

NIMD Annual Report  
2017  
(April 2017 to March 2018)

National Institute for Minamata Disease  
Ministry of the Environment  
Japan

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Report on Research and Other Activities in the  
Fiscal Year 2017

## 1. Pathomechanism Group

The aim of this study group is to understand the molecular mechanisms underlying methylmercury (MeHg) toxicity in humans. Focusing on the group focuses on the biological effects of MeHg, our goals are as follows: to understand the initial signs of MeHg poisoning, to evaluate MeHg toxicity, to develop methods protecting against MeHg-induced disorders, and to develop new treatment options to repair the damage caused by MeHg poisoning. In this study, we used techniques from biochemistry, molecular biology, and pathology in cell cultures and animal models to determine the differences in MeHg sensitivities in different organs and individuals. We analyzed stress responses and changes in the cellular signal transduction induced by MeHg exposure. In addition, to elucidate the mechanisms underlying MeHg toxicity, we investigated cell death and regeneration in nerve cells damaged by MeHg. We also studied the effect of food additives on Hg excretion after MeHg exposure. We are in the process of identifying drugs and treatments that suppress MeHg toxicity and promote nerve regeneration. This research was conducted during the fiscal year 2017.

### [Research theme and summary]

(1) Fundamental research on prevention and treatment of methylmercury toxicity

(Project research)

Masatake Fujimura

(Department of Basic Science)

We indicated that Fasudil, a ROCK inhibitor, was able to rescue MeHg-induced neurological symptoms and axonal degeneration in dorsal spinal root nerves by directing a phenotype change of microglia from the pro-

inflammation type to the anti-inflammation type in rats. Next, we demonstrated that MeHg exposure (8 ppm in drinking water) during gestation significantly decreased the number of mature neurons in the cerebral cortex through the suppression of proliferation of neural precursor cells in rats. Furthermore, we confirmed the effectiveness of vibration stimuli on the plantar part of the hind limb on a rat immobilization-induced muscle atrophy model. Moreover, we clarified that MeHg induced neural activity (c-fos) and subsequent cell death through the p38 MAPK and CREB pathway in differentiated SH-SY5Y cells.

This year, we presented our findings at four conferences, and published one peer-reviewed article.

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

(Fundamental research)

Masatake Fujimura

(Department of Basic Science)

We clarified that MeHg exposure induced the expression of thymidine glycol, a marker of oxidation, in the deep layer of the cerebral cortex vulnerable to MeHg neurotoxicity in mice. Next, we indicated that MeHg exposure during pregnancy induced synaptic pruning (decrease of synaptic vesicle proteins such as synaptophysin and IP<sub>3</sub>R<sub>1</sub>) in the cerebellum of rats.

This year, we presented our findings at four conferences (one conference as a representative), and published two peer-reviewed articles (one article as a representative).

(3) Study on changes in gene expression induced by methylmercury exposure, their effects on pathological conditions, and protection against toxicity

(Fundamental research)

Fusako Usuki

(Department of Clinical Medicine)

Nonsense-mediated mRNA decay (NMD), a cellular mechanism eliminating mRNAs that harbor premature translation termination codons (PTCs), was suppressed in methylmercury (MeHg)-treated mouse MeHg-susceptible myogenic cells, rat cerebral cortical neuronal cells (CNC) and rat astroglial cells (AGC). In order to know the causative factor of stress-induced NMD suppression, the role of phospho-eIF2 $\alpha$ /ATF4 pathway, a key pathway involved in adaptation to stresses, was investigated. The results demonstrated that phospho-eIF2 $\alpha$ -mediated translation repression plays a critical role. In addition, we uncovered that mTOR suppression-induced cap dependent translation inhibition and downregulation of NMD components UPF1, SMG7, and eIF4A3 were also involved in stress-induced NMD suppression. Stress-induced NMD suppression has potential to affect the condition of cells under environmental stresses through stabilization of NMD-targeted gene expression, which may be important to consider individual sensitivity to stresses. The manuscript on the mechanism of endoplasmic reticulum (ER) stress preconditioning-induced upregulation of membrane transporters that affect the cellular influx and efflux of MeHg was published in a peer-reviewed English journal (Usuki et al., *Scientific Reports*, **7** : 12390. doi:10.1038/s41598-017-09435-3, 2017).

Furthermore, to analyze the potential epigenetic effects of MeHg toxicity, we examined the expression of candidate genes in 36-day-old male rat samples upon

exposure to 8 ppm MeHg during fetal period. We performed DNA methylation analyses, western blot analyses, and immunohistochemistry of cerebellum, cerebrum, and hippocampus. We observed some samples showing the changes in these candidate genes. Further investigation is needed to determine if the changes in these candidate genes are due to real epigenetic changes.

(4) Study on the modifying factors in the toxicity of

methylmercury (Fundamental research)

Masaaki Nagano

(Department of Basic Medical Science)

In this study, we investigated the modifying factors (wheat bran, fructooligosaccharide (FOS), and glucomannan) in the toxicity of MeHg. So far, we have demonstrated that tissue Hg levels decreased via fecal excretion of Hg in FOS-fed mice.

In this past year, in order to confirm whether fecal excretion of Hg by FOS is via the metabolic activity of intestinal bacteria, antibiotics were administered to the basal diet- or FOS diet-fed mice. The Hg content in feces, for 4 days after MeHg administration, was significantly decreased in both the basal diet and FOS diet groups treated with antibiotics, as compared to the untreated groups. Fecal Hg contents in the FOS diet group, treated with antibiotics, did not differ from that of the basal diet group, treated with antibiotics. These results indicate that fecal excretion of Hg by FOS is not due to FOS itself being bound to Hg, suggesting instead that its mechanism may be via the metabolic activity of associated intestinal bacteria.

Furthermore, we established an acute MeHg intoxicated animal model using mice, in order to evaluate the protective effect of wheat bran or FOS for MeHg-induced neurological symptoms.

## 2. Clinical Group

### Research

Minamata disease (MD) is caused by severe mercury (Hg) poisoning. The incidence of comorbidities, such as cervical spondylosis or metabolic syndrome, tends to increase with age in these patients, making the diagnosis of MD difficult, as it relies exclusively on clinical neurological symptoms. Therefore, the development of objective methods to accurately identify methylmercury (MeHg) poisoning is necessary. In addition, as spasticity, dystonia (involuntary movements), and intractable chronic pain affect the quality of life (QOL) of MD patients, effective therapies are needed.

Our research group conducted experiments to evaluate the neurological function of patients with MD, using magnetoencephalography (MEG) and MRI. To develop an effective therapy for MD, we have performed repetitive transcranial magnetic stimulation (rTMS) therapy for ataxia, pain, numbness, and tremors observed in MD patients. Furthermore, we have continued botulinum toxin therapy for treating spasticity of MD patients.

The research conducted by our group during FY 2017 is outlined below:

#### [Research theme and summary]

(1) Research on the evaluation of human health effects and therapy against methylmercury exposure

(Project research)

Masaaki Nakamura

(Department of Clinical Medicine)

To develop an objective evaluation protocol using MEG and MRI, we compared somatosensory evoked fields (SEF) and morphological features in MD

patients and control subjects from the Kumamoto district (not polluted with MeHg). When the data of SEF were quantified on N20m amplitude, the stability of N20m latency, the direction of N20m and P35m, and frequency of somatic sensory area, we found an abnormality that was more common in the certified MD patients than in the control subjects. Furthermore, we removed noise from raw data of the MEG using the latest denoise script for a more precise frequency analysis.

Comparing the brain MRI findings of 72 control subjects with 12 MD patients, we found that the cerebellum, right crow spur fossa circumference of calcarine sulcus, right frontal operculum, and bilateral thalamus were significantly withered in MD.

With rTMS for MD patients, we observed improvements in ataxia, pain, numbness, and tremor. We continued botulinum toxin therapy for treating spasticity of the fetal-type MD patient. Furthermore, two additional MD patients began botulinum toxin therapy this year.

### Activities

In recent years, the compensation needs of MD patients have moved towards political resolution. The Department of Clinical Medicine actively organizes events on MD, in cooperation with related organizations. In addition, we organized conventional rehabilitation activities, including daycare facilities for fetal-type MD patients, rehabilitation technical schools, and care technical schools. We also examined the usefulness of vibration therapy for the relief of pain and spasticity associated with various chronic diseases of the nervous system, including MD. Furthermore, we

introduced a robot suit, HAL (Hybrid Assistive Limbs), which assists patients with standing and walking actions.

To examine the effectiveness of home care support for MD patients and their families, we conducted a project titled “Home support model study, including care prevention” (FY 2006–2008). Subsequently, the projects “Community development project for home care support, including healthcare practice” (FY 2009–2011) and “Community welfare promotion business for supporting MD victims” (FY 2012) were carried out to develop strategies for application of these concepts in the community. After the completion of these projects, we have continued supporting welfare activities in the cities of Minamata and Izumi.

Additionally, since pathological tissue specimens of MD autopsy cases are extremely valuable, we plan to digitize the pathological slides for permanent preservation and publish digitized pathological slides on the website for students and doctors training in pathology.

The following section includes an outline of the clinical practice conducted by this group in FY 2017:

**[Activities theme and summary]**

(1) Rehabilitation programs for patients with Minamata disease and dissemination of information on care and rehabilitation

Fusako Usuki

(Department of Clinical Medicine)

We continued to provide outpatients with rehabilitation, in the form of daycare, two days a week. The principal objective was to improve their QOL. In addition to vibration stimuli and repetitive facilitation therapy, a new device that administers repetitive peripheral magnetic stimulation was introduced this

year. The combined vibration stimuli, repetitive facilitation therapy, repetitive peripheral magnetic stimulation, and gait training with HAL were effective for a patient with fetal-type MD (FMD). The combined therapy improved the patient’s gait movements, such as velocity, step length, and physical cost index (PCI) (which indicates physical load), leading to improvement in the activities of daily living (ADL). The manuscript on a case with FMD whose gait movements were improved by an introduction of HAL was accepted in a peer-reviewed rehabilitation journal. Annual workshops on rehabilitation and assistance techniques are held in order to improve techniques used by specialized regional staff for patients. This year, the themes of organized workshops included “The mechanism of chronic pain and neurorehabilitation” as a rehabilitation technique and “Utilization of information technology (IT) to support the defect of communication” as a care technique. Based on the questionnaire results answered by attendees, the two workshops were well-received. We believe that the workshop is a useful outreach forum to share information on care and rehabilitation techniques that can be implemented in the community.

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

Masaaki Nakamura

(Department of Clinical Medicine)

We carried out a study titled “Home support model study, including care prevention” (FY 2006–2008) to identify support methods, including rehabilitation, prompting improvement of ADL for aging MD patients and their families. Following this project, we undertook two other projects titled “Community development project for home care support, including healthcare



practice” (FY 2009–2011) and “Community welfare promotion business for supporting Minamata disease victims” (FY 2012), to develop methods to implement these concepts in the community. Eventually, we continued implementing educational activities in the cities of Minamata and Izumi, to improve occupational therapy opportunities. Through this support, we strengthened the connection between our institute and the local community.

When we improved the program of the crafts classroom in Minamata, we received high satisfaction levels from the participants in a survey. We also instructed the inhabitants of Minamata on how to walk with fewer burdens using a safety walk navigator.

Furthermore, we publicized the work of the crafts classroom using the homepage and Facebook page of NIMD.

(3) Information transmission using the Minamata disease pathology specimens

Masumi Marumoto

(Department of Basic Medical Sciences)

Pathological tissue specimens of autopsy cases of MD are extremely valuable. Our institute has embarked on a mission to collect and store pathological tissue specimens of MD disease permanently. However, pathological tissue slides are difficult to preserve permanently, due to their fading with the passage of years. Therefore, our solution is to digitize these slides for permanent preservation and to publish the digitized files on our website for the use of pathology students and doctors.

### 3. Exposure and Health Effects Assessment Group

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

#### [Research theme and summary]

(1) Effect of glucose metabolism disorders on methylmercury toxicokinetics and toxicity

(Fundamental research)

Megumi Yamamoto

(Department of Basic Medical Science)

1) We have finished the neurobehavioral analysis of MeHg-treated KK-Ay mice of 12 weeks of age using the Dynamic Weight Bearing (DWB) test, and our results indicated that the DWB test would be a useful method for evaluation of neurobehavioral disturbance due to MeHg exposure in rodents. In addition, the pathological analysis using CD204 as a marker indicated that a lesion was detected in the sciatic nerve in MeHg-exposed KK-Ay mice, which displayed neurological symptoms. Based on this result, we conducted a pathological analysis using macrophage markers (CD68, CD163, CD204, HLA-DR) in peripheral nerves of certified (5) or uncertified (6) patients of MD. However, it was difficult to apply the

results of our experimental study using mice into a human study. Therefore, we have submitted the results of the experimental study using mice to a journal.

2) We are examining total Hg (THg) concentration in blood and tissues (brain, kidney, liver) of MeHg exposed BL/6 and KK-Ay mice (3 doses of MeHg, single administration) to evaluate the effect of glucose metabolism disorders on MeHg toxicokinetics.

(2) Research on the tissue localization of mercury and selenium in the mammals

(Fundamental research)

Masumi Marumoto

(Department of Basic Medical Science)

In conventional histopathological techniques, it was impossible to visualize the MeHg and Se. In exposed animals and humans, MeHg is slowly biotransformed to inorganic Hg. Se is a known antagonist of MeHg toxicity, however the underlying mechanisms are largely unknown, and histopathological distribution of Se has not been clarified. Therefore, the objective of this study was to reveal the tissue distribution of Hg and Se by using an X-ray probe microanalyzer that can detect both Hg and Se.

(3) Health risk assessment of high methylmercury exposure derived from whale

(Fundamental research)

Masaaki Nakamura

(Department of Clinical Medicine)

We attempted detection of protein associated with toxicity of MeHg, but not Se, to determine why clear health was not seen in adults of Taiji-cho. Among the plasma that we had in our possession of Taiji-cho

inhabitants, we performed two dimensional electrophoresis using the plasma samples of 4 subjects (two men and two women) with high Hg levels and 4 low level subjects (two men and two women). We ultimately obtained 14 spots which had a statistically significant difference of concentration between high and low level samples.

We also surveyed the effect of MeHg exposure on child development, by performing pediatric examinations at the Taiji-cho multi-purpose center, starting August 2015. This past year, we examined 26 first graders of Taiji-cho and Nachikatsuura-cho. The examinations were carried out in cooperation with Doshisha University, Tohoku University, Akita University, and Jin-ai Women's College.

From a diet survey in Taiji-cho, and Nachikatsuura-cho, the following things became clear: 1) the fish which was the most widely consumed was tuna. 2) Taiji-cho inhabitants mostly took in MeHg from consuming whale, while Nachikatsuura-cho inhabitants most commonly took in MeHg from tuna.

(4) Studies on fetal exposure to methylmercury and other elements coexisting with mercury

(Fundamental research)

Mineshi Sakamoto

(Department of Environmental Science and Epidemiology)

1) Toxic metals (Hg, Pb, and Cd) and essential elements (Se, Fe, Cu, Mn, and Zn) were measured in red blood cells (RBCs) and plasma, respectively. Distribution of the examined elements between RBCs and plasma was compared for maternal blood and cord blood to understand the role of the placenta. In addition, inorganic Hg concentrations in RBCs and plasma for maternal blood and cord blood were measured. In

contrast to Cd and Pb, Hg level in RBCs of cord blood was higher (160%) than that in maternal blood, suggesting that MeHg is transferred from mother to fetus via an active amino acid transport system. Inorganic Hg concentration in RBCs and plasma was lower in cord blood than in maternal blood. This suggests that inorganic Hg was partially trapped in the placenta. In addition, the Se level in plasma was lower in cord blood than in maternal blood. These findings suggest that the protective effects of Se against MeHg toxicity expected in fetuses may be weaker than those expected in mothers.

2) The MeHg transfer from maternal blood to umbilical cord (fetal) blood was compared with 1) the transfer of lipid components, amino acids, and elements. Furthermore, 2) Hg, as well as Se, vitamin E, and docosahexaenoic acid (DHA), substances that have a protective effect against MeHg toxicity in maternal blood and fetal blood, were compared in order to discern why a fetus is more susceptible to MeHg toxicity. The level of Se in cord blood was almost the same as that in maternal blood. Vitamin E and DHA were much lower in cord blood than in maternal blood. Consequently, the ratios of Se/Hg, vitamin E/Hg, and DHA/Hg were lower in cord blood than those in maternal blood. Therefore, both higher Hg levels in cord blood and the lower ratios of protective factors, such as Se, vitamin E, and DHA, may account for the higher risk of fetuses for MeHg toxicity. This result was published in *Environ. Int.* 2017; Jan; 152: 446-453. (IF = 7.088)

(5) Total mercury, methylmercury, and nutrients in edible deep-sea fish

(Fundamental research)

Kimiko Izumo

(Department of Environmental Science and  
Epidemiology)

Deep-sea fish have attracted attention recently as new foodstuffs in Japan. Some deep-sea fishes are reported to have a relatively high Hg concentration. On the other hand, fish is a source of the nutrients, such as Se and DHA, which are protective against MeHg toxicity. Considering both the risk and the benefit of fish consumption, we measure THg, MeHg, Se, and  $\omega$ -3 polyunsaturated fatty acids in deep-sea fishes, in which existing data is scarce. In addition, we examine the usefulness of gills, which are normally not considered food, for evaluation of MeHg concentration in the edible parts of fish.

Five species of deep-sea fish were obtained from several markets in Shizuoka. As a control, we used marbled rockfish (*Sebastiscus marmoratus*), a kind of demersal fish, from the Nagashima coast and not from Minamata bay. Samples of muscle, gill, and liver tissues were collected for each individual. The median of THg concentration in the muscle tissues of marbled rockfish from Nagashima was less than the Japanese regulatory standard of 0.4 ppm of THg, but in 3 out of 50 cases, the THg concentration exceeded the regulatory standard. We are planning to increase the sample numbers and collect marbled rockfish from other areas next year.

## 4. Social and Information Service Group

The city of Minamata was rebuilt as an environmental city. In 2013, The Conference of Plenipotentiaries at the Minamata Convention was held in Minamata and Kumamoto, focusing on mercury (Hg). In 2014, the Regional Revitalization Act was enacted. At present, the major challenge is regional revitalization. Therefore, our research group performed studies on regional revitalization under an agreement with the city of Minamata (2015). In addition, we verified historical survey materials based Minamata disease (MD). In addition, our goal was to elucidate the problems related to risk perception of methylmercury (MeHg) in society and to practice effective communication and dissemination of relevant risk information. Thus, this research group aimed to contribute to "regional revitalization" and "transmission of information about MD." The research carried out by this group in FY 2017 is outlined in the following paragraphs.

### [Research theme and summary]

(1) New development of community design starts at "power of municipality" for regional revitalization around Minamata disease-affected areas

(Fundamental research)

Hirofumi Iwahashi

(Department of International Affairs and Research)

In this study, we set out to develop a research basis for "community design of future thinking" towards the regional revitalization of the Minamata area. As a result, 2 journal articles were published. In the first paper, we analyzed 3 efforts by major ventures that have utilized the rich natural environment of rural mountain villages in the Minamata area. From the results of the analysis, we examined the policies of

each local government that are considered necessary for maturing towards regional revitalization. In the second paper, we analyzed efforts by the Minamata Eco-Town project. From the results of the analysis, we examined the policies of Minamata City, which are considered necessary for maturing towards regional revitalization.

Additionally, we made various efforts to advance policy recommendations made to Minamata City.

(2) Study on risk governance of adverse health effects of methylmercury

(Fundamental research)

Noriyuki Hachiya

(Department of Environmental Science and

Epidemiology)

A historic epidemiological analysis was performed on the data of health examination survey that had been conducted in Minamata from 1975 to 1981. Self-reported questionnaire data, collected as primary examination in the survey, were retrieved on 27,621 participants with recovery rates of 82.6% of participants, and 74.4% of the population base. In univariate analyses, apparent associations with fish consumption frequency were found on the age specific prevalence of 25 neurological complaints and on the experience of abnormal cat death not only in fishing villages but in central and mountain areas of the city. The results of multifactorial analyses revealed that fish consumption frequency, fishery engagement, residence in a fishing village, and duration of residence in Minamata, in addition to age, hypertension and diabetes, were strongly associated with the health impairments. A paper was published discussing a problem of the

unequal risk taking in the history of Minamata disease.

**[Activities theme and summary]**

- (1) Transmission of information on Minamata disease, and organization of documents and materials in the “Minamata Disease Archives”

Hirofumi Iwahashi

(Department of International Affairs and Research)

The Minamata Disease Archives collected data and papers about MD and Hg. These materials are expected to be used for ongoing research. In addition, we updated the exhibited contents of Birds View Space. Information was provided through the use of an exhibition room and a lecture hall. In addition, we held training sessions, and other meetings that cooperated with the “Minamata Disease Municipal Museum” and the “Kumamoto Prefecture Environmental Center.” Through these efforts, we promoted further understanding of MD and Hg.

- (2) Information service using hair mercury analysis

Masaaki Nagano

(Department of Basic Medical Science)

In 2016, 561 hair samples were collected from visitors at the “National Institute for Minamata Disease,” the “Minamata Disease Archives,” and from other organizations. The total Hg levels of the samples were measured. The analytical results were sent to each participant.

## 5. Nature Environment Group

Mercury (Hg) circulates through land, water, and the atmosphere, and its chemical form changes in each medium. In addition, a part of inorganic Hg released from anthropogenic and natural emission sources is transformed into methylmercury (MeHg) in natural environments, and MeHg is bioaccumulated in humans via the food web in aquatic environments. Our research group carried out integrated studies, including field survey, monitoring, and laboratory experiments, for understanding Hg cycling, transport, and chemical reactions in natural environments. Our main target area included Minamata Bay, as well as the Yatsushiro Sea and ocean area around the Japanese Islands. In addition, we conduct investigations in Hg-polluted areas all over the world.

The outline of our studies in this fiscal year is described below.

[Research theme and summary]

- (1) Research on mercury exchange in air-sea interfaces and accumulation in marine wildlife of mercury around the Japanese Islands, using an atmospheric mercury monitoring network

(Project research)

Kohji Marumoto

(Department of Environmental Science and  
Epidemiology)

We continued the Hg monitoring in the air and rainwaters at Minamata, Fukuoka, Hirado, and Omaezaki. In addition, the monitoring at the Awaji Island was started in June 2017. However, monitoring at Kashiwazaki in the Niigata prefecture was terminated in March 2017. The Hg concentration in rainwater did not differ from the past few years with the

exception rainwater collected in Minamata in 2015. On the other hand, the concentrations of gaseous elemental mercury (GEM) and particulate bound mercury (PBM) in the air of the Fukuoka city were lower in this year than in the past few years. We will focus on those year-to-year trends with further continuous monitoring.

We also investigated Hg methylation in seawater. In our previous study, we found that the MeHg concentration in the seawater of the Kuroshio Current region was highest at the depth of around 500 m, where the North Pacific Intermediate Water (NPIW) circulates. The highest MeHg concentrations in the NPIW were observed in other sea areas, such as the eastern Pacific Ocean and equatorial regions. However, the reason is still unclear. Thus, using the R/V Hakuho-Maru (Research cruise KH-17-03), we took seawater samples from the western part of the subarctic regions of the North Pacific Ocean, where the NPIW develops from the Kuroshio and Kamchatka currents. Total Hg and MeHg were measured to obtain their vertical profiles. In this cruise, we also investigated the vertical profile of dissolved gaseous mercury (DGM) by the on-site measurement and Hg emission flux from the sea surfaces. We found that the DGM concentrations were higher in deeper water than in surface and subsurface waters. From the result of the Hg flux measurement, we also found that atmospheric gaseous Hg was easily deposited onto the sea surface of this area.

These data obtained in this study were highly important for understanding global and regional Hg cycling.

- (2) Bioaccumulation of mercury and food web analysis of near-shore ecosystems in Minamata Bay,

Yatsushiro Sea, and other sea areas.

(Fundamental research)

Keisuke Mori

(Department of Environmental Science and  
Epidemiology)

In and around Minamata Bay, the sampling of fish and benthic organisms for Hg and stable isotopes, as well as genetic analysis, has advanced smoothly over the last year. We obtained many species of fish and benthos, and analyzed Hg levels of 200 samples. Route of Hg accumulation was clearly via benthos from the sediment, based on the genetic analysis of benthos and Zooplankton. Also, the isotope composition of fishes was different depending on food source, such as plankton or benthic algae, based on stable isotope analysis. The genetic sequence of fishes and benthos was used to determine the identity of almost 100 species. The creation of a data base, as an excel file, documented marine life that were caught in Minamata Bay. Minamata Bay fish specimens were transferred to the Seikai National Fisheries Research Institute, and the benthic specimens that were caught by dredge sampling, or intertidal biota surveys of Minamata Bay between 1997 and 2017, were transferred to the Kitakyushu City Museum. A biological list of rare creatures in Fukuro Bay was created, and is being utilized as an outcome of regional environmental conservation activities.

(3) Research on the behavior of mercury in the aquatic environment of Minamata Bay and its surrounding sea area

(Fundamental research)

Akito Matsuyama

(Department of International Affairs and Research)

1) Annual average values of dissolved total Hg and MeHg concentrations were  $0.38 \pm 0.10$ , and  $0.10 \pm 0.10$

ng/L respectively (measured 5 times / year). An annual average value of dissolved total Hg concentration at the Eco Park in the Minamata reclamation area was  $0.77 \pm 0.17$  ng/L (3 times /year).

2) We discovered that the inside of the small environmental incubator that was used for culture experiments on Hg was contaminated, so it was cleaned. Then, to reduce future Hg contamination, a special resin mat that was coated with charcoal was installed at the inlet and outlet. As a result, the Hg contamination level of the incubator was reduced and could be used for the room culture experiment. Additionally, we discovered that the divalent inorganic Hg that was added to seawater in the room culture experiment was adsorbed to suspended solids (SS) in the seawater. Therefore, to evaluate the chemical reaction of Hg methylation exactly, an analytical method of MeHg in SS was developed.

3) The collection plan for sediment core samples that were distributed in Yatsushiro Sea was reconsidered. As a result, 37 core sediment samples and 25 surface sediments were taken in 2017. Then, the core sediment samples were cut by 1 cm each. The concentration range of total Hg content in sediment was between 0.10 ppm to 3.94 ppm. In order to elucidate the movement of bottom sediment by seawater flow, a special machine was utilized. Unfortunately, some errors were found in the device, so the experiment has been suspended at this time. However, computer simulation of bottom sediment movement by particle size was performed, based on the results of particle size distribution of Minamata Bay sediment. As a result, very little movement of bottom sediment larger than the particle size of the clay and silt was observed by computer simulation. Moreover, we obtained analytical data of Multi-Collector that has good accuracy and less



variation, based on the results of analytical data on international reference material of marine sediment.

(4) Development of atmospheric mercury monitoring method for rapid and simple screening in mercury emission sources and their surrounding areas

(Fundamental research)

Kohji Marumoto

(Department of Environmental Science and Epidemiology)

To develop the estimation of Hg emission amounts from volcanic and geothermal areas, Hg in the air, at the rim of the Aso Nakadake 1<sup>st</sup> crater was measured. In addition, we also carried out the test flight of a drone with a sampling system of air Hg and quartz crystal microbalance for air Hg (QCM-Hg) in the air space above the crater. We found that the Hg concentration in the air space above the crater was less than 100 ng/m<sup>3</sup>. These values were almost 20 times higher than those at the rim of the crater, but were lower than the detectable concentration (1,000 ng/m<sup>3</sup>) of the QCM-Hg.

The experiment of the QCM-Hg measurement device was applied and tested as a personal exposure monitor for Hg contaminated areas. However, under the concentrations of 500 ~ 600 µg/m<sup>3</sup>, the frequency variation of the QCM-Hg became nonlinear within 30 minutes and came to plateau. This was due to saturation of the reaction surface. Thus, further investigation of effective methods for lengthier measurements by QCM-Hg in highly contaminated areas is needed.

(5) Fabrication of standard gaseous oxidized mercury generator and its application to fundamental studies of atmospheric chemical reactions related to methylmercury

(Fundamental research)

Satoshi Irei

(Department of Environmental Science and Epidemiology)

MeHg may be formed under atmospheric oxidation. If this were the case, MeHg would be formed by a series of reactions of organic compounds with gaseous oxidized mercury (GOM), which is formed by the atmospheric oxidation of gaseous elemental mercury (GEM). For the investigation of the secondary formation of atmospheric MeHg, and better understanding of the global Hg cycle, it is very important to more accurately understand physical and chemical pathways of GOM and GEM in the natural environment. In this research project we fabricated a permeation device for the standard gas of GEM and GOM, to evaluate the significance of uptake processes of GEM and GOM in atmospheric water (rain, fog, and cloud droplets) in laboratory studies and the accuracy and precision of ambient measurements performed in Japan by a Tekran 2537/1130 automated atmospheric Hg analyzer. Here, the progress of this project in the fiscal year of 2017, which is the first year of the 3-year project, and the future plan are reported.

The purchasing order of a permeation device, Dynacalibrator 345 (VG International, Switzerland), was approved in August, and then the instrument was ordered and received in December. The instrument, however, did not function initially. After communication with the manufacturer, the issue was identified as a bug in the program. The instrument was fixed by replacement of the program in late January. The instrument functions currently, but is undergoing further testing. A permeation tube for GEM was available from the manufacturer, thus, the instrument has been tested with the GEM tube. For the uptake experiments, custom-made flow tube was already

designed and on hand. Therefore, the uptake experiments for GEM will be performed as soon as the full functionality of the instrument was confirmed. A permeation tube for GOM is not available in the market and has to be prepared by hand. A high precision micro balancer (XPE56, METTLER TOLEDO) for the calibration of hand-made permeation tubes was purchased already. In near future the instrumental modification for GOM will be performed by comparing the gas concentration and permeation rate of the tube. Once the instrument is optimized for GOM, the laboratory experiments and evaluation of atmospheric measurements for GOM will be conducted.

## 6. International Contribution Group

Environmental pollution by mercury (Hg) is spreading, and is particularly serious in developing countries. To decrease the risk of further pollution, the Minamata convention was adopted by 140 participating nations. Based on this background, international contributing groups in National Institute for Minamata Disease (NIMD) plan mutual cooperation with foreign researchers for studies on Hg and for information exchange via the NIMD forum. Moreover, based on the needs of the developing countries facing Hg pollution, we used our experience, knowledge, and the latest technology via obtaining the mutual cooperation of JICA. In addition, we developed a new simple Hg analysis methodology that is focused on methylmercury (MeHg), and it was positioned in the Minamata convention as a commitment to the MOYAI initiative. In this report, the summary of studies and duties of the international contributing group of 2017 are reported.

### [Research theme and summary]

(1) Development of a simple method for the determination of monomethyl mercury in the least-developed countries

(Project research)

Koichi Haraguchi

(Department of International Affairs and  
Environmental Sciences)

Our research project consists of three major parts: 1) development of a simple method for the determination of Hg compounds; 2) certification of our management system for analytical quality, administrative, and technical operations to ISO/IEC 17025; and 3) development of reference materials for

evaluating the accuracy of Hg analysis for human biomonitoring. These are part of the MOYAI initiatives aimed at promoting to support the least developed countries (LDCs) with their efforts in Hg monitoring.

The drinking water standards, directions, and guidelines intended for human consumption are proposed by various countries and organizations, such as the Ministry of Health, Labor and Welfare, Japan, the Council of The European Union, and WHO. Commonly used methods for determination of Hg contamination in drinking water require several steps for extraction. One of the main problems in examination of drinking water is the operation of a complex preprocess of Hg extraction. An analytical method, based on gold extraction and thermal decomposition amalgamation atomic absorption spectrometry, has been developed that is capable of extracting and quantifying inorganic Hg in drinking water. This method involves only a few steps: extraction with gold coated silica and its separation. The detection limits were down to the 0.001 ng/ml level. This value is clearly below the range of values found in drinking water standards, directions, and guidelines. Furthermore, this new method requires few reagents, making it particularly attractive for the examination of drinking water at a remote site in LDCs.

The ISO/IEC 17025 standard (general requirements for the competence of testing and calibration laboratories) is one of the main standards used for testing and calibration laboratories for their quality system. We were accredited to the standard for testing laboratories of Hg in human hair and urine. The purpose of accreditation is to improve our data quality and to provide certified reference materials, such as

specified property values with associated uncertainties and trace abilities. We slightly modified the “Mercury Analysis Manual (MOE 2004)”, improving the detection limit for low concentration urine samples. After review by the accreditation organization, we were accredited to the ISO/IEC 17025 laboratory certification in February 2018.

The purpose of the development of a new human urine reference material is to support human biomonitoring for general populations with low exposure to elemental and inorganic Hg. Elemental and inorganic Hg exposure can result from dental amalgams, inhalation of indoor air, to broken thermometers, and fluorescence lights. The requirements for certified reference materials for assessing human Hg is growing with an increasing body of research from surveys to evaluate the effectiveness of the Minamata Convention. We collected human urine from Japanese males without occupational exposure to Hg compounds. The urine was immediately sterilized by  $^{80}\text{Co}$  radiation, filtered, and blended. We prepared 4,000 bottles of urine, each containing 4 g. The inter-bottle homogeneity was confirmed by measuring the concentrations of totalmercury (THg) and cadmium in the material and by subsequent statistical analysis. We plan to test for the stability of specified values next fiscal year. The present reference material will be of value for the analytical quality assurance of long-term exposure to low-concentration monitoring.

(2) Assessment of methylmercury exposure in Vietnamese

(Fundamental research)

Megumi Yamamoto

(Department of Basic Medical Science)

1) We published a simple method for MeHg analysis of biological samples in 2016 (Yoshimoto et. al., *J. Toxicol. Sci.* 2016: 41(4):489-500). We have applied this method in studying public health. The Japanese shrimp industry depends on importing shrimp from other countries. However, little information is available on Hg speciation and Se concentrations in commercial shrimp available in Japan. The concentrations of THg, MeHg, and selenium (Se) in the muscles of imported and domestic commercial shrimp from the Kumamoto and Kagoshima prefectures were determined to obtain information for assessing the risk of MeHg exposure. The THg and MeHg concentrations were significantly different from different countries, even in samples from the same species. In the present study, the Se/Hg molar ratios in shrimp were more than one, indicating that the shrimp examined would be low-risk seafood because of their low MeHg and high Se concentrations. This result was published in *J. Toxicol. Sci.* (2017) 42(4):509-517.

2) People are exposed to MeHg mainly through fish consumption, which is increasing in Vietnam. However, little information is available on estimating the health risk of MeHg exposure through fish consumption in Vietnam. The present study examined the association between Hg levels in hair and Se levels in toenails of 196 Vietnamese citizens and their fish consumption, using a dietary questionnaire to obtain information pertinent for assessing health risk due to MeHg exposure. The geometric mean of Hg levels in the hair of males and females was 617 ng/g and 575 ng/g, respectively. The Hg concentrations in hair for males and females tended to increase with age. The levels of Hg in hair and Se in toenails rose with an increased frequency of marine fish consumption, and both showed a significant positive correlation in subjects who consumed marine fish  $\geq$  once/week. This is the

first cross-sectional study to investigate the association between hair Hg levels and fish consumption in Vietnam. These findings provide valuable information for future assessments of the health risk of MeHg exposure through fish consumption. This result was published in *J. Toxicol. Sci.* (2017) 42(5):651-662.

#### [Activities theme and summary]

- (1) Examination of hair mercury in areas concerned with mercury pollution around the world

Masatake Fujimura

(Department of Basic Medical Science)

We performed Hg analysis in hair samples and assessed the survey information including gender, age, occupation, and residence in areas of Philippines (Quezon City) and Indonesia (North Halmahera area), concerned with Hg pollution (209 samples). Measured Hg data in hair indicated that Hg pollution was very small in the above-mentioned areas.

- (2) Cooperation of research in the international organization

Mineshi Sakamoto

(Department of Environmental Science and Epidemiology)

(Dispatch)

In total, 15 researchers were dispatched to 10 countries including Italy, France, Thailand, and Nicaragua, and conducted presentations at international conferences and collaborative studies on Hg surveys.

(Invitation)

A researcher was invited from a university in Indonesia to conduct a collaborative investigation from January 9–19, 2018, and two researchers were invited from a university in Brazil to conduct a collaborative study on March 4–19, 2018.

(JICA and other Trainings)

We conducted JICA trainings 4 times, and lectures for 46 participants from 18 countries. We also conducted lectures to 23 university students from the United States, as part of their summer program; 10 university students from Kumamoto University, as part of a JST project called “Sakura science”; and 4 university students as part of an internship named the “HIGO internship. The health effect of Hg, overview of the Minamata Convention, and NIMD’s efforts were mainly the topics lectured. NIMD also accepted a Vietnamese student from the Joint Graduate School of Kumamoto Prefectural University, to conduct research she has been working on since 2015, and as a result, she obtained a PhD degree. As for activities for the Minamata Convention on Mercury, we have contributed a human biomonitoring project, in collaboration with WHO, and a worldwide Hg monitoring project, conducted mainly by UNEP. We also have been accepted for re-designation as a WHO collaborative center from 2017-2020.

- (3) NIMD Forum and International Workshop

Mineshi Sakamoto

(Department of Environmental Science and Epidemiology)

NIMD Forum 2017 “Integrated Understanding of Biogeochemical Cycling of Mercury around Ocean Environments” was held in Minneapolis in the United States on November 12\_16, 2017. On November 14th, there was a joint forum with “The Society of Environmental Toxicology and Chemistry (SETAC).” The theme was “Integrated Understanding of Biogeochemical Cycling of Mercury around Ocean Environments.” The Forum was conducted by collaboration with the National Institute for

Environmental studies (Japan). The invited speakers were 5 foreign researchers (United States 1, Russia 1, Korea 3) and 4 Japanese researchers, including 3 from our Institute and the National Institute for Environmental studies. The topics were field surveys of marine environments using ultra-trace Hg analysis, Hg isotopic analysis to indicate the fate processes, bioaccumulation through marine food webs, and chemical fate and transport processes and models integrating multimedia environment such as air, sea, land, and sediments.

At ICMGP 2017 "The 13th International Conference on Mercury as a Global Pollutant" held in Providence RI in the United States on July 16–21, 2017, we provided promotional activities to introduce our research activities and techniques for measuring Hg level in the hair.

## 7. Publications and Scientific meetings

### [International Journals]

Usuki F, Fujimura M, Yamashita A: Endoplasmic reticulum stress preconditioning modifies intracellular mercury content by upregulating membrane transporters. *Sci. Rep.*, 2017; 7: 12390.

Sakamoto M, Chan HM, Domingo JL, Koriyama C, Murata K. Placental transfer and levels of mercury, selenium, vitamin E, and docosahexaenoic acid in maternal and umbilical cord blood. *Environ. Int.*, 2018; 111: 309-315.

Fujimura M, Usuki F: Site-specific neural hyperactivity via the activation of MAPK and PKA/CREB pathways triggers neuronal degeneration in methylmercury-intoxicated mice. *Toxicol. Lett.*, 2017; 271: 66-73.

Fujimura M, Usuki F: *In situ* different antioxidative systems contribute to site-specific methylmercury neurotoxicity in mice. *Toxicology*, 2017; 392: 55-63.

Yamamoto M, Khan N, Muniroh M, Motomura E, Yanagisawa R, Matsuyama T, Vogel CF: Activation of IL-6 and IL-8 expressions by methylmercury in human U937 macrophages involves RelA and p50. *J. Appl. Toxicol.*, 2017; 37: 611-620.

Marumoto K, Sudo Y, Nagamatsu Y: Collateral variations between the concentrations of mercury and other water soluble ions in volcanic ashes and volcanic activity during the 2014-2016 eruptive episodes at Aso volcano, Japan. *J. Volcanol. Geoth. Res.*, 2017; 341: 149-157.

Marumoto K, Takeuchi A, Imai S, Kodamatani H, Suzuki N: Mercury evasion fluxes from sea surfaces of the Tsushima Strait and the Kuroshio Current in the East China Sea. *Geochem. J.*, 2018; 52: 1-12.

Hoang VAT, Sakamoto M, Yamamoto M: Mercury and selenium levels, and their molar ratios in several species of commercial shrimp in Japan regarding the health risk of methylmercury exposure. *J. Toxicol. Sci.*, 2017; 42: 509-517.

Hoang VAT, Do HTT, Agusa T, Koriyama C, Akiba S, Ishibashi Y, Sakamoto M, Yamamoto M: Hair mercury levels in relation to fish consumption among Vietnamese in Hanoi. *J. Toxicol. Sci.*, 2017; 42: 651-662.

Tomiyasu T, Kodamatani H, Imura R, Matsuyama A, Miyamoto J, Akagi H, Kochman D, Kotonic J, Fajon V, Horvat M: The dynamics of mercury near Idrija mercury mine, Slovenia: Horizontal and vertical distributions of total, methyl, and ethyl mercury concentrations in soils. *Chemosphere*, 2017; 184: 244-252.

Hiraoka H, Nakahara K, Kaneko Y, Akiyama S, Okuda K, Iwawaki T, Fujimura M, Kumagai Y, Takasugi N, Uehara T: Modulation of unfolded protein response by methylmercury. *Biol. Pharm. Bull.*, 2017; 40: 1595-1598.

### [International meetings]

Usuki F, Fujimura M, Yamashita A: Suppression of nonsense-mediated mRNA decay under environmental

stresses. 57<sup>th</sup> Annual Meeting of Society of Toxicology, San Antonio, 2018. 3.

Sakamoto M, Chan HM, Domingo JL, Yamamoto M, Murata K: Methylmercury versus selenium, vitamin E, and docosahexaenoic acid in fetal circulation: comparison with maternal status. 13th International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Sakamoto M: Health impact and HBM in populations exposed to elemental mercury vapor and methylmercury. 13th International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Sakamoto M, Itai T, Marumoto K, Mori K, Horvat M, Poulain A, Kodamatani H, Tomiyasu. Mercury speciation in preserved sludge which is estimated to be remaining under the reclaimed land area of Minamata Bay Japan. 53<sup>rd</sup> Congress of the European Societies of Toxicology, Bratislava, 2017. 9.

Matsuyama A: Outline of the dredging project of Minamata Bay and current state of Minamata Bay. International Symposium on Management and Remediation of Hg Contaminated River in Korea, Pohang, 2017. 12.

Fujimura M, Usuki F: Recovery effect of a ROCK inhibitor, Fasudil, on axonal degeneration of dorsal spinal nerve root in methylmercury-intoxicated rats. 57<sup>th</sup> Annual Meeting of Society of Toxicology, San Antonio, 2018. 3.

Yamamoto M, Motomura E, Yanagisawa R, Hoang VAT, Mogi M, Mori T, Nakamura M, Takeya M, Eto

K: Evaluation of Neurobehavioral Disorders in Methylmercury-Exposed KK-Ay Mice by Dynamic Weight Bearing Test. 13th International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Yamamoto M: Experimental study on methylmercury toxicity and its prevention. International Conference on Translational Medicine and Health Sciences, Semarang, 2017.10.

Mori K, Kanaya G, Seo E, Itho H, Kojima S: Bioaccumulation of mercury on fishes in Minamata Bay, based on food web analysis and carbon and nitrogen isotope analysis. 13th International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Mori K, Kanaya G, Seo E, Itho H, Kojima S: Bioaccumulation of mercury on fishes in Minamata Bay, based on food web analysis and carbon and nitrogen isotope analysis. 3<sup>rd</sup> Asian Marine Biology Symposium, Kumamoto, 2017. 11.

Marumoto K, Takeuchi A, Suzuki N: Mercury distribution in seawaters, planktons and fishes collected from the Kuroshio Current region of the East China Sea. Society of Environmental Toxicology and Chemistry North America 38<sup>th</sup> annual meeting, Minneapolis, 2017.11.

Haraguchi K, Sakamoto M, Yamamoto M, Matsuyama A, Hung DT, Yamakawa A, Sano T, Yoshinaga J: Development of human hair reference material supporting biomonitoring of methylmercury. 13th International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.



Haraguchi K: Human biomonitoring for mercury exposure - including hair sampling demonstration. WHO WP regional workshop: Health sector involvement in implementation of the Minamata convention on mercury, Minamata. 2017. 5.

Haraguchi K: Human biomonitoring for mercury-state of the art, WHO SE regional workshop: Health sector involvement in implementation of the Minamata convention on mercury. Bangkok. 2017. 7.

Nagano M, Fujimura M, Kobayashi Y, Inaba K: The effect of wheat bran on tissue level and excretion of mercury after methylmercury exposure in mice. 57th Annual Meeting of Society of Toxicology, San Antonio, 2018. 3.

Hoang VAT, Sakamoto M, Yamamoto M: Mercury and Selenium concentrations in several species of commercial shrimps in Japan. 13th International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Hoang VAT, Do HTT, Agusa T, Koriyama C, Akiba S, Ishibashi Y, Sakamoto M, Yamamoto M: Hair mercury levels in relation to fish consumption among Vietnamese in Hanoi. 57th Annual Meeting of Society of Toxicology, San Antonio, 2018. 3 .

Hattori T, Marumoto K, Nagasaka H, Suzuki N: Continuous monitoring on atmospheric GEM, GOM and PBM concentrations and wet Hg deposition fluxes at Fukuoka in the northern Kyushu of Japan. 13<sup>th</sup> International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Morrison LA, Suzuki N, Takeuchi A, Yamakawa A, Marumoto K, Koga M, Nagasaka H, Hattori T, Kim R, Sheu GR, Peralta T, Chulathipyachat T, Chaisaard N, Luke W, Gay DA, Olson M, Schmeltz D, Prestbo EM: Exploring possibility of coordinated atmospheric mercury monitoring in Asia-Pacific region. 13<sup>th</sup> International Conference on Mercury as a Global Pollutant, Providence, 2017. 7.

Takeuchi A, Marumoto K, Obata H; Vertical distributions of dissolved gaseous mercury (DGM) concentrations in the sub-Arctic Pacific Ocean. Ocean Science Meeting 2018, Portland, 2018. 2.