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National Institute for Minamata Disease  
Ministry of the Environment  
Japan

## Table of contents

### Report on Research and Other Activities in Fiscal Year 2014

<b>1. Mechanism Group</b>	2
<i>Research:</i>	
(1) Research on selective cytotoxicity and sensitivity of individuals toward methylmercury	
(2) Study on the molecular, genetic, and biochemical factors that cause differences in stress responses to methylmercury	
(3) Experimental research on relief of methylmercury-induced neurotoxicity	
(4) Research on the effect of dietary fiber on mercury excretion after methylmercury exposure	
<b>2. Clinical Group</b>	4
<i>Research:</i>	
(1) Clinical research on Minamata disease, with a focus on the development of an objective assessment technique using MEG	
(2) Research on the improvement of treatment of Minamata disease	
<i>Other activities:</i>	
(1) Rehabilitation programs for patients with Minamata disease and care and rehabilitation outreach	
(2) Community development project for home care support, including health care practice	
<b>3. Risk Recognition and Information Services Group</b>	6
<i>Research:</i>	
(1) Study on the information and perception of the health risk of low-level exposures to methylmercury	
<i>Other activities:</i>	
(1) Organization of documents and materials on Minamata disease and dispatch of related information in the Minamata Disease Archives	
(2) Examination of hair Hg levels in areas concerned with Hg pollution around the world	
(3) Information service using hair Hg analysis	
<b>4. Social Group</b>	8
<i>Research:</i>	
(1) Historical study on risk management in Minamata disease episodes	
(2) Study on the role of local government and the viewpoint of local community of Minamata disease problem	
<b>5. Local and Global Environment Group</b>	9
<i>Research:</i>	

- (1) Marine ecosystem and mercury levels in the Yatsushiro Sea - Quantitative survey of the marine benthic community in the Yatsushiro Sea and mercury concentration in different fish species in the food web
- (2) Research on its influences and behaviors of mercury in an aquatic environment of Minamata Bay
- (3) Research on chemical reactions, transport, and deposition of atmospheric mercury
- (4) Study on natural emission sources of atmospheric mercury
- (5) Study on biomagnification of mercury from sediment to demersal fish
- (6) Mercury contamination by many small-scale gold ore smelters in the Talawaan River, North Sulawesi, Indonesia
- (7) Study on alkyl derivatization technique for speciation of mercury in biological and human samples

*Other activities:*

- (1) Cooperation of research and field survey in the international organization
- (2) NIMD Forum and International Workshop

**6. Environmental Health Sciences Group** ..... 13

*Research:*

- (1) Studies on methylmercury exposure in a whale-eating district
- (2) Evaluation of the effect of exposure to methylmercury and other elements during gestation and breast-feeding
- (3) Protective effect of selenium against methylmercury toxicity and co-existence of mercury and selenium in humans and sea-mammals
- (4) Evaluation of factors affecting sensitivity to methylmercury exposure—Application of disease model animals and knock-out animals

**7. Publications and Conference Presentations** ..... 15

Report on Research and Other Activities in  
Fiscal Year2014

## 1. Mechanism Group

The aim of this study group is to understand the molecular mechanisms underlying mercury toxicity in humans. To that end, the group focuses on the biological effects of mercury. Our goals are as follows: (1) to understand the initial signs of methylmercury (MeHg) poisoning (2) to evaluate MeHg toxicity (3) to develop protecting methods against MeHg-induced disorders and (4) to develop new treatments to repair the damage caused by MeHg poisoning. In this study, we used biochemical, molecular biological, and pathological techniques in cell cultures and animal models. To determine the differences in MeHg sensitivity among organs and individuals, we analyzed stress responses and changes in the activation of cellular signal transduction caused by MeHg exposure. In addition, to elucidate the mechanisms underlying MeHg toxicity, we investigated cell death and regeneration in nerve cells damaged by MeHg, and the effect of dietary fiber on mercury excretion after MeHg exposure. We also are in the process of identifying drugs that suppress MeHg toxicity and promote nerve regeneration.

This group conducted the following research during 2014 fiscal year. The summary of the results completed in the mid-term plan 2010 also are described:

### [Research theme and summary]

(1) Research on selective cytotoxicity and sensitivity of individuals toward methylmercury

(Project research)  
Masatake Fujimura

(Department of Basic Medical Science)

We analyzed mRNA expression levels of anti-oxidative enzymes for each neuronal cell of mice using a microdissection system. We observed lower mRNA expression of anti-oxidative enzymes, especially Mn-superoxide dismutase (SOD), in the deep layer of the cerebral cortex, which is vulnerable to MeHg toxicity, than in the other neuronal cells (in the shallow layer of cerebral cortex). A part of this finding has been published in a peer-reviewed article. Additionally, we analyzed the mechanism of decreased eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) expression associated with dysfunction of synaptic homeostasis in the cerebellum of rats under MeHg exposure during the fetal period. We discovered that MeHg exposure affected the expression level of eEF1A1 through the suppression of the TrkA-p70S6K pathway. In collaboration with external research institutes, we have presented our finding at four conferences and published two peer-reviewed articles.

In the past 5 years (fiscal years 2010 to 2014), we have presented our findings at 16 conferences (four conferences as representative) and published 11 peer-reviewed articles (three articles as representative).

(2) Study on the molecular, genetic, and biochemical factors that cause differences in stress responses to methylmercury (Fundamental research)

Fusako Usuki

(Department of Clinical Medicine)

From siRNA-mediated knockdown studies, we uncovered that stress-induced activating transcription factor 4 (Atf4) and selenoenzyme glutathione peroxidase 1 (GPx1) are the key factors for contributing to the protection against MeHg cytotoxicity

The level of thiol antioxidant barrier (sHp) in the

blood plasma of MeHg-exposed rats significantly decreased 2 weeks after MeHg exposure; however, no reactive oxygen metabolite (dROM) or biological anti-oxidant potential (BAP) in the plasma and no histopathological changes in the cerebellum were observed at this stage. To determine whether the early decrease in plasma sHp in MeHg-exposed rats is attributable to specific MeHg toxicity mechanisms, and whether this effect could be used as a relevant biomarker, we examined the plasma oxidative stress markers in rats exposed to 0.2% lead acetate (Pb) in drinking water for 8 weeks. We demonstrated that significant changes in dROM, sHp, and BAP could not be detected in those rats. We further examined the time course of changes in selenoprotein P1 (SeP1) levels and the activities of glutathione peroxidase 3 (GPx3) and glutathione reductase (GR) in MeHg-exposed rats. The results showed that the expression of SeP1 and GPx3 activity significantly decreased whereas non-selenoenzyme GR activity significantly increased. In contrast, the expression of SeP1 significantly increased in Pb-exposed rats.

The results indicated that the MeHg-induced relative selenium-deficient condition due to the high affinity of MeHg for the selenohydryl group and selenide blocked the syntheses of SeP1 and GPx3 through a posttranscriptional defect by executing nonsense-mediated mRNA decay. The decreases in selenoproteins (SeP1 and GPx3) seem to be specific signs of MeHg toxicity similar to the decrease in sHp.

In the mid-term plan 2010, we demonstrated the following findings. (1) An antioxidant capacity, selenium kinetics, ATF4, GPx1, and GRP78 are the key factors involved in MeHg toxicity (J Biol Chem 286: 6641-9, 2011; Scientific Reports 3:2346 doi: 10.1038/srep02346). The genetic polymorphism of GPx1, GRP78, SeP1, and TrxR1 may affect individual differences in MeHg toxicity. (2) The general mechanism of MeHg toxicity was observed in the cerebral cortical neuronal cells and astroglia cells.

In addition, we demonstrated that MeHg induced upregulation of glial cell line-derived neurotrophic factor (GDNF) and IL-6, which are related to neuronal survival and repair in astroglia cells. (3) Decreased plasma thiol antioxidant barrier and selenoproteins may be useful as potential biomarkers for ongoing methylmercury intoxication and an individual protective capacity

(3) Experimental research on relief of methylmercury - induced neurotoxicity (Fundamental research)

Masatake Fujimura

(Department of Basic Medical Science)

We indicated that a low concentration of MeHg (100 nM) suppressed the TrkA pathway, including the AKT and ERK pathways, and induced cell death, especially in neuronal cells, using the differentiating PC12 cells as a neuronal culture model. This finding has been presented at a conference and published in a peer-reviewed article. Next, we demonstrated that a low concentration of MeHg (10 nM) induced the expression of GSK-3 $\beta$  and suppressed cell proliferation in neural progenitor cells (NPCs) from rat embryonic brains. This finding has been presented at a conference. Furthermore, we started to establish chronic MeHg-intoxicated model rats to evaluate the ROCK inhibitor as a therapeutic drug for MeHg poisoning.

In the past 5-years (fiscal years 2010 to 2014), we have presented our finding at eight conferences (eight conferences as representative), and published four peer-reviewed articles (three articles as representative).

(4) Research on effect of dietary fibers on mercury excretion after methylmercury exposure

(Fundamental research)

Masaaki Nagano

(Department of Basic Medical Science)

In this study, we investigated the effect of wheat bran on Hg excretion from the tissue of MeHg-treated mice. So

far, we demonstrated that tissue Hg levels decreased in wheat bran-fed mice through the excretion of urinary Hg. In the current year, we examined the effects of the oxidized forms of glutathione and  $\gamma$ -glutamylcysteine on Hg excretion because previous LC-MS analysis showed that the oxidized forms of glutathione and  $\gamma$ -glutamylcysteine characteristically contained in the wheat bran diet. We showed that urinary Hg excretion increased in the glutathione diets-fed mice compared to the basal diet-fed group. The result suggested that the oxidized forms of glutathione and  $\gamma$ -glutamylcysteine contained in wheat bran may be one of factors which can accerelate urinary Hg excretion.

In fiscal year from 2012 to 2014, we demostated that wheat bran accelerated urinary Hg excretion and decreased tissue Hg levels in mice. The oxidized forms of glutathione and  $\gamma$ -glutamylcysteine contained in the wheat bran diet may be one of the main factors which can accelerate urinary Hg excretion.

## 2. Clinical Group

Minamata disease is caused by severe mercury poisoning. The incidence of comorbidities, such as cervical spondylosis or metabolic syndrome, tends to increase with age in these patients, making the diagnosis of Minamata disease based solely on clinical neurological symptoms difficult. Therefore, it is necessary to develop objective methods to accurately identify methylmercury (MeHg) poisoning, including Minamata disease.

Since spasticity, involuntary movements, such as dystonia, and intractable chronic pain are involved in the reduction of quality of life (QOL) in the Minamata disease patients, it is desirable to develop an effective therapy.

This research group conducted experiments to evaluate the neurological functions of patients with Minamata disease, using magnetoencephalography (MEG) and magnetic resonance imaging (MRI). Moreover, we set up a research group to examine the effective therapy for the above-mentioned symptoms.

The research conducted by this group during FY2014 is outlined as follows:

### [Research theme and summary]

(1) Clinical research on Minamata disease, with a focus on the development of an objective assessment technique using MEG (Project research)

Masaaki Nakamura  
(Department of Clinical Medicine)

The aim of this study was to develop an objective evaluation protocol using MEG and MRI to assess brain function and morphologic features. During this year, we tested the MEG method in 169 people, including 54

subjects from the Kumamoto district, an area that is not polluted with MeHg.

An examination of two-point discrimination using air-puff, the abnormal frequency was 34% in the Minamata district and 39% in the Kumamoto district, suggesting that it is necessary to evaluate carefully two-point discrimination in the chronic stage of MeHg poisoning. On the dipole waveform analysis of somatosensory evoked magnetic fields (SEF), many abnormal waveforms were observed in the Minamata district. Moreover, more information about sensory function was obtained by conducting a frequency analysis compared with the dipole waveform analysis.

We also have been creating an MRI database to compare brain atrophy in the Kumamoto and Minamata districts.

In the mid-term plan 2010, we examined the objective evaluation of brain function of MeHg intoxication using MEG and MRI and evaluated the pain and motor function by MEG to judge the therapeutic effect.

(2) Research on the treatment improvement of Minamata disease (Fundamental research)

Masaaki Nakamura  
(Department of Clinical Medicine)

To improve the QOL of Minamata disease patients, we started "the research group about the improvement in medical treatment of Minamata disease" to examine effective therapy.

In the mid-term plan 2010, we made the following preparations for full-scale clinical study in the next fiscal year: (1) explanation of the clinical significance of this research to a local medical association, (2)



distribution of a pamphlet and enforcement of lectures to provide a local medical association with the information on current medical treatment, (3) creation of patient registration papers and collection of patient information, (4) cooperation with the Kumamoto University, and (5) construction of a medical treatment organization.

### **Activities**

In recent years, the compensation claims from Minamata disease patients have moved toward a political resolution. The Department of Clinical Medicine actively conducts events about Minamata disease in cooperation with related organizations. In addition to undertaking conventional rehabilitation activities, including providing daycare for congenital and infantile Minamata disease patients, we organized rehabilitation and care technical schools. We also examined the usefulness of vibration therapy for the relief of pain and spasticity associated with various chronic nervous diseases, including Minamata disease. Furthermore, we introduced the robot suit, HAL (Hybrid Assistive Limbs), which assists standing and walking actions.

To examine the effectiveness of home care support for Minamata disease patients and their families, we conducted a project entitled “Home support model study including care prevention” (FY2006–2008). Following this project, the projects “Community development project for home care support including healthcare practice” (FY2009–2011) and “Community welfare promotion business for supporting Minamata disease victims” (FY2012) were carried out to develop methods to apply these concepts into the community. After these projects, we have continued supporting

welfare activities in Minamata and Izumi cities.

The following section includes an outline of the clinical practice conducted by this group in FY2014.

### **[Activities theme and summary]**

(1) Rehabilitation programs for patients with Minamata disease and care and rehabilitation outreach

(Other activities)

Fusako Usuki

(Department of Clinical Medicine)

We continued to provide outpatients with rehabilitation, in the form of daycare, twice or thrice per week. The principal objective was to improve their quality of life. Continuous vibration therapy relieved the severe pain and spasticity of three patients with fetal-type Minamata disease, and improved their activities of daily living (ADL). Vibration therapy also was used to relieve spasticity in seven chronic patients with cerebral vascular disorders in cooperation with another facility. In all cases, spasticity of the lower extremities and dorsiflexion power of the foot improved with the therapy. We now are preparing a manuscript on the results for the publication in a peer-reviewed journal.

In addition, we conducted training for standing and seating movements, which are known to effectively strengthen muscle power in the back, the abdomen, the lower extremities, and the neck. Further, we introduced a robot suit HAL (Hybrid Assistive Limbs) to one patient with fetal-type Minamata disease in order to expand, augment, and support physical capability when he performed standing and walking training. We are planning to evaluate the effectiveness of HAL in the rehabilitation program.

Annual workshops on rehabilitation and assistance techniques were held in order to improve techniques used by regional specialized staffs, to be applied to the patients. This year, the themes of the 7<sup>th</sup>

workshops organized included “Repetitive facilitation exercise — the progress and the application to cases” as a rehabilitation technique and “Introduction of robot suits to support motion of patients in rehabilitation and care programs” as a care technique. According to the results of a questionnaire given to attendees, the workshops were well received. We believe these workshops are useful outreach forums to share information on care and rehabilitation techniques that can be applied in the community.

In the mid-term plan 2010, we demonstrated that direct application of vibratory stimulation to the plantar fascia using a hand-held vibration massager is effective to relieve sole pain and spasticity of lower extremities of patients with fetal-type Minamata disease. Vibration therapy using a hand-held vibration massager is believed to be applicable for many patients with various chronic neurological disorders because the method is inexpensive and non-invasive.

(2) Community development project for home care support, including health care practice

(Other activities)

Masaaki Nakamura

(Department of Clinical Medicine)

We carried out a study entitled “Home support model study, including care prevention” (FY2006– 2008) to identify support methods including rehabilitation that lead to the improvement of ADL for aging Minamata disease patients and their families. Following this project, we performed two other projects entitled “Community development project for home care support including healthcare practice” (FY2009–2011) and “Community welfare promotion business for

supporting Minamata disease victims” (FY2012), to develop methods to apply these concepts into the community. After these projects, we continued conducting educational activities in Minamata and Izumi to improve occupational therapy in these areas. Through this support, we strengthened the connection between our institute and the local community.

In the mid-term plan 2010, this clinical practice spread not only in Minamata city but also in Izumi city, and we could promote cooperation with these areas.

### 3. Risk Recognition and Information Services Group

Intake of trace methylmercury (MeHg) from eating fish is of increasing concern worldwide; however, the information available is often difficult to interpret or mostly biased. This research group aims to identify the exact level of risk and the current state of MeHg intake in the diet, through the transmission of accurate and precise information.

The research carried out by this group in FY2014 is outlined as follows:

#### Research

##### [Research theme and summary]

(1) Study on information and perception of the health risk of low-level exposures to methylmercury.

(Fundamental research)

Noriyuki Hachiya

(Department of Environmental Science and Epidemiology)

A series of risk communication surveys have been conducted at different sites in Japan including measurement of hair mercury concentration as a biomarker of methylmercury exposure, and the perception of the health risk of methylmercury contained in ordinal fishery products was investigated before and after notification of the own hair mercury level with concerned information on the health risk. It was found that consumers in Japan were relatively tolerant to the risk of methylmercury, and enriched information on the risk resulted in improvement of reassurance. The apparent tolerance to the chemical was contrast with the attitude toward the health risk of radiation associated with the Fukushima nuclear accident, for example. Implications of these findings were discussed with an application of affect heuristic model in which cultural background or a sense of worth is assumed to have an important place in the risk perception. It was also suggested that the risk

communication program of methylmercury is useful to prompt understanding of the health risk of not only the chemical itself but also other hazardous environmental agents.

#### Activities

##### [Activities theme and summary]

(1) Organization of documents and materials on Minamata disease, and dispatch of related information in the Minamata Disease Archives (Other activities)

Noriyuki Hachiya

(Department of Environmental Science and Epidemiology)

The operations of the Minamata Disease Archives has been maintained effectively including the utilization of the lecture hall, periodical updates of the contents of the regular exhibition, and management of the materials and documents on Minamata disease. As the Prime Minister authorized administrative archives that hold historical materials and documents on Minamata disease, the Minamata Disease Archives has the consolidated contents and searching system listing more than 13,500 titles. The general policy for inspection and copy was defined based on the principles of the public use of the collection and of personal information protection. The annual number of visitors was 41,954 in 2014, and a total of 533,125 visitors have been welcomed to the archives since its establishment in 2001.

(2) Examination of hair Hg in areas concerned with Hg pollution around the world (Other activities)

Masatake Fujimura

(Department of Basic Medical Science)

This year, we analyzed Hg concentration in hair samples and gathered survey information regarding gender, age, occupation, and residence in areas of

Bolivia, the Philippines and French Guiana concerned with Hg pollution (433 samples). In addition, we participated in the COPHES project as a reference laboratory and evaluated standard hair samples. This result has been published in a peer-reviewed article.

In the past 5-year plan (fiscal years 2010 to 2014), we have presented our findings at two conferences (two conferences as a representative) and published two peer-reviewed articles (one article as a representative).

(3) Information service using hair Hg analysis  
(Other activities)  
Masaaki Nagano  
(Department of Basic Medical Science)

In 2014, 1,357 hair samples were collected from visitors at National Institute for Minamata Disease, the Minamata Disease Archives, and from other organizations, and were measured for total Hg. The analytical results were sent to each individual.

There were 7,107 individuals analyzed for hair Hg level between 2011 and 2014.

## 4. Social Group

More than half a century has passed since the official acknowledgement of Minamata disease. While the significant environmental pollution of methylmercury (MeHg) has become a historical episode in Minamata Bay and its surrounding sea areas, the social problems of Minamata disease epidemics from past pollution remain to be settled in the local communities. This research group is conducting a historical verification on risk management, a survey of present health problems of patients, and a study on social restoration of the affected areas.

### Research

#### [Research theme and summary]

(1) Historical study on risk management in Minamata disease episode (Fundamental research)

Noriyuki Hachiya

(Department of Environmental Science and Epidemiology)

Fish and shellfish in the Yatsushiro Sea in Japan had been extremely contaminated with MeHg discharged from an acetaldehyde factory during the 1950's and 1960's. To date, 2,277 residents have been certified officially as Minamata disease patient in the coastal areas of the sea. Additionally, about 60,000 or more residents of the districts have been authorized as applicants of the official medical support program because of the possible health effects of MeHg. A series of large-scale population-based health surveys were conducted on coastal residents of the Yatsushiro Sea and the Ariake Sea by Kumamoto Prefecture from 1971 to 1973. However, no analytical result has been clarified, but only a short summary was published by

the Kumamoto Prefecture. The historical data, found in the report of Kumamoto University summarized in 1977, were re-analyzed, and odds ratio was calculated on neurologic symptoms among residents. The total participants were 83,000. A significant increase in the odds ratio, compared with reference sites, was observed on perioral sensory impairment (7.34, 95% CI: 6.16–8.75), any sensory impairment persisting for one week or longer (3.16, 1.80–5.57), and dysarthria or slurred speech (2.45, 1.59–3.77) among residents in Minamata. Prevalence was observed to increase significantly for most neurological symptoms examined among residents in Minamata. Furthermore, a significant increase was also detected on perioral sensory impairment not only in residents of the northern Yatsushiro Sea (8.55, 6.96–10.50) but also the Ariake Sea (4.76, 3.91–5.81). These findings are consistent with widely distributed health damage not only in the Yatsushiro Sea but also in the Ariake Sea. It is pointed out that these epidemiological findings should be used effectively for countermeasures to Minamata disease.

(2) Study on the role of local government and the viewpoint of local community of Minamata disease problem (Fundamental research)

Hirofumi Iwahashi

(Department of International Affairs and Research)

This study examined the relationship between “Minamata city has continued environmental policies” and “Minamata city does not enact the citizen participation ordinance.” This study used the basic environment ordinance of Minamata city and its actual result. As a result of the examination, in Minamata city,

by emphasis on environmental policy, there is a tendency that the basic environmental ordinance is the basis for administrative management. This study concluded that Minamata city does not enact the citizen participation ordinance because the basic environment ordinance has helped the administrative management.

## 5. Local and Global Environment Group

Our research group implements total investigations that are focused on the mercury cycle or chemical changes of mercury in the environment based on Minamata disease that was caused by exposure of methylmercury (MeHg) in environment. Mercury circulates through land, water, and the atmosphere, and its chemical form changes in each domain. That is, mercury released as a metal becomes methylmercury and circulates to arrive in human bodies via the food chain. Based on this fact, our research group investigates the movement of methylmercury in the atmosphere, rain, seawater, sediment, soil, and life. Further, each primary researcher exchanges information with other researchers, establishing joint ownership of the results, because all of these elements are mutually related; this helps each individual research project. Outlines of typical outcomes of our group investigations for the past five years (2010-2014) are as follows:

### Research

#### [Research theme and summary]

(1) Marine ecosystem and mercury levels in Yatsushiro Sea - Quantitative survey of the marine benthic community in Yatsushiro Sea and mercury concentration in different fish species in the food web

(Fundamental research)

Keisuke Mori

(Department of Environmental Science and  
Epidemiology)

In and around Minamata Bay, sampling of fish and benthic organisms for mercury, stable isotopes, and genetic analysis has advanced smoothly throughout the year. We obtained many species of fish and benthos and

analyzed more than 50 species/600 individuals of fish. We were able to build a database on the mercury level of fish, including information of individual data and catching site.

The collaborative project, including genetic analysis of prey species found in fish guts, stable isotope analysis, and general surveys for fish and benthic organisms in the Yatsushiro Sea, also have progressed significantly, and we are expecting that these results will contribute largely to our understanding of the food web and bioaccumulation of mercury.

(2) Research on the influences and behaviors of mercury in an aquatic environment of the Minamata Bay (Fundamental research)

Akito Matsuyama

(Department of Environmental Science and  
Epidemiology)

Typical outcomes of the 5-year investigation are as follows:

1) Characteristics for mercury methylation and its seasonal variation in the Minamata Bay were obtained. We found that dissolved mercury concentration in the Minamata Bay may increase in the summer. Furthermore, there was diurnal-variation for the dissolved methylmercury concentration.

2) Influences of the dissolved methylmercury concentration presently in the Minamata Bay on fishes that are living were investigated. Fishes that were cultured in the Minamata Bay were not affected by the current concentration of dissolved methylmercury during this 2-year investigation.

3) The present mercury pollution state in the Minamata Bay was compared to the survey result that was performed by the Kumamoto Prefecture 25 years ago by visualizing the mercury concentration in Minamata Bay sediment. Our results showed that an average value of total mercury concentration in surface sediment decreased slightly compared with the average value 25 years ago. On the other hand, the average value of methylmercury concentration was almost 0.1% of the average value of total mercury concentration.

4) Seawater monitoring was performed around the Shinsui seawall. The results showed that rain had little influence on the Minamata Bay.

5) Environmental factors that may influence mercury methylation in seawater were chosen by multi-regression analysis using seawater-monitoring data of the Minamata Bay. Seasonal variation of dissolved MeHg concentration in the Minamata Bay was influenced by three environmental factors (DO(Dissolved Oxygen), NPOC(Non-Purgeable Organic Carbon), salinity  $R=0.702$ ). Moreover, the effectiveness of these environmental factors was confirmed by aquarium experiments.

(3) Research on chemical reactions, transport, and deposition of atmospheric Hg (Fundamental research) — Year to year trends in the concentrations of total Hg and mono-methyl Hg in rainwater —

(Fundamental research)

Kohji Marumoto

(Department of Environmental Science and

Epidemiology)

The following results were obtained by the 5-year research on chemical reactions, transport, and

deposition of atmospheric Hg:

1) Mono-methyl Hg (MMHg) concentrations in rainwater sampled in Minamata were higher in the winter than in the summer. In addition, wet MMHg depositions had the same seasonal variations. The results of the correlation analysis with other chemical components, such as major ions, and backward trajectory revealed that these variations of MMHg were caused by the influences of biogenic emission sources, long-range transport from the Asian continents, and photodecomposition of MMHg in the summer. These seasonal variations of MMHg also were observed in Hirado, located in the northwestern edge of the Kyushu Islands.

2) MMHg concentrations in rainwater observed in Minamata have decreased yearly since the monitoring was started in September 2008. The concentrations of non-sea-salt (nss-) potassium, which is predominantly emitted from biogenic sources and waste incineration, also had the same trend. However, the reasons are still unclear. Thus, long-term monitoring is needed for some time in the future.

3) Three-year monitoring on gaseous elemental Hg (GEM), gaseous oxidized Hg (GOM), and particle-bound Hg in fine particles (PBM2.5) in the atmosphere was conducted in Minamata, and the levels of these Hg species, their intraday variations, and seasonal variations were investigated. Two-year monitoring on atmospheric GEM, GOM, and PBM2.5 was also carried out in Hirado for one week in each season. In addition, the continuous monitoring of GEM in Fukuoka and of total gaseous Hg (TGM) in Minamata has been ongoing since 2011. The results of these monitoring efforts



revealed that GEM and TGM concentrations increased in specific meteorological conditions, such as the passage of a cold front extended from low-pressure systems, and that PBM concentrations increased when Asian dust events occurred.

- 4) The quality control for the monitoring of GOM and PBM was carried out by the comparison between our manual monitoring method and the continuous monitor (Tekran Inc.), which is used widely worldwide. In addition, we proposed that the manual monitoring method for PBM in large particles using the Tekran monitor and obtained good results on the validation of their observational data.
- 5) We continued the Hg monitoring in air and wet depositions at five sites in Japan and provided the data and monitoring skills to the Asian-Pacific Monitoring Network (APMMN).

(4) Study on natural emission sources of atmospheric mercury – Estimation of Hg evasion fluxes and Hg speciation in the Seto Inland Sea and the Genkai Sea –

(Fundamental research)

Kohji Marumoto

(Department of Environmental Science and

Epidemiology)

The following results were obtained by the 5-year research on natural emission sources of atmospheric Hg:

- 1) A new sampler for the determination of dissolved gaseous Hg (DGM) in seawater was developed to prevent volatile loss of DGM from samples. Using this sampler, more accurate measurement of DGM in seawater was achieved.
- 2) DGM concentration and Hg evasion flux were measured in the Minamata Bay from June 2012 to

May 2013. The results revealed that the annual Hg evasion flux from the sea surface of the Minamata Bay (Surface area: 3.82 km<sup>2</sup>) was 180 ± 210 g, corresponding to almost twice the input from atmospheric Hg deposition.

- 3) The detection limit of MMHg in seawater improved to about 1 pg/L (in the case of an 800 mL sample) with a minor change of the analytical method.
- 4) Observations of DGM, MMHg, and total Hg in the seawater of the Seto Inland Sea and the Genkai Sea were carried out. The results revealed that their levels were lower than those in the Minamata Bay.
- 5) The Hg emission fluxes from the soil surface in the volcanic area were observed using a flux chamber method and were more than 100-times higher than those from background soil surface. Thus, further research is needed to understand the impact of volcanic activities on atmospheric Hg.

(5) Study on biomagnification of mercury from sediment to demersal fish (Fundamental research)

Shoko Imai

(Department of Environmental Science and

Epidemiology)

We conducted experiments on the uptake and elimination of mercury using marine demersal fish to understand the mechanism of bioaccumulation in marine food webs. The redspotted grouper (*Epinephelus akaara*), the devil stinger (*Inimicus japonicus*), and the marbled flounder (*Pleuronectes yokohamae*) were used as test fishes. These fishes were fed foods contaminated with mercury and/or benthos, which had accumulated mercury, and subsequently, we observed the uptake of mercury by the fishes. The total concentration of mercury in fish increased with the

concentration of mercury in the food and the uptake period. Our results showed that in *E. akaara* and *I. japonicas* fed a concentration of 0.592 µg/g MeHg, the total mercury concentration was  $0.268 \pm 0.004$  µg/g and  $0.183 \pm 0.026$  µg/g, respectively. The total mercury concentration of *P. yokohamae* fed benthos that had accumulated mercury was  $0.180 \pm 0.026$  µg/g.

We collected zooplankton from the Seto Inland Sea and the Sea of Genkai and analyzed the total mercury concentration to understand mercury levels in the zooplankton. Total mercury concentrations in the zooplankton from the Seto Inland Sea were  $87.3 \pm 74.9$  ng/g (2013) and  $424.0 \pm 252.7$  ng/g (2014). The total mercury concentration was  $32.4 \pm 6.7$  ng/g in the zooplankton from the Sea of Genkai (2014). The total mercury concentration in the zooplankton from most sites was within the range reported in the previous study, but several points showed relatively high levels. It is thought that mercury concentrations in these plankton were high because the mercury concentration in the environment is high at these points.

(6) Mercury contamination by many small-scale gold ore smelters in the Talawaan River, North Sulawesi, Indonesia

(Fundamental research)

Keisuke Mori

(Department of Environmental Science and Epidemiology)

We re-checked the data set of the mercury content in sediment, fish, benthic organisms, and resident hair samples from three villages along the Talawaan River that were collected during 2011–2012. We also have made significant progress in the data processing. Some fishes showed considerably high mercury content, and the members of our counterpart in Indonesia were

informed of this situation for the local community.

(7) Study on alkyl derivatization technique for speciation of mercury in biological and human samples

(Fundamental research)

Koichi Haraguchi

(Department of International Affairs and Environmental Sciences)

The purpose of this study was to develop a simple method for the determination of MMHg. One of the main problems associated with the analysis of MMHg in the least developed countries (LDCs) is the procurement of analytically pure reagents and carrier gases. Therefore, there is an urgent need for the development of a new analytical method for the determination of MMHg that consumes fewer reagents to assist in the protection of human health in LDCs. A new analytical method has been developed based on thin layer chromatography (TLC) and thermal decomposition amalgamation atomic absorption spectrometry (TDA AAS), and it is capable of separating and quantifying mono-methyl and inorganic mercury. This method involves several steps, including acid leaching, dithizone extraction, and preparative TLC analysis. The MMHg isolated by TLC then can be measured as elemental mercury by TDA AAS.

## Activities

### [Activities theme and summary]

(1) Cooperation of research and field survey in the international organization

(Other activities)

Mineshi Sakamoto

(Department of International Affairs and Research and Department of Environmental Science and Epidemiology)

We conducted the technology transfer for mercury analysis in hair samples to a researcher from the Philippines and the technology transfer for total mercury and methylmercury analysis to two researchers from Vietnam. Methylmercury (MeHg) exposure assessment of inhabitants in Amazon River Basin was conducted at the Federal University of Para Western, Brazil. One researcher attended “JICA Project for Evaluation of Scientific Research Ability of CIRA and Information Collection of Mercury Pollution,” which was held 8–29 June 2014 in Managua City, Nicaragua. In total, 13 researchers were dispatched for the international conference (USA, Canada, Austria, UK, Vietnam, Korea, and Philippines) and field research (Nicaragua and Brazil). We conducted the JICA training and education on the health and environmental effects of mercury compounds 11 times (107 participants in total).

school students in Minamata city participated in this forum.

One researcher joined the “Asia-Pacific Mercury Monitoring Workshop,” which organized by the Asia-Pacific Mercury Monitoring Network (APMMN) and the United States Environmental Protection Agency (EPA) and held 10–12 September 2014 in Hanoi, Vietnam. Director-General Dr. Hiroshi Noda attended the “First Regional Forum of WHO Collaborating Centers in the Western Pacific,” which was held 13–14 November 2014 at the WHO Regional Office for the Western Pacific in Manila, Philippines.

## (2) NIMD Forum and International Workshop

(Other activities)

Mineshi Sakamoto

(Department of International Affairs and Research and  
Department of Environmental Science and  
Epidemiology)

The NIMD Forum 2014 “Evaluation of methylmercury exposure and health effects in humans” was held on 28th Oct at the Minamata Disease Archives, Minamata, Japan. Six researchers were invited to the Forum from the USA, one from Canada, one from Slovenia, and three from Japan. At the same time, the first anniversary forum of the Minamata Convention was held, which was organized by the National Institute for Minamata Disease (NIMD). In total, 230 first-year junior high

## 6. Environmental Health Sciences Group

Generally, there are two sub-populations susceptible to methylmercury (MeHg) exposure, namely those who are exposed to high levels of mercury and those who are more sensitive to the effects of mercury. The Environmental Health Sciences group is conducting epidemiological surveys in Taiji-cho, Wakayama Prefecture, where the population has been exposed to high concentrations of MeHg. The group's main research also concerns the population particularly sensitive to MeHg exposure, such as fetuses or people suffering from various diseases, to contribute to appropriate risk assessment of MeHg exposure.

### Research

#### [Research theme and summary]

(1) Studies on methylmercury exposure in a whale-eating district

(Project research)

Masaaki Nakamura

(Department of Clinical Medicine)

We performed neurological examination of 194 residents (117 males and 77 females) in 2010 and 2011 in Taiji, which is famous as the birthplace of traditional whaling in Japan, and clarified that the apparent health effect of MeHg was not seen in Taiji residents who were considered to be highly exposed to MeHg from ingesting MeHg-contaminated whale meat. To examine the defense mechanism against MeHg toxicity, we measured mercury (Hg) and selenium (Se) using plasma samples from 153 subjects. A significant positive correlation between plasma mercury and Se levels was observed, and the plasma Hg/Se molar ratios of all subjects were  $< 1$ . These findings suggested that sufficient Se intake might be one of the causes of the absence of adverse effects of MeHg exposure in this study. We have been performing a comprehensive search of the proteins that are altered by MeHg exposure using mass spectrometry in order to clarify the factors that modify

the toxicity of MeHg.

We made the final preparations for the study on the effect of MeHg exposure on child development in Tokyo on June 25, 2014, and performed pediatric examinations at the Taiji-cho multi-purpose center from August 3 to 5, 2014. The examinations were carried out in cooperation with Doshisha University, Tohoku University, Akita University, and Jin-ai Women's College.

In the mid-term plan 2010, we demonstrated that the apparent health effect of MeHg was not seen in Taiji residents and that sufficient Se intake might be one of the causes of the absence of adverse effects of MeHg exposure in this study. We have been continuing the survey on the effects of MeHg on child development in Taiji.

(2) Evaluation of the effect of exposure to methylmercury and other elements during gestation and breast feeding

(Fundamental research)

Mineshi Sakamoto

(Department of Environmental Science and Epidemiology)

In this study, we conducted simultaneous analyses of the fingernails, toenails, maternal blood, umbilical cord blood, and maternal hair segments to investigate how Hg concentrations in the nails are related to other biomarkers, especially at parturition. For the statistical analysis, logarithmically transformed Hg concentrations were used. Mercury in the fingernails and toenails at early pregnancy reflected the maternal Hg body burden level approximately 5 months retroactively. At parturition, Hg levels in the fingernails and toenails also showed strong correlations with those in cord blood. Mercury levels in fingernails and toenails at parturition also reflected MeHg levels throughout the third trimester of gestation. These results suggest that fingernails and toenails at parturition are useful

biomarkers for prenatal MeHg exposure for mothers and fetuses, especially during the third trimester of gestation. We also investigated the significance of fingernail and toenail mercury concentrations as biomarkers for prenatal selenium exposure.

(3) Protective effect of selenium against methylmercury toxicity and co-existence of mercury and selenium in humans and sea-mammals (Fundamental research)

Mineshi Sakamoto

(Department of Environmental Science and Epidemiology)

Our goal is to confirm mercury selenide (HgSe) formation in toothed-whale muscles with increasing stages of methylmercury (MeHg) demethylation. Total mercury (T-Hg), MeHg, and selenium (Se) were measured in the muscles of four toothed-whale species. In all species, the MeHg concentrations in the muscles increased with increasing T-Hg concentrations and tended to reach a plateau. In contrast, the MeHg percentages decreased from 90–100% to 20–40%. The T-Hg and Se concentrations showed strong positive correlations. The Se/I-Hg molar ratios rapidly decreased with increasing I-Hg concentrations and conversed a constant ratio of 1. These results indicated that demethylated MeHg in the muscles rapidly formed I-Hg/Se equimolar complexes. An X-ray absorption fine structure analysis of bottlenose dolphin muscles confirmed that the dominant chemical form in the Hg/Se complexes was HgSe. Further, an electron probe microanalysis of bottlenose dolphin muscles showed that mercury and Se coexisted as particles in the same cellular location, mainly in cells near the endomysium. These results indicate that toothed-whale muscles have a strong demethylation ability of MeHg, and the demethylated MeHg deposit as inert HgSe within their muscle cells and the high T-Hg accumulation in the muscle are explained by the increase of HgSe.

(4) Evaluation of factors affecting sensitivity to MeHg exposure—Application of disease model animals and knock-out animals (Fundamental research)

Megumi Yamamoto

(Department of Basic Medical Science)

- 1) We evaluated MeHg-induced peripheral neuropathy using a behavioral test (dynamic weight bearing [DWB] test) and pathological analysis. During MeHg exposure from early stage (12 w) to late stage (17 w), the weight balance of the forelimb/hindlimb in MeHg-exposed, KK-Ay mice changed from favoring the hindlimb to equal balance to favoring the forelimb. The DWB test will be useful for evaluating the behavioral changes due to MeHg exposure in this model. Peripheral neuropathy associated with MeHg could be evaluated by Masson trichrome staining, CD204 expression, and M2 macrophage markers in KK-Ay mice.
- 2) We examined inflammatory cytokine expression against MeHg exposure in U937 macrophages. In addition, we have succeeded in achieving appropriate experimental conditions for a knock-down experiment of NF- $\kappa$ B in U937 macrophages.
- 3) We aimed to develop a simple method for total mercury (T-Hg) and methylmercury (MeHg) analysis in biological samples using methyl isobutyl ketone (MIBK) instead of chloroform in the degreasing step of our previous report (Miyamoto et al., 2010). We have confirmed the accuracy of this modified method by determining MeHg concentrations in standard materials (codfish, swordfish, and hair). We used it to determine the MeHg and T-Hg concentrations and MeHg/T-Hg ratios in the tissues from five species of commercially sourced fish. In the muscle of all fish and squid analyzed, MeHg/T-Hg ratios were in the range of 96–98%, which was consistent with those in a previous report.

## 7. Publications and Scientific meetings

### [International Journals]

Sakamoto M, Chan HM, Domingo JL, Oliveira RB, Kawakami S, Murata K. Significance of fingernail and toenail mercury concentrations as biomarkers for prenatal methylmercury exposure in relation to segmental hair mercury concentrations. *Environ. Res.* 2015; 136: 289-294.

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Marumoto K, Imai S. Determination of gaseous mercury in seawater of Minamata Bay and estimation for mercury exchange across air-sea interface. Mar. Chem. 2015; 168: 9-17.

**[International meetings]**

Sakamoto M, Marumoto M, Yasutake A, Iwasaki T, yasunaga G, Fujise M, Nakamura M, Murata K, Chan HM: Mercury and selenium concentrations in tooth-whale meats and their element profile in blood of the inhabitants in a whaling town, Japan. The EcoHealth 2014 conference, Montreal, Canada, 2014. 8.

Sakamoto M, Murata K, Chan HM, Oliveila R, Doming J. Significance of nail mercury concentrations as a biomarker of prenatal methylmercury exposure in relation to segmental hair mercury concentrations at parturition. The 50th Congress of the European Societies of Toxicology (Eurotox2014), Edinburgh, UK, 2014. 9.

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Fujimura M, Usuki F: Low level of methylmercury inhibits cell proliferation through the activation of glycogen synthase kinase  $3\beta$  and subsequent degradation of cyclin E in cortical progenitor cells of rats. 54<sup>th</sup> Annual Meeting of Society of Toxicology, San Diego, US, 2015. 3.

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