

NIMD Annual Report
2016
(April 2016 to March 2017)

National Institute for Minamata Disease
Ministry of the Environment
Japan

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Report on Research and Other Activities in the
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1. Pathomechanism Group

The aim of this study group is to understand the molecular mechanisms underlying methylmercury (MeHg) toxicity in humans. Therefore, 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 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 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 mercury (Hg) excretion after MeHg exposure. We are in the process of identifying drugs and treatments that suppress MeHg toxicity and promote nerve regeneration. This group conducted the following research during the fiscal year 2016.

[Research theme and summary]

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

(Project research)

Masatake Fujimura

(Department of Basic Science)

We evaluated the therapeutic effects of a Rho kinase (ROCK) inhibitor in a rat model of chronic MeHg-intoxication. A ROCK inhibitor (Fasudil) improved MeHg-induced neurological symptoms and neuropathological changes in spinal cord. Next, we

examined the effects of MeHg exposure during fetal period in rats. A low level MeHg exposure (5 ppm) showed a trend on suppression of proliferation in neural precursor cells (NPCs) of the cerebral cortex. Furthermore, we established a rat model of muscle atrophy to evaluate the therapeutic mechanism of vibration stimuli in Minamata disease patients.

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

(Fundamental research)

Masatake Fujimura

(Department of Basic Science)

We analyzed mRNA and protein expression levels of anti-oxidative enzymes for each neuronal cell type in mouse cerebrum. We observed low expression levels of anti-oxidative enzymes including Cu, Zn-SOD, Mn-SOD, and GPx1 in the deep layer of cerebral cortex that is susceptible to MeHg toxicity as compared to that in other neuronal cells. Next, we investigated the site-specific expression of the signaling cascade related to neural activity in a mouse model of MeHg-intoxication. MeHg exposure selectively induced expression of site-specific markers of neural activity (c-fos and BDNF) through the MAPK and PKA/CREB pathway in the deep layer of cerebral cortex vulnerable to MeHg toxicity, compared to that in other neuronal cells.

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

(3) Study on changes in gene expression induced by methylmercury exposure, the effect of which on pathological conditions, and the protection against the toxicity

(Fundamental research)

Fusako Usuki

(Department of Clinical Medicine)

We investigated the mechanism of endoplasmic reticulum (ER) stress preconditioning-induced upregulation of methionine transporters that affect the cellular influx of MeHg, LAT1, LAT3, and SNAT2 and a membrane transporter that affects the efflux of MeHg, ABCC4. Here, we identified the role of the phospho-eIF2 α /ATF4 pathway in the upregulation of LAT1, SNAT2, and ABCC4 and the role of NMD suppression in LAT3 upregulation. Further, we showed that ER stress preconditioning amplified the expression of the membrane transporter. This could be due to the translation of the upregulated mRNAs caused by ATF4-dependent transcription and NMD suppression. These results suggest that the phospho-eIF2 α /ATF4 pathway activation and NMD suppression could present as therapeutic targets for alleviating MeHg cytotoxicity by enhancing Hg efflux besides inducing protective stress responses. We submitted these results to a peer-reviewed English journal and also presented at academic conferences.

Furthermore, to analyze the potential epigenetic effects of MeHg toxicity, we examined gene expression of ABCC4, SBP2, and neuroigin 4 in rat samples upon exposure to MeHg during fetal period. So far, however, no change in the expression of these candidate genes was observed. We are further investigating these candidate genes undergo epigenetic changes when model rats are exposed to a higher dose of MeHg during the fetal period.

(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, fructo-oligosaccharide and glucomannan) in the toxicity of MeHg. So far, we demonstrated that tissue Hg levels decreased in wheat bran-fed mice through urinary excretion of Hg. The composition analysis showed that betaine characteristically contained in wheat bran diet. In this fiscal year, we examined the effect of betaine on urinary Hg excretion. We showed that urinary Hg excretion increased only in the wheat bran diets-fed mice compared to the basal diet-fed mice. The result suggested that the effect of urinary Hg excretion was not caused by betaine in wheat bran.

Furthermore, we examined the effects of fructo-oligosaccharide (FOS) and glucomannan (GM) on tissue Hg concentration. We demonstrated that tissue Hg levels decreased in FOS-fed mice through fecal excretion of Hg. On the other hand, GM diet showed no effect.

2. Clinical Group

Research

Minamata disease (MD) is caused due to severe methylmercury (MeHg) 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, it is necessary to develop objective methods to accurately identify MeHg poisoning.

As spasticity, (involuntary movements) such as tremor and dystonia, and intractable chronic pain affect the quality of life (QOL) of MD patients, effective therapies are needed.

This research group conducted experiments to evaluate the neurological function of patients with MD, using magnetoencephalography (MEG) and MRI. Moreover, we set up another research group to examine effective therapy for the above-mentioned symptoms. Furthermore, we started repetitive transcranial magnetic stimulation (rTMS) therapy for ataxia, pain, numbness, and tremors observed in MD patients this year.

The research conducted by this group during FY 2016 is outlined below:

[Research theme and summary]

- (1) Research on evaluation of human health effect 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 morphologic 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 that the abnormality was more common in the certified MD patients than in the control subjects.

Spasticity, involuntary movements such as tremor and dystonia, and intractable chronic pain cause a reduction in the QOL of MD patients. To improve QOL of these patients, we established “the research group on the improvement in the medical treatment of Minamata disease” and engaged in “community medicine meeting” to examine effective therapy. Improvements in ataxia, pain, numbness, and tremor were observed when we initiated rTMS therapy for MD patients. Furthermore, we continued botulinum toxin therapy for treating spasticity of the fetal-type MD patient.

Activities

In recent years, the compensation claims from 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, and 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 methods for application of these concepts in the community. After these projects, we have continued supporting welfare activities in the cities of Minamata and Izumi.

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 2016:

[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 or three days a week. The principal objective is to improve their QOL. The combined vibration stimuli, repetitive facilitation therapy, and gait training with Hybrid Assistive Limb (HAL) were effective for a patient with fetal-type Minamata disease (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 reporting the effectiveness of vibration therapy on

spasticity of three cases with FMD was published in a peer-reviewed English journal (Usuki & Tohyama, *Medicine* 95 (15) e3385, 2016). In this paper, we also reported a decrease in amplitude of soleus H-reflex after the vibration therapy, suggesting the mechanism is involved in the suppression of spinal α -motor neuron excitability.

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 workshops organized included “Disuse syndrome in locomotor system ~ its mechanism and prevention” as a rehabilitation technique and “Practice of foot care designed to improve balance function and walking ability ~ to make healthy foot” as a care technique. Based on the questionnaire results answered by attendees, the workshops were well received. We believe that the workshops are 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 Minamata and Izumi to improve occupational therapy in these cities. Through these supports, we strengthened the connection between our institute and the local community.

Furthermore, we conducted a survey in Minamata city in the first half of the year to offer better crafts in future and used these analysis results for the activity during latter half of the year.

(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 the responsibility to save abundant pathological tissue specimens of MD permanently. However, pathological tissue slides are difficult to be preserved permanently as they fade with the passage of years. Therefore, our objectives are: to digitize these pathological slides for permanent preservation; and to publish the digitized pathological tissue slides on the website for the students and doctors training in pathology.

3. Exposure and Health Effects Assessment Group

There are two sub-populations susceptible to methylmercury (MeHg) exposure: 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 major research interest also focusses on the population that is particularly sensitive to MeHg exposure, such as fetuses or people with various diseases, to contribute to appropriate risk assessment of MeHg exposure.

Research

[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 completed the neurobehavioral analysis of MeHg-treated KK-Ay mice (12 weeks old) using Dynamic Weight Bearing (DWB) test. The results indicated that DWB test could be a useful method to evaluate neurobehavioral disturbance due to MeHg exposure in rodents. In addition, pathological analysis using CD204 as a marker indicated a lesion in the sciatic nerve in MeHg-exposed KK-Ay mice, which showed neurological symptoms. Based on these results, we are performing pathological analysis using macrophage markers (CD68, CD163, CD204, HLA-DR) in peripheral nerves of certified (5) or uncertified (6) patients with MD.

2) We are examining total Hg (T-Hg) concentration in the blood and tissues (brain, kidneys, and liver) of MeHg-exposed BL/6 and KK-Ay mice (10 mg Hg/kg

BW, single administration) to evaluate the effect of glucose metabolism disorders on MeHg toxicokinetics. We also examined the application of our novel analytical method to measure MeHg concentration in mammalian tissues (Yoshimoto et. al., *J. Toxicol. Sci.*, 2016). The results indicated that this method was applicable to the analysis of small amounts of T-Hg and MeHg concentration in tissues (15-100 mg, brain, kidney, liver, pancreas, spleen).

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

(Fundamental research)

Masumi Marumoto

(Department of Environmental Science and

Epidemiology)

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

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

(Fundamental research)

Masaaki Nakamura

(Department of Clinical Medicine)

We determined that presence of selenium hindered our analysis on health effects in adults of Taiji-cho last year. We conducted proteomic analysis using mass

spectrometry (MALDI-TOF/TOF) this year to search for the protein involved in MeHg toxicity except for selenium. However, the above search for the protein associated with MeHg toxicity other than selenium could not proceed as optimal conditions of the plasma sample purification and concentration for MALDI-TOF/TOF analysis could not be decided. In the following year, we would like to complete the listing of the candidate protein at the minimum.

We also surveyed the effect of MeHg exposure on child development by performing pediatric examinations at Taiji-cho multi-purpose center from August 2015. This year, we examined 47 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.

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

(Fundamental research)

Mineshi Sakamoto

(Department of International Affairs and Research)

1) MeHg versus selenium(Se), vitamin E(VE), and docosahexaenoic(DHC) acid in fetal circulation: comparison based on maternal status

The main objective of this study is to investigate the difference in the status of Hg, Se, VE, and DHA in fetal blood with those in maternal blood to evaluate high background sensitivity of fetus against MeHg toxicity. While Hg was higher in cord blood (7.26 ng/g) than in maternal blood (185%), the amount of selenium was similar in both cord blood (153 ng/g) and maternal blood. On the other hand, the amounts of VE (0.31 mg/dl) and DHA (57.9 µg/ml) were lower in cord blood than maternal blood (45% for VE and 22% for DHA). These results showed that the ratios of selenium/Hg, DHA/Hg, and VE/Hg were lower in fetal circulation

than those in maternal blood. Besides nearly twice the amount of Hg, the lower ratios of protective factors such as selenium, VE and DHA against Hg in fetal circulation might result in a higher susceptibility of the fetus against MeHg toxicity.

2) Hg speciation in preserved sludge, which is estimated to be remaining under the reclaimed land area of Minamata Bay Japan

MD is caused by MeHg, which is generated as a by-product of an acetaldehyde production process from Chisso plant in Minamata, Japan. The discharge of MeHg to Minamata Bay continued until the halt of acetaldehyde production in 1968 resulting in the sediment with high Hg concentrations. Approximately 780000 m³ of sediment containing more than 25 µg/g of T-Hg was dredged into a reclaimed land area during a project that was completed in 1990. The objective of this study is to retrieve information on the potential risk to Minamata Bay seawater from the re-spillage of the sludge due to accidents. We analyzed T-Hg and MeHg concentrations in the historical and preserved sludge samples obtained before the dredging project was initiated (it now exists under the current reclaimed land). We observed a nearly straight line when T-Hg and MeHg were plotted on a double logarithmic chart. The percent of MeHg tended to decrease with the increase in T-Hg in all the examined samples. According to the X-ray absorption fine structure analysis (XAFS) and pyrolysis analysis, most of the Hg was estimated to exist in the stable form of HgS.

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 research on regional revitalization under an agreement with the city of Minamata (2015). In addition, we verified historical survey material based on Minamata disease (MD). In addition, our goal is to elucidate the problems related to risk perception of methylmercury (MeHg) in society and to practice effective dissemination and communication about relevant risk information. Thus, this research group aims to contribute to "regional revitalization" and "transmission of information about Minamata disease."

The research carried out by this group in FY 2016 is outlined in the following paragraphs.

Research

[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 research bases for "community design of future thinking" towards the regional revitalization of Minamata area. Additionally, in pursuit of vision and implementation of the regional revitalization, we are trying to create a policy proposal.

As a part of the effort, we surveyed literature on "power of municipality" of Minamata area, specifically "policy suggestions of eco-towns in Kyushu." We presented some of them at the Japan Association of Regional Policy.

In addition, we held Future Sessions ten times with the aim of introducing policy proposal to Minamata City. At the Future Sessions, we retrieved opinions about this issue. We convened "Minamata area revitalization vision research meeting" six times in order to discuss the vision and implementation. As a result, the study report was completed. Based on the report, we presented policy proposal to Minamata City.

- (2) Study on risk governance of adverse health effect of methylmercury

(Fundamental research)

Noriyuki Hachiya

(Department of Environmental Science and Epidemiology)

Epidemiological studies were conducted on health effects of MeHg exposure through ordinary fish consumption in Nicaragua and on historical health survey data obtained from residents of Minamata from the late 1970s to early 1980s. The results of health risk assessment on the consumption of fish caught in Lake Managua of Nicaragua indicated no association between adverse health effects and hair Hg concentrations, an indicator of MeHg exposure. Alternatively, beneficial effects of fish consumption were suggested. In the historical epidemiological analysis of MD, data were rescued from the questionnaire collected from more than 22,000 participants in the past health survey whose data have

not been statistically analyzed. Implications of risk governance of MD will be discussed after epidemiological analysis on these data.

Activities

[Theme and summary]

- (1) Transmission of information on Minamata disease, and organization of documents and materials in 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 is provided through the use of exhibition room and lecture hall. In addition, we hold NIMD forum, and other meetings cooperated with Minamata Disease Municipal Museum and 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, 387 hair samples were collected from visitors at National Institute for Minamata Disease, the Minamata Disease Archives, and from other organizations. The total mercury levels of the samples were measured. The analytical results were sent to each participant.

5. Nature Environment Group

Hg circulates through the land, water, and atmosphere, and its chemical form changes in each medium. In addition, part of inorganic mercury(Hg) released from anthropogenic and natural emission sources is transformed into methylmercury(MeHg) in natural environments. MeHg is then bioaccumulated in humans via the food web in aquatic environments. Our research group executes investigations that are focused on the Hg cycle and its chemical changes in natural environments. Our main target area is Minamata Bay as well as the Yatsushiro Sea and the ocean area around the Japanese Islands. In addition, we also conduct worldwide investigations in areas polluted with Hg.

[Research theme and summary]

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

(Project research)

Kohji Marumoto

(Department of Environmental Science and
Epidemiology)

We continuously monitored atmospheric Hg. Other than total Hg (T-Hg) concentrations in the rainwater collected in 2015 in Minamata, Hg concentrations in the rainwater and atmosphere were not different over the past few years. In addition, Hg evasion fluxes from the sea surface and Hg speciation in seawater, plankton, and fishes of the East China Sea were examined. From the results of Hg evasion fluxes, annual Hg evasion amount from the East China Sea was calculated and found to be 49 ± 17 ton. This value is about twice the emission amounts from Japanese anthropogenic Hg emission sources. Therefore, re-evaluation of Hg emission inventories including natural emission

sources is needed in the future.

In the Kuroshio Current of the East China Sea, MeHg concentration in seawater was highest at a depth of around 500 m, where the North Pacific Intermediate Water (NPIW) circulates. However, the highest MeHg concentrations in the NPIW were observed around other sea areas such as the eastern Pacific Ocean and Equatorial regions. The reason for this is still unclear. Thus, further investigation is needed to elucidate MeHg production in the sea area where the NPIW is formed. The trophic magnification factors (TMS) were also calculated from data regarding T-Hg and the ratio of nitrogen isotopes in planktons and fishes collected in the Tsushima Strait (part of the Kuroshio Current region). The TMSs were 1.29 in autumn 2014 and 1.22 in summer 2015. The TMS in Tsushima Strait had no seasonal variations and was lower than that in other sea areas such as the Suruga Bay and the Sanriku Coast. Therefore, it is clear that noticeable Hg bioaccumulation does not occur in the marine ecosystem of the Tsushima Strait, even though this sea area is under the influence of Hg discharge from the Asian Continent.

- (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)

The sampling of fishes and benthic organisms for Hg and stable isotopes and genetic analysis advances smoothly throughout the year across Minamata Bay. We obtained several species of fishes and benthos, and analyzed Hg levels in 250 samples. The genetic

analysis of prey species in the gut of fishes collected in the sandy or muddy bottom showed the importance of feeding area for Hg accumulation. The stable isotope ratio of $\delta^{13}\text{C}$ indicated substantial differences between phytoplankton with POM and benthic algal complex. We started collaborative research to elucidate the transition route of MeHg to the coastal food web by Hg stable isotope ratio analysis, which was performed preliminarily on nine carnivorous fishes. We conducted 11 monitoring surveys on intertidal community structures every two years from 1977.

- (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)

Outcomes of this study carried out in 2016 is described below.

- (a) Based on the results of Hg monitoring in Minamata Bay, the average values of dissolved T-Hg concentrations and dissolved MeHg concentrations were 0.44 ± 0.16 ng/L and 0.06 ± 0.03 ng/L, respectively. The average value of dissolved T-Hg concentration in Minamata Bay seawater that was collected at the revetment of Minamata Bay was 0.89 ng/L. In addition, influences of the earthquake in Kumamoto Prefecture were not observed at revetment of Minamata Bay.
- (b) A seawater incubation experiment using dark condition and a small size environmental incubator was performed at our laboratory. The conditions of incubation experiment were as follows: temperature 30°C; salinity 1.5‰; and inorganic Hg concentration 5 ng/L. As a result, a maximum peak of dissolved MeHg concentration was observed 72

hours later. In addition, the ratio of dissolved MeHg to dissolved T-Hg exceeded 60%.

- (c) Sampling plan of sediments in Yatsushiro Sea was revised to 66 points from 55 points in comparison with a previous plan. In addition, to obtain a better understanding of the sediment moving at the sea bottom, a new observation device enabling hoisting of sediment was provisionally developed by the Kyusyu University and NIMD. Moreover, T-Hg concentration of the sediment sampled in Yatsushiro Sea during the last year was measured. As a result, Hg concentration in the sediment of -12.5 cm below the surface increased gradually, reaching a maximum concentration of 0.3 mg/kg on dry weight basis. On the other hand, T-Hg concentration deeper than -12.5 cm decreased gradually.

- (4) Development of atmospheric mercury monitoring method for rapid and simple screening in mercury emission sources and their surrounding areas - Impact assessment on mercury emitted from volcanos and geothermal areas (1) -

(Fundamental research)

Kohji Marumoto

(Department of Environmental Science and Epidemiology)

We measured Hg in the volcanic ash ejected from the Nakadake 1st crater of Mt. Aso during the period from November 2014 to February 2016. The results showed that the concentrations of Hg and other major ions such as SO_4^{2-} and Ca^{2+} in volcanic ash samples ejected from the Strombolian magnetic eruptive episode varied with the amplitude change of seismic signals, serving as one of the indicators of volcanic activity. In addition, Hg concentrations in volcanic ash from the phreatic-phreato eruptive episode were almost 100 times higher than the

magmatic eruptive episode. This could be because Hg in gas phase dissolves in volcanic water, called “Yudamari,” and concentrated Hg could be adsorbed onto volcanic ashfall. However, the actual reason is still unclear.

Furthermore, Hg behavior in the geothermal area of southern Kyushu was also investigated as the investigations around Mt. Aso volcanic area was stalled due to the Kumamoto earthquake that occurred on April 14 and 16, 2016. The concentrations of Hg was measured in the geothermal area; the results suggested that the concentrations around the spout was not as high as (10–30 ng/m³) the concentration in the surrounding areas (8–10 ng/m³), even though the concentration in the spout was extremely high, 22,000 ng/m³. Therefore, it is possible that Hg in the spout of the geothermal area does not spread widely. However, further research on the influences of Hg emitted from volcanic and geothermal areas is needed.

(5) Study on effect of mercury compounds on marine plankton food web

(Fundamental research)

Shoko Imai

(Department of Environmental Science and Epidemiology)

We collected zooplanktons from the west of Kume Island in August 2016 using plankton net (100 µm mesh) and measured the total Hg in the zooplanktons. The average concentration was 0.078 ± 0.034 µg/g-dry (Average \pm 1 s.d.), ranging from 0.033 to 0.114. These values were similar to those obtained in the Seto Inland Sea, Tsushima Strait, and Kagoshima Bay since 2013.

We also collected zooplanktons from the seawater of Minamata Bay several times. The collected seawater was size fractionated and T-Hg concentration in each size was analyzed. The results showed that T-Hg concentration increased with the increase in plankton

size. In Minamata Bay, the average Hg concentration in zooplanktons, with the size of 100 µm or above, is 0.205 ± 0.162 µg/g-dry.

(6) Development of mercury stable isotope analysis system and its application to the environmental / biological samples

(Fundamental research)

Takaaki Itai

(Department of International Affairs and Research)

This research was started this year to develop an analytical method for Hg stable isotope, that can serve as a useful tracer of the Hg biogeochemical cycle. The goal of this year was to set-up a laboratory and a multi-collector ICP-MS. The competitive bid was conducted fairly, and the installation of instrument was completed at the end of March. Important consumables, such as isotope reference solution (NIST3133) and common in-house standards, were shared by researchers from abroad. The isotope database was also created by surveying 107 literature references to facilitate isotope data analysis. Eighty percent of the data (n=2400) has been tabulated.

6. International Contribution Group

Environmental pollution by mercury(Hg) is spreading, and it is particularly concerning in the developing countries. To decrease the risk of environmental pollution, the Minamata Convention was adopted by 140 participating nations. Accordingly, international contribution groups in NIMD plan in mutual cooperation with foreign researchers studies on Hg and information exchange via the NIMD forum. Moreover, considering the needs of developing countries facing Hg pollution, we use our experience, knowledge, and latest technology via mutual cooperation with JICA. In addition, we developed a novel, simple Hg analysis methodology focusing on methylmercury (MeHg), and it is promised in the Minamata Convention as a commitment to the MOYAI initiative. Later, the summary of studies and duties of international contribution group 2016 were 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 four major parts: 1) development of a simple method for the determination of monomethyl mercury compound; 2) improvement of consignment analysis efficiency for low concentration samples that cannot be detected by simple method; 3) certification of our management system for analytical quality, administrative, and technical operations to ISO/IEC 17025 (general requirements for the competence of testing and calibration laboratories); and 4) development of reference materials for evaluating the accuracy of Hg analysis. These are part of the

MOYAI initiatives aimed at hastening ratification of the Minamata Convention on Hg among least developed countries (LDCs).

Human hair is a suitable medium for monitoring human intake of MeHg. Most of Hg in the hair exists in the form of monomethyl mercury; exposure to elemental or inorganic Hg can also occur due to the use of elemental Hg in the gold mining activities and with the use of some skin-lightening creams and soaps. For the groups, evaluation of actual MeHg exposure is possible by measuring monomethyl mercury and total mercury(T-Hg) in the hair. One of the main problems in monomethyl mercury monitoring in LDCs is the difficulty of obtaining pure reagent and a carrier gas. Commonly used methods for monomethyl mercury determination require several reagents for short-term storage. Additionally, use of the toxic reagents in the laboratories can be a drawback for waste treatment in LDCs. Therefore, new methods with lower reagent consumption are an important improvement in the human health protection efforts in LDCs. An analytical method based on thin-layer chromatography (TLC) and thermal decomposition amalgamation atomic absorption spectrometry (TDA AAS) has been developed that is capable of separating and quantifying monomethyl and inorganic Hg. This method involves several steps, including acid leaching, dithizone extraction, and preparative TLC analysis. The monomethyl mercury collected by TLC can then be measured as elemental Hg by TDA AAS. This method was validated by analyzing various hair samples (certified reference materials and various samples from Japanese females from beauty salons) and spiked-recovery tests. Satisfactory recoveries (90–109%) were obtained in all cases. The performance of the method suggested that sub-ng amount of Hg present in a few mg hair samples can be measured. This new

method, therefore, represents a simplified tool for monitoring human exposure to MeHg. Furthermore, this new method requires few reagents, making it particularly attractive for the determination of monomethyl mercury and protection of human health in a low technology environment.

The purpose of the development of a new human hair reference material is to support human biomonitoring for Hg speciation. The requirements for certified reference materials for assessing human Hg is growing with an increasing number of research from surveys to evaluate the effectiveness of the Minamata Convention. We collected human hair from Vietnamese males from a barbershop in Hanoi in 2016. Special precaution was taken to avoid contamination from grinding vessel during the preparation procedure. We prepared 1,000 bottles of hair, each containing 3 g, after sieving and blending of the hair powder. The inter-bottle homogeneity was confirmed by measuring the concentrations of total and monomethyl mercury, other trace elements (Cu, Zn, As, Se) 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 the 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 submitted and published our results concerning simple analysis of T-Hg and MeHg in seafood using heating vaporization atomic absorption spectrometry in *J. Toxicol. Sci.* (Yoshimoto et. al., 2016).

2) We have applied our developed method for MeHg analysis in studying public health. The concentrations of T-Hg, MeHg, and Se in the muscles of imported

and domestic commercial shrimps from the Kumamoto and Kagoshima prefectures were determined to obtain information for assessing the risk of MeHg exposure. The T-Hg 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 shrimps examined would be a low-risk seafood because of their low MeHg and high Se concentrations.

3) We have completed the collection of human specimen (hair and toenail) of control group as one of the general population in Hanoi, Vietnam. The determination of T-Hg concentration in hair (200 samples) was completed, and Se concentration analysis in toenails is currently undergoing.

[Activities theme and summary]

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

(Other activities)

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 Malaysia and Indonesia concerned with Hg pollution (254 samples). Furthermore, we proceeded with the preparations of hair for mercury analysis and dietary survey in the Marshall Islands.

This year, we published one peer-reviewed article.

(2) Cooperation of research in the international organization

(Other activities)

Mineshi Sakamoto

(Department of International Affairs and Research, and Department of Environmental Science and Epidemiology)
(WHO Collaboration Centre)

NIMD was redesignated as a WHO Collaboration Centre from January 9, 2017, to January 9, 2021. Terms of Reference as a WHO CC (JPN-49) are: 1) collaborate with WHO to develop or strengthen surveillance systems in relation to health and environmental issues associated with Hg pollution in selected countries; 2) by request of WHO, to conduct laboratory assessment of human and environmental exposure to Hg compounds; 3) collaborate with WHO to conduct assessment of human and environmental exposure in public health emergencies concerning Hg pollution in the region and provide necessary preventive and curative measures and responses. NIMD started contributing to Global Hg monitoring projects by request of WHO-EURO. NIMD conducted the mirror analysis (quality control check) of the hair, blood and urine samples (20 samples of each specimen) from four countries (China, India, Mongolia, and Kyrgyzstan).

We held NIMD Mercury Session in the 5th Conference on Prenatal Programming and Toxicity (PPTox) on November 15, 2016 (venue: Kitakyushu International Conference Center in Kitakyushu City, Japan).

The speakers were: Dr. Irina Zastenskaya from WHO European Centre for Environment and Health; two from Japan including one from NIMD.

(Dispatch)

Researchers conducted a dispatch of 18 researchers in eight countries such as Italy, Australia, Nicaragua, and the United States. We conducted presentations at international conferences, collaborative researches on Hg, and surveys on Hg values measured by Hg exposure. One researcher participated in the 2nd Regional Forum of WHO Collaboration Centre in

Western Pacific, November 28-29, 2016 in Manila, Philippine.

(Invitation)

We invited one researcher from Indonesia to conduct collaborative research.

(JICA and other Trainings)

We conducted JICA training, and lectures for 227 participants from 33 countries six times. Also, we conducted local satellite lecture (51 participants) twice at a conference in Minamata about the health effects of Hg, an overview of Hg treaty, and NIMD's efforts. NIMD also accepted one Vietnamese student from the Joint Graduate School of Kumamoto Prefectural University, to conduct research guidance, who has been working since 2015 and started collaborative research in Vietnam.

(3) 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 2016 (Pathomechanism of Methylmercury Toxicity: Various Approaches to the Problems) was held in Minamata Disease Archives, Minamata on December 6 and 7, 2016. There were a total of 14 speakers (USA: 3, France: 1, Sweden: 1, Luxembourg: 1, and Japan: 8, including four from NIMD) and approximately 80 people were in the audience. We also held NIMD Mercury Session as NIMD Forum during the 5th Conference on Prenatal Programming and Toxicity (PPTox) on November 15, 2016. The venue of the conference was Kitakyushu International Conference Center, Kitakyushu City, Japan. The speakers were: Dr. Irina Zastenskaya from WHO European Centre for Environment and Health and other two speakers were from Japan including one from NIMD.

7. Publications and Scientific meetings

[International Journals]

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[International Meetings]

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