolutely comparable to risks factors deriving from low birth weight, children exposed prenatally to methyl mercury may enjoy equal opportunities for acquiring skills necessary to obtain an adequate adaptation to adult life
5) As the author's also found in subanalyses, there may still be smaller or more subtle decreases in personal functioning. In a similarily recent study (Naomi Bresalu, In Press) IQ deficit associated with low birth weight (compared to normal birth weight) remained constant over the period of school attendance, and was independent of socioeconomic status and urban vs. sub-urban residence.

The first Faroese cohort at ages 7 and 14 years

Bivariate associations between important predictors and mercury

First, the distribution for age was similar at age 7 and age 14 (7 years: Mean = 6.9; SD .32
14 years: Mean = 13.8; SD .32)

From table 2 it can be seen that age is generally a much stronger predictor at 7 years, influencing both motor speed, attention, language,memory and visuo-constructive function than at 14 years where only a much weeker and less significant association is found with motor speed and attention. No significant association is seen with important cognitive areas like language, learning and memory and visuo-constructive function. The effect of age as a predictor is thus deceasing from age 7 years to 14 years.

The effect of sex is increasing and becomes more differentiated, being roughly the same between the two ages for motor speed, where boys do better. For attention boys do slightly better at age 7 (fewer having slow reactions) and girls do slighty better at age 14 (more boys have slow reactions).
For language there is no significant difference between the sexes at neither age. For memory and visuo-constructive abilities there is no sex difference at age 7, but a significant effect in the girls' favour at age 14. The effect of Sex increases and appears more complex; with changes in the girls' favour.

The mothers' score on the Raven test appears a general, strong and remarkably stable predcitor of the child's performance across age in all domains.

Cord blood mercury seems relatively stable though slightly decreasing in some areas in it's bivariate association with the outcome variables from age 7 to age 14.

The covariates shown in the table are generally not higly correlated, but children of mothers with a high score on the Raven test tend to have had less mercury in their cord blood at birth. Also a slight association is seen between age and mercury at age 14.

Table 2. Bivariate associations between some main outcome variables and predictors, and coorelation between predictors

			Age				Sex (0=G; 1=B)				Raven				Mercury			
Tests	Functional Domain		7 yrs	p	14 yrs	p	7 yrs	p	14 yrs	p	7 yrs	p	14 yrs	p	7 yrs	p	14 yrs	p
NES2, Fingertaps, both hands max	Motor Speed	r	0,205	***	0,090	**	0,199	***	0,193	***	0,074	*	0,106	**	-0,051		-0,065	
NES2, CPT, Reaction Time, mean	Attention	r	-0,291	***	-0,096	**	-0,083	*	0,085	*	-0,118	***	-0,108	**	0,100	**	0,110	**
BNT, Total (uncued + sem. + phon.)	Language	r	0,262	***	0,040		0,048		0,021		0,211	***	0,205	***	-0,174	***	-0,124	***
CVLT, Trial 1-5, Total no. correct	Learn. / Mem.	r	0,187	***	-0,009		-0,030		-0,231	***	0,183	***	0,215	***	-0,087	*	-0,065	
WISC-R, Block Design	Visuo-constr.	r	0,267	***	0,048		0,064		-0,114	***	0,188	***	0,195	***	-0,098	**	-0,015	
Age at 7 years in days		r					-0,021		-0,033		-0,005		-0,030		0,003		0,092	**
Sex		r	0,021		-0,033						0,001		-0,006		-0,048		0,040	
Maternal Raven Score		r	-0,005		-0,030		-0,001		-0,006						-0,129	***	-0,110	**
Log2 Blood-Mercury		r	0,003		0,092	**	0,048		0,040		-0,129	***	-0,110	**				

* p ≤ 0,05; ** p ≤ 0,01; *** p ≤ 0,001

Explanatory value of regression models at two ages.

Table 3. Percent of variance explained by multiple regression models for the outcome variables that showed statistically significant negative associations with cord blood mercury exposure at 14 years of age[a].

	Full model		Mercury		Pct. of total	
Test	7y	14y	7y	14y	7y	14y
NES2, Finger Tapping w. Both Hands	9.6	8.2	0.3	0.6	3.4	7.7
NES2, CPT, Reaction Time, mean	16.2	5.6	3.0	0.6	18.7	11.6
Boston Naming Test, with cues	18.9	10.0	1.4	0.5	7.5	4.7

[a]Results (R^2) are indicated for the full regression model with all covariates, and the partial result for the cord-blood mercury concentration alone, and the latter as percent of the former, at age 7 and 14 years.

Table 4. Additional analysis of two outcome variables not significantly associated with cord blood mercury exposure at 14 years of age

	Basic model		Mercury		Pct. of total	
Test	7y	14y	7y	14y	7y	14y
CVLT, Trial 1-5, Total no. correct	9.2	11.7	0.2	0.1	2.4	0.7
WISC-R, Block Design	15.5	7.3	0.3	0.2	2.0	2.9

From tables 3 and 4 it can be seen that the R^2 in general is higher at age 7 than at age 14. The same predictors explain up to 3 times as much of the variance in the outcome variables at age 7 years as they do at age 14. Of the shown outcome variables, verbal learning and memory is an exception, where the model explains more of the variance at age 14.

Even if less of the variance in several outcome variables can be explained by known predictors at the age of 14, the proportion of the explained variance that is attributable to mercury is over all relatively similar.

At the age of 7 years 8 to 10 outcome variables were significantly decreased with higher exposure to mercury, while only 3 were significantly related to mercury at age 14 (tables not shown). While the effect of mercury thus appeared to be somewhat weaker at 14 years than seven years before, the overall explanatory power of the statistical models was also more limited than it was previously. A multitude of events and incidents, beneficial and adverse, may have occurred during the time between the two examinations and may have attenuated the correlation between two sets of tests and decreased the association with early-life covariates.

For example, differences in time of onset of puberty, together with very high growth rates in this phase of development, may contribute unadjusted variability that overshadows the association with defined predictors, including MeHg exposure. In addition, the covariates and methylmercury exposure might particularly affect the rate at which skills are acquired at the younger age, while innate ability might be a more important predictor of performance at 14 years. Thus, although the neuropsychological performance results at age 14 were more affected by variability that could not be captured by adjustments using known covariates, the relative importance of MeHg exposure was about the same at the two examinations. In addition, analyses that incorporated test results at both examinations showed that the mercury-associated deficits had changed only little between age 7 and 14 years.

When considering the estimated effect size, comparison of regression coefficients with the standard deviations of the test scores is appropriate. Because of the logarithmic transformation of the exposure scale, effects must be expressed in terms of log-scale increases. We found that a doubling of the prenatal MeHg exposure at age 7 years corresponded to a decrease in performance in the range of about 5-10% of the standard deviation. The effect at age 14 years was similar in size (table 7).

Table 5. Mercury effects on five groups of neurobehavioral tests estimated in structural equation analysis with covariate adjustment with and without maternal fish intake during pregnancy.

Test group[a]	No adjustment for fish intake			Adjusted for fish intake		
	Goodness of fit (p[b])	Effect[c]	p	Goodness of fit (p)	Effect	p
Motor	0.062	-7.41	0.034	0.070	-9.37	0.0088
Attention	0.044	-8.40	0.030	0.049	-9.54	0.017
Spatial	0.0015	2.60	0.50	0.0013	1.04	0.80
Verbal	0.13	-5.97	0.080	0.14	-6.87	0.051
Memory	0.30	-2.86	0.40	0.27	-3.05	0.38

[a] Motor (the three NES2 finger tapping scores, CATSYS supination/pronation score, CATSYS maximum finger tapping score), Attention (Digit Spans, Spatial Span, CATSYS mean reaction time, NES2-CPT mean reaction time, number of false responses, and number of missed responses), Spatial (Children's category test, WISC-R Block Design, WISC+WAIS Block Designs, Copying total score, the Copying score for the last five designs, and number correctly recalled), Verbal (the two Boston Naming Test scores, the four California Verbal Learning Test scores, and the Similarities score), and Memory (the four CVLT scores and the Copying recall score);

[b] p-value in likelihood ratio test of the proposed model against the unrestricted model;

[c] Effect of true exposure doubling expressed in % of s.d. of latent response;

The regression coefficients obtained for mercury may be compared to those obtained for age. At age 7 years, a doubling in MeHg exposure corresponded to a loss in development by about 1-2 months. At age 14, age relationships for the neuropsychological tests are less steep, and a doubling in the MeHg exposure therefore corresponded to greater delays in development.

While most tests at age 14 showed a mercury association in the direction predicted, Spatial Span showed an unanticipated positive effect of increased prenatal MeHg exposures. This tendency caused a poor fit of one of the structural equation models. Although a chance finding cannot be excluded, one must also consider the possibility that MeHg exposure may be associated with intake of essential nutrients from seafood, and that, for example, long-chain n-3 fatty acids may have beneficial effects on brain development. Partial adjustment for this factor was provided by adding maternal fish intake during pregnancy as a covariate. This addition changed the mercury regression coefficients in the direction anticipated to better reflect the true adverse effect of methylmercury *per se*. This confounding had previously been considered as an explanation of the weak tendency of decreased (improved) visual evoked potential latencies at higher prenatal MeHg exposure levels. It therefore seems likely that the outcomes of the present study are affected by both contaminant toxicity and beneficial nutrient effects in the opposite direction, although the different seafood components may not affect different brain functions to the same degree. Furthermore, it is apparent that any beneficial nutrients would have compensated only partially for the neurotoxic effects of MeHg in this cohort. However, adjustment for fish intake was based only on maternal recall of the frequency of fish dinners during pregnancy, thus not allowing any precise assessment of essential nutrient intakes. If more precise indicators of nutrient supply had been available, statistical control for their beneficial effects might have revealed an even stronger MeHg effect.

To summarize

Reliable and stable results are more difficult to obtain with young children, and the likelihood of establishing adverse neurotxic effects from neurotoxicants is less.

By school age (7 years) most children are mature enough to cooperate adeqautely in a test situation.

The explanantory value of a regression model of predictors has decreased by age 14.

A multitude of events – adverse, beneficial, random and compensatory - ocurring between the two administrations may attenuate the relationship with predictors.

Also, some environmental predictors (shared factors) are known to decrease with age.

Despite these limitations at age 14, it has been possible to demonstrate multi-focal and permanent negative effects of mercury on brain function also at this age.

Joint Analysis of the Two Faroese Cohorts Assessing the Effect of Prenatal Mercury Exposure in 7 Year Old Children

Esben Budtz-Jørgensen[1], Pál Weihe[2] and Philippe Grandjean[3,4]

[1] Department of Biostatistics, Institute of Public Health, University of Copenhagen, Øster Farimagsgade 5B, 1014 København K, DK-2200 Copenhagen, Denmark

[2] Department of Occupational and Environmental Health, The Faroese Hospital System, Sigmundargota 5, PO Box 14, FO 100 Tórshavn, Faroe Islands

[3] Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Winslowparken 17, DK-5000 Odense, Denmark

[4] Department of Environmental Health, Harvard School of Public Health, Landmark 3-110E, 401 Park Drive, Boston, MA 02215 USA

Two birth cohorts have been formed in the Faroe Islands to examine the long-term effects of prenatal exposure to methylmercury, while taking into account confounders, including concomitant exposure to PCB. The first cohort was the largest by far, but had limited data on PCB exposure, while the second, smaller cohort included PCB analysis of maternal pregnancy serum and milk.

Information from two cohort studies was therefore combined in a structural equation analysis to achieve a more precise estimate of the effects of prenatal exposures to mercury and PCB. Cohort 1 consisted of 1022 children, recruited between 1986 and 1987, while the smaller Cohort 2 was recruited at birth over a 12-month period between 1994 and 1995. The cohorts were very similar in regard to average exposure levels and maternal seafood diets that included frequent consumption of fish, and occasional consumption of whale meat and blubber. In both cohorts, the prenatal mercury exposure was assessed based on mercury concentrations in maternal hair and cord blood. In Cohort 1, PCB in cord tissue was measured in half of the children, while the smaller Cohort 2 provided more detailed PCB measurements from almost all children.

At approximately 7 years, both sets of children underwent a detailed neurobehavioral examination. The joined data was analyzed using structural equation models. This approach is superior to standard regression techniques, when estimating the effects of predictors with measurement error. Thus, we allowed for imprecision in mercury concentrations, and an error component was also included for the cord tissue PCB concentration. In this way, the estimated mercury effect was adjusted for a PCB effect estimate based mainly on the more precise exposure measure of the smaller cohort. This analysis showed that mercury effects in the two cohorts were in proper agreement with one another. Although adjustment for PCB tended to decrease mercury effects, for some outcomes the mercury effect remained significant. When the information was pooled across outcomes, adverse effect where estimated for both mercury and PCB, but only the former effect was statistically significant.

Joint analysis of the two Faroese cohorts assessing the effect of prenatal mercury exposure in 7 year old children

Esben Budtz-Jørgensen,[1] Pal Weihe,[2] Philippe Grandjean[3,4]

[1]University of Copenhagen, Denmark
[2]The Faroese Hospital System, Torshavn, Faroe Islands
[3]University of Southern Denmark, Denmark
[4]Harvard School of Public Health, USA

Japan, February 2006

Outline

- Main results from the Faroese Cohort 1 analysis
- Statistical concerns
  - Measurement error in exposures variables
  - Measurement error in confounding factors
- The Faroese Cohort 2
- Structural equation models
- Joint analysis of cohort 1 and 2 data

The Faroese Cohort 1

EXPOSURE:
1. Cord Blood Mercury
2. Maternal Hair Mercury
3. Maternal Seafood Intake

RESPONSE:
Neuropsychological Tests

Age:	Birth	7 Years
Calendar:	1986-87	1993-94
Children:	1022	917

Multiple Regression Results: effect of 10-fold increase in exposure

	Cord Blood		Maternal Hair	
Response	β	p	β	p
NES2 Finger tapping				
Preferred hand	−1.10	0.05	−1.21	0.04
Non preferred hand	−0.39	0.46	−0.88	0.12
Both hands	−1.67	0.14	−2.69	0.02
NES2 Hand-Eye Coordination				
Error score	0.03	0.19	0.05	0.07
NES2 Continuous Performance Test				
Ln total missed	0.27	0.02	0.14	0.24
Reaction time	40.28	<0.001	22.68	<0.001
Wechsler Intelligence Scale				
Digit Spans	−0.27	0.05	−0.21	0.15
Similarities	−0.04	0.90	−0.27	0.53
Sqrt. Block Designs	−0.17	0.11	−0.11	0.32
Bender Visual Gestalt Test				
Errors on copying	0.67	0.15	0.64	0.21
Reproduction	−0.25	0.10	−0.07	0.68
Boston Naming Test				
No cues	−1.77	<0.001	−1.26	0.02
With cues	−1.91	<0.001	−1.36	0.01
California Verbal Learning Test				
Learning	−1.25	0.12	−1.15	0.18
Short-term repro.	−0.57	0.02	−0.51	0.05
Long-term repro.	−0.55	0.05	−0.49	0.09
Recognition	−0.29	0.15	−0.22	0.32

Statistical concerns

- Measurement error in prenatal mercury exposure
  mercury effect likely underestimated
- Adjustment for multiple comparisons?
- Limited adjustment for effects of PCB exposure
  - PCB measured in only half of the children
  - PCB measured in cord tissue

Consequences of confounder error

General case: Y: response, X: exposure, Z: confounder

True relationship: $Y \approx \alpha + \beta_x \cdot X + \beta_z \cdot Z$

Confounder variable has measurement error: $U = Z + \epsilon$

Naive analysis: replace Z by U

$$\tilde{\beta}_x \to \beta_x + \beta_z \cdot [cov(X,Z)/var(X)] \cdot [var(\epsilon)/var(U|X)]$$

Special case: X=true log(Hg), Z=true log(PCB)

$\beta_z < 0$ and $cov(X,Z) > 0$

Therefore: $\tilde{\beta}_x \to \tilde{\beta}_x < \beta_x$

Failure to adjust for measurement error in PCB concentration introduces bias in estimated mercury effect. Adverse mercury effect may have been overestimated.

The Faroese Cohort 2

Exposure:	Outcome:	Outcome:	Outcome:	Outcome:
Blood Hg Hair Hg Serum PCB	test scores	test scores	test scores	test scores

Age: Birth 3.5 years 4.5 years 5.5 years 7.5 years

Number of children: 182

Structure of the joint data

Cohort 1		Cohort 2	
Mercury	PCB	Mercury	PCB
blood/hair (N=900)	cord tissue (N=400)	blood/hair (N=170)	serum (N=170)

11 neuropsychological outcomes measured in *both* cohorts

Aim: to determine - with the greatest possible precision - the effect of prenatal mercury exposure.

In a joint analysis, cohort 2 contributes to the total sample size but also provides a superior assessment of PCB exposure.

Structural equation models

Consist of two parts

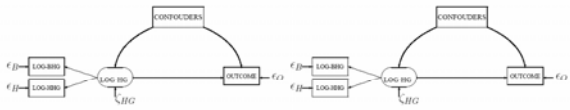
The measurement part:

Observed variables considered manifestations of a limited number of underlying (latent) variables. Obtained through *factor analytic models*.

The structural part:

Latent variables related to each other and to observed covariates. Obtained through *multiple linear regression models*.

Cohort 1 Cohort 2

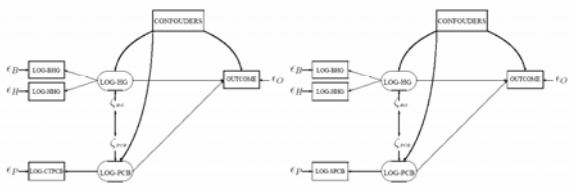


Effects of mercury exposure in two cohorts

	Cohort 1		Cohort 2		Test for	Cohort 1 & 2	
Outcome	β	95%-conf.	β	95%-conf.	equality	β	p
Boston Naming Test							
No cues	-2.06	-3.12; -0.99	-1.89	-4.32; 0.53	0.91	-2.03	<0.001
Cues	-2.18	-3.23; -1.12	-1.88	-4.26; 0.49	0.82	-2.13	<0.001
Wechsler Int. Scale							
Similarities	0.087	-0.75; 0.93	-0.568	-1.71; 0.57	0.37	-0.143	0.68
California Verb. Learn.							
Learning	-1.30	-3.10; 0.502	1.12	-3.15; 5.39	0.31	-0.935	0.27
Short-term repro.	-0.573	-1.10; -0.04	-0.241	-1.62; 1.14	0.66	-0.530	0.036
Long-term repro.	-0.613	-1.21; -0.02	-0.011	-1.31 ; 1.29	0.41	-0.509	0.066
Recognition	-0.234	-0.68; 0.21	0.765	-0.27; 1.80	0.083	-0.083	0.69

Estimated effect of 10 fold increase in prenatal exposure to mercury

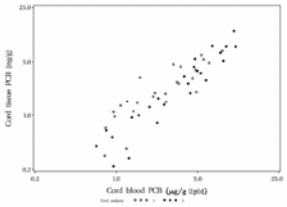
Cohort 1 Cohort 2



$var(\epsilon_P) = ?$ $var(\epsilon_P) = 0$

Imprecision in cord tissue measurement

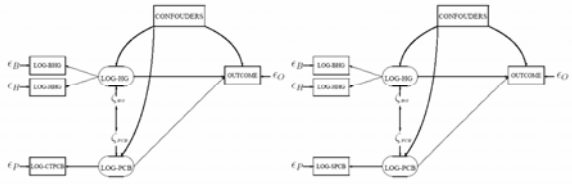
50 children in cohort 1 have PCB measurements in cord tissue *and* cord blood.



Model: $\log(\text{CT-PCB}) = \alpha + \beta \log(\text{CB-PCB}) + \epsilon_P$

Estimate: $var(\epsilon_P) = 0.03$ - meaning: $C.V.[\text{CT-PCB}] \approx 40\%$

Cohort 1 Cohort 2



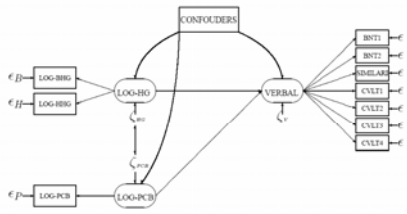
$var(\epsilon_P) = 0.03$ $var(\epsilon_P) = 0$

Effects of mercury and PCB exposure

		Mercury			PCB	
Outcome	β	$s.e.(\beta)$	p	β	$s.e.(\beta)$	p
Boston Naming Test						
No cues	-1.399	0.750	0.062	-1.250	1.00	0.21
Cues	-1.622	0.737	0.028	-1.053	0.977	0.28
Wechsler Int. Scale						
Similarities	-0.147	0.495	0.77	0.219	0.593	0.71
California Verb. Learn.						
Learning	-0.947	1.255	0.45	-0.015	1.636	0.99
Short-term repro.	-0.926	0.38	0.015	0.840	0.493	0.088
Long-term repro.	-0.347	0.401	0.39	-0.311	0.515	0.55
Recognition	-0.159	0.313	0.61	0.064	0.419	0.88

Estimated effect of 10 fold increase in prenatal exposure to mercury and PCB

Latent verbal outcome function



Fitted in both cohorts - effect parameters constrained to be equal

Effects of mercury and PCB on verbal function

		Mercury			PCB	
Outcome	β	$s.e.(\beta)$	p	β	$s.e.(\beta)$	p
Verbal function	-1.37	0.635	0.032	-0.581	0.885	0.51

Estimated effect of 10 fold increase in prenatal exposure to mercury and PCB

Conclusions - methodology

- Measurement error in predictor variables causes bias
- Structural equation models allow for
  - error in exposures
  - error in confounders
  - multiple comparisons

Conclusions - biology

- Mercury effects in two cohorts consistent
- Mercury effect remained significant after PCB adjustment
- PCB effect negative - but less certain

Structural equation models

$z_i = (z_{i1}, \ldots, z_{iq})^t$: covariates of subject i.
$y_i = (y_{i1}, \ldots, y_{ip})^t$: responses of subject i.

The measurement part:

$$y_i = \nu + \Lambda \eta_i + \epsilon_i$$

$\epsilon_i \sim N(0, \Omega)$

The structural part:

$$\eta_i = \alpha + B\eta_i + \Gamma z_i + \zeta_i$$

$\zeta_i \sim N(0, \Psi)$

Extensions: Multiple group analysis

$z_i = (z_{i1}, \ldots, z_{iq})^t$: covariates of subject i.
$y_i = (y_{i1}, \ldots, y_{ip})^t$: responses of subject i.

Parameters may depend on a group variable $g = 1, ..., G$

The measurement part:

$$y_i = \nu^g + \Lambda^g \eta_i + \epsilon_i$$

$\epsilon_i \sim N(0, \Omega^g)$

The structural part:

$$\eta_i = \alpha^g + B^g \eta_i + \Gamma^g z_i + \zeta_i$$

$\zeta_i \sim N(0, \Psi^g)$

Selenium as a Potential Protective Factor against Mercury Developmental Neurotoxicity

Anna L. Choi[1], Esben Budtz-Jørgensen[2], Poul J. Jørgensen[3], Pál Weihe[4] and Philippe Grandjean[1,5]

[1] Department of Environmental Health, Harvard School of Public Health, Landmark 3-110E, 401 Park Drive, Boston, MA 02215 USA

[2] Department of Biostatistics, Institute of Public Health, University of Copenhagen, Øster Farimagsgade 5B, 1014 Copenhagen K, DK-2200 Copenhagen, Denmark

[3] Institute of Clinical Research, Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense, Denmark

[4] Department of Occupational and Environmental Health, The Faroese Hospital System, Sigmundargota 5, PO Box 14, FO-100 Tórshavn, Faroe Islands

[5] Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Winslowparken 17, DK-5000 Odense, Denmark

Experimental studies have found that selenium (Se) may decrease mercury (Hg) toxicity. However, little is known about the potential protective effect of Se against Hg neurotoxicity in humans. We assessed the potential interaction between Hg and Se in a birth cohort. Singleton term infants were recruited at birth during a 12-month period between 1994 and 1995 in the Faroe Islands, where meals included frequent consumption of whale meat and fish. Hg and Se were measured in the 182 cord whole blood samples. Neurodevelopmental outcomes of the children were evaluated at 2 weeks, 6 months, 18 months, 30 months, 42 months, 54 months, and 66 months of age. We modeled each outcome as a function of Hg-Se exposure (with adjustments for potential predictors) and considered their potential interactions in several ways: 1) Hg-Se ratio (both expressed in nmol/L); 2) Hg exposure by low and high Hg-Se ratio (defined as below and above the median ratio); and 3) Hg exposure by low and high Se levels (defined as below and above the median Se level). Cord blood Hg levels had a geometric mean of 20.7 (range 1.90 – 101.8 μg/L). Cord blood Se levels ranged from 77.1 to 157.5 μg/L, with a geometric mean of 102.6 μg/L. The correlation between Hg and Se was 0.29 (p=0.0005). Overall, we found no evidence that Se was a significant protective factor against Hg neurotoxicity even though it was present in cord blood in an average molar excess of about 10-fold above Hg. Because Hg may readily cross the placenta and the fetal brain is regarded as particularly sensitive to neurotoxicant exposures, efforts to reduce mercury levels in contaminated seafood are deemed important.

Selenium as a Potential Protective Factor Against Mercury Developmental Neurotoxicity

Anna Choi[1], Esben Budtz-Jørgensen[2], Poul J. Jørgensen[3], Pál Weihe[4], Philippe Grandjean[1,5]

NIMD Forum 2006

[1]Harvard School of Public Health, [2]University of Copenhagen, [3]Odense University Hospital, [4]Faroese Hospital System, [5]University of Southern Denmark

Methylmercury

- Found in seafood and freshwater fish
- A neurotoxicant that can have adverse effects on the developing nervous system

Chronology of methylmercury toxicity

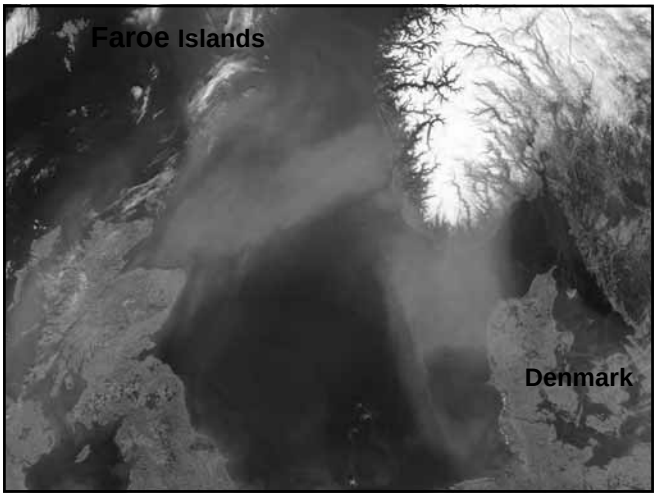
- 1940s: Occupational disease
- 1950/1960s: Minamata disease
- 1970s: new poisoning incidents
- 1990s: insidious toxicity
- Now: time for prevention

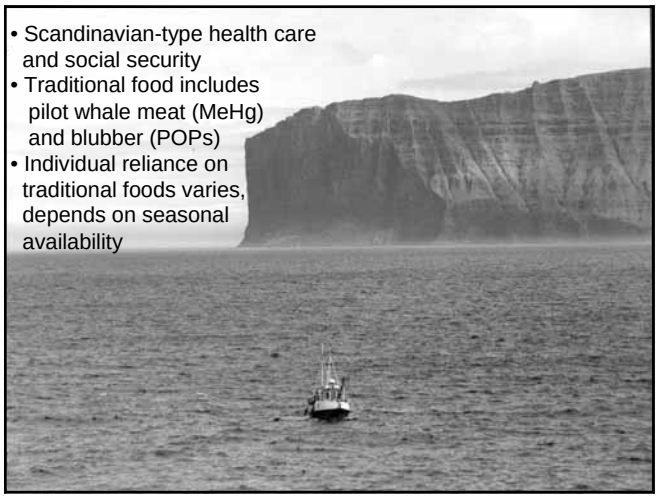
Selenium

- Found in seafood, plant food and meats
- A trace mineral essential to good health
- A constituent of selenoproteins:
  - An antioxidant
  - A catalyst for production of active thyroid hormones

Methylmercury-Selenium Interaction

- Se may decrease MeHg toxicity
  - In rats (Parizek et al, 1967)
  - In quails given MeHg in tuna diet (Ganther et al 1972)
  - With vitamin E to reduce MeHg in rats (Beyrouty and Chan, 2005)
- Little is known in humans





Why MeHg research in the Faroes?

- Exposure to MeHg from pilot whale meat is like a natural experiment - highest level 1000x the lowest
- Exposure only weakly associated with confounders
- Homogeneous, western culture
- High participation rate (88% at 14 yrs)

Faroese cohort studies

- Cohort 1 (N= 1022) born 1986-1987
- Cohort 2 (N = 182) born 1994-1995
- Cohort 3 (N = 547) born 1998-2000

Second birth cohort

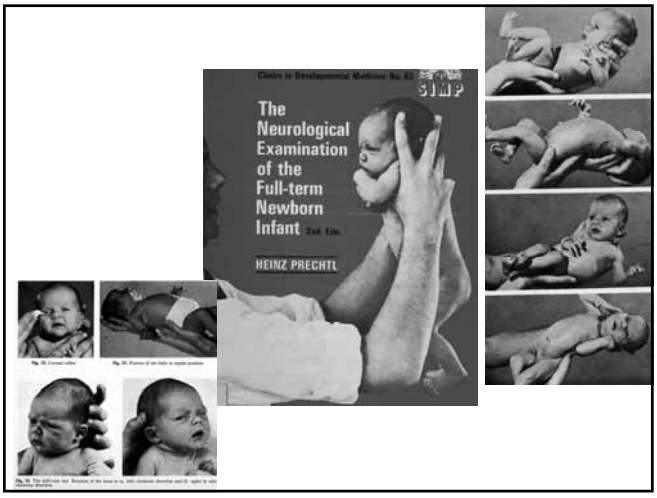
- Formed in 1994-1995 (N = 182)
- Data on early development and nutrition questionnaire were available
- Neurodevelopmental assessments were evaluated at several occasions

Considerations on assessing developmental neurotoxicity

- Neurotoxicity may not be immediately apparent
- Nervous system must mature to express relevant functions
- Test validity and appropriateness

Neurodevelopmental Outcomes

Age of child	*Assessments*
2 weeks	NOS
6 months	MDI, PDI
18 months	NOS, MDI, PDI
30 months	MDI, PDI, Copying, Bead Memory
42 months	NOS, MDI, PDI, Copying, Bead Memory, Boston Naming Test (BNT)
54 months	Copying, Bead Memory, Block Design, BNT
66 months	Copying, Bead memory, Block Design, Digit Span, BNT, California Verbal Learning Test



Exposure Biomarkers

- Cord blood Hg and Se levels
- Hg (µg/l, nmol/l)
- Se (µg/l, nmol/l)

Assessing Hg and Se Interactions

Outcome = f (Hg,Se, Hg*Se, covariates[a])

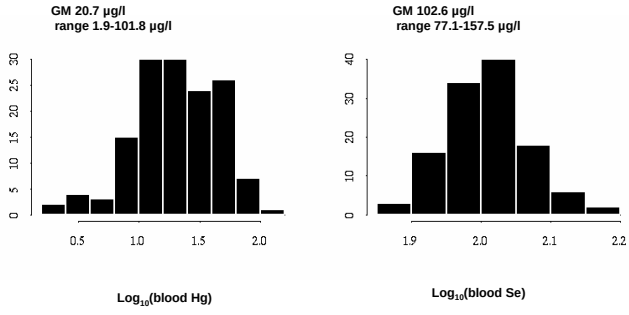
Hg*Se considerations:

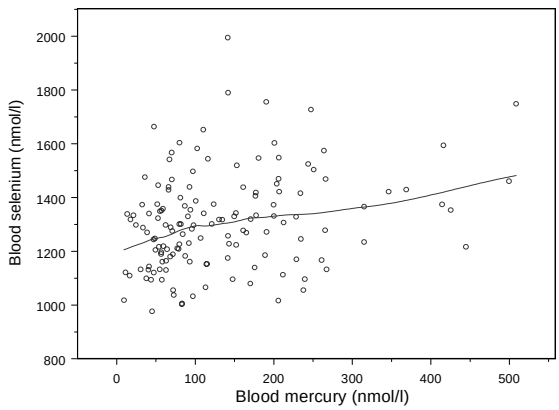
- Hg/Se ratio (nmol/l)
- Hg by low and high Hg/Se ratio (cutoff at median ratio)
- Hg by low and high Se levels (cutoff at median of Se)

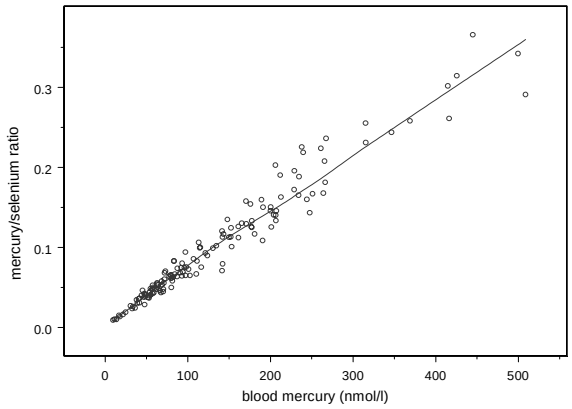
[a]potential confounders (p<0.10 with outcome)

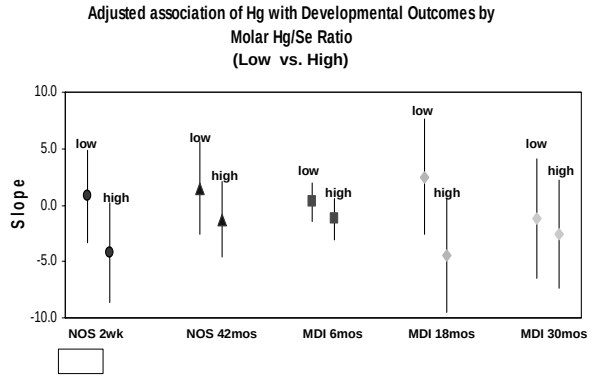
Characteristics of cohort

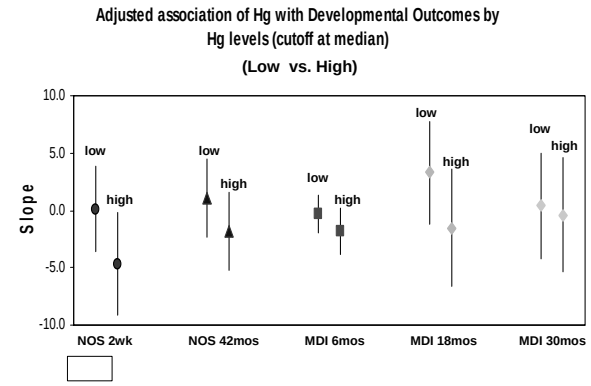
Maternal age (years)	28.0 ± 5.8
Previous births (0/1/≥2 [%])	29.7/29.1/41.2
Non-smoking during pregnancy	68.7%
No alcohol during pregnancy	87.4%
Fish meals/wk(0/1/2/≥3[%])	1.1/19.1/29.2/50.6
Whale meat meals/mo(0/1/ ≥2 [%])	39.9/25.3/34.8
Birth weight (g)	3672 ± 48.3
Gestational age (wks)	39.6 ± 1.2
Sex of child (boy/girl [%])	51.1/48.9











Conclusion

- On the average, Se was present in cord blood in a molar excess of about 10-fold above Hg
- Se concentrations suggested all children were Se sufficient
- No evidence that Se was a significant protective factor against MeHg neurotoxicity
- Preventive methods are needed to address MeHg exposures, rather than Se intakes

Work in Progress

- To incorporate evaluations at 7 years in Cohort 1 (N=1,022) for the assessment of the potential interaction between Hg and Se
- Possible paradigms of grouping the various outcomes to model the interactions

CHEF: Child Health and the Environment in the Faroes

Website: www.chef-project.dk

Lessons from an Animal Experiment of Combined Exposure to Methylmercury and PCBs on Neurobehavioral Influences in Mice

Norio Sugawara*, Kunihiko Nakai, Takashi Ohba, Tomoyuki Nakamura, Keita Suzuki, Satomi Kameo, Chieko Satoh, Hiroshi Satoh

Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, Japan

Abstract

Methylmercury (MeHg) and polychlorinated biphenyls (PCBs) are environmental pollutants that cause neurobehavioral deficits in human. Because the exposures to MeHg and PCBs occur through fish-eating, it is necessary to clarify the interaction of two pollutants. Therefore, we investigated the effects of perinatal exposure to MeHg and PCBs in mice. Female mice (C57BL/6Cr) were divided into four groups by the exposures : 1) vehicle control, 2) MeHg alone, 3) PCBs alone, 4) MeHg + PCBs. The MeHg exposed groups fed diet containing 5 ppm MeHg (as Hg) beginning 4 weeks prior to mating, and through pregnancy and lactation. The PCBs exposed groups received a commercial mixture of PCBs known as Aroclor 1254 at a dose of 18mg/kg body weight in 10 ml/kg corn oil by gavage every 3days from 5days after breeding and continued through PND20. Before weaning, assessment of eye opening revealed the interactive effect between MeHg and PCBs on PND12. We also observed the delays of grasp reflex on PND12 and 14. When the offspring were at 8 weeks olds, the group exposed to PCBs alone showed increasing number of defication and urination in an open field test. Analysis of the latency revealed the interaction between two exposures. Treatment with MeHg decreased the distance and interacted with PCBs exposure. Water maze test shows that treatment with MeHg prolonged the time to reach the platform, but this effect did not interact with PCBs exposure. Spontaneous locomotion activity was not affected by exposure at 9 weeks olds. These results showed that perinatal co-exposure to MeHg and PCBs does not produce additive or synergistic effects seen in previous in vivo studies. This phenomenon needs to be carefully considered.

Key words: methylmercury; PCBs; Aroclor 1254; neurobehavioral effect; open field test; perinatal exposure

Introduction

Methylmercury (MeHg) is a environmental pollutant that is widely distributed in the global ecosystem. Mercury exists originally in the nature, and is methylated in the environment, whether the origin is natural or anthropogenic. Thus formed, MeHg enters the aquatic food chain to become the predominant dietary source of mercury in humans. The highest levels of MeHg are

* Address correspondence to: Norio Sugawara
Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine
2-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
E-mail: nsuga@ehs.med.tohoku.ac.jp
Fax: +81-22-717-8106

found in predatory fish and sea mammals (Satoh, 2000).

The neurotoxicity of high levels of MeHg was well documented from the severe epidemic in the small Japanese fishing comunity, Minamata (Watanabe and Satoh, 1996). Since the developing fetal brain is highly susceptible to MeHg, severe neurotoxicity had been observed in the children whose mothers did not show clinically evident symptoms of MeHg poisoning (Harada, 1978).

In the 1990s, a new understanding has emerged regarding the adverse neurodevelopmental effects of MeHg. Studies on environmental exposure to MeHg demonstrated the effects of neurological and cognitive development in fish-eating population. In the Faroe Islands, children whose mothers were exposed to MeHg originated from pilot whale showed the deficits of language, attention, and memory, along with a trend for visuospatial and motor dysfunctions (Grandjean et al., 1997). At the age of 7, children perinatally exposed to MeHg showed a deficit of attention in the continuous performance test, and the deficit of motor function tests in the finger tapping tests (Grandjean et al., 1997). Neurophysiological tests also showed significant MeHg-associated delays of the latency of the auditory brain stem evoked potentials (Murata et al., 1999) In contrast to the Faroe Islands, children in the Seychelles Islands prenatally exposed to dietary MeHg from ocean fish did not show any neurological deficits associated with prenatal mercury exposure (Myers et al., 1997; Davidson et al., 1998).

The differences between two studies include the age of children at the time of testing, differences in the test batteries, genetic/ethnic differences, and potential differences in timing, magnitude, and duration of MeHg exposure. But, analysis of the umbilical cord in the Faroe Islands found PCB-associated deficits (Grandjean et al., 2001).

Because fish contains significant amounts of PCBs, co-exposure to MeHg and PCBs could occur through fish consumption. PCBs have also been recognized to be potent neurotoxicants when children prenatally exposed, and to cause a delay in neurodevelopment. In neonates, prenatal PCB exposure was associated with poorer performance on habituation and aoutonomic clusters of the neonatal behavioral assessment scale (Stewart et al., 2000). At 4 years of age, children prenatally exposed to PCBs showed poorer scores in the McCarthy scales that measure verbal and numerical memory (Jacobson et al., 1990). In addition, lower IQ scores were associated with prenatal PCB exposure (Jacobson et al., 1996) at 11 years old.

Children in the Faroe Islands were exposed to MeHg and PCBs. However the investigator failed to find detectable exposure to PCBs in the children's blood in the Seychelles (Davidson et al., 1998). Thus, it has been speculated that the effects in the Faroe Islands could be due to the additive or synergistic effects of MeHg and PCBs.

In vitro laboratory studies also support the hypothesis that the two chemicals may have additive or synergistic effects on nervous system function. In the striatal tissue punches from the rat brain, exposure to MeHg alone reduced tissue dopamine (DA) and increased media DA in a dose-dependent fashion. In addition, co-exposure to MeHg and PCBs resulted in greater effects on DA (Bemis and Seegal., 1999). The same group also found that low concentrations of MeHg and PCBs synergistically increased intracellular calcium concentrations, but intracellular calcium concentration reduced by higher concentrations or longer exposure times (Bemis and Seegal., 2000).

Several in vivo studies were performed to examined the effects of co-exposure to MeHg and PCBs during perinatal periods. Tanimura et al. (1980) firstly reported the effects of co-exposure. They found that mice exposed to both MeHg and PCBs during development showed impairments on cliff avoidance, visual placement, and hind-limb support tasks before weaning. In the open field test, the number of square traversed was increased in the MeHg-only exposed group on PND 21 and at 10 weeks, while the PCBs-only exposed group and the co-exposure to MeHg and PCBs showed decreased the number at 10 weeks old. Learning impairments were also observed in the PCB-exposed groups and MeHg plus PCBs group on the water-filled T-maze. Afterward, Roegge et al. (2004) reported a motor deficit on the rotating rod task in rats exposed to MeHg and PCBs with marginally significant changes of Purkinje cell height in the cerebellum (Roegge et al., in press). Widholm et al. (2004) also reported that co-exposure to MeHg and PCBs synergistically reduced the latencies in non-cued alteration task.

In this study, we investigated the neurobehavioral effects of co-exposure to MeHg and PCBs during perinatal periods. For the assessment of physical and neurobehavioral development, we observed some developmental landmarks and reflexes before weaning. Afterwards, we carried out an open field test, a water maze test, and a spontaneous locomotion activities to evaluate the effects of co-exposure to MeHg and PCBs.

Materials and methods

Animals and exposure

Male and female C57BL/6Cr mice were purchased from commercial breeder (Nippon SLC Co., Ltd., Hamamatsu, Japan). Upon arrival at the laboratory, the mice were housed individually in plastic cages (22 cm x 15 cm x 13 cm). They were kept in a temperature-controlled room (23 ± 2 °C) with a 12-h light-dark cycle (light phase, 0800-2000). Pellet food (Crea Japan Inc., Tokyo, Japan) and tap water were available ad libitum. Mating began 28 days after arrival in which each female was paired with an unexposed male mouse. The same male-female pairs were housed together for five days.

Mice were assigned to one of four exposure groups: 1) vehicle control, 2) MeHg alone, 3) PCBs

alone, 4) MeHg + PCBs (Fig. 1). The MeHg exposed groups fed diet containing 5 ppm MeHg (as Hg) beginning 4 weeks prior to breeding, and through pregnancy and lactation. The MeHg dose has been chosen considering our previous results indicating a clear neurobehavioural alteration following a chronic exposure to mice (data not shown). The PCBs exposed groups received a commercial mixture of PCBs known as Aroclor 1254 (AccuStandard, Inc., New Haven, USA, Lot# 124- 191) at a dose of 18mg/kg body weight in 10 ml/kg corn oil (Nacalai tesque, Inc., Kyoto, Japan) by gavage every 3days from 5days after breeding and continued through PND20. The doses have been chosen considering our previous results indicating a clear neurobehavioural alteration following a perinatal administration of PCBs (Sugawara et al., in press).

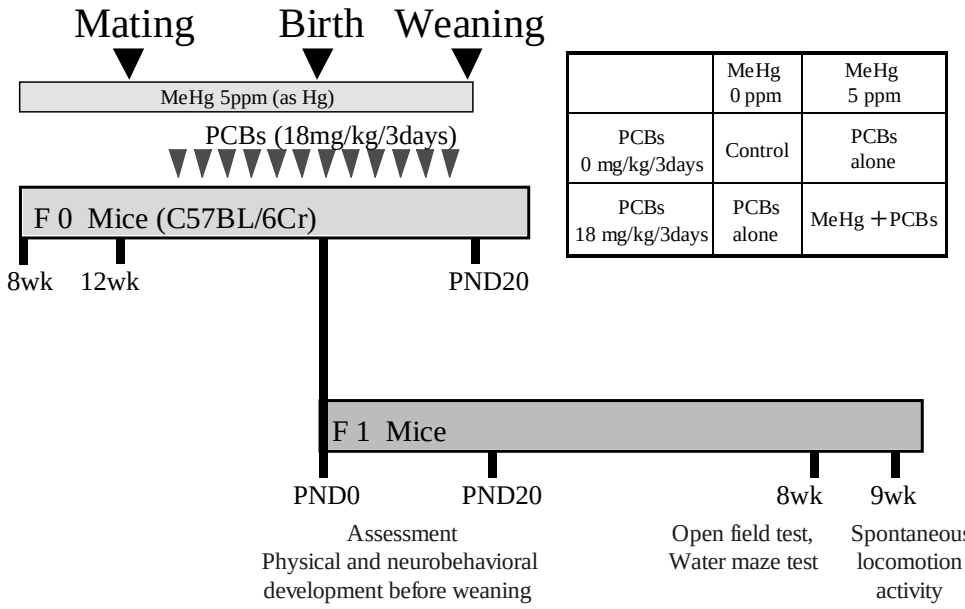


Fig.1 Experimental protocol

The day when the birth of offspring was observed in the morning was defined as PND0. On PND1, the litter size was adjusted to 5-6 and the all mice of both sexes were assigned to the assessment of development before weaning. The offspring were weaned at PND21. All the mothers were sacrificed, and number of implantation sites was counted. After weaning, we used only male mice to exclude the influence of the estrous cycle. Two or three males from each litter of each treatment group were used for three tests after 8 weeks old. All the animals were handled by the same experienced person so that they would become used to being handled. This study was carried out in accordance with the Guide of Animal Experimentation of Tohoku University Graduate School of Medicine.

Assessment of development before weaning

Before weaning, physical and neurobehavioral development was observed on PNDs4, 7, 10, 12, 14, and 16 for the mice of both sexes. For the assessment of physical development, pinna

detachment, hair growth, day of eye opening and incisor eruption were also recorded: pinna detachment (the pinna of both ears is completely detached); hair growth (hair growth of trunk is evaluated.); eye opening (eye opening present bilaterally); incisor eruption (the appearance of incisor eruption is evaluated by observer). For the assessment of neurobehavioral development, pups were tested according to the method as described by Fox (1965). The tests were conducted during the light phase between 1400 and 1900, each subject being tested at approximately the same time of the day. The following reflexes and responses were examined: grasp reflex [a pup holds itself from a steel wire (diameter 1.0 mm) with its forepaws]; righting reflex [a pup is capable of rapidly returning to its four feet when placed on its back]; walking [a pup moves using its four feet with the abdomen not touching the ground]; negative geotaxis [when a pup is placed on a board inclined at 45° with the head pointing downward, it has to turn around 180°]; cliff avoidance [a pup turns and crawls away from the edge of a cliff when placed beyond it with the forepaws].

Open-field test protocol

An open-field test was carried out when the male offspring were 8 weeks old as described by Kim et al. (2000). The apparatus was a square wooden device (50 x 50 x 33 cm) painted white. During the experiment, the field was illuminated at 800 lx with fluorescent light 1.5 m above the apparatus. Each mouse was transferred from the home cage directly to the center of the open field, and a small box made of opaque plexiglas was then placed over the mouse. After the box was removed, the movement of the animal was recorded for 2 min using a CCD camera. The movement was then analyzed using an image analyzer (AXIS 60 video-tracking system, Neuroscience Inc., Tokyo, Japan); the latency in the start of walking, distance travelled by the mouse, and mean walking speed were calculated over a period of 2 min. The numbers of incidences of defecation and urination in the open field were counted by the observer. Before each trial, the floor was cleaned with 70% ethanol followed by wiping with wet cotton to avoid possible bias due to odor clues left by previous subjects. To minimize possible effects of circadian changes on open-field behavior, all trials were carried out between 1000 and 1200.

Water maze test protocol

Several days later after the open-field test, the offspring were subjected to the water maze test as described by Kim et al. (2000). The water maze was consisted of a circular plastic pool with 100-cm-diameter. The pool was filled with tap water adjusted to approximately 20 °C and made opaque by adding milk to prevent the mice from seeing a submerged platform and to increase the animal-background contrast for digital analysis. A round, 10-cm-diameter platform was placed 0.5 cm beneath the water surface at the center of one quardant of the pool. Different visual cues were placed around the pool. Each mouse was released into the water at a fixed position, from the inside of the pool wall. A CCD camera, mounted at the center above the pool, recorded the movements of each mouse. The movements were then analyzed using an image analyzer; the time

taken and distance travelled by the mouse to reach the submerged platform, and the mean swimming speed were calculated. The mice were subjected to three trials on each of five days. When the mouse could not find the platform within 2 min, the test was terminated and the mouse was placed on the platform and left there for 20 sec. In this case, the time necessary to reach the platform was considered to be 2 min.

Count of spontaneous locomotion activity

Over a period of 24h, the spontaneous locomotion activity of offspring was counted in a separate plastic cage similar to the home cage. Each cage was placed in a small isolated chamber, which was kept at 23 ± 2 °C and illuminated at 150-200 lx with a small fluorescent light with a 12 h-light-dark cycle (light phase, 0800-2000). Each mouse was left in a cage for at least 12 h before the recording commenced. Each cage was placed 15 cm below an infrared sensor (Model NS-AS01, Neuroscience Inc.) connected to a host computer, and spontaneous locomotion activity data were collected at 1-min intervals using an analysis system (AB system, Neuroscience Inc.).

Statistical analysis

Data are presented as mean ± S.E.M. Two-way ANOVA (MeHg x PCBs) was performed to determine statistically significant differences in reproductive outcome, body weight, open-field test, water maze test, spontaneous locomotion activity data. With regard to data on physical and neurobehavioral development, the complete appearance of a somatic feature and adultlike responses were evaluated by the logistic regression for each time point. A value of $p<0.05$ was considered significant. The software package JMP 5.0.1 (SAS Institute, Inc., North Carolina, USA) was used to analyze the data.

Results

Reproductive outcomes

Number of implantation site, male surviving offspring on PND1 and gestational weight gain was not affected by treatment. Nevertheless, the MeHg main effect was significant for reduced litter size on PND1 ($F[1,38] = 8.27$, $P < 0.01$).

Physical and neurobehavioral development before weaning

Pinna detachment, hair growth and incisor eruption were not affected by treatments. Analysis of eye opening (Fig. 2) revealed the interaction between MeHg and PCBs on PND12 ($\chi 2[3] = 7.04$, $P < 0.01$). Grasp reflex (Fig. 3) was affected by MeHg exposure on PND12, 14 ($\chi 2[3] = 26.28$, $P < 0.01$; $\chi 2 [3] = 12.22$, $P < 0.01$, for each trial day). Development of negative geotaxis tended to delay by MeHg on PND7 ($\chi 2 [3] = 3.47$, $P < 0.10$). All the other responses (e.g., righting reflex and walking) were not affected.

The body weight of male offspring at 8 weeks old was altered by treatment (MeHg main effect $F[1,47] = 47.46$, $P < 0.01$; PCBs main effect $F[1,47] = 9.01$, $P < 0.01$; MeHg x PCBs interaction $F[1,38] = 5.61$, $P < 0.05$).

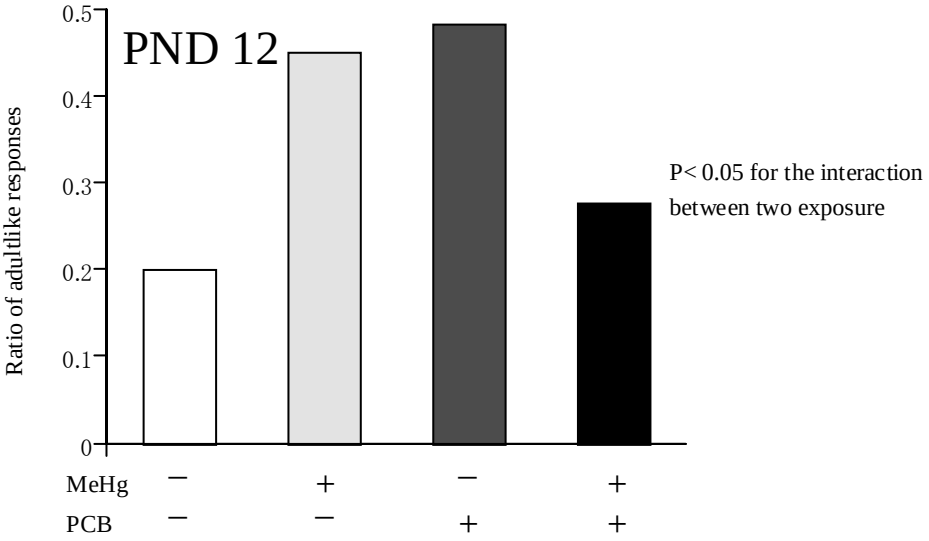


Fig.2 Assessment of eye opening on PND 12

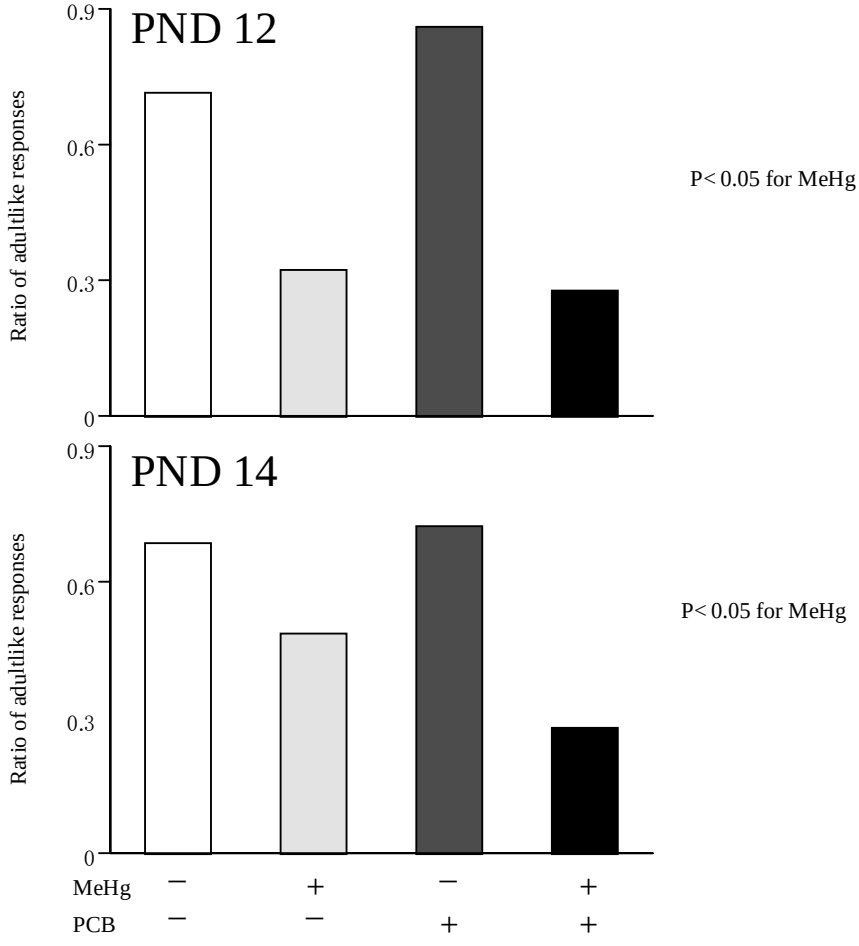


Fig.2 Assessment of grasp on PND 12,14

Open-field test

The results of the open-field test are shown in Fig. 4. The PCBs exposure group showed increasing number of defication ($F[1,48] = 4.73$, $P < 0.05$) and urination ($F[1,48] = 7.60$, $P < 0.01$). Analysis of the latency revealed the interaction between MeHg and PCBs (MeHg x PCBs interaction $F[1,48] = 4.07$, $P < 0.05$). Treatment with MeHg decreased the distance (MeHg main effect $F[1,48] = 5.63$, $P < 0.05$) and interacted with PCBs exposure (MeHg x PCBs interaction $F[1,48] = 6.33$, $P < 0.05$). No other effects on all the other parameters were observed.

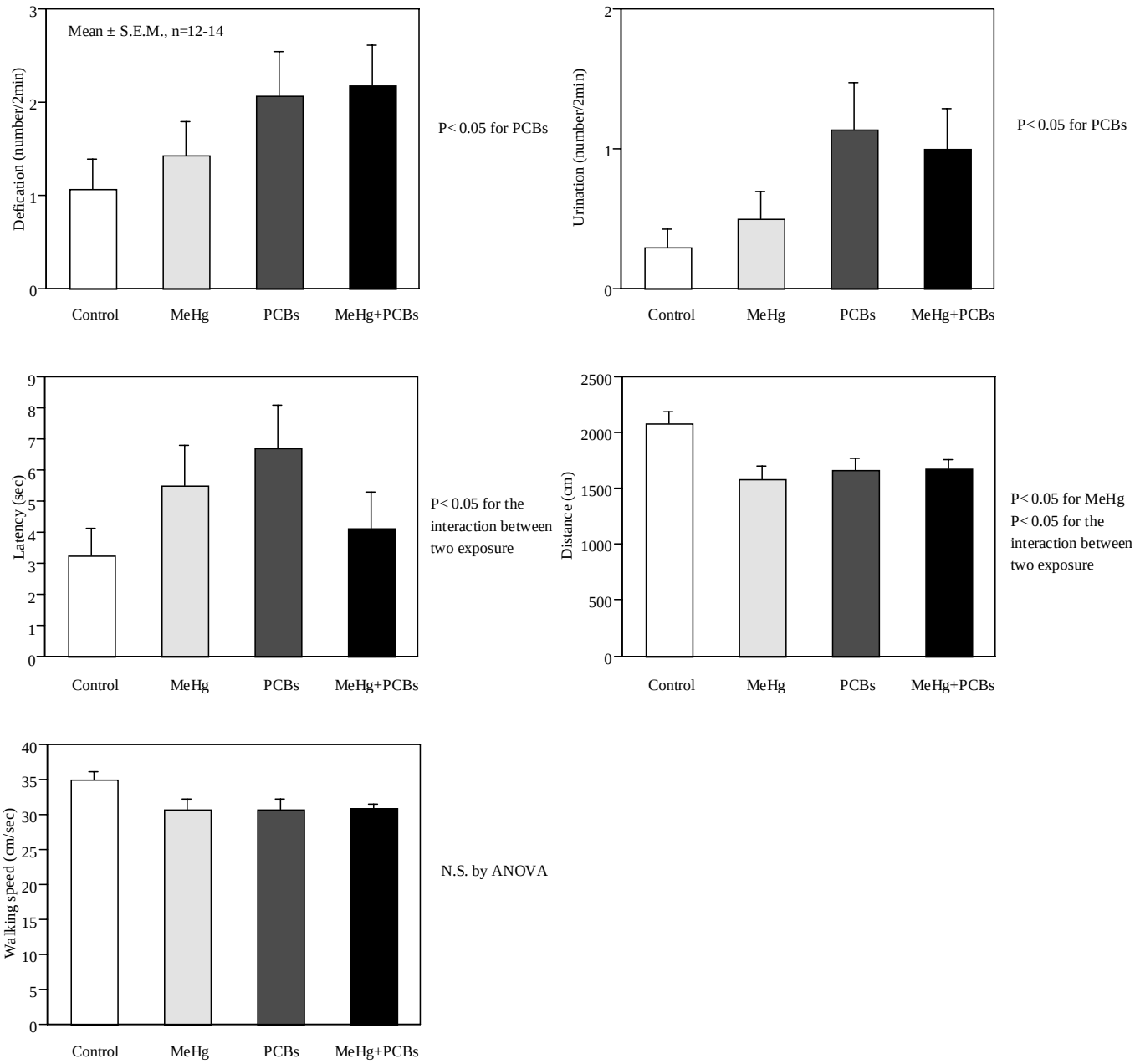


Fig.4 Open field test

Water maze test

Figure 5 shows the results of the water maze test on the fifth trial day. Swimming speed calculated from time and distance data was obtained in this test. Treatment with MeHg prolonged the time to reach the platform (MeHg main effect $F[1,48] = 4.64$, $P < 0.05$), but this effect did not interact with PCBs exposure . Treatment did not affect swimming speed. These results indicate that swimming speed is not directly related to time required to reach the platform.

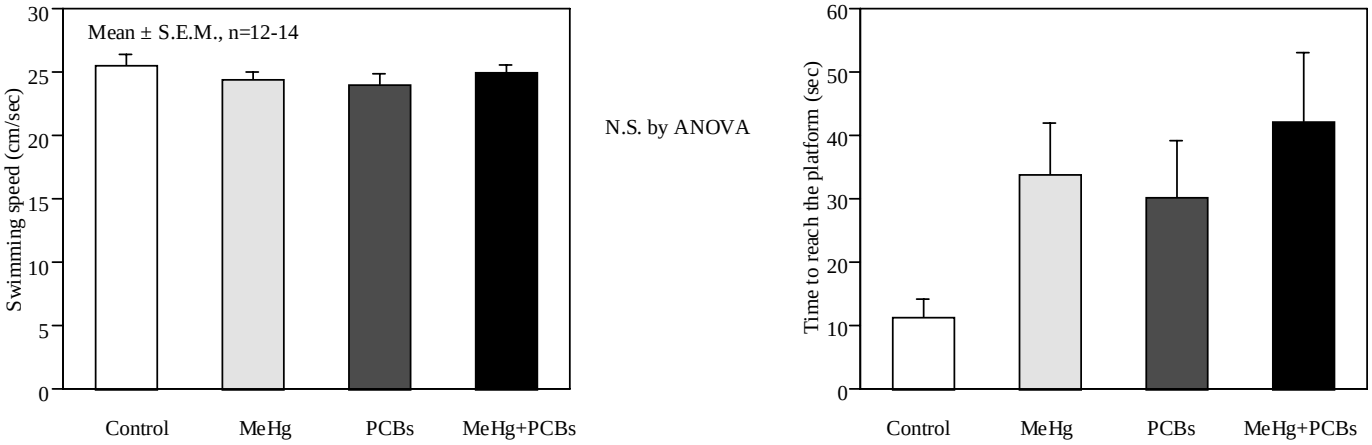


Fig.5 Water maze test

Spontaneous locomotion activity

Spontaneous locomotion activity was measured over a period of 24 h, and 6 h. The total number of movements was not affected by exposure. In either time domain, which was obtained by dividing 24 h by 6 h, the number of movements was not affected by the treatment.

Discussion

In the present study, we investigated the effects of co-exposure to MeHg and PCBs in mice. We used an open field test, a water maze test, and a spontaneous locomotion activity to evaluate the neurobehavioral effects of co-exposure to mice on 8 and 9 weeks olds. Previous study reported the effects of co-exposure in an open field test (Tanimura et al., 1980). However, mice were treated with higher dose MeHg (0.4, 4 mg/kg body weght) and PCBs (500 ppm in diet). We used the lower doses in MeHg (5 ppm as Hg in diet) and PCBs exposure (18mg/kg/3 days). Japanese Ministry of Health, Labour and Welfare announcesd the concentration of mercury in blue-fin tuna (mean 1.15 ppm, min 0.39- max 6.1).

About PCBs exposure level, coho and chinook salmon from Michigan lake (Manchester-Neesvig et al., 2001) showed the concentration of PCBs (1.5 mg/kg). The body burdenn of PCBs from the contaminated fish can only be estimated. Since Japanese was supplied about 200g seafood per day

(from Japanese Fisheries Agency), the body burden was almost the same in human (as 50kg body weight). The doses used in this study would likely also result in exposure that is higher than that observed in environmentally exposed human populations. However, as mentioned previously, the doses of MeHg and PCBs were lower than those used in previous study which investigated the neurobehavioral effects of co-exposure. This study can provide valuable information about the effect of co-exposure to MeHg and PCBs.

The litter size on PND1 was reduced by MeHg exposure. But, number of male offspring was not altered by treatment. In one previous study, perinatal exposure to MeHg (2.5ppm in diet) showed the reduced survival ratio on PND5/PND0 (Verschuuren et al., 1976). However, such an effects was not reported in another study of perinatal exposure to MeHg 6 ppm in drinking water (Newland et al., 1999). The difference in the findings of these studies might be due to the difference in the method or timing of exposure. The reduction of litter size on PND1 may be due to abortion or cannibalism of female offspring. But, male offspring which were applied to behavioral tests after 8 weeks old were not affected by biological selection.

The developmental effects of exposure to PCBs during gestation and lactation were assessed before weaning. On PND12, we observed interactive effect between two pollutants in eye opening. Previous reports showed the accelerated day of eye opening by MeHg-only exposure (Tachibana., 1989) and PCBs-only exposure (Goldey et al., 1995). Therefore, we considered that antagonistic effect exsit between two chemicals. In addition, the mice exposed to MeHg showed poorer adultlike responses in the grasp reflex on PND 12 and 14. There is no previous report to assess the grasp reflex of mice exposed to MeHg. However, many reports to investigate the motor function showed that perinatal exposure to MeHg delayed the development support our results.

In an open field test, we observed the interaction between two exposures in latency and distance. Previous reports showed prolonged latencies in MeHg-only and PCB-only exposure (Spyker et al., 1972; Sugawara et al. in press). But we showed that MeHg and PCBs antagonistically reduced latency. This antagonistic result resemble the report of Bemis and Seegal(2000), which investigated calcium concentration. But, they used 2,2'-dichlorobiphenyl as PCB congener. This result could not explain our data, where mixture of PCBs was used. Moreover, our antagonistic result of latency in an open field test doesn't necessarily mean reduction of toxicity in co-exposure to MeHg and PCBs. Shortened latency may reflect the excitability. This result should be interpreted cautiously.

In addition, we observed that MeHg-only exposure and MeHg and PCBs co-exposure reduced the distance traveled in an open field test. But, synergistic effects were not observed between two substances. It seems that co-exposure with PCBs doesn't necessarily worsen the effects of

MeHg-only exposure. There is a possibility that distance traveled in an open field test reflects the spontaneous locomotion activity. But, there is no significant difference in spontaneous locomotion activity evaluated in the home cage among groups. We considered that the result in the open field test is independent of spontaneous activity, and caused by emotional stress.

Spatial learning ability was examined in the water maze test. MeHg-only exposure prolonged the time to reach the platform as shown in Fig 5. Many factors can affect the time in this test, and swimming ability is one possible factor. However, because swimming speed has not been influenced, it is considered that a disturbance of spatial memory is a cause of the delay. Widholm et al. (2004) reported that co-exposure to MeHg and PCBs reduced the latency in incorrect trials of non-cued alteration task. But, spatial alteration performance was not impaired by treatment in the same task. They also reported that co-exposure did not impair the learning and memory in delayed spatial alteration task. This report supports our result that effects of co-exposure on spatial learning were not observed in water maze test. Moreover, it is thought that effects in latency reported by Widholm et al. shows emotional effects rather than spatial alteration performance.

There are a few studies to investigate the mechanism of effects in co-exposure to MeHg and PCBs. Bemis and Seegal (1999) reported that synergistic reduction of tissue dopamine (DA) in a dose-dependent fashion. DA is an important neurotransmitter for many cognitive processes including memory and attention. In vivo studies also showed that treatment with MeHg-only (Faro et al., 1997) and PCBs-only (Seegal., 2002) release DA. But, there is not in vivo study about co-exposure to MeHg and PCBs on DA release. In addition, changes of DA could not explain our antagonisitic effects of co-exposure to MeHg and PCBs.

In conclusion, we found that co-exposure to MeHg and PCBs during perinatal period caused the antagonistic effects in an assessment of development and an open field test. It seems that co-exposure with PCBs doesn't necessarily worsen the effects of MeHg-only exposure. Moreover, our antagonistic result of latency in an open field test doesn’t necessarily mean reduction of toxicity in co-exposure to MeHg and PCBs. These results should be carefully considered. The mechanism of antagonistic effects in co-exposure should be investigated.

Acknowledgments

The authors thank Mrs. Makiko Sakamoto and Ms. Yuka Susukida for excellent technical assistance. This work was supported by a grant from the Environmental Restoration and Conservation Agency (ERCA), and Grant-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology.

References

Bemis JC, Seegal RF. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro. Environ. Health Perspect. 1999 ; 107 : 879-885.

Bemis JC, Seegal RF. Polychlorinated biphenyls and methylmercury alter intracellular calcium concentrations in rat cerebellar granule cells. Neurotoxicology 2000 ; 21 : 1123-1134.

Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, Berlin M, Clarkson TW. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment. JAMA. 1998 ; 280 : 701-707.

Faro LR, Duran R, do Nascimento JL, Alfonso M, Picanco-Diniz CW. Effects of methyl mercury on the in vivo release of dopamine and its acidic metabolites DOPAC and HVA from striatum of rats. 1997 Ecotoxicol. Environ. Safety. 38, 95-8.

Fox WM. Reflex-ontogeny and behavioural development of the mouse. Anim. Behav. 1965 ; 13 : 234-40.

Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing. Toxicol. Appl. Pharmacol. 1995 ; 135 : 77-88.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PJ. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 1997 ; 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-Jorgensen E, Keiding N, White RF Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol. Teratol. 2001 ; 23 : 305-17.

Harada M. Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology 1978 ; 18 : 285-8.

Jacobson JL, Jacobson SW, Humphrey HE. Effect of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J.Pediatr. 1990 ; 116 : 38-45.

Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. New Engl. J. Med. 1996 ; 335 : 783-789.

Kim CY, Nakai K, Kasanuma Y, Satoh H. Comparison of neurobehavioral changes in three inbred strains of mice prenatally exposed to methylmercury. Neurotoxicol. Teratol. 2000 ; 22 : 397-403.

Manchester-Neesvig JB, Valters K, Sonzogni WC. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. Environ. Sci. Technol. 2001 ; 35 : 1072-7.

Morse DC, Plug A, Wesseling W, van den Berg KJ, Brouwer A. Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol. Appl. Pharmacol. 1996 ; 139 : 252-61.

Myers GJ, Davidson PW, Shamlaye CF, Axtell CD, Cernichiari E, Choisy O, Choi A, Cox C, Clarkson TW. Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study. Neurotoxicology 1997 ; 18 : 819-29.

Nakai K, Satoh H. Developmental Neurotoxicity Following Prenatal Exposures to Methylmercury and PCBs in Humans from Epidemiological Studies. Tohoku J. Exp. Med. 2002 ; 196 : 89-98.

Newland MC, Reile PA. Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. Toxicol. Sci. 1999 ; 50 : 106-116.

Roegge CS, Wang VC, Powers BE, Klintsova AY, Villareal S, Greenough WT, Schantz SL. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicol.Sci. 2004 ; 77 : 315-324.

Roegge CS, Morris JR, Villareal S, Wang VC, Powers BE, Klintsova AY, Greenough WT, Pessah IN, Schantz SL. Purkinje cell and cerebellar effects following developmental exposure to PCBs and/or MeHg. Neurotoxicol. Teratol. in press.

Satoh H. Occupational and Environmental Toxicology of mercury and Its Compouds. Ind. Healh. 2000 ; 38 : 153-164.

Seegal RF, Okoniewski RJ, Brosch KO, Bemis JC. Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: an in vivo microdialysis study. 2002 Environ. Health Perspect. 110, 1113-7.

Spyker JM, Sparber SB, Goldberg AM. Subtle consequences of methylmercury exposure: behavioral deviations in offspring of treated mothers. Science 1972 ; 177 : 621-3.

Stewart P, Reihman J, Lonky E, Darvill T, Pagano J. Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance. Neurotoxicol. Teratol. 2000 ; 22 : 21-29.

Sugawara N, Nakai K, Nakamura T, Ohba T, Suzuki K, Kameo S, Satoh C, Satoh H. Developmental and neurobehavioral effects of perinatal exposure to polychlorinated biphenyls in mice. Arch. of toxicol. in press.

Tachibana T.Behavioral teratogenic effect of methylmercury and d-amphetamine: meta-analysis and power analysis of data from the Collaborative Behavioral Teratology Study of National Center for Toxicological Research. Teratology 1989 ; 40 : 93-100.

Tanimura T, Ema M, Kihara T. Effects of combined treatment with methylmercury and polychlorinated biphenyls (PCBs) on the development of mouse offspring. In Advances in the study of birth defects, Volume 4: Neural and behavioural teratology. (T.V.N. Persaud, Ed.), University Park Press, Baltimore; 1980. pp163-198.

Verschuuren HG, Kroes R, Den Tonkelaar EM, Berkvens JM, Helleman PW, Rauws AG, Schuller PL, Van Esch GJ. Toxicity of methylmercury chloride in rats. II. Reproduction study. Toxicology 1976 ; 6 : 97-106.

Watanabe C, Satoh H. Evolution of our understanding of methylmercury as a health threat. Environ. Health Perspect. 1996 ; 104 : 367-79.

Widholm JJ, Villareal S, Seegal RF, Schantz SL. Spatial alteration deficits foloowing developmental exposure to Aroclor 1254 and/or methylmercury in rats. Toxicol.Sci. 2004 ; 82 : 577-589.

WHO World Health Organization Environmental Health Criteria 101: Methylmercury. Geneva ; 1990.

WHO World Health Organization Environmental Health Criteria 140: Polychlorinated biphenyls and terphenyls (Second Edition), Geneva ; 1993.

Effects of "Spike" (Short-Term Peak) Exposure to Methylmercury on Mercury Accumulation and Neuronal Degeneration in the Brain

Shun'ichi Honda[1], Mineshi Sakamoto[2*], Akiyoshi Kakita[3] and Komyo Eto[4]

[1]Department of Epidemiology, National Institute for Minamata Disease

[2] Department of International Affairs and Environmental Sciences, National Institute for Minamata Disease

[2]Brain Research Institute, Niigata University

[4] Director-General, National Institute for Minamata Disease

Abstract

The National Academy of Science recommended a study as follows: "Because a pilot whale, on average, has substantially higher concentrations of methylmercury (MeHg), traditional Faroese whale dinners may result in a "spike" (short-term peak) in blood MeHg levels. The magnitude of this peak has not been measured directly, but special studies could be designed for this purpose." The purpose of the present study was to evaluate the effects of the "spike" in MeHg exposure on mercury (Hg) accumulation and neuronal degeneration in the brain using rats. Six groups of male Wistar rats were orally administered daily (0.3, 1.5 and 3.0 mg/kg/day) and intermittent (2.1, 10.5 and 21 mg/kg/week) doses of MeHg, respectively. Changes in Hg accumulation in the blood were observed for 5 weeks. The different forms of exposure did not cause any difference in final body weights. Blood Hg concentrations increased gradually in the group exposed daily. On the other hand, the intermittently-dosed groups showed repeated rapid increase and decline in Hg blood concentrations. Hg concentrations in the brain on the days of dissection were almost the same between the groups given daily or intermittent dosages. However, Hg brain levels were higher in rats treated with 0.3 mg/kg/day and 2.1 mg/kg/week, respectively, for 3 weeks than in those treated 1.5 mg/kg/day and 10.5 mg/kg/week for 5 weeks. The severity of the degeneration in the sensory nerve fibers on the days of dissection was almost the same between 2 dosage groups. However, more serve degeneration was seen in rats treated with 1.5 mg/kg/day and 10.5 mg/kg/week for 5 weeks than in those treated with 0.3 mg/kg/day and 2.1 mg/kg/week, for 3 weeks. These results suggested that the intermittent MeHg exposure which caused the "spike" in blood mercury levels would not produce any difference in Hg accumulation in the brain and neuronal damage in the sensory nerve fibers compared to daily exposure with the same amount of MeHg. Furthermore, we examined the effects of the "spike" in MeHg exposure during the gestation period. Two groups of mated Wistar rats were orally administered daily (1.0 mg/kg/day) and intermittent (5.0 mg/kg/5 days) doses of MeHg for 20 days from gestational days 1 to 20. The brain Hg concentrations and neuronal damage to the fetus on gestational days 21 were also compared between the groups.

Key words: Brain, Sensory nerve fibers, Methylmercury, Intermittent exposure, Spike

[*] Corresponding author. Tel: +81-966-63-3111, Fax: +81-966-61-1145, E-mail: sakamoto@nimd.go.jp

1. Introduction

The humans most susceptible to methylmercury exposure are fetuses and neonates, whose developing nervous systems appear to be more sensitive than those of adults[1,2]. Many large cohort studies have been conducted around the world in order to scientifically identify susceptible group to methylmercury exposure and the relationship between methylmercury exposure and its adverse effect to human health, particularly fetus and infant. The best known of these studies are so-called "the Faroe Islands study[3]" and "the Seychelles Islands Study[4,5]". The total mercury concentrations in maternal hair in the Seychelles Islands were slightly higher that those in the Faroe Islands, confirming the methylmercury exposure level in the Seychelles was higher. However, only the Faroe Islands study showed adverse effects, i.e., the association between fetus exposure to methylmercury during the pregnant period and the neuropsychological performance of the infant after birth. This result made researchers have various opinions and possibilities to carry out further experiment. One possibility is the difference of methylmercury exposure patterns based on fish and seafood consumption patterns between these islands[1,6]. The main fish consumed by Faroese is pilot whale a few times a month, and Seychellois is mainly large other fish daily. This difference indicates that Faroese are intermittently affected by a relatively high concentration of methylmercury contained in the muscle tissues of pilot whales, while Seychellois are continuously exposed to a relatively low concentration of methylmercury from the muscle tissue of market fish. The National Toxicology Council also mentions that the difference of fish and seafood consumption pattern suggests a pattern of peaking exposure which might represent a moderate increase above baseline concentrations[1]. The present study aimed at identifying a difference in effect between spiky exposure (short-term peak) and continuous exposure to methylmercury.

2. Materials and methods

Two series of experiments were carried out: 1) one on adult male rats and 2) then on pregnant rats (Fig. 1).

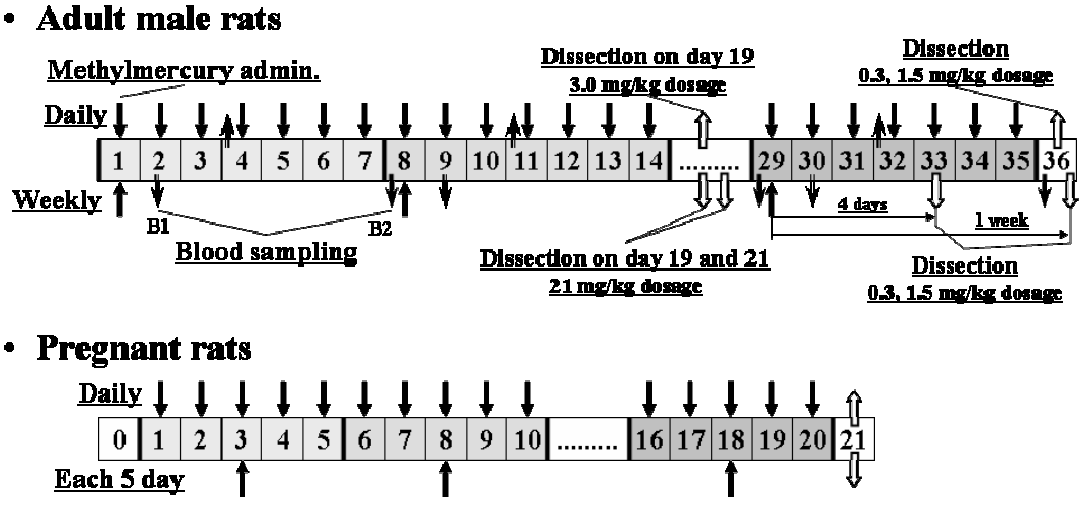


Fig.1 Experimental protocol

In the first experiment, adult male Wistar rats 8 weeks of age were used. We orally administrated 0.3, 1.5 and 3.0 mg·methylmercury/kg·body weight/day to the continuous exposure group (n = 5 in each group) and 2.1, 10.5 and 21.0 mg·methylmercury/kg·body weight/week to the intermittent exposure group (n = 5 in each group) whose concentrations were 7 times higher than those of the continuous exposure group. The accumulation and patterns of methylmercury had been traced by measuring the total mercury concentrations in the blood collected from the tail veins of the rats as follows:

- Once in mid-week from the continuous exposure group;
- Once after 24 hours (B1 group) and before (B2 group) the administration from the intermittent exposure group

Dissections were carried out as follows:

- Continuous exposure group: on day 36 after 1 day from the last administration (except high-dose group);
- B1 group: on day 33 after 4 days from the last administration (except high-dose group);
- B2 group: on day 36 after 1 week from the last administration (except high-dose group).

The days for dissection in B1 and B2 groups were the days when the brain generation would peak. The rats in high dose group were dissected on the days when their body weights were under the beginning of the experiment. The pathological experiment was carried out using the spinal cord and nerve fibres collected from the rats in the intermittent exposure group. Tissues were stained with Glial fibrillary acidic protein stain, Klüver-Barrera stain, and Trichromatic stain.

In the experiment with pregnant rats, 9-week-old female Wistar rats mated with adult male of same age. The day when vaginal sperm was first observed in a smear was taken as pregnant day 1. The oral doses of methylmercury were 1.0 mg·methylmercury/kg·body weight/day in the continuous exposure group (n = 3) and 5.0 mg·methylmercury/kg·body weight/week in the intermittent exposure group (n = 3). The exposure day was daily in the continuous exposure group and once 5 days in the intermittent exposure group. All rats were dissected on the pregnant day 21, and the brains of mothers and fetuses were collected.

The tissues and blood were firstly dissolved with water and homogenised. The dissolved samples were digested with HNO_3HClO_4 and H_2SO_4 (1+1+5) at 240°C for 30 minutes. Total mercury concentrations were analysed by cold vapour atomic adsorption spectrometry[7].

3. Results

Figure 2 shows changes in body weights in the adult male rats. In the low-dose group (0.3 mg/kg/day and 2.1 mg/kg/week), the body weights steadily increased from the beginning to the end of the experiment, whereas the body weights of continuous and intermittent exposure groups in the medium-dosage group (1.5 mg/kg/day and 3.0 mg/kg/week) declined from day 27 and day

15, respectively. In addition, the high-dose group (3.0 mg/kg/day and 21 mg/kg/week) shows that the body weights were unsteady at the beginning of the experiment, decreased sharply after day 18 and were finally less than the original weights. Therefore, the high-dose group was dissected immediately after the body weights were less than originals. Figure 3 shows the changes in body weights in the experiment on pregnant rats. The body weights in the continuous exposure group steadily increased, whereas those in the intermittent group slightly decreased during the first-three administrations. The overall tendency of body weight increase in both groups was the same.

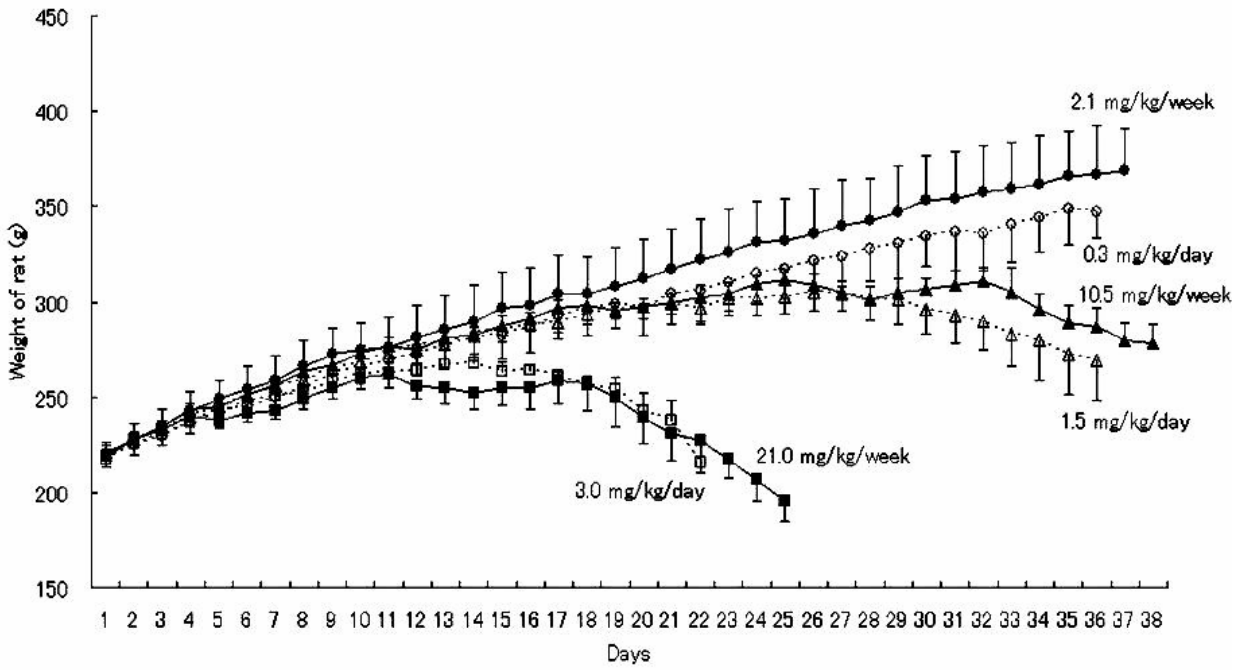


Fig.2 Changes in body weights of the adult male rats

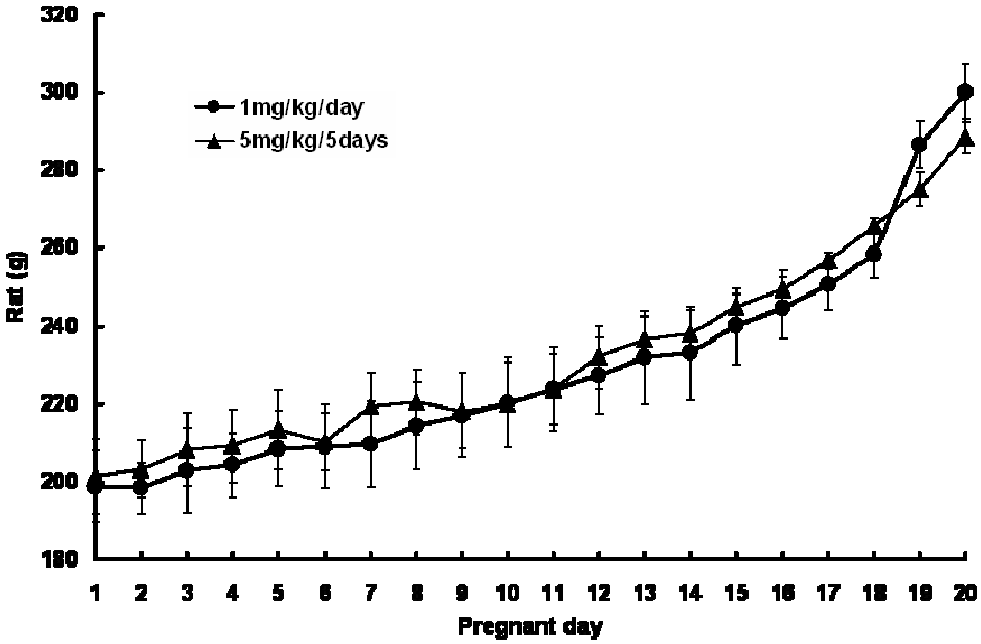


Fig.3 Changes in body weights of the pregnant rats

Figure 4 shows the total mercury concentrations in the blood in the experiment of adult male rats. The total mercury concentrations of the rats in the continuous exposure group steadily increased, whereas those in the intermittent exposure group sharply increased after the exposure and steadily decreased until next exposure. However, this relationship disappeared when the body weights were decreasing in the medium-and high-dose group.

Table 1 shows the total mercury concentrations in the brains of adult male rats. There was no significant difference in the total mercury concentrations in the brains between the continuous or intermittent exposure. Table 2 shows the total mercury concentrations in the brains of the pregnant rats and fetuses. The total mercury concentrations in the brains of the intermittent exposure group were slightly higher than those in the continuous exposure group. The ratio of the total mercury concentrations between the mother and fetus with the continuous and intermittent exposure was 1: 1.79 and 1: 1.88, respectively, and these ratios were on average[1)].

Myelinated nerve fibres were eliminated and gliosis appeared at the posterior column- posterior horns in the spinal cord, and was observed at all parts from the cervical spine to the lumbar spine (Fig. 5-A). This was the lesion of central nerves as well as the dorsal roots. In the dorsal root ganglion, 50% of the ganglion cells were eliminated, and the collagen fibres and satellite cells increased (Fig. 5-B). The myelinated fibres were injured, and the Schwann cells and fibroblasts were increased (Fig. 5-C). The myelin sheath was degenerated and the nucleus components were increased in the sciatic nerve (Fig. 5-D). Table 3 shows the degeneration level of the sensory nerve fibres in the adult male rats. The highest degeneration was found the rats in the medium-dose group (1.5 mg/kg/day and 10.5 mg/kg/week). The level of degeneration in the high-dose group (3.0 mg/kg/day and 21.0 mg/kg/week) was lower than that in the medium-dosage group. This reason was that the high-dose group was dissected immediately after the last administration, despite the fact that lesion is generally confirmed after a period. There was no difference between the continuous and intermittent exposure groups in the level of degeneration of the sensory nerve fibres. In addition, there was no significant difference between the continuous and intermittent exposure groups in the level of degeneration among the cerebrum, cerebellum and brain stem.

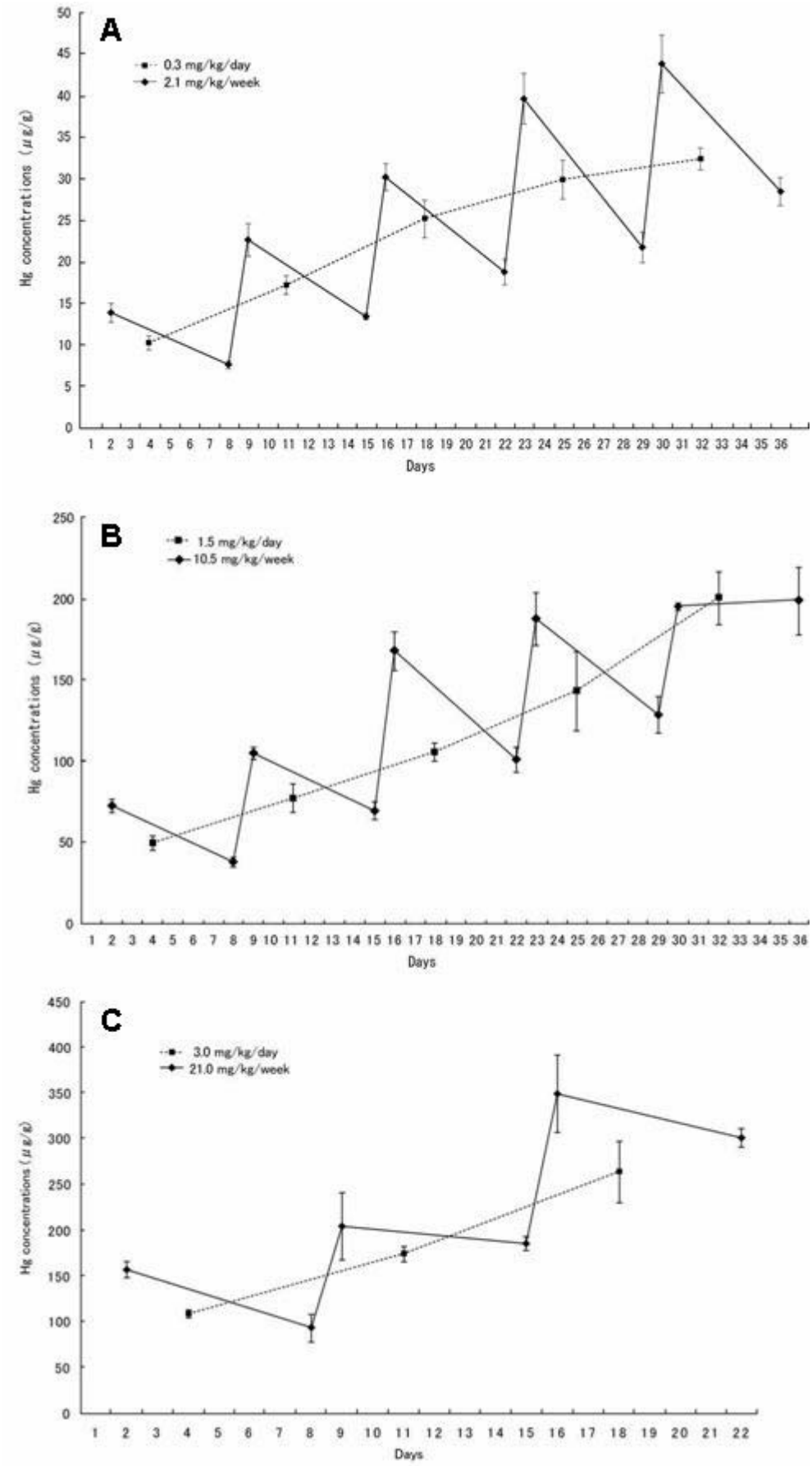


Fig. 4 Changes in total mercury concentrations in the blood (A: Low-dose group (0.3 mg/kg/day and 2.1 mg/kg/week), B: Medium-dose group (1.5 mg/kg/day and 10.5 mg/kg/week), C: High-dose group (3.0 mg/kg/day and 21 mg/kg/week))

Table 1 Mercury concentrations in the brains of adult male rats

MeHg dosage	T-Hg concentrations in the brains (μg/g)
0.3 mg/kg/day	1.69 ± 0.12 (Dissected on Day 36)
2.1 mg/kg/week	1.66 ± 0.16 (B1: Dissected on Day 33) 1.59 ± 0.06 (B2: Dissected on Day 36)
1.5 mg/kg/day	12.8 ± 1.58 (Dissected on Day 36)
10.5 mg/kg/week	12.8 ± 1.58 (B1: Dissected on Day 33) 11.0 ± 0.96 (B2 Dissected on Day 36)
3.0 mg/kg/day	18.0 ± 0.56 (Dissected on Day 19)
21.0 mg/kg/week	20.3 ± 2.15 (B1: Dissected on Day 19) 20.2 ± 1.76 (B2: Dissected on Day 22)

Table 2 Mercury concentrations in the brains of pregnant rats and fetuses

	T-Hg concentrations in the brains (μg/g)	
MeHg dosage	Pregnant rats	Fetuses
1 mg/kg/day	2.11 ± 0.26	3.79 ± 0.56
5 mg/kg/5days	2.15 ± 0.23	4.04 ± 0.12

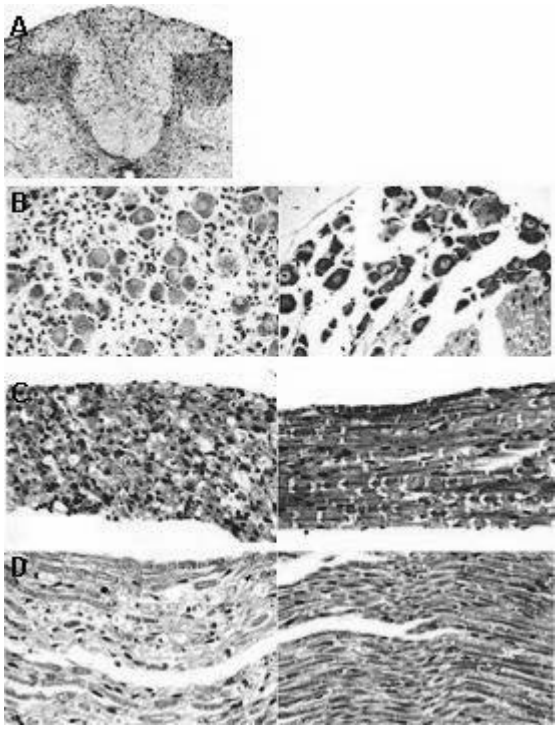


Fig. 5 Degeneration of the spinal cord and its nerves in the methylmercury-treated adult male rat (left) and control rat (right). A: Bilateral posterior horns and a posterior column (Glial fibrillary acidic protein stain); B: Posterior root ganglion (Klüver-Barrera stain); C: Posterior root nerve (Klüver-Barrera stain); D: Sciatic nerve (Trichromatic stain).

Table 3 Level of degeneration of the sensory nerve fibres in the adult male rats. 0: within normal; 0.5: slight; 1: mild; 2: moderate; 3: severe

	Level of degeneration				
MeHg dosage	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
0.3 mg/kg/day	0	0	0	0	0
2.1 mg/kg/week	0.5	0	0	0.5	0.5
1.5 mg/kg/day	3	3	3	3	3
10.5 mg/kg/week	3	3	3	3	3
3.0 mg/kg/day	2	2	2	2	2
21.0 mg/kg/week	1	1	1	1	1
Control	0	0	0	0	0

Figure 6 shows the relationship between continuous and intermittent exposure with the same mercury intake. The body burden of mercury by a continuous and intermittent exposure was simulated by the following equation[2]:

$$A_T = \left(a/\left(ln2/T_{1/2}\right)\right)\times\left(1-exp\left(\left(-ln2/T_{1/2}\right)\times t\right)\right) + Co\times exp\left(\left(-ln2/T_{1/2}\right)\times t\right)$$

where:

A_T = body burden of mercury

a = daily mercury exposure level

C_0 = body burden of mercury at mercury exposure

$T_{1/2}$ = days of biological half time = 70

T = days of mercury exposure

The body burden of mercury with intermittent exposures crosses up and down on that of continuous exposure. Based on the total mercury concentrations (Faroe Islands study: 4.5 ppm[3]; Seychelles Islands study: 6.8 ppm[4,5]; Japanese study: 1.65 ppm[8]; Minamata disease patients: 338.4 ppm[9]) in the maternal hair, the mercury body burden in each country case was simulated in Fig. 7. As the comparison with the reference dose (RfD) established by the United States Environmental Protection Administration (USEPA) and the tolerable weekly intake (TWI) established by the Food Safety Commission in Japan, both mercury body burdens in Faroese and Seychellois exceed RfD and TWI; however, these body burdens are far less than the threshold of the fetal lowest-effect level.

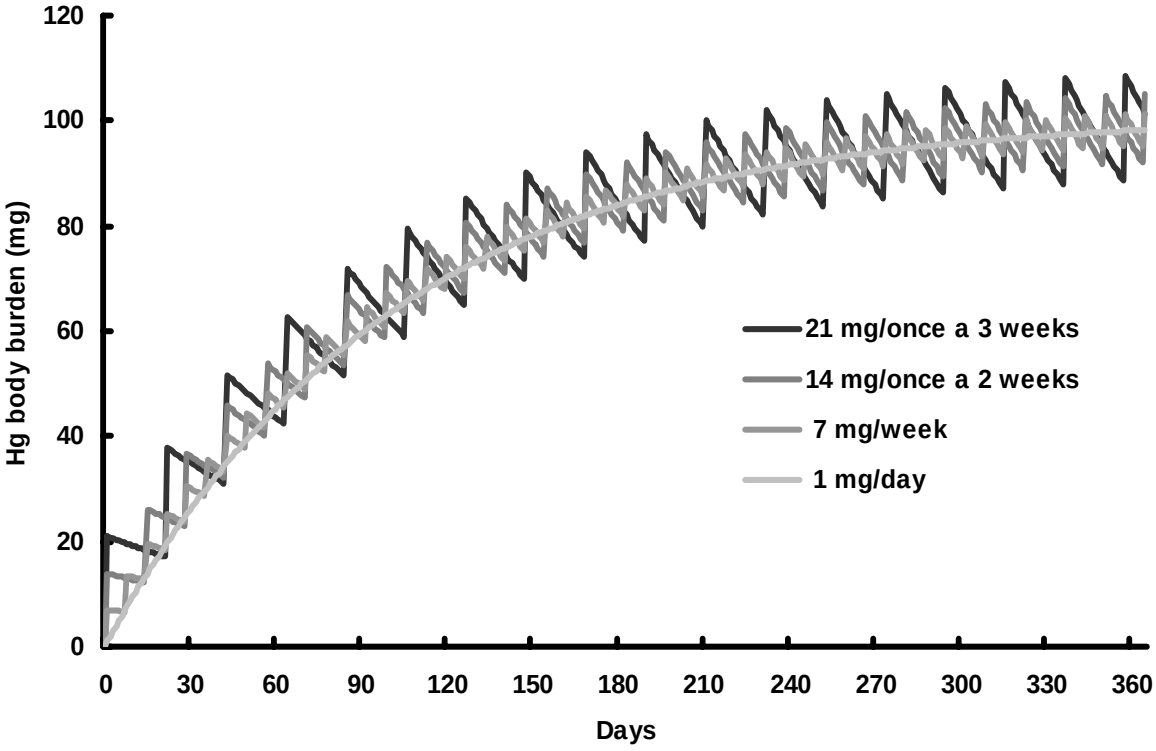


Fig. 6 Body burden of mercury on continuous and intermittent exposure

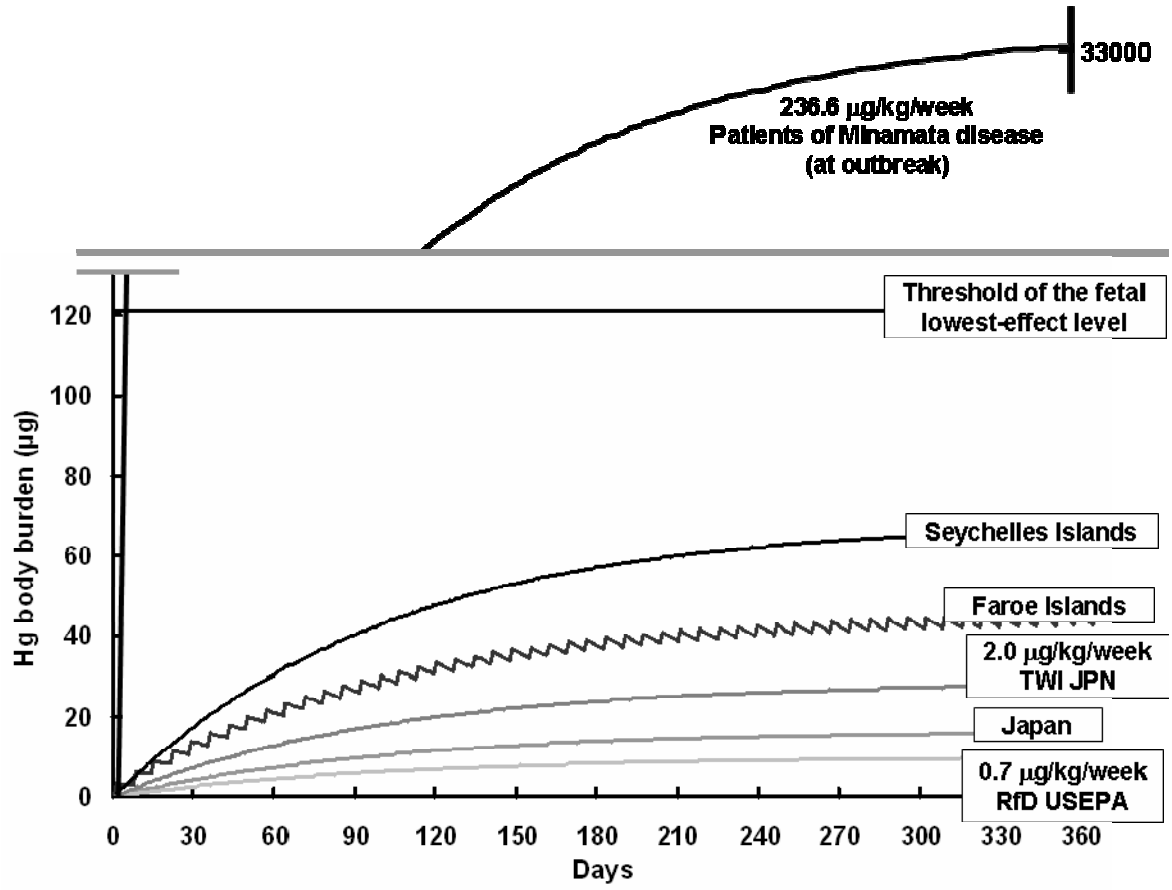


Fig. 7 Simulation of body burden of mercury of Faroese, Seychellois, Japanese and Minamata disease patients

4. Discussion

Fetuses are the most susceptible group to methylmercury exposure. Its one of the main reason is long methylmercury exposure at low concentration, due to the fact that childbearing-age and pregnant women in coast areas frequently consume fish and seafood. There are many cohort studies focusing on the identification of a relationship and association between methylmercury exposure and its adverse effects on human health, particularly neural development of fetuses; however, these studies show similar or different results. The study attempted to determine the difference in methylmercury accumulations between continuous and short-term peak exposure, the so-called spiky exposure, by animal experiments, based on the difference of methylmercury exposure between the Faroe Islands and Seychelles Islands studies.

Generally, a decrease in rat body weights precedes the appearance of symptom on methylmercury poisoning. Rat body weight in the low-dose group increased steadily, whereas those in the medium- and high-dose group were unsteady and decreased finally. Therefore, we should refer the result of mercury concentration in the blood in the low-dose group for human case whose body weight is not affected by methylmercury exposure. The spiky exposure, which is thought to be

occurred in the Faeroe Islands, was confirmed by the intermittent exposure experiment (Fig. 4). A different pattern of changes in body weight was confirmed between the medium- and high-dose groups. However, there was same level of degeneration between those two dose groups.

The mercury concentrations in the blood in the continuous exposure group were steadily increasing, while those in the intermittent exposure group increased sharply after the administration and then were decreasing until the next administration. In addition, the mercury concentration in the intermittent group crossed up and down on that in the continuous exposure group. Body burden of mercury between the continuous and intermittent exposure groups was almost same finally, when total mercury intake was same. This result can explain that there was no significant difference between continuous and intermittent exposure groups on lesion of tissues and nerve fibres.

There was no significant difference in the level of degeneration between the continuous and intermittent exposure. Although individual differences must be taken into account, the degeneration in the medium-dose group was greater than in the high-dose group, and was confirmed not to be dose-dependent. Therefore, long-period exposure at high concentration methylmercury contributes to high level of degeneration of tissues and nerve fibres.

The total mercury concentrations in the brains of the fetuses in the intermittent exposure group were slightly higher that those in the continuous exposure group. This might indicate that the intermittent exposure to methylmercury causes relatively more adverse effects on developing central nerves as well as other organs in fetuses. However, this experiment must be repeated to accumulate data and conclude whether intermittent exposure has an adverse effect.

5. Conclusions

The present study focused on the possibility of intermittent methylmercury exposure, based on the results of the cohort studies in Faroe Islands and Seychelles Islands. The animal experiments indicated no significant difference in mercury accumulation and level of degeneration in adult male rats under either the continuous or intermittent exposure. However, there was a slight difference in the total mercury concentrations in the brains of rat fetuses between the continuous and intermittent exposure through their mothers. More of the same experiments must be conducted in order to conclude that the fetus is affected differently by continuous or intermittent exposure to methylmercury.

References

1) National Research Council. Toxicological Effects of Methylmercury. Washington DC: National Academy Press; 2000.

2) International Programme on Chemical Safety. Environmental Health Criteria 101, Methylmercury. Geneva: World Health Organization; 1990
3) Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PJ. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol. 1997;19: 417-428.
4) Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, Berlin M, Clarkson TW. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. JAMA. 1998;280:701-707.
5) Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari E, Sloane-Reeves J, Wilding GE, Kost J, Huang LS, Clarkson TW. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet. 2003;361:1686-1692.
6) Grandjean P, White RF, Weihe P, Jorgensen PJ. Neurotoxic risk caused by stable and variable exposure to methylmercury from seafood. Ambul Pediatr. 2003;3:18-23.
7) Japan Public Health Association. Preventive Measures against Environmental Mercury Pollution and Its Health Effects. Tokyo Japan: Japan Public Health Association; 2001. http://www.nimd.go.jp/english/image/manual.pdf.
8) Yasutake A, Hachiya N. Methylmercury exposure in current Japanese: estimation form hair analysis. Proc KSEH · Minamata Forum 2005. 2005; 4-15.
9) Katsuna M (Ed.). Minamata disease. Study group of Minamata disease. Kumamoto University, Japan. 1968.

Correlations between Mercury Concentrations in Umbilical Cord Tissue and other Biomarkers of Fetal Exposure to Methylmercury in Japanese Population

Mineshi Sakamoto[1*], Tuyoshi Kaneoka[2], Katsuyuki Murata[3], Kunihiko Nakai[4], Hiroshi Satoh[4], Hirokatsu Akagi[5]

[1]*Department of Epidemiology, National Institute for Minamata Disease, Kumamoto 867-0008, Japan*

[2]*Department of Obstetrics and Gynecology, Fukuoka University Graduate School of Medicine, Fukuoka 814-0180, Japan*

[3]*Division of Environmental Health Sciences, Department of Social Medicine, Akita University School of Medicine, Akita 010-8543,Japan*

[4]*Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Miyagi 980-8575, Japan*

[5]*International Mercury Laboratory Inc., Kumamoto 867-0034, Japan*

Abstract

Methylmercury (MeHg) is one of the most risky substances to affect humans through fish consumption, and the fetus is known to be in the highest susceptible group. Our objective in this study is to examine the relationships of total mercury (THg) and MeHg concentrations between umbilical cord tissue and other tissues as biomarkers of fetal exposure to MeHg in the Japanese population. In total, 116 paired samples were collected in 3 Japanese districts, the Tsushima Islands, Fukuoka City, and Katsushika Ward of Metropolitan Tokyo. THg was measured for hair and THg and MeHg were measured in cord tissues, maternal blood and cord blood. The relationships in Hg concentrations among tissues were similar among districts. Therefore, we analyzed the relationships using all the samples. More than 90% of Hg in cord tissue, cord blood, and maternal blood were MeHg. THg and MeHg in cord blood was about 2 times higher than in maternal blood. A strong correlation coefficient was found between THg and MeHg in cord tissue. The cord tissue THg and MeHg showed a strong correlation with cord blood Hg, which is recognized as the best biomarker for fetal exposure to MeHg. The findings of this study indicate the significance of cord tissue THg and MeHg as a biomarker for fetal exposure to MeHg at parturition.

Keywords: mercury, methylmercury, exposure, umbilical cord tissue, umbilical cord blood, maternal blood, hair, parturition, biomarkers, fetus, parturition

A part of this work was supported by a grant for Comprehensive Research of Minamata Disease from the Ministry of Environment, Japan. This study was approved by the Ethics Committee of the National Institute for Minamata Disease (No. 19-55-4 NIMD).

* All correspondence to: Mineshi Sakamoto (address per above), Phone: +81-966-63-3111 (Ext. 312), Fax: +81-966-61-1145, E-mail: sakamoto@nimd.go.jp

1. Introduction

Fetuses are known to be a high-risk group for methylmercury (MeHg) exposure because of the high susceptibility of the developing brain itself (Choi 1989; Sakamoto et al. 1993; WHO 1990) . Moreover MeHg easily crosses the blood-placenta barrier, accumulating more in the fetus than the mother (Choi 1989; Sakamoto et al. 2004, 2002; Stern and Smith 2003; WHO 1990). Therefore, the effect of MeHg exposure on pregnant women is an important issue for elucidation, especially in Japanese and some other populations which consume much fish and sea mammals (Grandjean et al. 2005, 1997; Myers et al. 2003, 1995; NRC 2000; WHO 1990).

The epidemic called “Minamata disease” is well known as the first instance on record of severe MeHg poisoning caused by man-made environmental pollution, which occurred mainly among fishermen and their families in and around Minamata City. It originated from the consumption of large amounts of fish and shellfish contaminated with MeHg discharged from a chemical plant (Irukayama and Kondo 1966). The principal symptoms included neurological disorders such as sensory disorder, cerebellar ataxia, contraction of the visual field, hearing impairments and disequilibrium (Takeuchi et al. 1962). The first patient was reported in 1953, and the number of patients rapidly increased after 1955. Up to the present, more than 2000 people have been certified to have Minamata disease. Furthermore, many fetuses exposed to MeHg through the placenta of the exposed mother showed severe cerebral palsy-like symptoms, while their mothers had mild or no manifestation of the poisoning (Harada 1978). Outbreaks of the typical fetal-type Minamata disease occurred during 1955-59 when the mercury pollution appears to have been most severe, judging from the incidence of patients (Harada 1978) and the MeHg concentration in the umbilical cords of inhabitants of the area (Nishigaki and Harada 1975). During the period, a deceased male birth ratio associated with increased male fetal death was observed in Minamata City pollution, indicating its severity and widespread distribution (Sakamoto et al. 2001). This landmark epidemic was the first to bring worldwide attention to the high risk of fetal exposure to MeHg.

Thereafter, large prospective cohort studies have been conducted in the Seychelles (Myers et al. 2003, 1995) and Faroe Islands (Grandjean et al. 2005, 1999, 1997), where fish or sea mammal consumption is high. Though the MeHg exposure level was similar in these studies, different conclusions were reached (NRC 2000). The exposure level in the epidemic in Minamata is thought to be much higher than in those studies. Therefore, the study of Minamata disease is still very important, and it will provide more obvious health effect information by using recently-developed neurological test batteries. Fortunately, Japanese people have a custom of preserving a small piece of the dried umbilical cord tissue as a birth memento in a wooden or plastic small box deep inside a chest of drawers. MeHg in the tissue will not be reduced by microorganism, because it is completely

dried. By measuring the Hg concentrations in this preserved dry cord sample, we can estimate the individual MeHg exposure level at birth. Grandjean et al. (2005) revealed that cord tissue Hg as well as cord blood Hg was useful as a predictor of the effect of fetal exposure to MeHg. However, the preserved cord tissues had often been treated with mercurochrome at the period when cut off at parturition. The mercurochrome can easily dissociate into Hg ions in the solution and the ions act as a disinfectant. Then the cord tissue treated with mercurochrome shows very high Hg concentrations when THg is measured. Accordingly, we need to measure not THg but MeHg in the preserved cord tissue in Japan, especially in the samples treated back in those days. Some studies (Akagi et al. 1998; Nishigaki and Harada 1975) have revealed exposure of the fetus to MeHg in the Minamata area by measuring it in the preserved cord tissue. The present study investigates the relationships between THg and MeHg in cord tissue and the relationships between other biomarkers of fetal exposure to MeHg in the Japanese population to evaluate the significance of the Hg concentrations in cord tissue.

2. Materials and methods

2.1. Subjects and sampling

In total, 116 healthy Japanese pregnant women without any special exposure to mercury, ranging in age from 19 to 41 yr (average 30.0 ± 5.0), gave informed consent to take part in the present trial. The samples were collected in the Tsushima islands in Nagasaki Prefecture (30 cases), Fukuoka City in Fukuoka Prefecture (68 cases) and Katsushika, a special Ward of Metropolitan Tokyo (18 cases). Blood samples from the mothers and umbilical cord were collected immediately after birth in 1996. Whole length of maternal hair and cord tissue near the fetus were also collected at parturition. Samples were stored at -80 ºC until analysis. About 1 cm of umbilical cord from the fetus side was rinsed in physiological saline solution to remove blood and body fluid and pressed between paper towels to remove the saline solution. Further, they were dried at 40 ºC for three days and were kept in desiccators. No further weight loss occurred after the dehydration, ensured by weighing for 3 days. The dried tissues were then cut into small pieces by scissors and around 0.1-0.2 g of the tissues were moistened with 0.5 ml of water one day prior to the Hg analysis. This study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD).

2.2. Mercury analysis

THg in the samples was determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to the method of Akagi et al. (2000). The method involves sample digestion with HNO_3 $HClO_4$ and H_2SO_4 (1+1+5), followed by reduction to elemental Hg vapor by $SnCl_2$. The detection limit was 0.01 ng/g. MeHg in the samples was determined by gas chromatography with electron capture detection (GC-ECD) according to the method of Akagi et al. (2000) The method involves sample digestion with KOH-Ethanol and subsequently, under a slightly acidic condition, the fatty content is

removed using n-hexane. After extraction with dithizone-toluene, methylmercury is back-extracted with a slightly alkaline sodium sulfide solution. The excess sulfide ions are then removed as hydrogen sulfide by purging with nitrogen gas after slight acidification with HCl solution. MeHg is then re-extracted with a small portion of dithizone-toluene, the extract is washed with NaOH solution to remove the excess dithizone, and then slightly acidified with HCl and analyzed by GC-ECD. The detection limit was 0.01 ng/g. Accuracy of THg was ensured by using reference blood material Level 2, MR9067 (Seronorm201605: Nycomed Co., Oslo, Norway): the THg determined averaged 7.5 µg/L, as compared to the recommended range of 6.8-8.5 µg/L by ICP-SFMS. The precision of the method, expressed as coefficient of variation, was 0.8%. Analysis of a MeHg standard solution containing 5 µg /L gave a recovery of almost 100%. The precision and accuracy of THg and MeHg were repeatedly verified by inter-laboratory calibration exercises including the analysis of standard reference material such as IAEA-085, 086 and 142 (Horvat M et al. 1988).

2.3. Statistics

THg and MeHg concentrations among the districts were analyzed by one-way analysis of variance (ANOVA). Similar correlations were observed between the Hg concentrations in each tissue among the districts. Therefore, all the data were combined and the associations between THg and MeHg among samples were studied by Pearson's correlation analysis. Logarithmic transformation was used to correct the skewed distribution of the Hg concentrations. The differences in Hg concentrations between paired samples were determined by paired *t*-test. A p value less than or equal to 0.05 was considered to demonstrate statistical significance.

3. Results

Table 1 presents the geometric means of THg in hair and THg and MeHg in cord tissue, maternal

Table 1 Geometric means and 25th–75th percentiles of total mercury (THg) in hair and total and methylmercury (MeHg) in cord tissue, maternal blood and cord blood in three districts in Japan

(ng/g)	Hair	Cord tissue		Maternal blood		Cord blood	
Districts	THg	THg	MeHg	THg	MeHg	THg	MeHg
Tsushima	1453	64.0	54.6	4.62	3.98	9.13	8.38
n=30	(1157 – 1950)	(46.7 – 87.9)	(38.1 – 71.8)	(3.02– 7.55)	(2.75 – 5.95)	(6.37 – 12.4)	(5.84 – 10.7)
Fukuoka	1954	97.0	89.3	4.9	4.61	9.27	8.93
n=68	(1170 – 2293)	(80.6 – 125)	(75.8 – 121)	(3.65 – 6.44)	(3.61 – 5.73)	(7.03 – 13.9)	(6.57 – 11.5)
Katsushika	2120	137.7	130.8	7.91	7.54	13.9	11.4
n=17	(1810 – 2990)	(94.5 – 207)	(95.5 – 197)	(5.42 – 11.3)	(5.21 – 10.3)	(8.87 – 20.9)	(8.13 – 20.1)
Total	1624	91.7	83.1	5.18	4.77	9.81	9.32
n=115	(1175 – 2195)	(63.5 – 127)	(57.1 – 122)	(3.63 – 7.34)	(3.5 – 6.54)	(6.96 – 13.6)	(6.56 – 13.4)
MeHg (%) Mean ± SD			90.6 ± 10.4		92.5 ± 8.1		95.2 ± 5.5

The mean MeHg percentage (MeHg / THg ×100)

blood and cord blood in three districts in Japan. There were significant differences in all the Hg concentrations among the districts ($p<0.01$). The geometric means of Hg concentrations in all tissues were highest in Katsushika Ward of Metropolitan Tokyo, followed by Fukuoka City and the Tsushima Islands. All the data were combined, because similar correlations were observed among the Hg concentrations in all tissues among the districts as shown in Figures 1-3. Significant correlations of THg and MeHg concentrations were observed among the biomarkers as shown in Table 2. THg and MeHg in cord tissue showed strong correlations with those in maternal and cord bloods (Table 2). However, the correlation coefficients of THg in maternal hair and THg and MeHg in other biomarkers were comparatively low and scattered as shown in Table 2 and Figure 3. As shown in Table 2 and Figure 1, the geometric means of THg and MeHg in cord blood were 9.81 and 9.32 ng/g, respectively, and the concentrations were about 2 times higher ($p<0.01$) than those of maternal blood (5.18 and 4.77 ng/g). THg and MeHg in cord tissue showed strong correlation coefficients (Table 2 and Figure 2), and the geometric means of THg and MeHg in cord tissue were 91.7 and 83.1 ng/g, respectively. The mean MeHg percentage (MeHg / THg ×100) in cord tissue was 90.6 ±10.4 %. MeHg percentages in cord blood and maternal blood were 95.2% and 92.5%, respectively (Table 1), and the mean MeHg percentage in cord blood was significantly ($p<0.01$) higher than that of maternal blood.

Table 2 Correlation coefficients of logarithmic total mercury (THg) in hair and total and methylmercury (MeHg) in cord tissue, maternal blood and cord blood in the total of three districts in Japan

	Hair	Cord tissue		Maternal blood		Cord blood	
r	THg	THg	MeHg	THg	MeHg	THg	MeHg
Hair–THg	1						
CT–Hg	0.641	1					
CT–MeHg	0.626	0.975	1				
MB–THg	0.648	0.809	0.799	1			
MB–MeHg	0.654	0.846	0.839	0.981	1		
CB–THg	0.651	0.848	0.815	0.888	0.885	1	
CB–MeHg	0.646	0.873	0.839	0.882	0.888	0.992	1

All the correlation coefficients were significant ($P<0.01$)

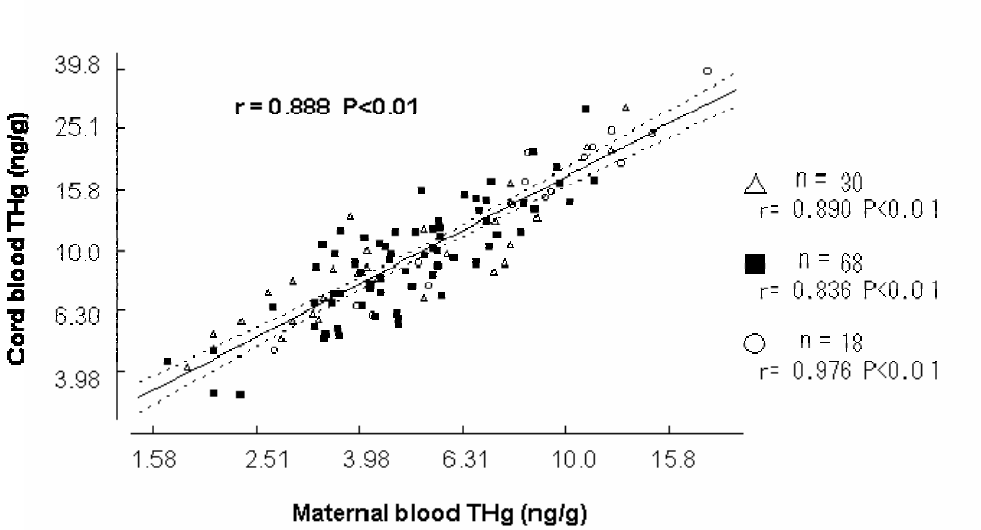


Figure 1. Correlation between total mercury (THg) in maternal blood and cord blood. △: Tsushima Islands; ■: Fukuoka City; ○: Katsushika Ward of Metropolitan Tokyo. Dotted lines represent 95 percent confidence intervals for the regression line.

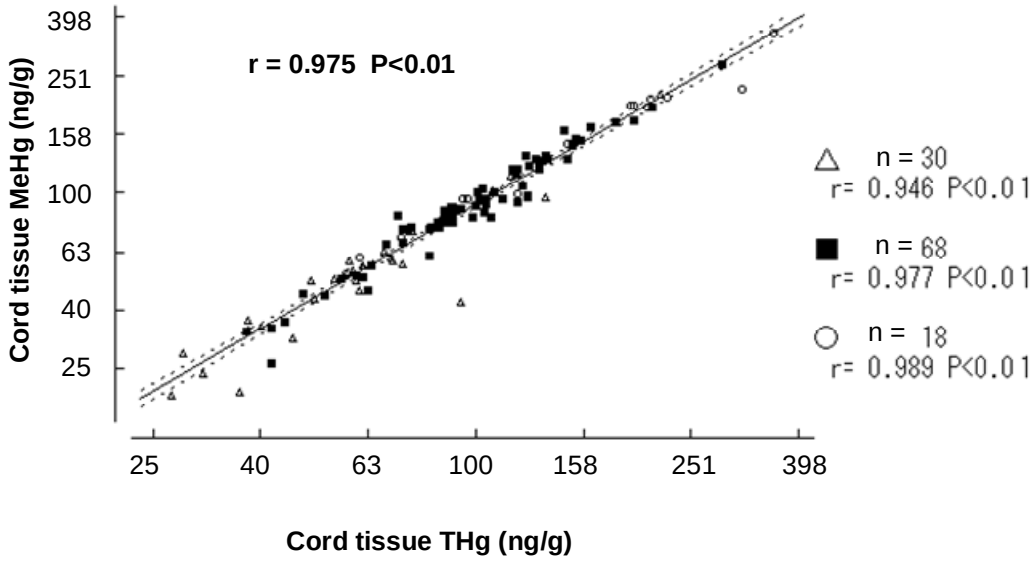


Figure 2. Correlation between total mercury (THg) and methylmercury (MeHg) in cord tissue. △: Tsushima Islands; ■: Fukuoka City; ○: Katsushika Ward of Metropolitan Tokyo.Dotted lines represent 95 percent confidence intervals for the regression line.

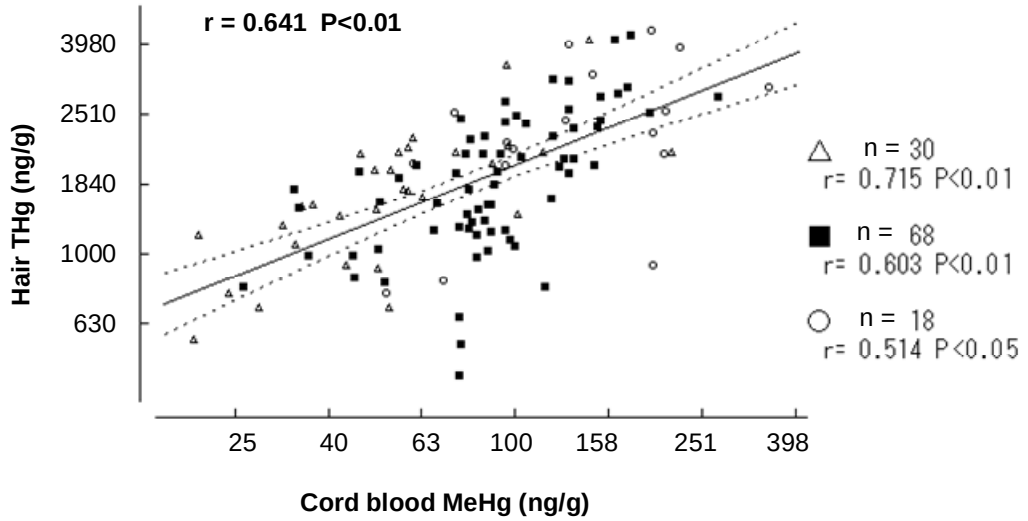


Figure 3. Correlation between cord tissue methylmercury (MeHg) and hair total mercury (THg). △: Tsushima Islands; ■: Fukuoka City; ○: Katsushika Ward of Metropolitan Tokyo. Dotted lines represent 95 percent confidence intervals for the regression line.

4. Discussion

MeHg is one of the most risky substances for the fetal brain, and most of the human exposure to MeHg is through maternal fish consumption. MeHg easily passes through the placenta as a cysteine conjugate during intrauterine life (Aschner and Clarkson 1987; Kajiwara et al. 1996). The National Research Council (NRC 2000) recommended cord blood Hg as the best biomarker for fetal exposure to MeHg. In addition, cord tissue Hg concentration was revealed to be useful as a predictor of the effect of fetal exposure to MeHg (Grandjean et al. 2005). The MeHg concentration in preserved umbilical cord was also used as a biomarker of fetal-type Minamata disease patients (Akagi et al. 1998; Nishigaki and Harada 1975). The present study investigated the relationships between THg and MeHg in cord tissue and other biomarkers of fetal exposure to MeHg in the Japanese population to evaluate the significance of Hg in the tissue.

The differences in MeHg exposure levels in various geographic areas were similar to the data from a recent Japanese hair mercury survey (Yasutake et al. 2004). The high Hg concentrations in Katushika Ward of Metropolitan Tokyo may also be explained by the high amount of tuna consumption in Tokyo and nearby Tokyo Metropolitan Prefecture (Yasutake et al. 2004). The correlations between the Hg concentrations in biomarkers were similar among areas. The geometric mean of THg in cord tissue in this study was 91.7 ng/g, and the level was approximately half that of the Faroe Islands study (Dalgard et al. 1994; Grandjean et al. 2005).

Thanks to the traditional Japanese custom of preserving umbilical cord tissue at the time of the infant's birth, we may use this dried cord tissue to estimate past MeHg exposure. The cord tissue is formed mainly during the second and third trimester, and cord blood is the blood circulating in the fetal body at parturition. Judging from the data on the biological half-life of MeHg of about 45 days (Smith and Farris 1996), not only fetal blood but also cord tissue Hg will reveal the average MeHg burden of the fetus during the third trimester. In addition, rapid brain growth occurs primarily during the third trimester in humans (Dobbing and Sands 1979) and the brain at the period is known to be most vulnerable to the toxicity of MeHg (Rice and Barone 2000). Strong correlation coefficients were observed between cord blood THg, which is recommended as the best biomarker for fetal exposure to MeHg by the National Research Council (NRC 2000). The strong correlation coefficient between THg and MeHg in cord tissue and the high MeHg percentage (about 90%) also suggest that cord tissue MeHg as well as THg concentrations are useful biomarkers for prenatal MeHg exposure.

Under the steady-state condition, the hair/blood mercury ratio is about 250 (WHO 1990). However, in the present study, the ratio between maternal hair/maternal blood was about 350, presumably due to the lower hematocrit (Htc, the ratio of the volume of red blood cells to the total volume of blood) during gestation (Bollini et al. 2005), especially in the last trimester as plasma volume increases. The low maternal Htc during gestation will explain the higher hair/blood ratio, because about 90% of Hg exists in red blood cells in a population that consumes much fish (Group 1970; Sakamoto et al. 2002; Svensson et al. 1992). The difference in MeHg % between maternal blood (92.5%) and cord blood (95.2%) may also be explained by the low Htc in the former blood and the high Htc in the latter blood as indicated by Sakamoto et al. (2002).

Maternal hair and maternal blood Hg concentrations are also important biomarkers for fetal MeHg exposure. Originally, maternal biomarkers reflect the exposure of the mother herself, and there is a certain variability between the maternal and fetal MeHg levels. Our recent study indicated that individual cord/maternal Hg concentrations in red blood cells varied from 1.08 to 2.19 in 53 pairs mother-infants at parturition, which show the individual differences in MeHg concentrations between maternal and fetal circulations at late gestation (Sakamoto et al. 2004). Stern and Smith (2003) also summarized the variability of cord/maternal blood Hg level ratio. Grandjean et al. (2005, 1997) revealed significant associations between the adverse effects and the Hg level in cord blood and cord tissue, but the effects were not well associated with the level in maternal hair. In the present study, THg and MeHg in cord showed strong correlations with those in maternal and cord blood. However, the correlations of THg in maternal hair and either THg or MeHg in other biomarkers were comparatively low, and the 95% confidence interval of the intercept for the regression line did not

include zero. This may have been due to the fact that we used whole length of hair for Hg analysis in the present study, while Hg levels in newly formed hair reflect those in blood (Phelps et al. 1980) . In this way, the Hg concentrations in whole hair do not exactly reflect the Hg level in blood at parturition. In addition, another reason for the scattered distribution would be decrease in Hg level by artificial hair waving (Dakeishi et al. 2005; Yamamoto and Suzuki 1978)

Some of the ratios among biomarkers calculated from our study were similar to those calculated from the Faroe Island study (Grandjean et al. 2005, 1997), suggesting that the ratios are applicable to estimate the past Hg levels in other tissues at parturition in a population in which the MeHg exposure level is comparatively high. The mean cord tissue Hg level of fetal-type Minamata disease patients (median MeHg = 1.63 μg/g, (Akagi et al. 1998)） was about 8 times higher than that in the Faroe Islands (geometric mean THg = 0.21 μg/g; Grandjean et al. 1999, 1997), and about 20 times higher than our present result (geometric mean MeHg = 0.083 ng/g). The estimated mean THg in maternal blood, cord blood and maternal hair of the fetal-type of Minamata disease patients were approximately 100 ng/g, 200 ng/g and 32 μg/g, respectively. However, the estimation of the maternal hair THg will be more uncertain as we mentioned earlier.

The findings of this study support the use of umbilical cord THg and/or MeHg as biomarkers of fetal exposure to MeHg. Further, the MeHg concentration in preserved cord tissue will be useful as the only biomarker available as a predictor of the retrospective dose-response (or dose–effective) study in the Minamata district even up to today.

References

Akagi H, Castillo ES, Cortes-Maramba N, Francisco-Rivera AT, Timbang TD. 2000. Health assessment for mercury exposure among schoolchildren residing near a gold processing and refining plant in Apokon, Tagum, Davao del Norte, Philippines. Sci Total Environ 259(1-3):31-43.

Akagi H, Grandjean P, Takizawa Y, Weihe P. 1998. Methylmercury dose estimation from umbilical cord concentrations in patients with Minamata disease. Environ Res 77(2):98-103.

Aschner M, Clarkson TW. 1987. Mercury 203 distribution in pregnant and nonpregnant rats following systemic infusions with thiol-containing amino acids. Teratology 36(3):321-328.

Bollini A, Hernandez G, Bravo Luna M, Cinara L, Rasia M. 2005. Study of intrinsic flow properties at the normal pregnancy second trimester. Clin Hemorheol Microcirc 33(2):155-161.

Choi BH. 1989. The effects of methylmercury on the developing brain. Prog Neurobiol 32(6):447-470.

Dakeishi M, Nakai K, Sakamoto M, Iwata T, Suzuku K, Liu X, et al. 2005. Effects of hair treatment

on hair mercury – The best biomarker of methylmercury exposure? Environ Health Prev Med 10(4):208-212.

Dalgard C, Grandjean P, Jorgensen PJ, Weihe P. 1994. Mercury in the umbilical cord: Implications for risk assessment for Minamata disease. Environ Health Perspect 102(6-7):548-550.

Dobbing J, Sands J. 1979. Comparative aspects of the brain growth spurt. Early Hum Dev 3(1):79-83.

Grandjean P, Budtz-Jorgensen E, Jorgensen PJ, Weihe P. 2005. Umbilical cord mercury concentration as biomarker of prenatal exposure to methylmercury. Environ Health Perspect 113(7):905-908.

Grandjean P, Budtz-Jorgensen E, White RF, Jorgensen PJ, Weihe P, Debes F, et al. 1999. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. Am J Epidemiol 150(3):301-305.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19(6):417-428.

Group SE. 1970. Methylmercury in fishes. A toxicological-epidemiological evaluation. Report of a group of experts.

Harada M. 1978. Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology 18(2):285-288.

Horvat M, Stegnar P, Byrne AR, M. D. 1988. In: Trace elements－analytical chemistry. A study of trace elements in human placenta, blood and hair from Yugoslav central Adriatic. Berlin:de Gruyter & Co.

Irukayama K, Kondo T. 1966. [Studies on the organomercury compound in the fish and shellfish from Minamata Bay and its origin. VII. Synthesis of methylmercury sulfate and its chemical properties]. Nippon Eiseigaku Zasshi 21(5):342-343.

Kajiwara Y, Yasutake A, Adachi T, Hirayama K. 1996. Methylmercury transport across the placenta via neutral amino acid carrier. Arch Toxicol 70(5):310-314.

Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari E, et al. 2003. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 361(9370):1686-1692.

Myers GJ, Davidson PW, Cox C, Shamlaye CF, Tanner MA, Choisy O, et al. 1995. Neurodevelopmental outcomes of Seychellois children sixty-six months after in utero exposure to methylmercury from a maternal fish diet: pilot study. Neurotoxicology 16(4):639-652.

Myers GJ, Marsh DO, Davidson PW, Cox C, Shamlaye CF, Tanner M, et al. 1995. Main neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet: outcome at six months. Neurotoxicology 16(4):653-664.

Nishigaki S, Harada M. 1975. Methylmercury and selenium in umbilical cords of inhabitants in the Minamata area. Nature (London) 258:324-325.

NRC. 2000. National Research Council. Toxicological Effects of Methylmercury. Washington, DC: National Academy Press.

Phelps RW, Clarkson TW, Kershaw TG, Wheatley B. 1980. Interrelationships of blood and hair mercury concentrations in a North American population exposed to methylmercury. Arch Environ Health 35(3):161-168.

Rice D, Barone S, Jr. 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108 Suppl 3:511-533.

Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, Satoh H. 2004. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ Sci Technol 38(14):3860-3863.

Sakamoto M, Kubota M, Matsumoto S, Nakano A, Akagi H. 2002. Declining risk of methylmercury exposure to infants during lactation. Environ Res 90(3):185-189.

Sakamoto M, Nakano A, Akagi H. 2001. Declining Minamata male birth ratio associated with increased male fetal death due to heavy methylmercury pollution. Environ Res 87(2):92-98.

Sakamoto M, Nakano A, Kajiwara Y, Naruse I, Fujisaki T. 1993. Effects of methyl mercury in postnatal developing rats. Environ Res 61(1):43-50.

Smith JC, Farris FF. 1996. Methyl mercury pharmacokinetics in man: a reevaluation. Toxicol Appl Pharmacol 137(2):245-252.

Stern AH, Smith AE. 2003. An assessment of the cord blood:maternal blood methylmercury ratio: implications for risk assessment. Environ Health Perspect 111(12):1465-1470.

Svensson BG, Schutz A, Nilsson A, Akesson I, Akesson B, Skerfving S. 1992. Fish as a source of exposure to mercury and selenium. Sci Total Environ 126(1-2):61-74.

Takeuchi T, Morikawa N, Matsumoto H, Shiraishi Y. 1962. A pathological study on Minamata disease in Japan. Acta Neuropathologica 2:40-57.

WHO. 1990. Methylmercury. Environmental Health Criteria 101, Geneva: World Health Organization.

Yamamoto R, Suzuki T. 1978. Effects of artificial hair-waving on hair mercury values. Int Arch Occup Environ Health 42(1):1-9.

Yasutake A, Matsumoto M, Yamaguchi M, Hachiya N. 2004. Current hair mercury levels in Japanese for estimation of methylmercury exposure. J Health Sci 50(2):120-125.

Imprecision of Cord Tissue Mercury and Other Biomarkers of Prenatal Methylmercury Exposure, and the Implications for Exposure Limits

Philippe Grandjean,[1,2] Esben Budtz-Jørgensen,[1,3] Poul J. Jørgensen,[4] and Pál Weihe[1,5]

[1]Institute of Public Health, University of Southern Denmark, DK-5000 Odense, Denmark

[2]Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA

[3]Department of Biostatistics, Institute of Public Health, University of Copenhagen, DK-2200 Copenhagen, Denmark

[4]Institute of Clinical Research, Odense University Hospital, DK-5000 Odense, Denmark; and [5]Faroese Hospital System, FR-100 Tórshavn, Faroe Islands

Abstract

Following the discovery of Minamata disease as methylmercury poisoning, attempts were made to use the methylmercury concentration in preserved umbilical cords as indicator of the level of prenatal exposure. Although formal validation of this biomarker was missing, children with congenital Minamata disease had significantly higher methylmercury concentrations in preserved cords. Subsequent studies in the Faroe Islands have shown that the total mercury concentrations in cord tissue and cord blood correlate well. The cord grows substantially during the third trimester, and the mercury concentration may therefore represent the average exposure during this time, where toxic effects to brain development may result in functional abnormalities. In a prospective study of a birth cohort from the Faroe Islands, we used as the main exposure biomarkers the mercury concentrations in cord blood and maternal hair obtained at parturition. We supplemented these biomarkers with mercury analyses of umbilical cord tissue from 447 births. In particular when expressed in relation to the dry weight of the tissue, the cord mercury concentration correlated very well with that in cord blood. Structural equation model analysis showed that these two biomarkers have average total imprecisions of about 30%, which is much higher than the laboratory error. The imprecision of the dry-weight-based concentration was lower than that of the wet-weight-based parameter, and it was intermediate between those of the cord blood and the hair biomarkers. In agreement with this finding, regression analyses showed that the dry-weight cord mercury concentration was almost as good a predictor of methylmercury-associated neuropsychological deficits at age 7 years as was the cord blood mercury concentration. Similar findings were obtained at age 14 years. Cord tissue is easy to collect, and cord mercury analysis can be used as a valid measure of prenatal methylmercury exposure. However, appropriate adjustment for the imprecision should be considered. Using the documented imprecision levels for different exposure biomarkers examined in this study, benchmark dose levels can be adjusted. The results suggest that current exposure limits would be lower, had exposure imprecision been taken into regard.

Introduction

Exposure assessment is a crucial aspect of environmental epidemiology, but remains an inexact science, where validity must be optimized within the confines of efficiency and practicality. Dietary questionnaires constitute a crucial instrument in nutritional epidemiology (Marshall 2003), but they are less useful for food contaminants, because their concentrations usually vary much more than those of essential nutrients. Instead, environmental epidemiology is relying to an increasing extent on measurements of contaminant concentrations in human tissue samples (Grandjean 1995). Such exposure biomarkers are generally thought to constitute valid measures when laboratory error is carefully controlled. Studies incorporating exposure biomarkers therefore rarely take into account the measurement imprecision.

The ideal exposure biomarker should show a clear-cut relationship to the degree of exposure (Grandjean et al. 1994), but the reality is often that up to several imprecise measures may be available, none of them necessarily an accurate indicator of the true exposure. In regard to methylmercury, substantial information is now available on daily intake levels (European Food Safety Authority 2004), and experimental studies in human volunteers have demonstrated how the dietary intakes may be translated into mercury concentrations in blood (Sherlock et al. 1984) or hair (Hislop et al. 1983). However, these two commonly used exposure biomarkers show only scattered associations (Budtz-Jørgensen et al. 2004a), thus suggesting that their total imprecisions significantly exceed routine laboratory errors.

In the first etiologic studies of the so-called Minamata disease, researchers took advantage of the local tradition of saving a dried piece of umbilical cord. Using the cord mercury concentration as an exposure biomarker, much higher levels were found in Minamata disease patients compared to control groups (Harada 1977). These retrospective exposure assessments have later been extended (Dalgård et al. 1994; Akagi et al. 1998). More recently, mercury was analyzed in a selection of umbilical cords collected from a British birth cohort (Daniels et al. 2004). A sample of umbilical cord is easily collected in connection with births, and the validity of determining mercury as an exposure biomarker therefore deserves to be assessed. However, several factors may affect the characteristics of a cord sample, including blood vessel contractions (Yao and Lind 1974) and varying amounts of Wharton's jelly (Scott and Wilkinson 1978; Sloper et al. 1979). The cord mercury concentration is therefore usually expressed the result in terms of dry weight (Dalgård et al. 1994; Akagi et al. 1998).

The most frequently used sample for methylmercury exposure assessment is scalp hair, especially in field studies (Grandjean et al. 2002). Sampling of hair is noninvasive and painless, and it is a feasible and efficient procedure under most field study conditions. Depending on the rate of hair growth, the mercury concentrations along the hair shaft can represent a calendar of past exposures. Yet, environmental mercury vapor may bind to the hair (Yamaguchi et al. 1975), while hair permanent treatments can remove much of the endogenous mercury from the hair (Yamamoto and Suzuki 1978; Yasutake et al. 2003). Also, hair color or structure may affect the incorporation of mercury into the hair (Grandjean et al. 2002).

The blood concentration is generally considered the appropriate indicator of the absorbed dose and the amount systemically available. This biomarker is also subject to possible variation. Methylmercury binds to hemoglobin, and the high affinity to fetal hemoglobin results in a higher mercury concentration in cord blood than in maternal blood (Sakamoto et al. 2004). Further, whole-blood mercury concentrations are affected by the hematocrit, and some researchers therefore prefer to measure the mercury concentration in erythrocytes (Sakamoto et al. 2004), although this procedure is more cumbersome. Routine analyses for total mercury concentrations also include inorganic mercury, but cord blood mercury is almost entirely on the methylated form, for which the placenta does not constitute a barrier (Kelman et al. 1982).

In the absence of a gold standard, statistical correlations can be used to ascertain interrelationships between biomarkers. However, all biomarkers are subject to imprecision, and such data will not provide the validation desired. Factor analysis may be used to determine the total imprecision, i.e., the combination of laboratory imprecision and preanalytical variation, of each biomarker (Budtz-Jørgensen et al. 2003). The predictive validity of the biomarkers may also be assessed from their associations with known outcome variables (Grandjean et al. 1999). An extended analysis can be carried out using a structural equation model, where confounders and effect variables are included with adjustment for imprecision (Budtz-Jørgensen et al. 2002).

We determined the cord mercury concentration as an exposure biomarker in comparison with more commonly used biomarkers of prenatal methylmercury exposure from maternal seafood consumption. The cord tissue samples were obtained along with cord blood and maternal hair in connection with a prospective birth cohort study initiated in the Faroe Islands (Grandjean et al. 1992). The children were examined in regard to possible developmental neurotoxicity effects at age 7 years (Grandjean et al. 1997), and the exposure biomarkers could therefore also be compared in regard to their predictive validity.

Methods

Cohort formation and biomarker analyses

A birth cohort of 1022 subjects was formed from consecutive births between 1 March, 1986 and the end of 1987 at the three Faroese hospitals (Grandjean et al. 1992). In connection with each birth, we collected umbilical cord tissue, cord blood, and maternal hair. The study was carried out in accordance with the Helsinki convention and with the approval of the ethical review committee for the Faroe Islands and the institutional review board in the US.

Cord blood and maternal hair were analyzed in connection with the cohort formation (Grandjean et al. 1992). Because the full hair length corresponding to the complete pregnancy duration had originally been analyzed, we also determined the mercury concentration in the proximal 2-cm segment as an indication of methylmercury exposure during the third trimester (Grandjean et al. 2003). For some cohort members, one or more specimens were not available, and some hair samples were sufficient only for the full-length analysis. The analytical quality data suggested a highly acceptable imprecision of approximately 5%.

In regard to the analysis of cord tissue, the wet weight of the sample was determined before freeze-drying for 48 hours, followed by assessment of the dry weight. Acid mineralization took place in a microwave oven, and the mercury analysis was performed by flow-injection cold-vapor atomic absorption spectrometry (FIMS-400 and AS-90, Perkin-Elmer, Wellesley, MA, USA). A total of 447 samples were available for analysis. Quality assurance results suggested that an imprecision of this analysis similar to the one for the other biomarkers. The cord water content of the cord was mostly about 85-90%, with a total range of 62% to 95%. In 10 split samples, the wet-weight-based mercury concentration showed an average CV of 17%, while concentrations in previously analyzed split freeze-dried samples showed an average CV of 4 % (Dalgård et al. 1994), i.e., similar to the normal laboratory error.

Clinical follow-up

Follow-up of this cohort included an extensive neurobehavioral examination at age 7 years, where five main outcome tests were selected to represent different brain functions (Grandjean et al. 1997): Finger Tapping with the preferred hand (motor speed); Continued Performance Test reaction time (attention); Bender Visual Motor Gestalt Test (visuospatial); Boston Naming Test (language); and California Verbal learning test – Children short-term reproduction (verbal memory). Based on the associations

with exposure biomarkers, the main effects were seen in attention and language, with lesser impact on motor speed, verbal memory, and visuospatial performance.

Statistical analysis

Following descriptive analyses, logarithmic transformations were used for mercury concentrations that showed skewed distributions, and geometric means were calculated. Interrelationships between the transformed exposure biomarkers were determined by correlation coefficients.

A structural equation model analysis was then carried out using only the exposure biomarkers (Budtz-Jørgensen et al 2002). In a structural equation model, each of these markers (M-Hg) was assumed to be manifestations of the true (unobserved) exposure (Hg):

$$\log(M\text{-}Hg) = \alpha_m + \lambda_m \log(Hg) + \varepsilon_m$$

We chose to express the true exposure on the scale of the cord blood concentrations. Thus, the factor loading (λm) is fixed at 1 for this biomarker, and the intercept (αm) is zero. In an additional equation, Hg was assumed to depend on the frequency of maternal pilot whale dinners during pregnancy, as indicated by a dietary questionnaire.

In this type of analysis, measurement errors (εm) in different markers are usually assumed to be independent. However, we anticipated dependence between error terms in the two hair measurements and between errors in the cord-based measurement. To adjust for such local dependence, we allowed εm for the three cord measures to be associated, and likewise we introduced correlation between the εm terms for the two hair concentrations.

In this analysis, error standard deviations in natural log-transformed variables can be interpreted as error CVs in the untransformed concentrations. In addition, meaningful comparisons of the biomarkers can be obtained from their estimated correlations with the true exposure.

Children with incomplete information on the five exposure variables were included in a missing data analysis based on the maximum likelihood principle (Little and Rubin 1987). Compared to standard complete case analysis, this approach is more powerful and less likely to yield biased results. Under the usual assumption that the likelihood ratio test statistic follows a $\chi 2$ distribution, the hypothesis of pairs of error terms being of similar size can be tested.

Using the main outcomes at age 7 years, we then carried out multiple regression analyses that included the same set of confounders that was originally selected (Grandjean et al. 1997). Instead of the cord blood mercury concentration (Grandjean et al. 1999; Budtz-Jørgensen et al. 2002), we now used a cord tissue mercury concentration as the exposure variable. The mercury effect is expressed in terms of the change in the response variable relative to the standard deviation of the response that was associated with a doubling in the mercury concentration (Grandjean et al. 1999).

Using the main outcomes at age 7 years, we then carried out multiple regression analyses that included the same set of confounders that was originally selected (Grandjean et al. 1997). Instead of the cord blood mercury concentration (Grandjean et al. 1999; Budtz-Jørgensen et al. 2002), we now used a cord tissue mercury concentration as the exposure variable. The mercury effect is expressed in terms of the change in the response variable relative to the standard deviation of the response that was associated with a doubling in the mercury concentration (Grandjean et al. 1999).

Results

All exposure biomarkers showed wide ranges, where the highest concentration approached 1000-fold the lowest (Table 1). The medians were very close to the geometric means. The correlations between the biomarkers showed that mercury concentrations in cord tissue and cord blood were closely associated, as were the two hair parameters (Table 2). Overall, the dry-weight cord measurement showed stronger correlations with other mercury biomarkers than the wet-weight concentration.

Table 1 Geometric means, 25^{th}-75^{th} percentiles, and total ranges of prenatal methylmercury exposure biomarkers used in a Faroese birth cohort

Exposure biomarker	N	Geometric Mean	Interquartile Range	Total Range
Cord blood (μg/L)	996	22.35	13.1-40.4	0.90-351
Cord (μg/g dry weight)	447	0.210	0.132-0.36	0.000-1.28
Cord (μg/g wet weight)	422	0.0249	0.0149-0.044	0.0024-0.23
Full length hair (μg/g)	1019	4.17	2.52-7.7	0.17-39.1
Proximal hair (μg/g)	683	4.46	2.76-14.6	0.34-40.5

Table 2 Pair-wise correlation coefficients for logarithmic transformations of biomarkers of prenatal methylmercury exposure used in a Faroese birth cohort

	Cord blood	Cord (dry)	Cord (wet)	Hair (full-length)	Hair (proximal)
Cord (dry)	0.940	1			
Cord (wet)	0.907	0.942	1		
Hair (full-length)	0.784	0.732	0.690	1	
Hair (proximal)	0.837	0.781	0.730	0.926	1

The structural equation model provided an excellent fit to the data ($p = 0.46$ for difference between observed and predicted values). The cord blood measurement was the most precise exposure marker, and the dry-weight cord tissue measure was only slightly inferior, as reflected by the correlations with the true exposure (Table 3). The imprecision of the cord-blood concentration was less imprecise than that of the other exposure biomarkers ($p < 0.05$). An additional pair-wise comparison showed that the dry-weight-based cord tissue concentration also had a lower imprecision than the wet-weight parameter ($p < 0.05$). Separate analyses were carried out with different selections of biomarkers with and without adjustment for local dependence. The results obtained were very similar to those shown in Table 3, thus supporting the robustness of the model. Likewise, exclusion of outliers changed the results only minimally, although the imprecision of the cord tissue analysis decreased slightly.

Table 3 Factor loading (λ), standard deviation of the error term (ε) and correlation to the estimated true exposure calculated for five biomarkers of prenatal methylmercury exposure

Biomaker sample	Factor loading	Error standard deviation	Correlation to truth
Cord blood	(1)	0.29	0.94
Cord (dry)	0.89	0.33	0.91
Cord (wet)	0.87	0.40	0.87
Hair (full-length)	0.84	0.45	0.83
Hair (proximal)	0.88	0.37	0.89

Regression analyses were then carried out to compare the predictive validity of the exposure biomarkers in regard to adverse effects on neurobehavioral development at age 7 years. The regression coefficients (Table 4) for cord tissue concentrations generally showed results similar to those previously obtained for cord blood (Grandjean et al. 1999), although some are based on much smaller cohort subgroups with complete data for the cord tissue biomarkers. For four out of five outcome

variables, the cord concentration measured in terms of dry weight appeared to be a better predictor than the one expressed in regard to the wet weight.

Table 4 Numerical change (expressed as percent of the standard deviation) in five different response variables associated with a doubling in cord-tissue mercury concentrations after adjustment for confounders. For comparison, data for cord blood are also shown (Grandjean et al., 1999). The direction of all effects is toward increasing deficit at higher exposures.

	Cord tissue				Cord blood	
	Dry weight		Wet weight			
Response	N	Bate (p)	N	Beta (p)	N	Beta (p)
Motor speed	411	3.00 (0.47)	388	1.38 (0.74)	820	5.37 (0.05)
Attention	89	29.6 (0.01)	72	27.3 (0.03)	390	15.9 (<0.0001)
Visuospatial	406	1.70 (0.66)	384	1.63 (0.69)	818	3.83 (0.15)
Language	402	11.3 (0.006)	379	10.1 (0.01)	791	10.5 (<0.0001)
Verbal memory	392	7.45 (0.08)	370	8.04 (0.07)	797	6.64 (0.019)

Calculations of cord-blood based BMDLs suggested a level of 58 μg/L, and with an uncertainty factor of 10, the resulting 5.8 μg/L blood was considered an appropriate reference dose (exposure limit), which corresponds to a daily methylmercury intake of 0.1 μg/kg body weight (NAS, 2000). However, because cord-blood mercury is an imprecise biomarker of fetal exposure to methylmercury, adjustment for an imprecision of about 25% is needed. It results in a BMDL of 43 μg/L (Table 5). In addition, cord blood contains a higher methylmercury concentration than does maternal blood. By comparison of hair/blood ratios (Grandjean et al. 1992), cord blood seems to contain about 33% more mercury. While even higher ratios have also been reported (Sakamoto et al 2004), we assumed that the cord-blood concentration is 50% higher than the one in maternal blood. Because of these two factors, the exposure limit should correspond to a blood concentration of 2.9 μg/L, which is half of the exposure limit calculated by the NAS (2000). The similar JECFA (2003) calculations were based on BMDLs expressed in terms of the hair-mercury concentrations. Because of the greater imprecision of hair-based BMDLs, adjustment causes a larger decrease. This adjustment takes into account some of the uncertainty of the hair:blood mercury concentration ratio. Because detailed date are available from the Faroes data only, adjusted JECFA calculations were done using this data set. The results of the two sets of calculations are similar (Table 5).

Table 5 Calculation of exposure limits for methylmercury using benchmark dose levels (BMDLs) based on exposure biomarkers and adjusted for statistical imprecision.

	NAS[1]	Updated	JECFA[2]	Updated
BMDL				
Maternal hair (μg/g)	-	-	12	6[3]
Cord blood (μg/L)	58	43[3]	-	-
Maternal blood (μg/L) [4]	-	29	48	24
Uncertainty factor				
Hair-to-blood ratio	-	-	2	1.5
Individual vulnerability	-	-	3.2	6.4
Total	10	10	6.4	10
Exposure limit (μg/L blood)	5.8	2.9	7.5	2.4
Converted to μg/kg*d	0.10	0.05	0.15	0.04

[1] NAS (2000); [2] JECFA (2003), using Faroes data only; [3] BMDL from Budtz-Jørgensen et al. (2004); [4] Converted by using average hair-blood mercury concentration ratios.

Discussion

An imprecise exposure assessment will tend to underestimate the true effect of the exposure and may also complicate confounder adjustment (Carroll 1998). Validation of exposure biomarkers therefore is a key to environmental epidemiology studies. However, even superb laboratory repeatability results cannot substantiate the validity of a biomarker in regard to a causative exposure and the associated disease risk. A valid exposure marker must reflect the actual exposure, which is usually unknown.

The present study has employed different statistical strategies to explore this issue. The results show that analysis of cord blood or cord tissue is likely to provide better precision than maternal hair. Our previous application of structural equation models showed that the imprecision in hair-mercury analyses is substantial and can result in underdetermination of neurotoxic impacts of methylmercury exposures (Grandjean et al. 2003). Other authors have shown a highly scattered association between maternal hair-mercury concentrations and subsequent mercury concentrations in the child's brain

obtained at autopsy (Huang et al. 2003). These data are in accordance with the measurement error for the hair-mercury parameter found in the present study using a structural equation model. Furthermore, the regression coefficients obtained from using the two cord mercury parameters as exposure variable approximate the results obtained for cord blood (Grandjean et al. 1997; 1999). Similar results were found using the similar outcome data from the recently completed 14-year examinations (Debes et al. 2006).

Given the large imprecision of the hair-mercury parameter and its known variation with hair type and hair color (Grandjean et al. 2002), a better exposure biomarker for prenatal methylmercury is desirable. Cord blood has been recommended as the best available parameter (National Research Council 2000). The umbilical cord offers advantages because it is easy to sample by non-invasive means, the tissue otherwise being discarded after parturition. The cord is formed mainly during the second and third trimesters, and it reaches two thirds of its full length already by the end of the second trimester (Kaufmann and Scheffen 1998). Assuming a biological half-life of about 45 days for methylmercury (Smith and Farris 1996), the cord-mercury concentration is likely to represent a measure of the average mercury burden during the third trimester. It will likely be less sensitive to short-term changes than will the cord-blood mercury concentration. Because of variations in the content of blood and Wharton's jelly, the dry-weight based mercury concentration would seem to be a more precise parameter than the level expressed on a wet-weight basis.

The analytical reproducibility data document that the dry-weight based mercury concentration is more precise than the one expressed on a wet-weight basis. Although these laboratory comparisons were based on the intraindividual variability, the interindividual variation in water content is probably greater. In agreement with this finding, the structural equation model shows that the dry-weight cord parameter has a better correlation to the true mercury exposure. Likewise, the predictive validity in regard to neurobehavioral deficits as age 7 years also favors the dry-weight biomarker.

The findings on biomarker imprecisions also need to be considered in light of the literature on methylmercury neurotoxicity. The fact that all exposure biomarkers are much more imprecise than suggested by laboratory quality data suggests that dose-effect relationships may have been underestimated, not only in the Faroes cohort (Grandjean et al. 2003). Substantial imprecision of an exposure parameter also means that inclusion of confounders in the regression analysis may add to the bias toward the null hypothesis (Budtz-Jørgensen et al. 2003).

Other pollutants in seafood, such as polychlorinated biphenyls (PCBs) may also affect the neurobehavioral outcomes (Grandjean et al. 2001) and may also be measured with substantial imprecision. However, structural equation modeling has shown that, even if substantial imprecision is assumed in regard to the Faroese data, PCB exposure does not explain the mercury-associated deficits (Budtz-Jørgensen et al. 2002). Also, as expected for a persistent pollutant as PCB, this exposure is more closely associated with the hair-mercury concentration as a long-term measure of seafood intake, although this marker is clearly inferior to the cord-blood concentration as a marker of methylmercury exposure.

The issue of biomarker imprecision is crucial in regard to dose-response relationships and calculation of exposure limits. Neither of the two major risk assessments carried out by the National Academy of Sciences (2000) and the World Health Organization (JECFA, 2003) considered this factor. In addition, the two reports differed in several less important respects. For example, NAS included three prospective cohort studies, while JECFA excluded one of them. The NAS chose a 'most sensitive' effect, while JECFA chose an 'average'. The NAS applied an uncertainty factor of 10 (individual differences and incomplete data base), while JECFA used 3.2 (for differences in kinetics) and an additional factor of 2 for the blood-hair mercury concentration ratio. Overall, the JECFA limit is higher than the one calculated by NAS, but these two approaches are so similar that EFSA (2004) refrained from choosing one above the other. However, inclusion of measurement imprecision results in a limit that is 50% below the one calculated by the NAS.

The findings of this study support the use of cord blood as the best available exposure biomarker for methylmercury. Cord tissue is clearly an appropriate alternative, especially when the mercury concentration is measured in relation to the dry weight. When proxy exposure parameters (such as exposure biomarkers) are applied adjustment for imprecision should always be considered.

Acknowledgments

This study was supported by the U.S. National Institute of Environmental Health Sciences (ES09797) and the Danish Medical Research Council. The contents of this paper are solely the responsibility of the authors and do not represent the official views of the NIEHS, NIH or any other funding agency. We gratefully acknowledge the technical support by B Andersen. The present paper is an updated version of Grandjean et al. (2005).

References

Akagi H, Grandjean P, Takizawa Y, Weihe P. 1998. Methylmercury dose estimation from umbilical cord concentrations in patients with Minamata disease. Environ Res 77:98-103.

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

Budtz-Jørgensen E, Keiding N, Grandjean P, Weihe P, White RF. 2003. 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 95:385-393.

Budtz-Jørgensen E, Keiding N, Grandjean P. 2004b Effects of exposure imprecision on estimation of the benchmark dose. Risk Anal 24: 1689-96.

Carroll RJ. 1998. Measurement error in epidemiologic studies. In: Armitage, P., Colton, T. (Eds.), Encyclopedia of biostatistics. Wiley, Chichester, pp. 2491-2519.

Crump KS. 1995. Calculation of benchmark doses from continuous data. Risk Anal 15: 79-89.

Dalgård C, Grandjean P, Jørgensen PJ, Weihe P. 1994. Mercury in the umbilical cord: Implications for risk assessment for Minamata disease. Environ Health Perspect 102:548-550.

Daniels, JL, Longnecker MP, Rowland AS, Golding J, The ALSPAC Study Team. 2004. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology 15:394-402.

Debes F, Budtz-Jørgensen E, Weihe P, White RF, Grandjean P. 2006. Impact of prenatal methylmercury toxicity on neurobehavioral function at age 14 years. Neurotoxicol Teratol (in press).

European Food Safety Authority. 2004. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to mercury and methylmercury in food (EFSA-Q-2003-030). Brussels. http://www.efsa.eu.int/science/contam/contam_opinions/259_en.html (accessed 12 March, 2006).

Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E, Videro T. 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch Environ Health 47:185-195.

Grandjean P. 1995. Biomarkers in epidemiology. Clin Chem 41: 1800-1803.

Grandjean P, Brown S, Reavey P, Young D. 1994. Biomarkers of chemical exposure: state of the art. Clin Chem 40:1360-1362.

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, Budtz-Jørgensen E, White RF, Jørgensen PJ, Weihe P, Debes F, Keiding N. 1999. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. Am J Epidemiol 150:301-305.

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, Budtz-Jørgensen E, Keiding N, Weihe P. 2003. Underestimation of risk due to exposure misclassification. Eur J Oncol Suppl. 2:165-172.

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.

Grandjean P, Budtz-Jørgensen E, Jørgensen PJ, Weihe P. 2005. Umbilical cord mercury concentration as biomarker of prenatal exposure to methylmercury. Environ Health Perspect 113: 905-8.

Harada M. 1977. Mental deficiency due to methylmercury poisoning. (English summary) Brain and Development 6:378-387.

Hislop JS, Collier TR, White GP, Khathing D, French E. 1983. The use of keratinised tissues to monitor the detailed exposure of man to methylmercury from fish. In: “Clinical Toxicology and Clinical Chemistry of Metals” (Brown SS, Ed.). New York: Academic Press, pp.145-148

Huang LS, Cox C, Wilding GE, Myers GJ, Davidson PW, Shamlaye CF, Cernichiari E, Sloane-Reeves J, Clarkson TW. 2003. Using measurement error models to assess effects of prenatal and postnatal methylmercury exposure in the Seychelles Child Development Study. Environ Res 93:115-122.

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 March, 2006)

Kaufmann P, Scheffen I. 1998. Placental development. In: Polin RA, Fox WW, eds. Fetal and Neonatal physiology, 2nd ed, vol. 1. Philadelphia: Saunders, pp.59-70.

Kelman BJ, Walter BK, Sasser LB. 1982. Fetal distribution of mercury following introduction of methylmercury into porcine maternal circulation. J Toxicol Environ Health 10:191-200.

Little RJA, Rubin DB. 1987. Statistical analysis with missing data. Wiley, New York.

Marshall JR. Methodologic and statistical considerations regarding use of biomarkers of nutritional exposure in epidemiology. J Nutr 133 Suppl 3:881S-887S.

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

Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, Satoh H. 2004. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ Sci Technol 38:3860-3863.

Scott JM, Wilkinson R. 1978. Further studies on the umbilical cord and its water content. J Clin Pathol

31:944-948.

Sherlock J, Hislop J, Newton D, Topping G, Whittle K. 1984. Elevation of mercury in human blood from controlled chronic ingestion of methylmercury in fish. Human Toxicol 3:117-131.

Sloper KS, Brown RS, Baum JD. 1979. The water content of the human umbilical cord. Early Hum Dev; 3:205-210.

Smith JC, Farris FF. 1996. Methyl mercury pharmacokinetics in man: a reevaluation. Toxicol Appl Pharmacol 137:245-252.

Yamaguchi S, Matsumoto H, Kaku S, Tateishi M, Shiramizu M. 1975 Factors affecting the amount of mercury in human scalp hair. Am J Publ Health 65:484-488.

Yamamoto R, Suzuki T. 1978. Effects of artificial hair-waving on hair mercury values. Int Arch Occup Environ Health 42:1-9.

Yao AC, Lind J. 1974. Placental transfusion. Am J Dis Child 127:128-141.

Yasutake A, Matsumoto M, Yamaguchi M, Hachiya N. 2003. Current Hair Mercury Levels in Japanese: Survey in Five Districts. Tohoku J Exp Med 199:161-169.

Effects of Breast Feeding on Neuropsychological Development in a Community with Methylmercury Exposure from Seafood

Tina Kold Jensen[1], Philippe Grandjean[1,2], Esben Budtz Jørgensen[3], Roberta F. White [4], Froði Debes[1,5] and Pál Weihe[5]

[1] Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Winslowparken 17, DK-5000 Odense, Denmark

[2] Department of Environmental Health, Harvard School of Public Health, Landmark 3-110E, 401 Park Drive, Boston, MA 02215 USA

[3] Department of Biostatistics, Institute of Public Health, University of Copenhagen, Øster Farimagsgade 5B, 1014 Copenhagen K, DK-2200 Copenhagen, Denmark

[4] Department of Environmental Health, Boston University School of Public Health, 715 Albany Street, Talbot Building, Boston, MA 02118 USA

[5] Department of Occupational and Environmental Health, The Faroese Hospital System, Sigmundargota 5, PO Box 14, FO-100 Tórshavn, Faroe Islands

Breastfeeding has been associated with an advantage to infant neurobehavioral development, possibly in part due to essential nutrients in breast milk. However, breast milk may be contaminated by environmental neurotoxicants, such as methylmercury. In the Faroe Islands, where maternal consumption of pilot whale may cause transfer of marine toxicants into breast milk, a cohort of 1022 consecutive singleton births was generated during 1986–1987.

Methylmercury exposure was assessed from mercury concentrations in cord blood and in the hair of the child at age 12 months, and the duration of breastfeeding was recorded. At approximately 7 years of age, 917 (90%) of the children underwent detailed neurobehavioral examination. After adjustment for confounders, breastfeeding was associated with only marginally better neuropsychological performance on most tests. These associations were robust even after adjustment for cord-blood and hair mercury concentration at age 1 year. Thus, in this cohort of children with a relatively high prenatal toxicant exposure and potential exposure to neurotoxicants through breast milk, breastfeeding was associated with less benefits on neurobehavioral development than previously published studies though not associated with a deficit in neuropsychological performance at age 7. In conclusion, although the advantage may be less, Faroese women can still safely breastfeed their children.

Effects of breast feeding on neuropsychological development in a community with methylmercury exposure from seafood

Tina Kold Jensen, Philippe Grandjean,
Esben Budtz Jorgensen, Roberta F White
and Pal Weihe
University of Southern Denmark,
Department of Environmental Medicine

Breastfeeding benefits for the child

- Breastfeeding reduces the risk for a wide range of infectious diseases
- Good evidence that breastfeeding reduces the risk of asthma and allergy, obesity, cancer and diabetes
- Positive influence on attachment and behavior

Breastfeeding benefits for the child

- Breastfeeding is also associated with an advantage in subsequent mental development
- A meta-analysis adjusted for appropriate key cofactors showed an adjusted benefit of 3.16 IQ points for breastfed compared with formula-fed children
- Children with low birth weight exhibited larger benefits
- **Residual social confounding possible**

Breastfeeding benefits for the mother

- Less postpartum bleeding
- Earlier return to pre-pregnancy weight
- Improved bone strength
- Reduced risk of ovarian and premenopausal breast cancers

Background

- Among the essential nutrients in human milk of particular importance for brain development, long-chain polyunsaturated fatty acids are thought to be of special relevance
- For these fatty acids, seafood is an important source, and breastfeeding could therefore be particularly advantageous in fishing communities

Possible adverse effects of breastfeeding

- Breast milk may be contaminated by environmental pollutants of which some are neurotoxic
- The human brain is particularly sensitive to toxicity during gestation and infancy

Problems

- With essential nutrients and neurotoxicants originating from the same food items, the resultant effect of breastfeeding may be difficult to predict
- The toxicants may also have been transferred to the fetus through placenta in utero, thereby affecting both prenatal and postnatal development

Recommendation

- The World Health Organization has extended its recommendation of the length that the infant should be breastfed exclusively from 4 to 6 months or longer
- It this also safe in high exposed populations?

The Faroes

- The population in the Faroe Islands consumes pilot whale meat and/or blubber which is shared in the communities where the whales are caught
- This Nordic community (45,000 inhabitants) has large variations in seafood intake but limited social differences (limited confounding)

The Faroese cohort 1

- All singleton births from 1 Marts 1986 to the end of 1987 at all three Faeroese hospitals
- Sample of 1022 included (75.1% of all births)
- 9 dead before examination
- Examined in 1993-94 at age 7 years
- 917 completed examination (90.3% of eligible children)
- 7 excluded because of neurological diseases unrelated to mercury exposure
- 910 included in the final analyses

Neuropsychological tests

- Neurobehavioral Evaluation System (NES) Finger Tapping Test (manual motor speed)
- NES Hand-Eye Coordination Test (manual coordination)
- NES Continuous Performance Test, missed responses and reaction time (vigilance/attention)
- Bender Visual Motor Gestalt Test, copy and recall, with Göttingen scoring system (visuospatial function and non-specific measure of brain damage)

Neuropsychological tests

- Wechsler Intelligence Scale for Children – Revised (WISC-R) Digit Spans (forward only) (attention and tracking)
- WISC-R Similarities (reasoning and cognitive flexibility)
- WISC-R Block Designs (visuospatial organization and reasoning)
- California Verbal Learning Test (children) (short-term memory)
- Boston Naming Test (language)

Exposure data

- Mercury concentration in cord blood (N=894)
- Maternal hair mercury at delivery (N=914)
- Child hair mercury at 12 months (N=527) and at 7 years (N=903)
- PCB measured in 436 stored samples of cord tissue from the 443 children examined in 1993

Breastfeeding data

- Visiting district health nurses filled in a questionnaire on development and information on total months of breastfeeding and months on breastfeeding exclusively (N=572)
- Mothers filled in a questionnaire about breastfeeding at examination 7 years later
- District nurse information overruled mothers when both were available (agreed +-1 in 56% and 74%)(N=889)

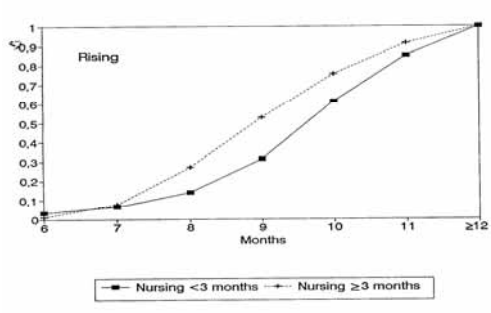
Statistical analyses

- Multiple linear regressions for each of the neuropsychological response variables
- Total number of months of breastfeeding square root transformed
- Mercury data transformed by use of logarithm
- Number of missed responses on the Continuous Performance Test converted to the natural logarithm of the score +1
- Block Designs score transformed to the square root of the score +1

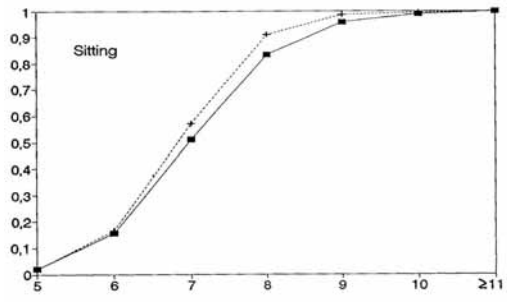
Confounders in all analyses

- Sex and age of the child, medical risk factors
- Mothers Raven score, mother and father professionally trained, father employed at time of examination
- Child in day-care, year of examination
- In NES tests: familiarity with computer games and CPT analysis only for examination year 1993
- Analyses were performed both with and without adjustment for mercury concentration in cord blood/hair at 12 months

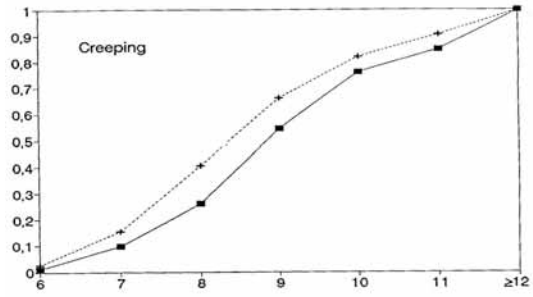
Cumulated distribution of the age at which Faroese infants were able to rise to a standing position, as related to duration of breastfeeding



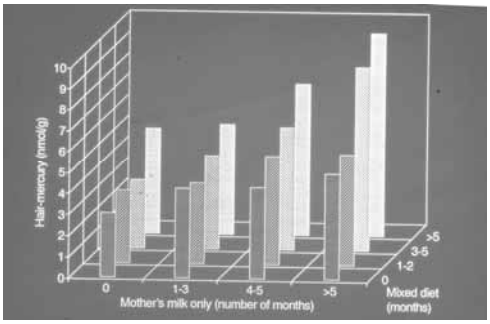
Cumulated distribution of the age at which Faroese infants were able to sit without support, as related to duration of breastfeeding



Cumulated distribution of the age at which Faroese infants were able to creep, as related to duration of breastfeeding



Mercury concentration (mean) in hair of 12-months-old infants in relation to the length of the nursing period



Results

Increased hair mercury *at 12 months* was especially related to poor performance at age 7 on:

- NES finger tapping
- NES Continuous Performance Test
- Boston Naming Test

Could this be due to a latent toxic effect of breastfeeding which becomes apparent as the nervous system matures?

Results

- 8% not breastfed, 56% fully breastfed more than 3 months and 55% breastfed more than 6 months in total
- Longer breastfeeding was associated with maternal Raven score, parental school and professional education, maternal smoking during pregnancy and child's medical risk factors

Adjusted test results for 905 children

Test	Breastfeed exclusively >4 months	Increase for each month of total breastfeeding
NES 2 hand-eye coordination Error score	0.006	-0.001
NES 2 continuous performance test Reaction time	8.08	0.26
Block design	0.17*	0.004
Boston naming test with cues	0.81*	0.05*

Results

- Stronger effect of breastfeeding expected if adjusted for mercury exposure because of their negative effects
- Results not affected apart from Boston naming
- Still longer reaction time in NES2 Continuous Performance test

Advantages

- Large and intensive study
- High participation rate
- Homogeneous society with low alcohol intake in pregnancy
- Large exposure intervals

Possible shortcomings

- Possible residual confounding by social factors (not likely) or overadjustment
- Very few women did not breastfeed
- Imprecision in assessment of duration of breastfeeding and in potential exposure assessment
- PCB exposure

Conclusion

- In this cohort of children with a relatively high prenatal mercury exposure and potential exposure through breast milk, breastfeeding was not associated with any deficit in neuropsychological performance at age 7
- Breastfeeding appeared not as beneficial as previously reported by other investigators in non exposed populations

Conclusion

- Women can still be advised to breastfeed their children even in this community with relatively high exposures to pollutants
- However, newly published results have found a negative effect of breastfeeding on early postnatal growth in children with high exposure to methylmercury, and it therefore remains to be determined whether breastfeeding should be limited at high contamination levels

Future studies

- Three generation studies of reproduction
- PCB and mercury
- Sex ratio
- Semen quality
- Time to pregnancy
- Pregnancy outcome

"He has been a strong boy since infancy. He breastfed until he was two years old"

84-year-old Klavdiya Valisyevna Yeltsina, on her son, Russian president Boris Yeltsin, in "Semya", Moscow" (Source: World Press Review, September 1992)

"There is no finer investment for any community than putting milk into babies"

Winston Churchill

Tohoku Study of Child Development, a Cohort Study to Examine the Effects of Perinatal Exposure to Methylmercury, PCBs or Dioxins on Child Development; the Association of Neonatal Neurobehavioral Status with Maternal Hair Mercury Concentration and Fish Intake.

Keita Suzuki[1*], Kunihiko Nakai[1], Takashi Ohba[1], Katsuyuki Murata[2], Mineshi Sakamoto[3], Hiroshi Satoh[1]

[1]*Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine*

[2]*Department of Environmental Health Sciences, Akita University School of Medicine*

[3]*Department of Epidemiology, National Institute for Minamata Disease*

Abstract

Aims- We have been performing a cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) on child development. In the present study, we report the protocol of this study and some preliminary results about the association of neonatal neurobehavioral status with maternal hair mercury concentration and fish intake.

Protocol- Healthy pregnant women were registered between January 2001 and September 2003 in urban area of Tohoku district, Japan. Maternal peripheral blood, placenta, cord, cord blood and breast milk were collected for chemical analysis. Maternal hair samples were also taken for MeHg analysis. Maternal diet including the fish intake was assessed with a semi-quantitative food frequency questionnaire (FFQ). For the assessment of neurobehavioral development, Brazelton Neonatal Behavioral Assessment Scale (NBAS) was performed when children were three days old, and other test including Bayley Scales of Infant Development second edition, Fagan Test for Infant Intelligence, Kaufman Assessment Battery for Children were performed with growth of the children.

Results- 599 mother-infant pairs were registered in a cohort study. Among all subjects, analytical subjects were 529 mother-infant pairs whose data on hair mercury concentration, FFQ, NBAS and their characteristics were available. Maternal hair mercury concentration was associated with the decreased score of motor cluster. On the other hand, maternal fish intake was associated with the increased score of the same cluster. These findings suggested that the fish intake had two aspects of a potential risk and a benefit for the neonatal neurobehavioral development.

* Address correspondence to Keita Suzuki
Environmental Health Sciences, Tohoku University Graduate School of Medicine,
2-1, Seiryo, Aoba, Sendai, Miyagi 980-8575, Japan
mail (keita@mail.tains.tohoku.ac.jp)

Introduction

Negative effects of perinatal exposure to methylmercury (MeHg) and environmentally persistent organic pollutants including polychlorinated biphenyls (PCBs) on child development are great concern worldwide (Nakai and Satoh, 2002). There have been reports on the association of prenatal MeHg exposure with poorer cognitive functions in New Zealand (Kjellstorm et al, 1986), Faroe Islands (Grandjean et al, 1997), and Madeira Islands (Murata et al, 1999), although the cohort study in Seychelles Islands did not observe the negative effect of prenatal MeHg exposure on the development (Davidson et al, 1998). Several epidemiological studies have also reported on the effects of perinatal exposure to PCBs on neurobehavioral development. The cohort studies in North Carolina (Rogan et al, 1986), Michigan (Jacobson et al, 1985, 1990), New York (Darvill et al, 2000; Stewart et al, 2000), The Netherlands (Patandin et al, 1999; Vreugdenhil et al, 2002), Germany (Winneke et al, 1998; Walkowiak et al, 2001) and Faroe Islands (Grandjean et al, 2001) demonstrated negative associations of perinatal exposure to PCBs with cognitive function of children. These chemicals accumulate in humans mostly through the consumption of food, especially fish and shellfish. From the nutritional perspective, fish is usually recommended for pregnant women because it is rich in nutrients such as polyunsaturated fatty acids (PUFA) essential for brain development. Therefore, from the perspective of risk assessment, these health hazard issues are particularly of importance in fish-eating populations.

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to MeHg and PCBs on child development. In the present study, we report the protocols of study and preliminary results about the association of neonatal neurobehavioral status with maternal hair mercury concentration and fish intakes.

Protocols

The protocols of the cohort study have been described previously (Nakai et al, 2004), but will be briefly outlined. The study had been performing in the urban area of Tohoku district, Japan. Healthy pregnant women were recruited with their informed consent at obstetrical wards of two hospitals. To establish an optimal study population, only infants born at full-term (36 to 42 weeks of gestation) without the congenital anomalies or diseases were included. Pregnancy and delivery should have been completed without overt signs of serious illness or complications. The study protocol has the approval of the ethical committee of Tohoku University Graduate School of Medicine. Fig.1 shows the outline of registration and sample collection. Various samples were collected for the determination of chemicals including the total mercury concentration and PCBs. For example, maternal hair samples were taken at two days after delivery from the back of head near the occipital protuberance, and the proximal three cm from the scalp were used for analysis of total mercury concentration. The total hair mercury concentration (hair Hg) was measured by cold vapor atomic absorption (Akagi, 1991) at National Institute of Minamata Diseases. To

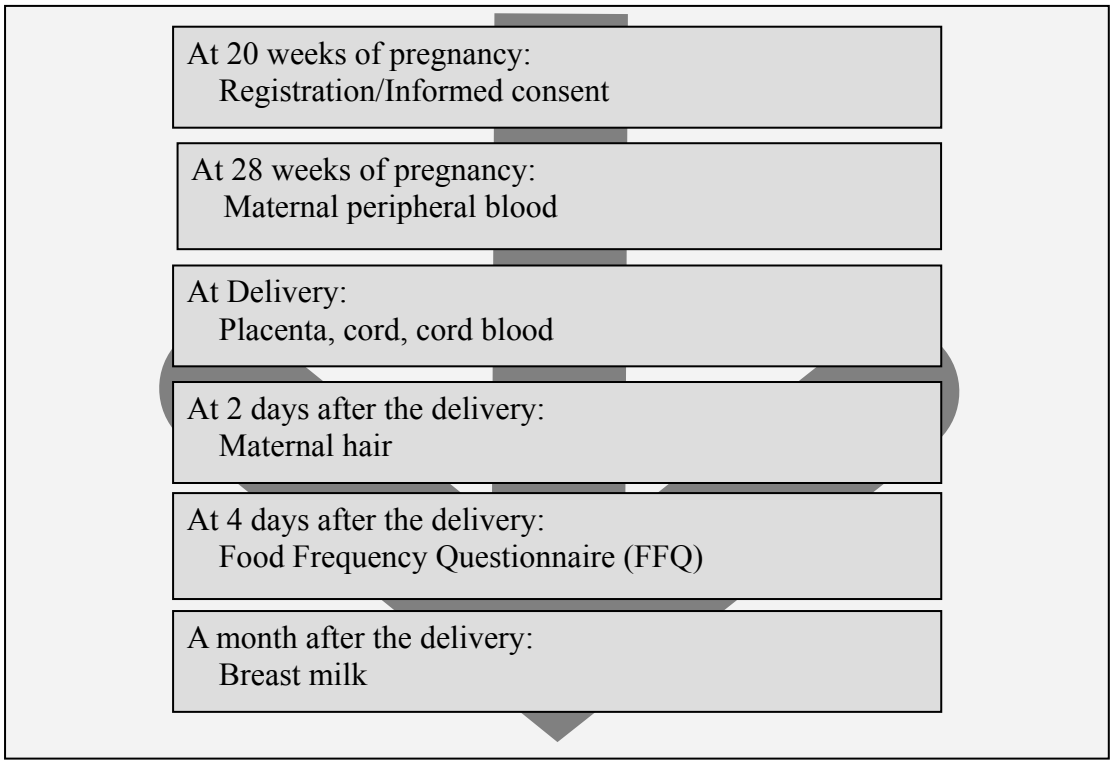


Fig.1 The registration and sample collection

estimate the maternal diet including the fish intake, the semi-quantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes (Date et al., 1996) and 13 additional items regarding fish and shellfish was administered at four days after delivery. Trained investigators showed the real size photograph of each food, then, mothers answered the frequency and the amount of the intake per meal. Fig.2 shows the outline for the assessment of neurobehavioral development. The Neonatal Behavioral Assessment Scale (NBAS) (Brazelton, 1974) was performed when children were three days old. All examiners had been trained in the NBAS training center at Nagasaki University School of Medicine, Japan. When children were seven months old, the Bayley Scales of Infant Development (BSID) (Bayley, 1993), Kyoto Scales of Psychological Development (KSPD) (Ikuzawa, 1985) and Fagan Test for Infant Intelligence (FTII) (Fagan and Shepherd, 19987) were performed. BSID and KSPD also performed when children were 18 months old. When children were 42 months old, Kaufman Assessment Battery for Children (Kaufman et al, 1983) and CATSYS (Despres et al. 2000) were performed.

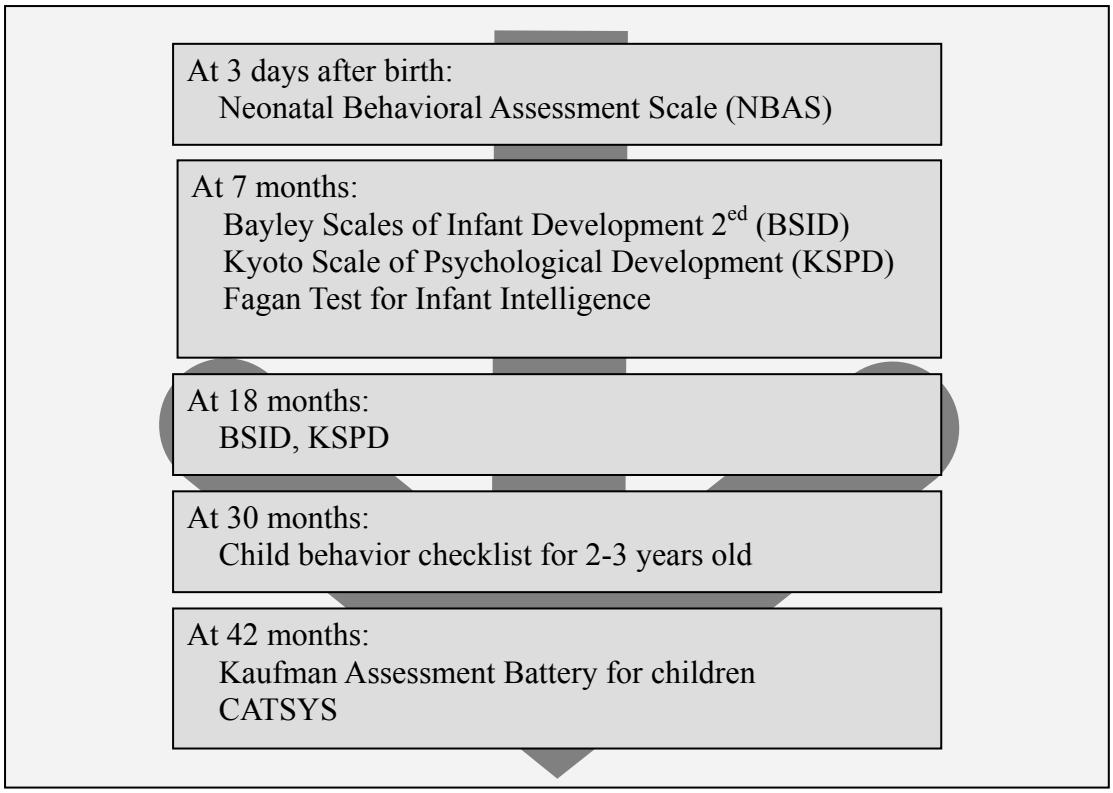


Fig.2 The measurement of neurobehavioral development

Results and Discussion

During the registration period from January 2001 to September 2003, 599 mother-infant pairs were registered in a cohort study. The follow up examination has been administrated when children are three days, seven months, 18 months, 30 months, and 42 months old, respectively, and the percentages of families who participated in each examination were always over 80% (data not shown).

Among all participants, analytical subjects were 529 mother-infant pairs whose data about hair Hg, FFQ, NBAS, and their characteristics are available. In the statistical analysis, the multiple regression analyses were performed for adjustment of covariates. The two multiple regression models were made based on the fish intake. In the model 1, seven clusters of NBAS were set as a dependent variables and total hair mercury concentration, total fish intake and covariates as independent variables. In the model 2, the intake of 13 categorized fish was set as independent variables instead of total fish intake. Potential covariates were selected from maternal and neonatal characteristics (Table 1).

Table 1. Maternal and neonatal characteristics

	Mean (SD)	Min	Max
Maternal characteristics			
# Maternal age at the time of delivery	31.3 (4.3)	20.0	42.0
# Educational status (under 12y/over 13y)	128/396		
# Alcohol drinking during pregnancy (n/y)	416/113		
# Smoking (no/ceased/yes)	427/82/20		
# Delivery type (spontaneous/caesarian)	457/72		
# Parity (first/others)	269/260		
# Total energy intake (kcal/day)	1600 (642)	399	6539
Infant characteristics			
# Gender (male/female)	275/254		
# Gestational age (weeks)	39.6 (1.2)	36.0	42.0
# Birth weight (g)	3074 (330)	2412	4176
Birth length (cm)	49.0 (1.8)	44.0	55.0
Head circumference (cm)	33.5 (1.3)	28.0	37.0
# Apgar score 1m after birth	8.2 (0.7)	1.0	10.0
# Cord blood T3 (ng/ml)	0.53 (0.15)	0.28	1.82

These items were used as a covariates at a multiple regression analyses.

There were moderate to strong correlations between birth weight and head circumference (pearson's r = .57), and birth weight and birth length (pearson's r = .70). In consideration of multicollinearity, birth length and head circumference were excluded from the covariates.

Table 2 and 3 shows the distribution of total hair Hg concentration and maternal fish intakes, respectively. The mean hair Hg was 2.2 μg/g (SD1.1), and the mean total fish intake was 25.2 kg/year (SD17.1). Table 4 shows the result of model1. Maternal hair Hg was associated with the decreased score of motor cluster ($\beta = -0.32$, $p < .05$) (Fig. 3). On the other hand, total fish intake was associated with the increased score of the same cluster ($\beta = 0.23$, $p < .01$) (Fig. 4). As results of model2, hair Hg was associated with the decreased score of motor cluster ($\beta = -0.25$, $p < .01$). Silvery blue fish intake was associated with increased score of motor cluster ($\beta = 0.08$, $p < .05$) and range of state cluster ($\beta = 0.09$, $p < .01$), respectively (data not shown). The association of hair Hg with the decreased score of motor cluster was observed. It is suggested that methylmercury affects adversely neonatal neurobehavioral development. On the other hand, we found the positive association of motor cluster with total fish intake and silvery blue fish intake. It is suggested that the beneficial nutritive factors of fish intake contribute to neonatal neurobehavioral development. Indeed, silvery blue fish consisted of pacific saury, mackerel, sardine, and caperine, and it is known as rich in PUFA. These findings suggested that the fish intake had two aspects of a potential risk and a benefit for the neonatal neurobehavioral development. We will readdress this health issue after the completion of PCBs determination and when children become older.

Table 2. Total hair mercury concentration

	Mean	SD	Median	Min	Max
Maternal hair	2.20	1.14	1.95	0.29	9.35

(μg/g)

The hair sample was used 3cm from scalp.

Table 3. Maternal fish intake

	Mean	SD	Median	Min	Max
Total fish intake	25.19	17.06	21.72	0.91	143.78

(Kg/year)

Table 4. Multiple regression model[1]

	Habituation	Orientation	Motor	Range of state	Regulation of state	Autonomic stability	Reflex
Hair Hg[1]	0.29	0.31	-0.32*	0.30	-0.12	-0.01	0.47
Total fish intake[1]	-0.26	-0.03	0.23**	-0.01	0.11	-0.07	0.07

(β value)

* p < .05, ** p < .01

[1]Log transformations, $Log_{10}X$, were used on the value of hair Hg and total fish intake.

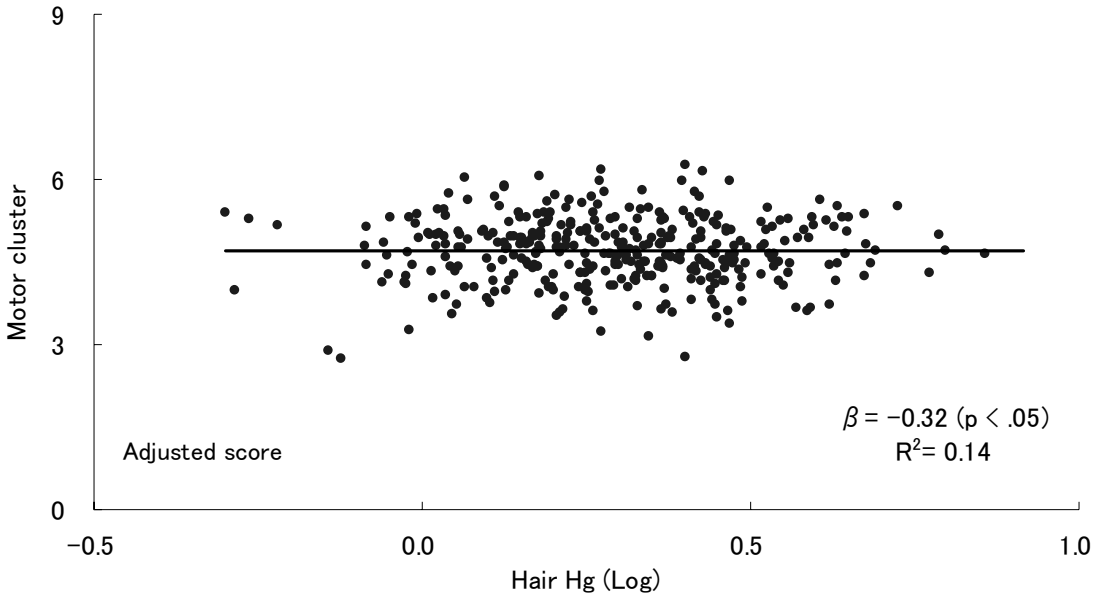


Fig. 3. The association between hair mercury and motor cluster

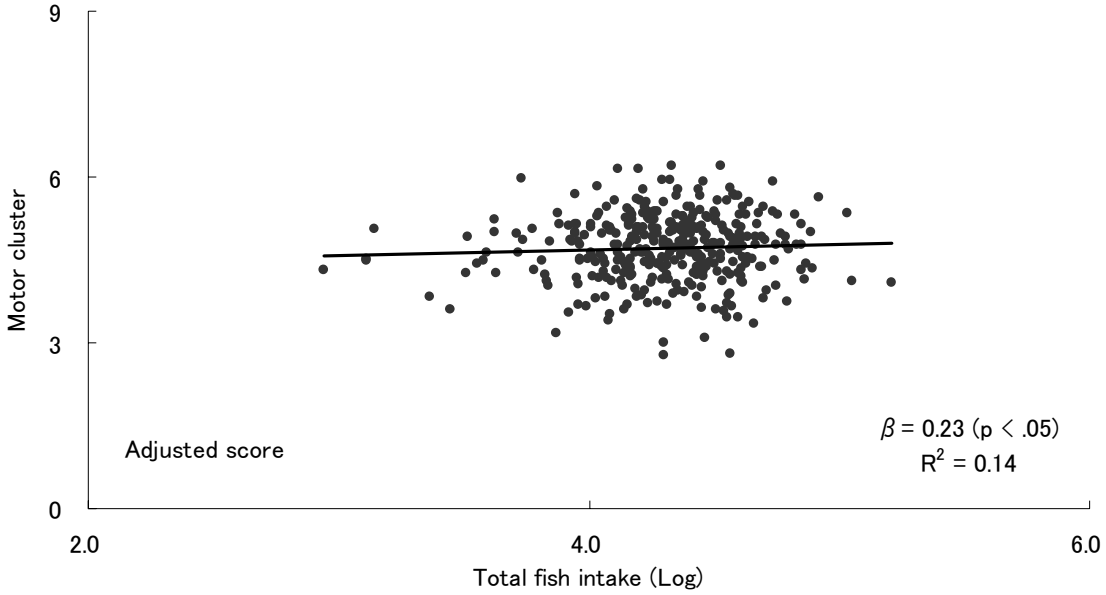


Fig. 4. The association between total fish intake and motor cluster

Acknowledgement

We thank all families who participated in the study. This research was founded by grant from the Japan Ministry of Health, Labor and Welfare, Research on Risk of Chemical Substances.

Reference

Akagi, H. and H. Nishimura (1991). Specification of mercury in the environment. Advances in mercury toxicology. T. Suzuki, N. Imura and T. W. Clarkson. New York, Plenum Press: 53-76.

Bayley, N. (1993). Bayley Scales of Infant Development 2nd ed. The Psychological Corporation, San Antonio.

Brazelton, T. B. (1973). Neonatal Behavioral Assessment Scale. Spastics International Medical Publications, Heinemann Medical, J. P. Lippincott, London Philadelphia.

Darvill, T., E. Lonky, et al. (2000). Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 21: 1029-38.

Davidson, P. W., G. J. Myers, et al. (1998). Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. JAMA 280: 701-7.

Despres, C., D. Lamoureux, et al. (2000). Standardization of a neuromotor test battery: the CATSYS system. Neurotoxicology 21: 725-35.

Fagan JF. & Shepherd PA. (1987). Fagan Test of Infant Intelligence: Training manual. Infant Corp, Cleaveland.

Grandjean, P., P. Weihe, et al. (2001). Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 23: 305-17.

Grandjean, P., P. Weihe, et al. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19: 417-28.

Ikuzawa M. (1985) Kyoto Scale of Psychological Development, Nakanishiya, Kyoto.

Jacobson, J. L., S. W. Jacobson, et al. (1990). Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol 12: 319-26.

Jacobson, S. W., G. G. Fein, et al. (1985). The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 56: 853-60.

Kaufman, A. S., M. R. O'Neal, et al. (1987). Introduction to the Kaufman Assessment Battery for Children (K-ABC) for pediatric neuroclinicians. J Child Neurol 2: 3-16.

Kjellstorm, T., P. Kennedy, et al. (1986). Physical and Mental Development of Children with Prenatal Exposure to Mercury from Fish. Stage 1: Preliminary tests at age 4. National Swedish Environmental Protection Board. report Number 3080, Solna.

Murata, K., P. Weihe, et al. (1999). Delayed evoked potentials in children exposed to methylmercury from seafood. Neurotoxicol Teratol 21: 343-8.

Nakai, K. and H. Satoh (2002). Developmental neurotoxicity following prenatal exposures to methylmercury and PCBs in humans from epidemiological studies. Tohoku Journal of Experimental Medicine 196: 89-98.

Nakai, K., K. Suzuki, et al. (2004). The Tohoku Study of Child Development: A cohort study of effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children. Tohoku Journal of Experimental Medicine 202: 227-37.

Patandin, S., C. I. Lanting, et al. (1999). Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 134: 33-41.

Rogan, W. J., B. C. Gladen, et al. (1986). Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 109: 335-41.

Stewart, P., J. Reihman, et al. (2000). Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance. Neurotoxicol Teratol 22: 21-9.

Vreugdenhil, H. J., C. I. Lanting, et al. (2002). Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 140:

48-56.

Walkowiak, J., J. A. Wiener, et al. (2001). Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 358: 1602-7.

Winneke, G., A. Bucholski, et al. (1998). Developmental neurotoxicity of polychlorinated biphenyls (PCBS): cognitive and psychomotor functions in 7-month old children. Toxicol Lett 102-103: 423-8.

Neuromotor Dysfunction in Patients with Fetal Minamata Disease

Katsuyuki Murata[1], Mineshi Sakamoto[2], Xiaojie Liu[2], Shigeru Takaoka[3], Miwako Okamoto[4], Toyoto Iwata[1], Miwako Dakeishi[1] and Komyo Eto[2]

[1] Department of Environmental Health Sciences, Akita University School of Medicine

[2] National Institute for Minamata Disease

[3] Neurology & Rehabilitation, Kyoritsu Clinic

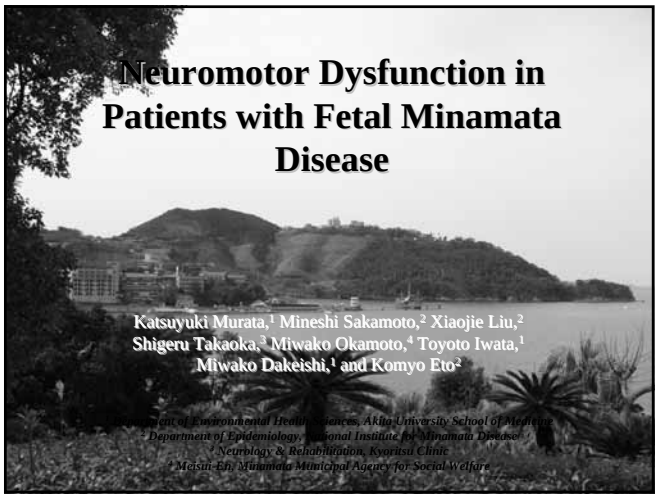
[4] Meisui-En, Minamata Municipal Agency for Social Welfare

Objectives: All children identified as suffering from the most severe form of fetal Minamata disease (i.e., methylmercury poisoning) expressed mental retardation, primitive reflexes, cerebellar ataxia, disturbances in physical growth, dysarthria, and limb deformities. The diagnosis of Minamata disease was easy in typical and severe cases, but was difficult in "mild" cases ranging from partly damaged to healthy. This study was carried out to identify the specific hand tremor to methylmercury, by using hand tremor and postural sway.

Methods: Hand tremor and postural sway with power spectral analysis (fast Fourier transform method) were assessed in 12 patients with fetal Minamata disease (mean age 49, 45~55 years; 8 males) and 28 control subjects (mean age 50, 40~56 years; 15 males), residing in Minamata.

Results: In analysis of covariance with two covariates (age and sex), hand tremor intensities at 1-6 Hz with right and left hands were significantly larger in the 12 patients than in the 28 control subjects ($P<0.05$), although the total tremor intensity, tremor intensity at 6-10 Hz or at 10-14 Hz did not differ between the two groups ($P>0.1$). Especially, the proportion of tremor intensity at 1-6 Hz was significantly higher in the patients (42.6% for right hand and 38.3% for left hand) than in the control subjects (30.7% and 33.7%, respectively). Only five patients with fetal Minamata disease could stand quietly on a platform under eyes-open condition. The transversal sway at 0-1 Hz, sagittal sway at 1-2 Hz, and sway area were significantly larger in the 5 patients than in the 28 control subjects ($P<0.05$).

Conclusions: It is suggested that neuromotor dysfunction, assessed by hand tremor and postural sway, in patients with fetal Minamata disease is characterized by a very slow sway of body.



Neuromotor Dysfunction in Patients with Fetal Minamata Disease

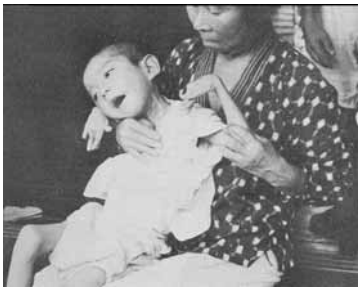
Katsuyuki Murata,[1] Mineshi Sakamoto,[2] Xiaojie Liu,[2] Shigeru Takaoka,[3] Miwako Okamoto,[4] Toyoto Iwata,[1] Miwako Dakeishi,[1] and Komyo Eto[2]

[1] Dept of Environmental Health Sciences, Akita University School of Medicine
[2] Department of Epidemiology, National Institute for Minamata Disease
[3] Neurology & Rehabilitation, Kyoritsu Clinic
[4] Meisin-En, Minamata Municipal Agency for Social Welfare

Background

Children identified as suffering from the most severe form of fetal Minamata disease (i.e., methylmercury poisoning) expressed mental retardation, primitive reflexes, cerebellar ataxia, disturbances in physical growth, dysarthria, and limb deformities. The diagnosis of Minamata disease was easy in typical and severe cases, but was difficult in "mild" cases ranging from partly damaged to healthy.

Objective



This study was carried out to identify the specific tremor to methylmercury, by using hand tremor and postural sway.

Subjects

- **Twelve registered patients with fetal Minamta disease (mean age 49, 45~55 years; 8 males), who have been admitted at the *Meisui-En* (Minamata Municipal Welfare Institute for Minamta disease patients), were examined as a case group**
- **Twenty-eight age- and sex-matched subjects (mean age 50, 40~56 years; 15 males), residing in Minamata City, were examined as a control group**
- **The study was carried out with their or guardian's informed consent and approval of the ethical committee at the Akita University School of Medicine**

Methods (1)

Hand tremor and postural sway were assessed:

One trained examiner examined hand tremor using the Neurobehavioral Test System (CATSYS 2000). Hand tremor was measured successively for each hand for 16.4 s: the subject was instructed to hold a light stylus as he/she would hold an ordinary pen, with their elbows bent at a right angle and free of body contact or any obstacles. The stylus was held horizontally, parallel to the abdomen at about 10 cm in front of the navel, and the index finger was positioned about 1 cm from the tip of the stylus.

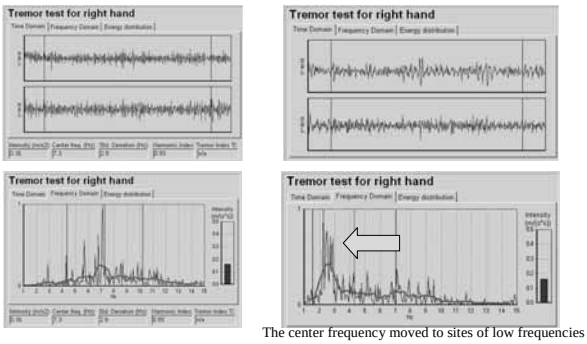
Methods (2)

Hand tremor and postural sway were assessed

One trained examiner examined hand tremor using the Neurobehavioral Test System (CATSYS 2000). Postural sway was measured on a flat floor. The subject was instructed to stand quietly on a platform for 66 s under eyes-open condition. Main parameters measured under eyes-open condtion were sway area, transversal sway (Dx), and sagittal sway (Dy). The spectral analysis of Dx and Dy was conducted with the fast Fourier transform (FFT) analysis to identify the specific sway.

Results 1-1

Hand tremor test



The center frequency moved to sites of low frequencies

Control **Case**

Results 1-2

Result of tremor test

	12 patients		28 controls	t-test (*p*)	ANCOVA* (*p*)
Right hand					
Intensity, total	0.169 ± 0.095		0.129 ± 0.045	0.1797	0.1174
1- 6Hz	0.105 ± 0.061		0.060 ± 0.014	0.0285	0.0016
6-10Hz	0.095 ± 0.068		0.099 ± 0.039	0.8704	0.6964
10-14Hz	0.052 ± 0.034		0.046 ± 0.025	0.5901	0.7957
Proportion (%)					
1- 6Hz	42.6 ± 7.3	>	30.7 ± 6.1	<0.0001	<0.0001
6 10Hz	36.8 ± 8.8	<	47.5 ± 5.8	<0.0001	<0.0001
10-14Hz	20.6 ± 6.4		21.8 ± 4.6	0.5002	0.4098
Center frequency (Hz)	5.72 ± 1.92		7.14 ± 0.71	0.0288	0.0007
Left hand					
Intensity, total	0.162 ± 0.093		0.120 ± 0.050	0.1654	0.1098
1- 6Hz	0.091 ± 0.044		0.065 ± 0.027	0.0810	0.0475
6-10Hz	0.104 ± 0.090		0.088 ± 0.038	0.5653	0.5368
10-14Hz	0.052 ± 0.020		0.044 ± 0.023	0.3656	0.5386
Proportion (%)					
1- 6Hz	38.3 ± 6.3	>	33.7 ± 5.0	0.0190	0.0136
6-10Hz	38.9 ± 7.6	<	44.2 ± 5.0	0.0118	0.0104
10-14Hz	22.9 ± 6.0		22.1 ± 3.3	0.6808	0.6754
Center frequency (Hz)	6.19 ± 1.81		6.99 ± 0.70	0.1659	0.0407

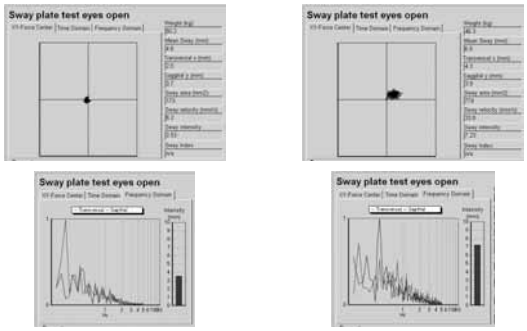
*Adjusted for age and sex (Analysis of covariance)

Results 1-3

In analysis of covariance with two covariates (age and sex), hand tremor intensities at 1-6 Hz in right and left hands were significantly larger in the 12 patients than in the 28 control subjects (*P*<0.05), although the total tremor intensity, tremor intensity at 6-10 Hz or at 10-14 Hz did not differ between the two groups (*P*>0.1). Especially, the proportion of tremor intensity at 1-6 Hz was significantly higher in the patients (42.6% in right hand and 38.3% in left hand) than in the control subjects (30.7% and 33.7%, respectively); as the result, the mean center frequency was significantly lower in the patients than in the control subjects.

Results 2-1

Postural sway test with eyes open



Control **Case**

Result 2-2

Results of postural sway test with eyes open

	5 patients	28 controls	t-test (*p*)	ANCOVA* (*p*)
Dx	4.28 ± 1.30	3.19 ± 1.05	0.0474	0.0709
Dx, 0-1Hz	5.43 ± 1.67	3.92 ± 1.33	0.0309	0.0483
Dx, 1-2Hz	0.88 ± 0.52	0.74 ± 0.31	0.3904	0.2737
Dx, 2-4Hz	0.42 ± 0.33	0.24 ± 0.09	0.2908	0.0088
Dy	5.94 ± 3.79	4.45 ± 1.38	0.4330	0.2003
Dy, 0-1Hz	7.42 ± 5.18	5.41 ± 1.63	0.4382	0.1719
Dy, 1-2Hz	1.09 ± 0.70	0.70 ± 0.35	0.2790	0.0428
Dy, 2-4Hz	0.61 ± 0.48	0.29 ± 0.14	0.2142	0.0039
Area	880 ± 736	352 ± 199	0.1861	0.0032

*Adjusted for age and sex (Analysis of covariance)

Results 2-3

Only five patients with fetal Minamata disease could stand quietly on a platform under eyes-open condition. The transversal sway (Dx) at 0-1 Hz and 2-4 Hz, sagittal sway (Dy) at 1-2 Hz and 2-4 Hz, and sway area were significantly larger in the 5 patients than in the 28 control subjects (*P*<0.05).

Discussion

- **Narabayashi and Ohye (1978) suggested that the tremor generation system might be concerned with the cerebellar afferent pathway.**
- **Yamanaga reported that the tremor frequency (center frequency) was significantly lower in patients with aquired Minamta disease in the normal controls (*Tohoku J Exp Med* 141: 13-22, 1983), and suggested that the tremor in Minamata disease may be related to lesions of the granular cell in the affected cerebellar output.**

TABLE 1. *Tremor frequency and amplitude in normal subjects*

Age	Frequency (Hz) Mean	s.d.	Range	Amplitude Mean	s.d.	Range	Number
3- 9	10.306	0.480	(9.662-10.833)	1.101	0.233	(0.883-1.488)	5
10-19	10.656	0.752	(9.564-12.102)	1.267	0.315	(0.797-2.013)	13
20-29	10.227	0.895	(8.198-12.492)	1.286	0.438	(0.709-2.722)	33
30-39	10.546	0.662	(9.222-11.809)	1.200	0.351	(0.615-2.066)	24
40-49	10.130	0.936	(8.491-12.102)	0.993	0.281	(0.708-1.294)	15
50-59	9.033	0.788	(7.612-10.150)	1.036	0.368	(0.715-1.671)	20
60-69	8.174	1.069	(6.832-10.150)	1.087	0.488	(0.486-2.763)	25
70-79	7.502	0.357	(6.051- 9.076)	1.039	0.314	(0.726-1.927)	16
80-89	6.847	0.627	(5.953- 7.905)	1.174	0.438	(0.822-2.020)	6
3-86	9.469	1.418	(5.953-12.492)	1.143	0.382	(0.486-2.763)	

TABLE 2. *Tremor frequency and amplitude in Minamata disease*

	Age (years)		Tremor frequency Postural Hz	Postural (mV)	Action
1	33	M	8.198	(1.163)	8.695
2	37	F	7.417	(1.165)	7.692
3	38	M	7.417	(3.008)	8.0
4	38	F	4.489	(0.836)	4.651
5	41	F	6.636	(0.814)	—
6	44	M	7.122	(2.025)	7.142
7	47	M	7.808	(3.165)	8.333
8	49	M	7.515	(2.061)	8.0
Mean±s.d.	40.8±5.4		7.075±1.140	(1.834±0.908)	7.501±1.348

***From* Hiroaki Yamanaga: *Tohoku J Exp Med* 141: 13-22, 1983**

Conclusion

Our data suggest that neuromotor dysfunction in patients with fetal Minamata disease was characterized by a low frequency (less than 6 Hz) of hand tremor. The tremor in Minamata disease may have been involved in lesions of the granular cell in the affected cerebellar output.

Retrospective Assessment of Prenatal Exposure to Methylmercury from Whaling Records

Maria Skaalum Petersen[1], Pál Weihe[2], Philippe Grandjean[1]

[1]Department of Environmental Medicine, University of Southern Denmark,
Winsløwparken 17, 5000 Odense C, Denmark, and
[2]Department of Occupational Medicine and Public Health,
Faroese Hospital System, Sigmundargøta 5, 100 Tórshavn, Faroe Islands

Introduction

Disease outcomes may become apparent only after a substantial latency period, at which time retrospective exposure assessment becomes necessary to reveal possible causal factors. Methylmercury has been hypothesized to cause degenerative disease of the nervous system in the elderly. In the Faroe Islands, where methylmercury exposure primarily originates from ingestion of whale meat, the existence of detailed historical whaling records can be potentially used for retrospective assessment of methylmercury exposure. This information can then be used for epidemiological studies, e.g., of possible associations between prenatal methylmercury exposure and degenerative diseases in old age. We have now developed a methodology for such assessment and have made this information available before conducting actual studies in this field.

In regard to exposures to methylmercury, the average methylmercury concentration in the whale meat is much higher than in other types of seafood consumed in the Faroes, and assessment of traditional dietary habits of eating boiled or dried whale meat can therefore be used for exposure evaluation.

Substantial information on whale catches in the Faroes has been recorded through several centuries. This subsistence whaling is conducted using small boats when pods approach the coasts. The pod is chased into a fiord, and the whales are then beached and killed, according to traditional practices.[1,2] The meat and blubber are shared locally according to ancient regulations. The whale catch is therefore communal and non-commercial. The meat is normally consumed for dinner or lunch over an extended period. Slices of the dried meat are often consumed as a snack (like pemmican).

Although whale meat and blubber are now more easily shared outside the individual communities using modern transportation facilities, inclusion of traditional food in the diet depended almost entirely on local access in the past. Thus, dietary habits are determined by seafood availability (i.e., access to whale) and less by limited differences in socioeconomic conditions.

We have therefore examined the possibility of using the available whaling records for retrospective assessment of methylmercury exposure on a community scale. This information can then be used for epidemiological studies, e.g., of possible associations between prenatal methylmercury exposure and degenerative diseases in old age.

Assumptions

Some assumptions must be made before these data can be used to estimate the methylmercury exposures on a community scale, or, rather, the likelihood of exposure. Whales were normally caught only during the summer months.[1,2] During the past, when refrigeration was not possible, the meat was stored after salting or drying. Although salted meat might be kept for extended periods, the majority of the supplies was probably consumed prior to next year's whaling period, i.e., mainly during the winter months. The size of most whale catches would also suggest that supplies only in rare cases would last longer than one year. One must also assume that old supplies from a previous year would most likely be discarded when a new catch was made. To simplify the calculations, we made the assumption that whale meat supplies were properly prepared and stored, but that supplies would last for a maximum of two years.

Mercury concentrations in whale meat have not indicated any clear changes during recent decades, where chemical analyses are available.[3,4] Likewise, modeling studies suggest that methylmercury concentrations may have been only slightly lower during the early decades of the 20th century.[5] Although the mercury concentrations in whale meat can vary, we used an overall average concentration of 2 μg/g[3,4] to calculate the total methylmercury dose available for each inhabitant from the whale meat allocation. In regard to other pollutants, organochlorine substances became important only after about 1950, due to the burgeoning use of PCBs, DDT, later on supplemented by additional persistent substances.[6,7] The present report focuses on the first half of the 20th century, and these substances are therefore not considered.

The detailed information on whale catches in the Faroes provides the exact location of each whale kill, the date, and the number of whales and the total weight.[8] The whale meat (and blubber) was shared according to detailed rules within the local districts.[9,10] The share was not necessarily divided evenly. The district, in which the kill took place, would get a full share and some neighboring districts only a half share, depending on the distance from the place where the whale catch occurred. However, the men who participated in the actual whaling received a larger share. Most of the local, able men probably participated in the catch, and this additional contribution to household supplies is therefore thought to be of limited consequence. The assumption was therefore made that each inhabitant received a share calculated from the total amount of whale meat landed divided by the number of inhabitants in the community.

Thus, in conjunction with census lists, the assignment of whale meat per resident can be computed from the size of the whale catches, the detailed rules for distribution within each district, and the number of residents. Because the census was carried out every five years and varied only little between the years, we used the population number from the census closest in time to the whale catch. For example, for the period 1 August 1918 to 1 July 1923, we used the census data from 1 February 1921.

While allowance for the formal requirement of sharing with neighboring districts can be easily incorporated, any further dissemination of whale meat is disregarded. We believe that this

assumption is appropriate, because tunnels and regular ferries were not part of the Faroese transportation system during the early decades of the past century. In relative terms, dissemination beyond the local districts was therefore considered negligible. Again this factor may contribute imprecision to the exposure assessment, but probably only to a limited extent.

Anecdotal evidence and historical accounts suggest that whale meat was consumed for dinner about three times a week on average. Our own analyses of hair samples collected in the past show that hair-mercury concentrations up to 90 μg/g occurred, but average exposures may have been closer to 30 μg/g. Using the toxicokinetic model developed by the (U.S.) National Academy of Sciences,[11] this average level would, at steady state, correspond to average daily intakes of about 3 μg/kg body weight. For a subject weighing about 60 kg, this number would translate to a daily intake approximating of 200 μg of mercury. With an unchanged average mercury concentration of 2 μg/g in whale meat,[3,4] this dose level corresponds to approximately 100 g of whale meat per day, or 3 kg per month. This consumption level appears in accordance with historical evidence. A higher consumption rate of 5 kg per month would be possible, if whale meat was eaten for dinner most days of the week, but families with easy access to fish or mutton may, on the other hand, have eaten whale meat only once or twice per week.

Methylmercury absorption in the gut is virtually complete, and the elimination half-time (first-order kinetics) is about 45 days.[11] Previous estimates of the elimination half-life was 70 days,[13] which would suggest longer retention times. When the supplies have run out, it takes at least four biological half-lives (i.e., six months), for a subject to decrease the accumulated methylmercury burden to approach background levels. Accordingly, the increased methylmercury body burden after a large whale catch can last for a total maximum of 2.5 years.

Methods

From the whaling records, we have included each whale catch from 1911 and onwards with the date and the total size of the catch. In our spreadsheet, we indicate the district, i.e., where the whales were landed. For each whaling district, each of the villages, in which the whale catch was distributed, is listed in accordance with the regulations in force. The amount of whale meat that each village received is then calculated. The number of inhabitants of each of these villages is retrieved from the census data, which then allows calculation of the amount of whale meat allocated to each subject.

These data can then be applied to calculate body burdens of individual residents. In regard to prenatal methylmercury exposure, the assessment is based on the mother's residence and the whaling data that relate to the pregnancy period. Because residence can be considered rather stable, we assume that the mother's residence at the time of childbirth also applied to the previous two years. Given the biological half-life of methylmercury, all subjects are assumed to have been born at term. The three months prior to the parturition will therefore constitute the third trimester of pregnancy, where methylmercury could in particular cause functional changes.[11] However, the

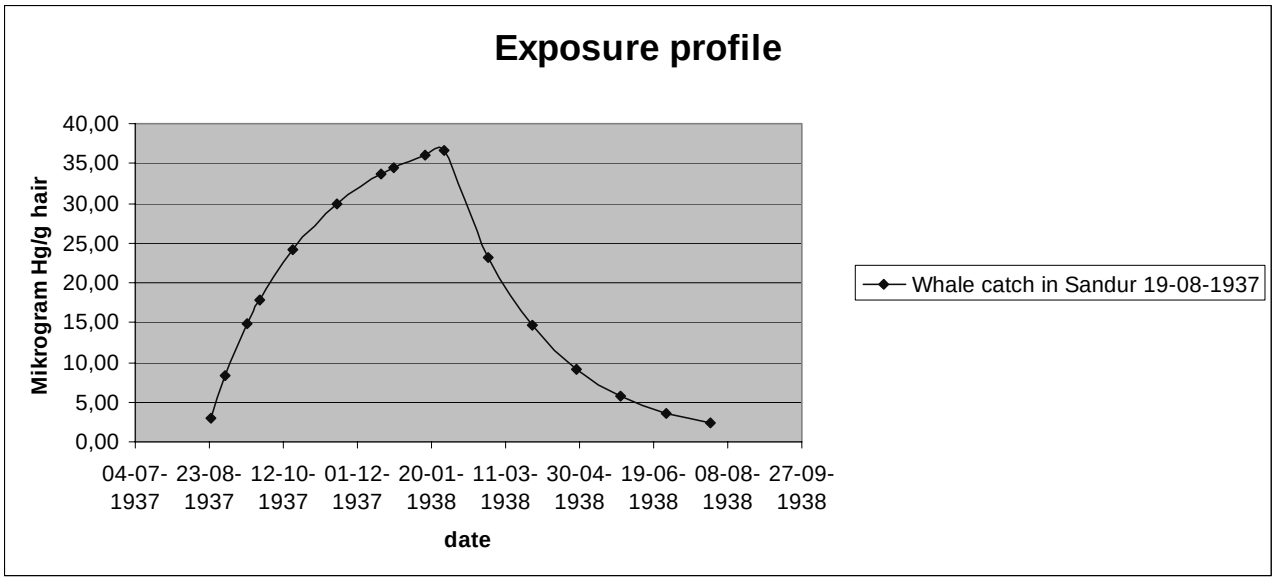
average for the whole pregnancy (area under the curve) may also be relevant, as may the calculated dose level at the beginning of the third trimester.

Although many of a district's whale catches have occurred at considerable time intervals, some districts have experience many catches within a short period of time. In the latter case, the supplies have not run out before the next catch occurred or, alternatively, the supply may have run out, but the contribution from the previous catch (the methylmercury burden) has not yet reached background levels. Account must therefore be taken of the contributions of all relevant whale catches prior to the time period of interest. In regard to prenatal exposures, all whale catches up to two years prior to the birth date are therefore considered.

Certain districts at certain time periods could be classified as virtually unexposed, due to the absence of whale landings for extended periods of time. Likewise, highly exposed districts with ample and almost continuous supplies of whale meat, can be identified. In some cases, low and high exposures have existed during the same time periods, thus allowing comparisons of subjects of the same age, but with widely different prenatal exposure potentials.

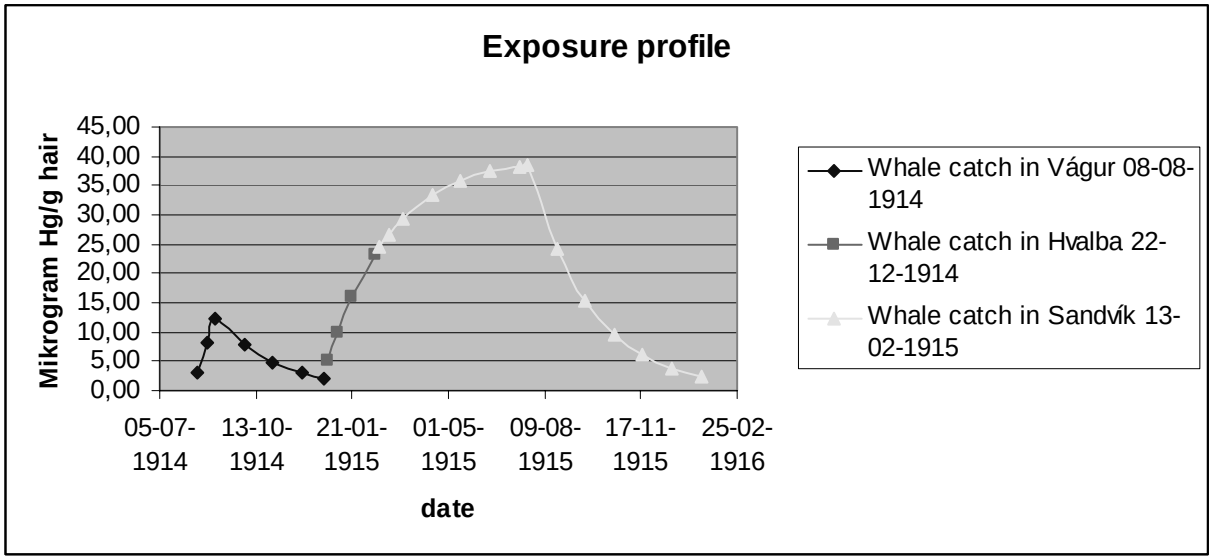
A more refined model to estimate actual exposure levels takes into account the build-up of methylmercury in the body while the calculated community supplies last (assumed consumption of 3 kg/month and maximum storage time of two years) and the decay given an elimination half-life of 45 days. In all these calculations, methylmercury concentrations of the whale meat is assumed to be the same, i.e., similar to the current average concentration of 2 μg/g[12]. Given the pace of mercury kinetics in the oceans,[14] this concentration is unlikely to have changed much during the last several decades.

The exposure assessment can be illustrated by some examples. Consider a subject born in Skálavík on 25 December 1937. The prenatal exposure assessment requires consideration of whale catches during the two years prior to the birth date. One relevant whale catch in the district was on 19 August 1937 in Sandur (see below graph). This catch will cause methylmercury exposures that



will last through 28 January 1938, i.e., it will include the last four months of this pregnancy. The previous catch in this district was in 1933, but no contribution would be considered from that catch to the body burden of methylmercury during the pregnancy.

As a second example, consider a subject born on 20 October 1915 in Sumba on the island of Suderoy. Three relevant whale catches in the Suderoy district happened on 8 August 1914, 22 December 1914, and 13 February 1915. The two most recent ones add to the supplies already available from the first catch. According to the calculations described above, the supplies from the second catch will last until 15 March 1915, but the elimination will not be complete before the subsequent catch. The third whale catch will therefore result in mercury burdens that do not start from zero. The calculations must therefore take into account the contribution from the previous catch/catches, when the elimination of accumulated methylmercury burden has not reached background levels.



In regard to our study of Parkinson's disease in the Faroe Islands, we have recorded the birth date and the birth place for each patient. The prenatal exposure of each subject can then be determined from the spreadsheets according to the above methods for calculations. In addition, the project calls for drawing of controls from the population registry for each patient. These subjects will be matched only by sex and age, and their place of birth will therefore determine their prenatal exposure. We have therefore developed spreadsheets to cover all Faroese districts for the relevant time period. As soon as these subjects have been drawn, retrospective exposure assessment can be easily completed using the above methodology decided upon a priori.

Conclusions

The whaling data available from the Faroes will allow estimation of approximate individual exposures to methylmercury based on the community-based shares of each whale catch. By assuming a relatively constant average methylmercury concentration, a regular consumption of the meat at a rate of 3 kg/month, and that supplies will last for no longer than two years, body burden

profiles can be generated for residents of each district, while taking into account the average biological half-life of methylmercury in the body (45 days). For assessment of prenatal exposure, the mother's exposure profile is calculated from the whaling data up to two years prior to the child birth. These numbers will allow calculation of average exposure during the whole pregnancy, during the third trimester, or the body burden at the beginning of the third trimester (i.e., three months before child birth). These numbers can then be used to compare prenatal exposures of subjects with a particular disease, such as Parkinson's disease, as compared to controls.

Acknowledgments

We are indebted to Dr. Dorete Bloch, Director of the Museum of Natural History, Tórshavn, Faroe Islands, for sharing with us her insight into whaling practices and distributions of meat and blubber. Dr. Anna Choi provided useful statistical comments, and Jennifer Asetta edited a draft version.

References

1. Bloch D, Desportes G, Hoydal K, Jean P. Pilot Whaling in the Faroe Islands, July 1986-July 1988. North Atlantic Studies 1990; 2: 36-44.
2. Johansen T, Olafsson A. The Faroe Islands: A Brief Introduction. Faroese Government Office, Copenhagen, 1999.
3. Julshamn K, Andersen A, Ringdal O, Morkore J. Trace elements intake in the Faroe Islands. I. Element levels in edible parts of pilot whales (Globicephalus meleanus). Sci Total Environ 1987; 65: 53-62.
4. Dam M. Mercury in the Faroe Islands – a review of available data. Fróðskaparrit 2004; 52: 85-133.
5. Booth S, Zeller D. Mercury, food webs, and marine mammals: implications of diet and climate change for human health. Environ Health Perspect 2005; 113: 521-6.
6. Eisenreich SJ, Capel PD, Robbins JA, Bourbonniere R. Accumulation and diagenesis of chlorinated hydrocarbons in lacustrine sediments. Environ Sci Technol 1989; 23: 1116-26.
7. Vartiainen T, Mannio J, Korhonen M, Kinnunen K, Strandman T. Levels of PCDD, PCDF and PCB in dated lake sediments in Subarctic Finland, Chemosphere 1997; 34: 1341-50.
8. Whales and whaling in the Faroe Islands. www.whaling.fo
9. Bekendtgørelse (nr. 137) om Reglement for Grindefangst på Færøerne 14. Juni 1909
10. Reglement for Grindefangsten paa Færøerne 1. Juni 1940
11. National Research Council. Toxicological Effects of Methylmercury. National Academy Press, Washington, DC, 2000.
12. Andersen A, Julshamn K, Ringdal O, Morkore J. Trace elements intake in the Faroe Islands. II. Intake of mercury and other elements by consumption of pilot whales (Globicephalus meleanus). Sci Total Environ 1987; 65: 63-8.
13. Smith JC, Farris FF. Methyl mercury pharmacokinetics in man: a reevaluation. Toxicol Appl Pharmacol 1996; 137: 245-52.
14. Booth S, Zeller D. Mercury, food webs, and marine mammals: implications of diet and climate change for human health. Environ Health Perspect 2005; 113: 521-6.

IPCS Assessments of the Risks from Exposure to Methylmercury

Antero Aitio* and Tim Meredith

International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Abstract

The International Programme on Chemical Safety has assessed the health risks from exposure to mercury or its compounds 12 times over 30 years in the series "Environmental Health Criteria Document" and reports of the "Joint FAO WHO Expert Committee on Food Additives and Contaminants". The assessments are performed by independent international experts; the process is fully open and transparent and special attention is paid to possible conflicts of interest. The intent of the programme is to provide assistance to Member States in establishing safe levels of exposure for national risk management decisions. For methylmercury, the key effect is neurotoxicity, especially developmental neurotoxicity, and the IPCS assessments have throughout the years been based mostly on observations in humans, and in the recent assessments, on observations in the most sensitive population group, i.e., developing children.

Introduction

IPCS was established in 1980 as a cooperative programme of WHO, ILO & UNEP. Its objectives were and remain to establish the scientific basis for safe use of chemicals, and to strengthen national capabilities & capacities for chemical safety. The main activities of IPCS are risk assessment and establishment of guidance values, as well as development and harmonization of risk assessment methods, collection and assessment of information on poisoning incidents, their prevention and management, as well as collection of human data. A more recent addition to the work area of IPCS is prevention of, preparedness for, and surveillance, alert and response to chemical incidents and emergencies.

IPCS hazard and risk assessments are based on the work of internationally-acknowledged experts with global participation. In this work, special attention paid to potential conflicts of interest, and key elements of the process are thorough peer reviews. The processes are fully transparent and based on publicly-available process and assessment guidelines (IPCS 1987, 1994, 2002, JECFA 2001 a, b). IPCS risk and hazard assessments are available in printed form, free of charge on the web (http://www.who.int/pcs and/or http:// www.inchem.org) and also on CD [from the Canadian Centre for Occupational Safety and Health (clientservices@ccohs.ca)]

*Present address: Institute of Occupational Health, Helsinki, Finland

IPCS risk assessment priorities are determined by the probability of exposure, and/or significant toxicity/ecotoxicity; the priority setting involves international consultation.

IPCS risk assessment products

IPCS makes hazard/risk assessments in two different settings, first, risk assessment of priority chemicals independent of the source and route of exposure. These assessments are made in the series of Concise International Assessment Documents (CICADs); between 1976 and 2005 some 230 assessments were published in the series of Environmental Health Criteria Documents (EHC); no new EHCs have been started thereafter. EHCs and CICADs consider exposure from all sources and via all routes and describe all effects, with an emphasis on key effects. The EHCs contain a hazard but not risk assessment, CICADs a sample risk characterization. IPCS also publishes International Chemical Safety Cards, two-page summaries of the key hazardous features of chemicals and ways of avoiding hazardous exposures, as well as succinct assessments of the acute hazards of pesticides in the series WHO Classification of Pesticides by Hazard.

The second set of assessment documents comprises, as targeted risk assessments, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and Joint FAO/WHO Expert Committee on Food Additives (JECFA) documents (risk characterization of chemicals in food), chemical risk assessments for WHO air quality guidelines and chemical risk assessments for WHO drinking water quality guidelines.

WHO/IPCS has assessed mercury and methylmercury in both assessment series, EHCs/CICADs and JECFA (IPCS 1976, 1989, 1990, 1991, 2003; JECFA 1971, 1972, 1978, 1989, 2000, 2004).

The process for EHCs and CICADs include, after priority setting by an external advisory body, a call for information, published on the web, production of the first document draft by an expert author or at present, more often, by a group of authors, an extensive international peer review, revision of the document by the author with consideration of all comments received, and finalization and approval in a meeting with special emphasis on the verification of the appropriateness of the author's responses to the peer review comments. In order to guarantee transparency and constancy, there is a publicly-available written guideline for the assessment and procedure. For all participants in the process, a declaration of interests is required.

The latest EHC on mercury/methylmercury was prepared in 1990, thereafter it has been considered that exposure to methylmercury is almost exclusively through the diet, and the assessments have been carried out in the Joint FAO/WHO Expert Committee on Food Additives and Contaminants, JECFA.

JECFA develops the priority lists for its assessments from recommendations of the Codex Committee on Food Additives and Contaminants, previous Expert Committees, and direct requests from governments, other interested organizations, and producers of substances that have been evaluated previously. For contaminants and additives scheduled, JECFA publishes a call for information, and then selects an expert to author the first draft document. The first draft is peer reviewed by a designated member of the FAO/WHO expert panel, and the product of the collaborative effort of the author and the panel member is brought to the JECFA meeting for revision and final approval. There are publicly-available written guidelines for the assessment (IPCS 1987) and for the procedure, and everyone involved fills in and signs a declaration of interests form.

JECFA Committees consist of 'Members' and 'Secretariat'. Members are responsible for making the decisions. The Secretariat consists of the WHO and FAO Joint Secretaries, other employees of WHO and FAO who assist with preparation for the meeting, and WHO Temporary Advisers (among them, the authors of the first draft documents) and FAO Consultants. Experts who participate as WHO Members must be Members of a WHO Expert Advisory Panel. JECFA is not a standing committee. Therefore, JECFA can make decisions only during the time of the meeting itself. The Secretariat conveys the decisions of the Committee to the executive heads of FAO and WHO. The Secretariat cannot modify or amend the interpretation of data by JECFA. The only modifications to reports and monographs that may be made by the Secretariat are those of an editorial nature; changes of a substantive nature can only be referred to a subsequent meeting for consideration.

The aim of JECFA is the derivation of an acceptable or tolerable intake level. From a detailed assessment of the toxicological database, the most sensitive, critical endpoint is identified from experimental or epidemiological studies. This endpoint then serves as the basis for the derivation of the acceptable or tolerable intake level, via the application of uncertainty factors applied to the critical endpoint, by taking available mode-of-action, toxicokinetic and toxicodynamic data, as well as uncertainties in the assessment, into account.

Mercury assessments in EHCs and CICADs

Environmental Health Criteria Document 1 (IPCS 1976) stated that pathological findings demonstrate that methyl- and ethyl-mercury compounds are primarily neurotoxic and produce similar types of lesion in man. The main pathological features consist of the destruction of neurological cells in the cortex, particularly in the visual areas of the occipital cortex, and various degrees of damage to the granular layer in the cerebellum. Damage to the peripheral nerves may occur as indicated by clinical signs but no definitive pathological observations are available for man. Recent studies on rats have confirmed the findings of Hunter et al. in 1940 that the earliest neurological effects in these animals is

damage to the peripheral sensory nerves. Later the disease affects other parts of the central and peripheral nervous systems. There is now evidence that the primary site of the disease is the cell bodies in the dorsal root ganglia with secondary deterioration in their fibres. It concluded that the most common symptoms and signs of methylmercury poisoning are paraesthesiae, constriction of the visual fields, impairment of hearing, and ataxia. The effects are usually irreversible but some improvement in motor coordination may occur. From information in humans, it considered that the concentrations of total mercury associated with the earliest effects in the most sensitive group in the adult population (5% prevalence) were: blood mercury 20-50 µg/100 ml, hair mercury, 50-125 µg/g, which correspond to a daily intake of 3-7 µg/kg bw. It further noted that the estimates apply only to adults and that prenatal life may be the stage of the life-cycle most sensitive to methylmercury.

The more recent EHC assessment (IPCS 1990) concluded that the nervous system is the principal target tissue for the effects of methylmercury in adult human beings. The sensory, visual, and auditory functions, together with those brain areas, especially the cerebellum, concerned with coordination, are the most common functions to be affected. At high doses, methylmercury affects the peripheral nervous system. From the quantitative point of view, the conclusion was that a concentration of methylmercury in blood about 200 µg/litre, corresponding to 50 µg/g of hair, is associated with a low (5%) risk of neurological damage to adults, but at maternal hair mercury levels above 70 µg/g there is a high risk (more than 30%) of neurological disorder in the offspring and estimated that a 5% risk may be associated with a peak mercury level of 10-20 µg/g in maternal hair.

Mercury assessment in JECFA

In its meeting in 1970, JECFA concluded that methylmercury compounds produce serious and sometimes fatal neurotoxicity and embryopathy (JECFA 1971). The data did not allow a tolerable intake level to be established. The Committee, however, took notice of a number of points described as being alarming: (1) the epidemics of poisoning; (2) the high sensitivity of the foetus; (3) the occurrence in non-epidemic areas of mercury levels approaching those associated with poisoning.

In 1972, JECFA concluded that the most reliable data for the toxicological evaluation of mercury in fish derive from adults with neurological involvement and that clinical data from Japan indicate that the foetus is more sensitive than the mother. The Committee estimated that the lowest mercury level at the onset of poisoning in adults with neurological involvement is 50 µg/g hair and 0.4 µg/g blood cells. An estimated intake of 0.3 mg Hg/day over prolonged periods, mainly as methylmercury, was considered to cause poisoning. JECFA thus established a Provisional Tolerable Weekly Intake (PTWI) of 0.3 mg total mercury per person, of which no more than 0.2 mg should be present as methylmercury. This corresponds to a provisional tolerable weekly intake of 5 µg/kg body weight of

total mercury (considering a 60kg person), of which no more than 3.3 µg/kg bw should be methylmercury compounds (JECFA 1972).

In 1978, JECFA revisited the question and considered the new studies that had become available. However, the Committee concluded that the new studies did not provide a basis for a changed PTWI, which was thus held at 5 µg/kg bw for total mercury, and 3.3 µg/kg bw for methylmercury (JECFA 1978).

In 1989 JECFA again assessed new data and confirmed the existing PTWI of 200 µg for methylmercury per person (3.3 µg/kg). However, JECFA also stated that pregnant women and nursing mothers are at a greater risk but existing data do not allow setting a PTWI for these groups (JECFA 1989).

In its 53rd meeting in 2000, JECFA maintained the PTWI of 3.3 µg/kg bw and considered that the studies in the Faroe Islands and the Seychelles did not provide consistent evidence of neurodevelopmental effects in children of mothers whose intake of methylmercury yielded hair burdens of 20 µg/g or less. The Committee could not evaluate the risks for the complex and subtle neurological end-points that would be associated with lower intakes. In the absence of any clear indication of a consistent risk in these recent studies, the Committee recommended that methylmercury be re-evaluated when other evaluations and relevant data from the two main cohort studies become available (JECFA 2000).

This re-evaluation was published in 2004. In this meeting, the Committee re-confirmed that neurotoxicity is the most sensitive end-point. In humans, the indices of neurotoxicity include neuronal loss, ataxia, visual disturbances, impaired hearing, paralysis and death. Both the central and peripheral nervous systems show signs of damage. A dose-response analysis was made from maternal hair mercury and neurological deficits in the child; from the Seychelles study (where no effects were observed), a NOAEL was used, while for the two other main studies, a BMDL was derived. Because of a marked uncertainty in the New Zealand study, finally only the Faroe and Seychelles studies were used. The average NOAEL/BMDL derived was 14 mg/kg hair. From an average hair/blood mercury ratio of 250, and application of a one-compartment model to the absorption, distribution and excretion of methylmercury in the body, it was considered that this corresponds in steady state to a daily intake of 1.5 µg/kg (JECFA 2004).

In the derivation of the tolerable intake, JECFA did not apply any uncertainty factor for the toxicodynamics, as the basis for the assessment was three different, sensitive populations. The

uncertainty of the hair/blood ratio was estimated to be 2.0. The uncertainty factor for interindividual differences in kinetics used was 100.5 = 3.2, the default value.

Thus the total uncertainty factor applied was 2 x 3.2 = 6.4, and the
PTWI = 1.5 µg/kg/d x 7 d/w/ 6.4 = 1.6 µg/kg

JECFA further noted that fish are an important part of a balanced, nutritious diet and this should be appropriately considered in public health decisions to set limits for methylmercury concentrations in fish.

References

IPCS (1976) Environmental Health Criteria 1 Mercury. Available at: http://www.inchem.org/documents/ehc/ehc/ehc001.htm

IPCS (1987) Environmental Health Criteria 70. Principles for the safety assessment of food additives and contaminants in food. Available at: http://www.inchem.org/documents/ehc/ehc/ehc70.htm

IPCS (1989) Environmental Health Criteria 86. Mercury - Environmental Aspects. Available at: http://www.inchem.org/documents/ehc/ehc/ehc086.htm

IPCS (1990) Environmental Health Criteria 101 Methylmercury. Available at: http://www.inchem.org/documents/ehc/ehc/ehc101.htm

IPCS (1991) Environmental Health Criteria 118. Inorganic mercury. Available at: http://www.inchem.org/documents/ehc/ehc/ehc118.htm

IPCS (1994) Environmental Health Criteria 170. Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits. Available at: http://www.inchem.org/documents/ehc/ehc/ehc170.htm

IPCS (2002) Guidelines for the preparation of Concise International Chemical Assessment Documents (CICADs) May 2002 Available at: http://www.who.int/ipcs/publications/cicad/en/CICAD_Guidelines_sept-2002.pdf

IPCS (2003) Concise International Chemical Assessment Document 50. Elemental mercury and inorganic mercury compounds: human health aspects. Available at: http://www.inchem.org/documents/cicads/cicads/cicad50.htm

JECFA (1971) Evaluation of food additives. Fourteenth report of the joint FAO/WHO Expert Committee on Food Additives. 24 June- 2 July, 1970. WHO Technical Report Series 462. Available at: http://whqlibdoc.who.int/trs/WHO _TRS_462.pdf

JECFA (1972) WHO Food Additives Series, 1972, No. 4. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbonate, and octyl gallate. Available at: http://www.inchem.org/documents /jecfa/jecmono/v004je02.htm

JECFA (1978) Mercury. In: Summary of toxicological data of certain food additives and contaminants. WHO Food additives series 13. Available at: http://www.inchem.org/documents/jecfa /jecmono/v13je14.htm

JECFA (1989) Toxicological evaluation of certain food additives and contaminants Prepared by the 33rd meeting of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, 21-30 March 1989. WHO Food Additives Series 24. Cambridge University Press. available at: http://www.inchem.org /documents/jecfa/jecmono/v024je12.htm

JECFA (2000) Safety evaluation of certain food additives and contaminants. Prepared by the fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food additives Series 44. available at: http://www.inchem.org/documents/jecfa/jecmono/v44jec13.htm

JECFA (2001a) Residues of veterinary drugs in food WHO procedural guidelines for the Joint FAO/WHO Expert Committee on Food Additives. Available at: http://www.who.int/ipcs/food/jecfa/ procedural_guidelines%20_drugs.pdf

JECFA (2001 b) Contaminants. Guidelines for the preparation of working papers for the joint FAO/WHO expert committee on food additives. Available at: http://www.who.int /ipcs/food/jecfa /en/contaminant_guidelines.pdf

JECFA (2004) Methylmercury (Addendum) In: Safety evaluation of certain food additives and contaminants. Prepared by the Sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food additives Series 52. available at: http://www.inchem.org /documents/jecfa/jecmono/v52je23.htm

Proceedings of
NIMD Forum 2006

平成１８年３月３１日発行

発行　環境省　国立水俣病総合研究センター
Published by National Institute for Minamata Disease

〒867-0008　熊本県水俣市浜 4058-18
TEL: 0966-63-3111
FAX: 0966-61-1145

4058-18, Hama, Minamata, Kumamoto 867-0008, Japan
TEL: +81-966-63-3111
FAX: +81-966-61-1145
http://www.nimd.go.jp

March 2006

問い合わせ先：　国際総合研究部
Inquiry: Department of International Affairs and Environmental Sciences
kokusai@nimd.go.jp