NIMD Annual Report 2015

（April 2015 to March 2016）

National Institute for Minamata Disease

Ministry of the Environment

Japan

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Report on research and Other Activities in Fiscal year 2015

1. Pathomechanism Group

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

This group conducted the following research during 2015 fiscal year.

[Research theme and summary]

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

(Project research)

Masatake Fujimura

(Department of Basic Medical Science)

We established a chronic MeHg intoxicated animal model using rats, in order to evaluate the therapeutic effects of a Rho kinase (ROCK) inhibitor for MeHg-induced neurological symptoms. The neurological dysfunction and neuropathological changes in this animal model continued 6 weeks after cessation of MeHg administration. Next, we demonstrated that Glycogen synthase kinase-3β (GSK-3β) inhibitors (Lithium and SB-415286) prevented MeHg-induced suppression of cell proliferation in Neural precursor cells (NPCs) from rat embryonic brains. Furthermore, we started an investigation concerning the therapeutic mechanism of vibration stimuli in Minamata disease using animal model.

This year, we presented our findings at two conferences as representatives, and published two peer-reviewed articles as representatives.

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

(Fundamental research)

Masatake Fujimura

(Department of Basic Medical Science)

We analyzed mRNA expression levels of anti-oxidative enzymes for neuronal cells of mice by using a microdissection system. We observed reduced mRNA expressions of anti-oxidative enzymes, especially Cu, Zn-SOD and Mn-SOD in the deep layer of cerebral cortex vulnerable MeHg toxicity, than in the other neuronal cells (in the shallow layer of cerebral cortex and hippocampus). Next, we investigated the causing factor of neurological dysfunction induced by low-dose MeHg exposure (5 ppm in drinking water) during fetal period. We clarified that the causing factor were due to hypoplasia of neurite outgrowth and synaptic homeostasis in cerebellum through the suppression of TrkA-p70S6K-eEF1A1 pathway. Furthermore, we examined the effects of long-term MeHg exposure (1 year) using rats. We clarified that very low dose MeHg exposure (0.4 ppm in drinking water) selectively decreased the expression level of phosphorylated CREB in hippocampus.

In this year, we presented our findings at 8 conferences (4 confidences as a representative), and published 2 peer-reviewed articles.

(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 examined mRNA expression of membrane transporters that potentially affect cellular influx and efflux of MeHg: methionine transporters L-type amino acid transporter (LAT) 1, LAT3, and sodium-coupled amino acid transporter 2 (SNAT2), and the ATP-binding cassette transporter cassette C subfamily 4 (ABCC4), which is related to the efflux of glutathione conjugates. Real-time PCR analyses demonstrated that MeHg exposure induced small changes in the expression of methionine transporters and upregulated ABCC4 in a dose-dependent manner. Mild endoplasmic reticulum (ER) stress preconditioning, which protects cells against MeHg toxicity, upregulated gene expression of all of these transporters. Among the four membrane transporters, ABCC4 upregulation was much higher than that of the others. The intracellular Hg content reflected the changes in the expression of these membrane transporters.

Furthermore, we examined epigenetic changes using blood DNA obtained from patients with fetal-type or infantile-type Minamata disease and from age-matched controls. We selected candidate genes that have potential epigenetic effects of MeHg during the fetal period. We are going to investigate whether these candidate genes undergo epigenetic changes using model rats exposed to MeHg during the fetal period.

Our paper entitled “Decreased plasma thiol antioxidant barrier and selenoproteins as potential biomarkers for ongoing methylmercury intoxication and an individual protective capacity” was published in Archives of Toxicology (Arch Toxicol 2015; doi:10. 1007: /s00204-015-1528- 3).

(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 methylmercury. So far, we demonstrated that tissue Hg levels decreased in wheat bran-fed mice through the excretion of urinary Hg. The composition analysis showed that the oxidized forms of glutathione and γ-glutamylcysteine characteristically contained in the wheat bran diet. Furthermore, the crude protein of the wheat bran diet was 1.3-fold of the basal diet. In the current year, we examined the effects of the oxidized forms of glutathione, γ-glutamylcysteine and crude protein 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 group. The result suggested that the effect of urinary Hg excretion was not caused by the oxidized forms of glutathione, γ-glutamylcysteine and crude protein in wheat bran.

2. Clinical Group

Research

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

Because spasticity, involuntary movements such as dystonia, and intractable chronic pain are involved in the reduction of quality of life (QOL) in Minamata disease patients, effective therapies are needed.

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

The research conducted by this group during FY2015 is outlined as follows:

[Research theme and summary]

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

(Project research)

Masaaki Nakamura

(Department of Clinical Medicine)

The aim of this study was to develop an objective evaluation protocol using MEG and MRI to assess brain function and morphologic features. In this year, we tested the MEG method in 136 people, including 64 subjects from the Kumamoto district, which is not polluted with MeHg. In the officially certified Minamata disease patients, many unusual patterns of dipole waveform of SEF were observed when these patients were compared with subjects from the Kumamoto district. We also measured pain-related neural activity by using MEG and PNS-7000 to evaluate the pain objectively.

The MRI results of the subjects from the Kumamoto district (101 people) and the officially certified Minamata disease patients (12 people) showed that atrophy of the cerebellum was seen in Minamata disease patients. We have also been creating a resting state fMRI database to compare brain networks in subjects from the Kumamoto and Minamata districts.

Spasticity, involuntary movements such as dystonia, and intractable chronic pain are involved in the reduction of QOL in Minamata disease patients. To improve QOL of these patients, we started “the research group on the improvement in medical treatment of Minamata disease” and “community medicine meeting” to examine effective therapy. We also began the administration of botulinus treatment for spasticity and the transcranial magnetic stimulation for the intractable pain in Minamata disease patients.

Activities

In recent years, the compensation claims from Minamata disease patients have moved toward a political resolution. The Department of Clinical Medicine actively organizes events on Minamata disease in cooperation with related organizations. In addition to undertaking conventional rehabilitation activities, including providing daycare for congenital and infantile Minamata disease patients, we organize 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 Minamata disease. Furthermore, we introduced a robot suit HAL (Hybrid Assistive Limbs) to the patients, which assists with standing and walking actions.

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

Since pathological tissue specimens of autopsy cases of Minamata disease are extremely valuable, we are going to digitize these pathological slides for permanent preservation and publish the digitized pathological tissue slides on the website for students and doctors learning pathology.

The following section includes an outline of the clinical practice conducted by this group in FY2015:

[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 was to improve their quality of life. Continuous vibration therapy to the plantar fascia using a hand-held vibration massager relieved the severe sole pain and spasticity of lower extremities of patients with fetal-type Minamata disease, and improved their activities of daily living (ADL). A decrease in amplitude of soleus H-reflex after vibration therapy was observed, suggesting that α-motor neuron excitability was suppressed. The manuscript with the results for the publication in a peer-reviewed English journal was revised.

In addition, we conducted training for standing and seating movements, which are known to effectively strengthen muscle power in the back, the abdomen, the lower extremities, and the neck. Furthermore, we introduced a robot suit, HAL, to one patient with fetal-type Minamata disease in order to expand, augment, and support physical capability when he was performing standing and walking training. HAL was effective in improving the actions of standing and walking.

Annual workshops on rehabilitation and assistance techniques are held in order to improve techniques used by regional specialized staff, to be applied to the patients. This year, the themes of the 8th year of workshops organized included “Possible prevention of dementia by doing exercise” as a care technique. Based on the results of a questionnaire given to attendees, the workshop was well received. We believe the workshop is a useful outreach forum to share information on care and rehabilitation techniques that can be applied 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 entitled “Home support model study, including care prevention” (FY2006–2008) to identify support methods, including rehabilitation, that lead to the improvement of ADL for aging Minamata disease patients and their families. Following this project, we undertook two other projects entitled “Community development project for home care support including healthcare practice” (FY2009–2011) and “Community welfare promotion business for supporting Minamata disease victims” (FY2012), to develop methods to apply these concepts in the community. After these projects, we continued carrying out educational activities in Minamata and Izumi to improve occupational therapy in these cities. Through this support, we strengthened the connection between

our institute and the local community.

(3)Information transmission using the Minamata disease pathology specimens

Masumi Marumoto
(Department of Basic Medical Sciences)

Pathological tissue specimens of autopsy cases of Minamata disease are extremely valuable. Our institute permanently retains many pathological tissue specimens of Minamata disease. However, pathological tissue slides are difficult to preserve permanently, as they fade with the passage of time. Therefore, our objectives are to digitize these pathological slides for permanent preservation and to publish the digitized pathological tissue slides on our website for students and doctors learning pathology.

3. Exposure and Health Effects Assessment Group

Generally, there are two sub-populations susceptible to methylmercury (MeHg) exposure: those who are exposed to high levels of mercury, and those who are more sensitive to the effects of mercury. 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 main research also concerns the population particularly sensitive to MeHg exposure, such as fetuses or people suffering from disease, which contributes to 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 have finished the neurobehavioral analysis of MeHg-treated KK-Ay mice of 12 weeks of age using the DWB test. In this experiment, the balance between forelimb and hindlimb changed from the early to the late stages of MeHg exposure. This was accompanied by the worsening of symptoms (neurological disturbance), forelimb<hindlimb, forelimb≒hindlimb, and forelimb>hindlimb, respectively. This result indicates that the DWB test will be a useful method for evaluating neurobehavioral disturbance due to MeHg exposure in rodents. The pathological analysis using CD204 as a marker indicated that lesions were detected in the sciatic nerve, cerebrum, and cerebellum in MeHg-exposed KK-Ay mice.

2) To confirm the observation concerning astrocyte swelling in KK-Ay mice in a previous report, conducted a preliminary experiment using electron microscopy in 12, 16, 20, and 24 week-old KK-Ay mice. Astrocyte swelling was observed around the hippocampus and basal ganglia in 24-week-old KK-Ay mice.

3) In terms of epidemiological study for the assessment of MeHg exposure in patients of diabetes mellitus, official procedure in institutions concerned is currently going on.

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

(Fundamental research)

Masumi Marumoto

(Department of Basic Medical Science)

Using conventional histopathological techniques, it is impossible to visualize methylmercury and selenium. In animals and humans exposed to methylmercury, histopathological distribution of methylmercury and selenium has not been determined. Therefore, the objective of this study is to reveal the tissue distribution of mercury and selenium by using an X-ray probe microanalyzer (EPMA) that can detect them.

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

(Fundamental research)

Masaaki Nakamura

(Department of Clinical Medicine)

We examined defense mechanisms for methylmercury (MeHg) toxicity of selenium using the blood samples of 130 people who received a neurological examination in 2010 and 2011.

A significant positive correlation was found between the mercury and the selenium concentrations in the plasma and blood cells, and the mercury and selenium concentrations of the blood cells were higher than those of plasma in all subjects. Because mercury:selenium molar ratios in blood cells (0.026–0.636) were higher than those in plasma (0.004–0.175), mercury was considered to be trapped by selenium mainly in blood

cells.

Because the increase of PON1 activity after selenium intake was observed in the Inuit residents of Canada who eat seal and walrus containing high concentrations of MeHg, we measured the plasma PON1 activity in this population. There was no significant correlation between the selenium level and the PON1 activity, suggesting that defense mechanisms for MeHg were different in whales and seals.

As for the survey on the effect of MeHg exposure on child development, we performed pediatric examinations at Taiji-cho multi-purpose center in August, 2015. Because there were few subjects in Taiji-cho, we also performed the investigation in Nachikatsuura-cho, the neighboring town of Taiji-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 its coexisting elements

(Fundamental research)
Mineshi Sakamoto
(Department of International Affairs and Research, and Department of Environmental Science and Epidemiology)

1) Implications of mercury concentrations in umbilical cord tissue in relation to maternal hair segments as biomarkers for prenatal exposure to methylmercury

In the present study, we investigated the relationships between Hg concentrations in cord tissue and those in maternal hair segments, maternal blood, and cord blood as biomarkers that are useful for predicting fetal exposure to MeHg. Our study suggests that the Hg concentration in cord tissue is a useful biomarker for prenatal MeHg exposure in the fetus, and reflects the maternal MeHg body burden during late gestation. The conversion factor from MeHg concentration in cord tissue (dry-weight basis) to maternal hair Hg (0–1 cm from the scalp) was calculated to be 24.09, which will be useful for evaluating the maternal MeHg exposure levels at birth in retrospective cohort studies using preserved umbilical cord tissue in Japan.

2) Effect of stable and episodic/bolus patterns of methylmercury exposure on mercury accumulation and histopathologic alterations in the nervous system

NRC (2000) pointed out that the more episodic/bolus exposure pattern in the Faroe Islands (with heavier dose per occasion) could mean a more adverse impact on neuronal development than the more constant exposure in the Seychelles. These two different intake patterns of MeHg can also occur in general populations of other countries. However, the effects of the two different MeHg exposure patterns on Hg accumulation in blood and brain are yet to be validated. This study aimed to compare the effects of stable and episodic/bolus patterns of methylmercury (MeHg) exposure on mercury (Hg) accumulation in adult rats, fetal rats, and a human model, and to observe neurological alterations in rats. Our results suggest that the two patterns of MeHg exposure (stable and bolus) do not cause differences in Hg accumulation in the blood and brain, nor do they cause neurological alterations, when the total doses are the same.

4. Social and Information Service Group

The city of Minamata was rebuilt as an environmental city. In 2013, The Conference of Plenipotentiaries on the Minamata Convention on Mercury was held in Minamata and Kumamoto. In 2014, the Regional Revitalization Act was enacted. Now, major challenge is the regional revitalization. Therefore, this research group did research for regional revitalization under an agreement with the city of Minamata (2015). In addition, this group performed historical verification based on surveys of Minamata disease-related materials. In addition, is to elucidate the problems related to risk perception of 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 the "transmission of information about the Minamata disease."

The research carried out by this group in FY2015 is outlined as follows:

Research

[Research theme and summary]

(1) New development of community design as a starting point for "power of regional autonomy" for regional revitalization centered on Minamata disease-affected areas.

(Fundamental research)

Hirofumi Iwahashi

(Department of International Affairs and Research)

In this study, we set out to research a foundation for the community design of future thinking for the regional revitalization of the Minamata area. In addition, in pursuit of the vision and implementation of the regional revitalization, we made efforts to create a policy proposal book of future thinking for revitalization.

To be more specific, we first conducted a survey on two themes in order to create a research infrastructure. One is a "factor analysis of the success or failure of the Eco-Town 3 area in Kyushu." The other is a "feature and problems of landscape resources of the Minamata area." The latter theme was presented at the Japanese Society of Landscape Designs.

Second, in order to create a vision of regional revitalization, drawn out the idea of the citizen to the regional revitalization in future sessions. This initiative was presented at the Japan Association of Regional Policy Studies and published in this Association's journal.

Third, in order to study the vision and implementation of the regional revitalization, it was held twice a "Minamata area revitalization Vision Research Group".

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

(Fundamental research)

Noriyuki Hachiya

(Department of Environmental Science and Epidemiology)

A literature survey was conducted on the genotoxicity and carcinogenicity of methylmercury, and problems with the evaluation of possible human carcinogenicity of the chemical were discussed. In another study, the validity of the Food Frequency Questionnaire (FFQ) method for the evaluation of methylmercury exposure through specific fishery products was investigated with the population exposure observed in a traditional whaling site in Japan. The estimated exposure level based on the FFQ was found to be in accordance with another estimation directly calculated from hair mercury concentration. To provide historical perspectives of the risk management of Minamata disease, a series of population-based health surveys conducted in Minamata and its neighboring sites in the early 1970s were reviewed and discussed from an

epidemiological viewpoint.

Activities

[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 has actively collected data and materials associated with Minamata disease and mercury. These data and materials will be utilized for the presently ongoing research. In addition, an information service will be provided via the exhibition room and lecture hall.

This project is beneficial for promoting a better understanding of Minamata disease, communicating the lessons learned from Minamata disease and mercury exposure, and contributing to the development of research in these fields.

(2) Information service using hair Hg analysis

Masaaki Nagano
(Department of Basic Medical Science)

In 2015, 1,246 hair samples were collected from visitors at the National Institute for Minamata Disease, the Minamata Disease Archives, and from other organizations. The total Hg of the samples was measured. The analytical results were sent to each individual.

5. Nature Environment Group

Our research group implements investigations that are focused on the mercury cycle or chemical changes of mercury in the environment. Mercury circulates through land, water, and the atmosphere, and its chemical form changes in each domain. Furthermore, mercury is transformed into methylmercury through some kinds of bacteria in the environment and enters human bodies via the food chain. Based on these facts, our research group investigates the movement of methylmercury in the atmosphere, rain, seawater, sediment, soil, and life. Our main target area is Minamata Bay. In addition, we conduct investigations in the Yatsushiro Sea and the ocean area around Japan and East Asia, and in all mercury-polluted areas of the world.

[Research theme and summary]

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

(Project research)

Kohji Marumoto
(Department of Environmental Science and Epidemiology)

To obtain knowledge on Hg exchange in air-sea interfaces and the bioaccumulation of Hg in marine ecosystems, the continuous monitoring of atmospheric Hg and the observation of Hg evasion fluxes from the sea surfaces of the Seto Inland Sea, the Genkai Sea, and the East China Sea were carried out in this study. In addition, total Hg and mmHg in seawater and total Hg in plankton and fish in the Genkai Sea were measured.

The total Hg concentrations in the wet depositions obtained in the Minamata site were slightly higher in the fiscal year of 2015 than during the past few years. However, the concentrations at the other sites were constant. It is possible that Hg in volcanic gases emitted from the active volcanos such as the Kuchinoerabu Islands, the Suwanose Islands, and Mt. Aso affects the wet Hg depositions. Thus, further investigation is needed.

On the other hand, Hg evasion fluxes in summer in the Genkai Sea were four times higher than in fall in 2014, because the sea surfaces were disturbed by the force of strong winds originating from low-pressure systems. In addition, the dissolved mmHg concentrations in the seawater were lower in the surface layer than in the depths of the thermocline. These vertical profiles of dissolved mmHg in fall 2014 and summer 2015 were similar with those of PO_4^{3-} concentrations. Therefore, the production of phytoplankton and remineralization may be related to the vertical profile of dissolved mmHg.

From the measurements of the total Hg and $\delta^{15}N$ in plankton and fish, trophic magnification slope (TMS) was calculated. It is obvious that the TMS in the Genkai Sea was lower than that in other sea areas, such as the Suruga Bay and the offshore of the Sanriku.

(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, sampling of fish and benthic organisms for mercury and stable isotopes analyses, as well as genetic analysis, has advanced smoothly throughout the year. We obtained many species of fish and benthos, and analyzed 100 fish and 100 samples of benthic organisms. The results of genetic analysis of prey species found in fish guts and the stable isotope analysis contributed the construction

of foodweb in Minamata Bay. We finished sample treatments and data analysis on monitoring surveys on intertidal community structures in 2013 and 2015, respectively. We also sampled sediments, benthic organisms, and fish in three stations in the Amakusa area for comparing studies on bioaccumulation of mercury.

(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 Environmental Science and Epidemiology)

Outcomes of this 2015 study are described below.

1) Based on the results of mercury monitoring in Minamata Bay, the average values of dissolved total mercury concentrations and dissolved methylmercury concentrations were 0.38±0.03 ng/L and 0.06±0.02 ng/L, respectively. The average value of dissolved total mercury concentration in Minamata Bay seawater that was taken at the revetment of Minamata Bay was 0.33±0.11 ng/L.

2) A seawater incubation experiment using glucose as an additive for a carbon source was performed at Kyusyu University.As a result, an effectiveness of glucose that was added to the incubation experiment was not recognized as for mercury-methylation in this study.

3) Based on the results of 3D diffusion simulation of discharged sediment from Minamata Bay to Yatsushiro Sea, the sediment sampling plan in Yatsushiro Sea, which includes the Amakusa sea area, was established. Then, surface sediment (four samples) and core sediment (21 samples) were taken at Yatsushiro Sea as a first sampling campaign during the end of December.

(4) Development of an atmospheric mercury monitoring method for rapid and simple screening of mercury emission sources and their surrounding areas －Impact assessment on mercury emitted from the eruption of Mt. Aso (1)－

(Fundamental research)

Kohji Marumoto

(Department of Environmental Science and Epidemiology)

Volcanic and geothermal activity are one of the emission sources of atmospheric Hg. The large-scale eruptions of Mt. Aso, which is located in the center of the Kyushu islands in Japan, occurred from November 2014 to May 2015, and sporadic eruptions continue. To evaluate the impact of Hg emitted from volcanic activity, the atmospheric Hg around the Mt. Aso volcanic area and Hg in the volcanic ash were measured. From the results of the Hg observations in the surrounding area of Mt. Aso, it was observed that the atmospheric Hg concentrations were almost the same as the average values in Japan. On the other hand, it is obvious that the time series behavior of Hg concentrations in the volcanic ashes varied in conjunction with the amplitude of the volcanic tremors. In addition, the concentrations largely changed depending on eruption types. The mercury emission amounts via the ashfall were estimated at 3.6 ± 1.9 kg from the period from November 2014 to May 2015.

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

(Fundamental research)

Shoko Imai

(Department of Environmental Science and Epidemiology)

We collected zooplankton from the Seto Inland Sea and the Sea of Genkai, and analyzed the total mercury concentration to determine mercury levels in the zooplankton. Total mercury concentrations of the zooplankton from the Seto Inland Sea were 0.087 ± 0.075 ng/mg-dry weight dw in 2013, 0.424 ± 0.253 ng/mg-dw in 2014, and 0.037 ± 0.031 ng/mg-dw in

2015. Total mercury concentrations of the zooplankton from the Sea of Genkai were 0.032 ± 0.007 ng/mg-dw in 2014 and 0.340 ± 0.133 ng/mg-dw in 2015. The total mercury concentration of the zooplankton from most sites was within the range reported in the previous study. However, total mercury concentration in the zooplankton varied depending on the sampling year. We will examine relationships between species composition and total mercury concentrations in plankton.

6. International Contribution Group

Environmental pollution by mercury is spreading, and environmental pollution is particularly serious in developing countries. To decrease the risk of environmental pollution, the Minamata convention was adopted by 140 participating nations. Based on this background, international contribution groups in NIMD plan mutual cooperation with foreign researchers for studies on mercury and for information exchange via the NIMD forum. Moreover, based on the needs of the developing countries facing mercury pollution, we use our experience, knowledge, and the latest technology via obtaining the mutual cooperation of JICA. In addition, we develop a new simple mercury analysis methodology that is focused on methylmercury, and it is promised in the Minamata convention as a commitment to the MOYAI initiative. After that, the summary on studies and duties of international contribution group 2015 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 Research)

Our research project consists of three major parts:

1) Development of a simple method for determination of monomethyl mercury compound, 2) improvement of consignment analysis efficiency for the low concentration samples not detected by the developed method, and 3) development of reference materials for use in evaluation of accuracy of mercury analysis. These are part of the MOYAI initiative aimed at helping to speed up the ratification of the Minamata Convention on Mercury among least developed countries (LDCs). One of the main problems in monomethyl mercury monitoring in LDCs is the difficulty of obtaining pure reagent and carrier gas. Commonly used methods for methylmercury determination require several reagents for short-term storage. Additionally, the use of toxic reagents can be a drawback in laboratories in LDCs. Therefore, new methods with lower reagent consumption are an important improvement in 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, which is capable of separating and quantifying monomethyl and inorganic mercury. Briefly, this method involves the following steps: acid leaching, dithizone extraction, TLC separation, and scraping the separated spots. Finally, the released elemental mercury from scraped TLC is measured by TDA AAS standard procedure. The fact that less reagent and no career gas is needed makes this method a valuable contribution to human health protection efforts in LDCs.

(2) Assessment of methylmercury exposure in the Vietnamese population

(Fundamental research)

Megumi Yamamoto

(Department of Basic Medical Science)

We have started to collaborate with the National Hospital of Dermatology and Venereology in Vietnam to collect human specimens (hair and nail) of control group as one of the general population. The official procedure, including the approval of the ethical committee in institutions, is currently ongoing. First sampling (hair and toenails) of the control group will be finished by early March.

To develop an easy and cost-effective analytical method for total mercury (T-Hg) and methylmercury (MeHg) in biological samples using heating vaporization atomic

absorption spectrometry, we modified the pretreatment process of biological samples, such as solubilization, degreasing, and solvent extraction, of our previous method for MeHg analysis. As a result, the procedure (using MIBK instead of chloroform) became simpler and faster than our previous method. Analytical values of certified reference materials for hair and fish using the present method agreed well with certified values.

Next, this method was used to determine T-Hg and MeHg concentrations in muscle and other tissue of several commercial seafood species. The MeHg/T-Hg ratios in muscle of fish examined in this study were in the range of 96−98%, indicating that this result is consistent with a previous report. We confirmed the accuracy of the presented method using muscle in fish with another MeHg analytical method equipped with gas chromatography–electron capture detector.

[Activities theme and summary]

(1)Technical transfer of mercury monitoring techniques necessary for knowing as for mercury pollution in Lake Managua Nicaragua, and survey of mercury exposure in the population around the Lake Managua (International cooperation based on the relationship between JICA and NIMD)

Akito Matsuyama

(Department of Environmental Science and Epidemiology)

1) Accuracy and precision of DMA80 and NIMD method of total mercury analysis in fish and sediment were checked by using certified reference material (DORM2,ERM580,IAEA405). The results showed that there are no problems with analytical results of all the reference material. In addition, the first plan of environmental sampling was prepared in the first dispatch of this project.

2) Hearing survey and questionnaire survey were performed with a family from a fishing village in Typitapa. Finally, we collected questionnaires (1046 sets) and hair samples (1046 samples)

3) We obtained ample data on land use around Lake Managua and the hydrology of the groundwater. Proposal of setting of working group that was organized by representative of related agencies as for this project was submitted to Ministry of the Environment, and was accepted.

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

Masatake Fujimura

(Department of Basic Medical Science)

This year, we did not perform Hg analysis in hair samples for several reasons, including the delivery delay of samples from the Philippines and the missing samples from French Guiana. However, we presented our past findings at an exhibition this year.

(3) Cooperation of research in the international organization

Mineshi Sakamoto

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

(Dispatch)

NIMD researchers conducted a dispatch of 10 in six countries, including Brazil, South Korea, and the United States. We conducted presentations at international conferences, and collaborative research on mercury was presented.

(Invitation)

We are planning to invite one researcher from the University of Ottawa in Canada to conduct collaborative research, and one student from the Federal University of Para Western in Brazil to conduct a technology transfer of mercury measurement technology on the 22–25th of March, 2016.

(JICA Trainings)

We conducted the JICA trainings six times, as well as lectures for 69 participants from 30 countries. In addition, a local lecture was conducted twice in

Minamata as a satellite (51 participants), and we discussed the health effects of mercury, the mercury treaty, and NIMD's efforts. NIMD also accepted one Vietnamese student from the Joint Graduate School of Kumamoto Prefectural University to conduct research guidance, which has been underway since last year, and started collaborative research in Vietnam.

(4) NIMD Forum and International Workshop

Mineshi Sakamoto
(Department of International Affairs and Research, and Department of Environmental Science and Epidemiology)

NIMD Forum 2015 was held as a special session of the 12th International Mercury Conference, from June 14–19 in Jeju, South Korea. The theme of the special session was "Identifying human populations at risk from methylmercury exposure and health effects." A total of seven researchers (five researchers from USA, Canada, Brazil, and Slovenia, one Japanese researcher, and one NIMD researcher) performed 15 minutes of the presentation including a question-and–answer session in each; it finished successfully. In the workshop that took place before the opening ceremony, two NIMD researchers had given lectures. During the conference, NIMD showcased their booth, posted posters of their activities and Minamata disease, and aired the Minamata Disease commentary video. In addition, NIMD distributed the Korean version of the NIMD brochure for general visitors. Hirokatsu Akagi, a former director of International Affairs and Research, was awarded the Lifetime Achievement Award as a contributor and gave a memorial lecture. For safety reasons related to a MERS epidemic, hair mercury measurements, which had been carried out in previous years, were not done this year.

7. Publications and Scientific meetings

[International Journals]

Sakamoto M, Itai T, Yasutake A, Iwasaki T, Yasunaga G, Fujise Y, Nakamura M, Murata K, Man Chan H, Domingo JL, Marumoto M: Mercury speciation and selenium in toothed-whale muscles. Environ. Res., 2015; 143: 55-61.

Usuki F, Fujimura M: Decreased plasma thiol antioxidant barrier and selenoproteins as potential biomarkers for ongoing methylmercury intoxication and an individual protective capacity. Arch. Toxicol., 2015; doi:10.1007/s00204-015-1528-3.

Fujimura M, Usuki F: Low concentrations of methylmercury inhibit neural progenitor cell proliferation associated with up-regulation of glycogen synthase kinase 3β and subsequent degradation of cyclin E in rats. Toxicol. Appl. Pharmacol., 2015; 288: 19-25.

Kariyazono Y, Taura J, Hattori Y, Ishii Y, Narimatsu S, Fujimura M, Takeda T, Yamada H: Effect of in utero exposure to endocrine disruptors on fetal steroidogenesis governed by the pituitary-gonad axis: a study in rats using different ways of administration. J. Toxicol. Sci., 2015; 40: 909-916.

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Shao Y, Yamamoto M, Figeys D, Ning Z, Chan HM. Proteomic Analysis of Cerebellum in Common Marmoset Exposed to Methylmercury. Toxicol. Sci., 2015; 146: 43-51.

Tomiyasu T, Minato T, Wilder LGR, Kodamatani H, Kono Y, Hidaka M, Oki K, Kanzaki R, Taniguchi Y, Matsuyama A: Influence of submarine fumaroles on the seasonal changes in mercury species in the waters of Kagoshima Bay, Japan. Mar. Chem., 2015; 177: 763-771.

Miyashita C, Sasaki S, Saijo Y, Okada E, Kobayashi S, Baba T, Kajiwara J, Todaka T, Iwasaki Y, Nakazawa H, Hachiya N, Yasutake A, Murata K, Kishi R: Demographic, behavioral, dietary, and socioeconomic characteristics related to persistent organic pollutants and mercury levels in pregnant women in Japan. Chemosphere, 2015; 133: 13-21.

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Marumoto, K., Hayashi, M., Takami, A: Atmospheric mercury concentrations at two sites in the Kyushu Islands, Japan, and evidence of long-range transport from East Asia. Atmos. Environ., 2015; 117: 147-155.

Song S, Selin NE, Soerensen AL, Angot H, Artz R, Brooks S, Brunke EG, Conley G, Dommergue A, Ebinghaus R, Holsen TM, Jaffe DA, Kang S, Kelly P, Luke WT, Magand O, Marumoto K, Pfaffhuber KA, Ren X, Sheu GR, Slemr F, Warneke T, Weight A, Weiss-Penzias P, Wip DC, Zhang Q: Top-down constraints on atmospheric mercury emissions and implications for global biogeochemical cycling. Atmos. Chem. Phys., 2015; 15: 7103-7125.

[International meetings]

Sakamoto M, Murata K, Chan HM, Oliveila R, Domingo JL: Significance of fingernail and toenail mercury concentrations as biomarkers for prenatal methylmercury exposure. The 12th International Conference on Mercury as a Global Pollutant, Jeju, 2015. 6.

Sakamoto M, Itai T, Nakamura M, Sawada M, Domingo JL: Detoxification of methylmercury by formation of mercury selenide in muscle of toothed-whale. EUROTOX2015, Porto, 2015. 9.

Sakamoto M: Fetuses as a high-risk group to methylmercury exposure. 9th Congress of Toxicology in Developing Countries, Natal, 2015. 11.

Fujimura M, Usuki F: Inhibition of the Rho/ROCK pathway prevents neuronal degeneration in vitro and in vivo following methylmercury exposure. ASIATOX 2015, Jeju, 2015. 6.

Takahashi T, Fujimura M, Usuki F, Nishizawa M, Shimohata Y: Blood-brain barrier dysfunction caused by vascular endothelial growth factor upregulation in a rat model of subacute methylmercury intoxication. Brain and Brain PET 2015, Vancouver, 2015. 6.

Usuki F, Fujimura M: Mild endoplasmic reticulum stress preconditioning upregulates gene expression of membrane transporters. 55th Annual Meeting of Society of Toxicology, New Orleans, 2016. 3.

Fujimura M, Usuki F: Low in situ expression of antioxidative enzymes in cerebellar granule cells susceptible to methylmercury in a rat model of Minamata Disease. 55th Annual Meeting of Society of Toxicology, New Orleans, 2016. 3.

Nagano M, Fujimura M, Inaba K: Wheat bran enhances urinary elimination and reduces mercury levels in blood and brain after methylmercury exposure in mice. 55th Annual Meeting of Society of Toxicology, New Orleans, 2016. 3.

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 activation of nuclear factor kappa B. The 12th International Conference on Mercury as a Global Pollutant, Jeju, 2015. 6.

Yoshimoto K, Yamamoto A, Koriyama C, Ishibashi Y, Tabata M, Nakano A, Yamamoto M. Total Mercury and Methylmercury Analysis in the Muscle and Gonads of Seafoods using Heating Vaporization Atomic Absorption Spectrometry. 55th Annual Meeting of Society of Toxicology, New Orleans, 2016. 3.

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Hachiya N: Frequency of neurological signs in health surveys conducted in the early 1970's in coastal areas of the Yatsushiro Sea in Japan. The 12th International Conference on Mercury as a Global Pollutant , Jeju, 2015, 6.

Marumoto K: Variations in mono-methyl mercury concentrations during a rain event at a site in Minamata, Japan. The 12th International conference on mercury as a global pollutant, Jeju, 2015. 6.

Marumoto K, Imai S: Observation of dissolved gaseous mercury and mercury evasion flux in surface seawater of some sea areas in western Japan. The 12th International conference on mercury as a global pollutant, Jeju, 2015. 6.

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