

NIMD Annual Report
2019
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National Institute for Minamata Disease
Ministry of the Environment
Japan

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Report on Research and Other Activities
in the Fiscal Year 2019

1. Pathomechanism Group

The aim of the study group is to understand the molecular mechanisms underlying methylmercury (MeHg) toxicity in humans. Our goals are as follows: 1. to understand the initial signs of MeHg poisoning, 2. to evaluate MeHg toxicity, 3. to develop methods to protect against MeHg-induced disorders, and 4. to develop new treatment options for MeHg poisoning. In this study group, we use techniques from the fields of biochemistry, molecular biology, and pathology in cell cultures and in animal models, in order to achieve the above objectives.

This group conducted the following research during the fiscal year 2019.

[Research theme and summary]

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

Masatake Fujimura

(Department of Basic Medical Sciences)

We found that MeHg exposure causes allodynia in the plantar of rats as well as in patients with Minamata disease. Moreover, it was clarified that Fasudil, a ROCK inhibitor, attenuated the MeHg-induced allodynia by directing microglia phenotype change from the pro-inflammation to the anti-inflammation type.

This year, we presented the findings of our previous research at two conference, and published one peer-reviewed article.

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

Masatake Fujimura

(Department of Basic Medical Sciences)

Using rats, we found that gestational exposure to MeHg increased blood corticosterone, which has a neuropathic effect, and conversely decreased estradiol, which has an

anti-neuropathic effect. We also clarified that increased plasma thiol antioxidant capacity levels were useful as biomarkers to predict the severity of subsequent neurological symptoms in MeHg-intoxicated rats

This year, we presented the findings of our previous research at six conferences, and published three peer-reviewed articles.

- (3) 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 effect of functional food ingredients (wheat bran (bran), fructooligosaccharide (FOS), and glucomannan) in the toxicity of MeHg. Last year, we had demonstrated that daily intake of bran or FOS significantly reduces tissue Hg levels after subchronic exposure to MeHg at a low dose in mice. In the current year, we examined their effect on tissue Hg levels after subchronic exposure to MeHg at a high dose in mice. At the end of the experiment, total Hg levels in the blood, brain and kidney were significantly reduced in the bran-fed group compared to that in the control group. On the other hand, the FOS-fed group showed lower total Hg levels in the kidney than the control group.

In the mid-term plan 2015 (2015-2019), we demonstrated the following findings. (1) Dietary bran or FOS reduced the Hg levels in the brain after single administration of MeHg in mice. Dietary glucomannan showed no effect. Daily intake of bran enhanced the excretion of Hg in both the feces and urine in mice. On the other hand, daily FOS intake enhanced fecal Hg excretion in mice, and its mechanism may be via a modification of the metabolic activity of associated intestinal bacteria. (2) Dietary bran, but not FOS, reduced the Hg levels in the brain after subchronic exposure of MeHg at low and high

doses in mice. (3) These results suggest that daily intake of bran or FOS could affect the fate of MeHg and may reduce tissue Hg levels after MeHg exposure in animals and humans.

(4) Research on selective cytotoxicity of methylmercury focusing on sulfur nucleophiles (Fundamental research)

Takamitsu Unoki

(Department of Basic Medical Sciences)

MeHg can covalently modify cellular proteins, causing their dysfunction and thereby exerting deleterious effects. Recent findings indicate that reactive sulfur species (RSS), which are highly nucleophilic molecules, easily react with MeHg to form dimethylmercury sulfide, a less toxic sulfur adduct than MeHg. Metabolomic analyses revealed that RSS increases in an age-dependent manner in the rat brain. These results support the contention that the embryo and juvenile rats are more sensitive to MeHg toxicity than adult rats, owing to low RSS levels during the early stages of development. The site-specific analysis of the adult rat brain revealed that cerebellar RSS levels are lower than those of the hippocampus. Microscopically, RSS levels of the granular cell layer were lower than those of the molecular layer in the cerebellum. Taken together with the finding that the brain RSS were consumed during MeHg exposure, these data suggest that RSS distribution is a key factor in the differential susceptibility of regions of the brain to MeHg.

(5) Research on the mechanism of the late stage of methylmercury neurotoxicity (Fundamental research)

Akio Sumioka

(Department of Basic Medical Sciences)

Exposure to MeHg induces oxidative stress in the brain and consequently causes neurodegeneration. Therefore, we investigated the mechanism of the late stage of MeHg neurotoxicity. For this, we developed a sensor vector for

MeHg toxicity and studied the MeHg-mediated dysfunction of the synapse and axon.

A. Research on the development of a sensor vector for MeHg toxicity

To monitor the process through which MeHg induces neuronal degeneration, we developed a sensor vector for detecting MeHg toxicity. Using genetic engineering, we utilized deficient selenocysteine insertion during selenoprotein translation mediated by MeHg exposure. In 2019, we constructed thioredoxin reductase-fused fluorescent protein cDNA and measured MeHg toxicity as a fluorescent signal in a cell culture model. Furthermore, we confirmed the low efficiency of selenocysteine insertion, which disturbs a sensor vector and tested several causative factors.

B. Research on MeHg-mediated excitotoxicity

Excitotoxicity is focused on which oxidative stress is a significant risk factor. We investigated the impairment of the excitatory synapse owing to MeHg to understand the molecular mechanism of MeHg toxicity. In 2019, we administered MeHg water to mice and observed individual differences over time for abnormal behavior. Next, we introduced a primary culture of cerebellar granule cells and discovered that MeHg exposure induced AMPA-type glutamate receptor impairment, which suggests a new mechanism for MeHg toxicity.

C. Research on MeHg-mediated neuronal toxicity by the tau protein

We also focused on tau pathology for which oxidative stress is one of the significant risk factors. Phosphorylation and aggregation of tau by MeHg were examined to understand the molecular mechanism of MeHg toxicity. In 2019, we administered MeHg water in transgenic mice showing hyperphosphorylation of the tau protein and observed increased sensitivity to MeHg in these mice. Furthermore, we demonstrated MeHg-mediated tau deficiency in cerebellar granule cells. This result suggests that MeHg may impair an axon as the primary localization of tau protein.

2. Clinical Group

Research

Minamata disease (MD) is an intoxication of the central nervous system caused by ingestion of seafood from the methylmercury (MeHg)-contaminated areas of Japan. The diagnosis of MD is usually not difficult in typical and severe cases; however, it is difficult in mild cases. Thus, objective methods are critical to clinically diagnose atypical or mild symptoms in the chronic stages of MD. We have been evaluating the brain function in MD using magnetoencephalography (MEG) and magnetic resonance imaging (MRI).

In addition, effective therapies are required for intractable chronic pain, tremor, ataxia, and spasticity, as they affect the quality of life of patients with MD. To develop an effective therapy for MD, we evaluated the neurological function of patients with MD using MEG and MRI. Based on the brain dysfunction, we performed repetitive transcranial magnetic stimulation (rTMS) therapy for pain, ataxia, and tremor. Furthermore, we used botulinum toxin therapy for treating spasticity in patients with MD.

The research conducted by our group during FY 2019 is outlined below.

[Research theme and summary]

(1) Research on health effects of methylmercury exposure in humans and therapeutic development (Project research)

Masaaki Nakamura
(Department of Clinical Medicine)

To develop an objective evaluation protocol using MEG and MRI, we compared somatosensory-evoked fields and morphological features of patients with MD and control subjects from the Kumamoto district (not polluted with MeHg). We quantified the somatosensory-evoked fields considering N20m amplitude, N20m latency stability, N20m and P35m direction, and somatosensory area

frequency after removing noise from MEG raw data using the latest denoise script. Abnormalities in these parameters were more frequent in patients with certified MD than in the control subjects. To increase the sensitivity of this method, we have been developing the script quantitating the extent of the network of the somatosensory area.

We evaluated the potential value of machine learning-based analysis to diagnose MD using quantitative MRI features. Twelve patients with MD (6 adult-type, 3 child-type, and 3 fetal-type MD) and 74 age-matched control patients were included in this retrospective study. The model using axial diffusivity-random forest and axial diffusivity-support vector machine was the best for discriminating the control from the patients with MD. Moreover, the features selected by random forest generally corresponded to brain lesions in MD. These findings suggest that machine learning-based MRI analysis using random forest or support vector machine and regional axial diffusivity parameters may be a useful method in diagnosing MD.

We observed improvements in ataxia and tremor in patients with MD treated with rTMS. We propose that the evaluation of M1 excitability using resting-state functional MRI can be effective in determining the optimal TMS stimulation conditions for cerebellar ataxia.

(2) A study of treatment for pain in patients with Minamata disease (Fundamental research)

Souhei Iwanaga
(Department of Clinical Medicine)

As lumbago or neuropathic pain results in the reduction in activities of daily living (ADL) in chronic-stage MD, it is important to develop an effective therapy. rTMS has recently been used to treat various serious pathological conditions and diseases. High-frequency rTMS for neuropathic pain treatment is estimated as level A in the

European guideline. The purpose of this research was to determine the optimal conditions of rTMS or spinal magnetic stimulation for neuropathic pain in MD. This year, I mastered the maneuver of magnetic stimulation, and received approval for research from the Kyushu University Certified Institutional Review Board for Clinical Trials.

Activities

In recent years, political measures have been undertaken to facilitate the rehabilitation of patients with MD. The Department of Clinical Medicine, in cooperation with related organizations, actively organizes events on MD. In addition, we organized conventional rehabilitation activities, including daycare facilities for fetal-type patients with MD, and annual workshops on rehabilitation and assistance techniques. We also examined the usefulness of vibration therapy for pain relief and spasticity associated with various chronic diseases of the nervous system, including MD. Furthermore, we introduced a robot suit, walking assistant machine which assist patients during gait training.

To examine the effectiveness of home care support for patients with MD 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 conducted to develop strategies for the 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.

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

Moreover, we started to examine the difference in

moment-to-moment changes in ADL using the clinical entities of MD to objectively evaluate ADL in chronic MD.

The following section includes an outline of the clinical practice conducted by this group in FY 2019:

[Activity theme and summary]

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

Souhei Iwanaga

(Department of Clinical Medicine)

We provided rehabilitation in the form of daycare for outpatients twice per week until last year. To ensure easy access to rehabilitation, we have started to provide this service in the morning and afternoon four times a week from this year. We also accepted six new users, including five certified patients with MD. In addition, we visited the Orange Hall three times a month, which is located in southern Minamata and used to improve the health and welfare of elderly people, including patients with MD. At the Orange Hall, we conducted physical and cognitive function evaluations, ADL evaluations, and health exercise classes.

Annual workshops on rehabilitation and assistance techniques were held to improve the skills of specialized regional staff for assisting patients with MD. This year, the themes of the organized workshops included “Thinking about rehabilitation medicine from an emotional perspective” as a rehabilitation technique and “The Healthy Life Span Extension Act and Measures to Combat Frail and Sarcopenia” as a care technique. Based on the questionnaire responses of attendees, the two workshops were well received. We believe that workshops are a useful outreach forum to share information on care and rehabilitation techniques that can be implemented across the community.

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

Masaaki Nakamura

(Department of Clinical Medicine)

We conducted a study titled “Home support model study, including care prevention” (FY 2006–2008) to identify support methods, including rehabilitation and improvement of ADL for aging patients with MD 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 MD victims” (FY 2012) to develop methods for implementing these concepts in the community. Subsequently, 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 craft class program based on responses to a questionnaire, we received high satisfaction levels from the participants in a subsequent survey. We also examined cognitive function in the elderlies of Minamata City using the Odor Stick Identification Test for Japanese and a touch panel-type test, MSP-1100.

Furthermore, we publicized the work of the craft class on the homepage and Facebook page of our institute.

(3) Information dissemination using 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 a duty to permanently preserve several pathological tissue specimens of MD. However, pathological tissue slides are difficult to preserve permanently as they fade away with the passage of years. Therefore, our objectives are to digitize these pathology slides for permanent preservation and to publish the digitized pathology tissue slides on a website for the students and doctors learning pathology.

(4) Examination of moment-to-moment changes in the activities of daily living (ADL) using the clinical entities of Minamata disease

Liu Xiao Jie

(Department of Environment and Public Health)

Although a rapid decrease in physical activity and ADL occurs in patients with MD, an objective evaluation of ADL has not been performed until now. The purpose of this study was to clarify the differences in moment-to-moment changes in ADL using the clinical entities of MD by a comparison of ADL over the course of 5 years.

We estimated the permanent changes in daily life performance ability in 21 patients with certified MD (9 fetal-type MD patients [F-MDP] and 12 adult-type MD patients [A-MDP]). Regarding the NM and N-ADL scores, there were decreases in both F-MDP and A-MDP, but statistical significance was observed in only A-MDP. In addition, no significant changes in daily life status were observed in F-MDP, but there were significant decreases in the scores of walking, feeding, sitting, rolling over, and excretion in A-MDP.

3. Exposure and Health Effect Assessment Group

Generally, two subpopulations are susceptible to methylmercury (MeHg) exposure: those who are exposed to high mercury (Hg) levels and those who are more sensitive to its effects. The exposure and health effect assessment group conducted epidemiological surveys in Taiji-cho, Wakayama Prefecture, where the population has been exposed to high MeHg concentrations. The group's research also concerned segments of the population particularly sensitive to MeHg exposure, namely fetuses or people suffering from various diseases, to contribute to the 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 Environment and Public Health)

1) As an experimental study to elucidate the toxicokinetics of MeHg in the pathological condition of glucose metabolism disorders, 12-week-old KK-Ay diabetic mice and BL/6 normal mice were exposed to a single administration of MeHg (three doses), and blood and tissues (brain, kidney, liver, and pancreas tissues) were sampled over time (4, 7, 11, and 14 days). Total Hg (T-Hg) in the samples was analyzed, and the data were analyzed using the Kp value (tissue Hg concentration/plasma Hg concentration) for the comparison of MeHg distribution in each tissue in KK-Ay and BL/6 mice. We found that the Kp value in the brain tissue of KK-Ay mice was higher than that of BL/6 mice at the early stage of MeHg exposure, but the trend was reversed with the passage of time. In addition, we have completed the analysis of the toxicokinetics of MeHg using other pharmacokinetic parameters.

2) To clarify the transfer of MeHg from a mother to a baby under the pathological condition of glucose metabolism disorder, a female mouse model of glucose metabolism disorder will be established, and the conditions for delivery will be examined. We are currently feeding mice a high-fat diet to establish a mouse model with glucose metabolism disorder that is suitable for research purposes.

3) We are collaborating with the University of Occupational and Environmental Health on a research concerning "Hg distribution in biological samples (hair, blood, and placental and umbilical cord tissues) of mothers and infants under the condition of glucose metabolism disorders (gestational diabetes mellitus and pregnancy associated with diabetes mellitus)." We are analyzing T-Hg in the obtained samples (2018: 28 samples, 2019: 33 samples; 61 samples in total).

(2) Research on the localization of mercury and selenium in mammalian tissue (Fundamental research)

Masumi Marumoto

(Department of Basic Medical Science)

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

(3) Health risk assessment of high methylmercury exposure derived from whales (Fundamental research)

Masaaki Nakamura
(Department of Clinical Medicine)

We searched for proteins associated with MeHg toxicity to elucidate why the adverse effects of MeHg exposure were not clearly observed in the adult inhabitants of Taiji-cho. We performed two-dimensional gel electrophoresis using plasma samples from four subjects with high Hg levels and four subjects with low Hg levels (two men and two women per group; all inhabitants of Taiji-cho). Using mass spectrometry analysis, we identified 14 proteins with significant differences in concentration between the high and low Hg-level samples. Among them, we focused on three proteins (transthyretin (TTR), adiponectin, and apolipoprotein E). In plasma samples from 120 Taiji-cho residents, TTR and adiponectin were positively and negatively correlated with Hg, respectively. When we evaluated cell survival in the presence of MeHg and TTR using U87-MG human neuronal glioblastoma cells, the protective effect of TTR against MeHg was not observed.

We have been performing a detailed analysis of a survey regarding the effect of MeHg exposure on child development, which was conducted at the Taiji-cho multi-purpose center from 2012 to 2017. Auditory and visual circuits in boys were affected by prenatal MeHg exposure. However, neuropsychological indicators, including IQ, were unaffected by MeHg exposure, suggesting that in this study, MeHg exposure level might have minimal effect on neurodevelopment.

We also collected 155 umbilical cord samples to examine the sequential changes and late effects of prenatal MeHg exposure in Taiji-cho residents.

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

Mineshi Sakamoto
(Special Advisor to the Director-General)

1) Hg speciation in preserved historical sludge to estimate risks from the sludge entrapped under the reclaimed area of Minamata Bay, Japan

This work was supported by JSPS KAKENHI (Grant Number JP15K00533).

The objective of this study was to obtain data on potential MeHg risks caused by sludge leakage into the reclaimed area at Minamata Bay, Japan, by analyzing preserved sludge and current sediment samples. The T-Hg (wet basis) was 0.18 ppm in the control (n = 1), 6.1 ppm (range: 0.83–12.6) in current sediments (n = 5), and 241 ppm (range: 22.4–3620) in preserved sludge (n = 4). MeHg percentages were 0.41%, 0.12% (range: 0.051–0.21), and 0.03% (range: 0.014–0.049) for the control, current bay sediments, and preserved sludge, respectively. %MeHg decreased exponentially with an increase in T-Hg concentration for all samples. X-ray absorption fine structure analysis suggested that the main chemical form of the preserved sludge is β -HgS. Results indicate that although MeHg pollution had been severe during the Minamata epidemic, with high T-Hg concentrations in the preserved sludge, the main chemical form of the sludge was β -HgS, which is insoluble, poorly bioavailable, and exhibits low MeHg content (0.049%). These results suggest that in the unlikely event of sludge leakage from the reclaimed area, the risk of exposure to MeHg is very low.

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<https://www.ncbi.nlm.nih.gov/pubmed/31648069>

2) Reanalysis of Hg and selenium in historical samples of Minamata disease: elevated Se levels in MeHg pollution

This work was supported by JSPS KAKENHI (Grant Number JP19K12353).

We reanalyzed T-Hg and Se concentrations in preserved historical samples collected during the period of heavy MeHg pollution to reveal the elevated Se levels associated

with Hg concentrations in the samples. Materials: 1) Cat 717, which showed neurological symptoms in experiments at Chisso factory in 1960, after it consumed a diet mixed with a distilled residual solution from the acetaldehyde manufacturing process, which contained T-Hg at 43 ppm and no Se. 2) Red sea bream collected from Minamata Bay in 1956. 3) Minamata Bay sediments (n = 2) collected in 1958 and 1980. 4) Shellfish (*Hormomya mutabilis*, n = 17) collected from Minamata Bay in 1959. All the control samples were collected recently. Cat 717 showed 40 ppm T-Hg and 0.5 ppm Se in the cerebrum, 126 ppm T-Hg and 8 ppm Se in the liver, and 49 ppm T-Hg and 2 ppm Se in the kidney, with the liver Se level being approximately 20 times that in the control. The two Minamata Bay sediments showed 401 ppm T-Hg and 21 ppm Se as well as 3620 ppm T-Hg and 19 ppm Se, with the Se levels approximately 200 times those in the control. The muscle of the red sea bream showed 44 ppm T-Hg and 3.2 ppm Se, with the Se level approximately three times that in the control. Furthermore, T-Hg and Se levels in the shellfish showed a significant positive correlation coefficient ($r = 0.89$). Although more data are required, these results suggest that heavy MeHg pollution in Minamata Bay may have led to elevated Se levels in the environmental samples.

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

Kimiko Izumo

(Department of Environment and Public Health)

Seafood is a major source of exposure to MeHg in humans, and in recent years, the world's supply of seafood has continued to increase. In this study, we examined the possibility of screening and monitoring for MeHg using the gills, which are usually discarded, instead of the edible parts to evaluate MeHg concentration in edible fish. However, seafood is also a source for the intake of health-beneficial nutrients such as Se. Recently, deep-sea fish have attracted attention as new foodstuff in Japan. Several deep-sea fish species are known to have high Hg

concentration; but there remain many others with insufficient information on Hg concentration. In addition to obtaining data on Hg concentrations mainly in deep-sea fish, we attempted to analyze the nutrients derived from fish to assess the risks and benefits of seafood intake.

We examined the correlation of T-Hg concentrations in the muscle and gill of the same individual in six fish species: splendid alfonsino (*Beryx splendens*), alfonsino (*Beryx decadactylus*), hardhead grenadier (*Coelorinchus japonicus*), marbled rockfish (*Sebastes marmoratus*), yellowbar red rockfish (*Sebastes albofasciatus*), and scorpionfish (*Helicolenus hilgendorfi*). A significant strong positive correlation was observed between T-Hg concentrations in the muscle and gill in each fish species. In addition, multiple regression analysis was performed to estimate muscle T-Hg concentration using gill T-Hg concentration and body weight as explanatory variables in a group totaling all individuals of the six fish species. The results suggest that such an estimation is possible. For marbled rockfish, we examined the correlation between MeHg concentration in the muscle and T-Hg concentration in the gill, and a strong positive correlation was observed. This result, at least in marbled rockfish, suggests that it is possible to estimate MeHg concentration in edible sites by measuring T-Hg concentration in the gill. T-Hg and Se were also measured in the muscle of marbled rockfish, alfonsino, hardhead grenadier, and southern hake (*Merluccius australis*). The molar concentration of Se was higher than that of T-Hg in all individuals, except for one case. Fish may have more benefit as a source of Se intake than risk as a source of MeHg exposure. However, in this study, we measured Se in only a small number of fish and did not examine the seafood intake in humans. Therefore, more research is needed in the future.

4. Social and Information Service Group

The Social and Information Service Group, Minamata Disease Archives, along with the Minamata Disease Municipal Museum and the Environmental Education and Intelligence Center, have been focusing on disseminating information and educational activities to increase the understanding of Minamata Disease (MD) and environmental problems. In addition, based on an agreement for Comprehensive Cooperation with Minamata City (2015), we submitted a policy proposal book to Minamata City (No. 1703281, official document of NIMD, dated March 28, 2017).

To implement the policy proposals, our group has developed cooperative relationships with Minamata City and other counterparts and has been aiming for research that can contribute to the region. Furthermore, we have been involved in organizing the MD-related documents stored by our center, conducting historical research, and transmitting risk information, including information regarding health effects, after scientific verification. Through these activities, we have contributed to the local and international community. The outline of each research project and activity of our group in the fiscal year 2019 is as follows.

[Research theme and summary]

(1) The Sociological Study on the Social Environment of Fetal Minamata Disease Victims (Fundamental research)

Rie Harada

(Department of International Affairs and Research)

Minamata Disease Archives interviewed patients with MD, their families, caregivers, medical staff, and supporters from fiscal year 2003 to 2014. From the data of 95 cases, we intensively extracted information regarding cases of patients with fetal-type MD who had much information on supplementary materials, and analyzed and

determined the actual conditions of the social environment, medical care, nursing care, and social life.

Through analysis of the content of the interview from the perspective of motivating patients with fetal-type MD, it was found that the patients' family and those around them were making efforts to improve their lives when medical, welfare, and administrative services were not delivered.

The second finding was that the patients had undergone a dramatic development in their physical and mental health after being admitted to a medical facility. In contrast, the psychological burden of leaving the family during childhood was strong.

In addition, some patients became adults without having a good relationship with their parents or siblings because they had been separated from their families during their childhood.

Moreover, a peculiarity of the social environment of patients with fetal-type MD is their excessive exposure to the media. Although exposure to the media and participation in anti-pollution movements place a burden on the patients and their families, journalists and supporters are involved in the lives of the patients to prevent them from being isolated and to improve the social and mental maturity of patients with fetal-type MD.

It was found that these social environments affected the establishment of the identity of patients with fetal-type MD.

(2) Epidemiological studies on methylmercury exposure and associated health risks (Fundamental research)

Akane Anai

(Department of International Affairs and Research)

In this study, previously obtained data were used to examine "simple methods for estimating MeHg exposure" and "long-term health risks and health effects owing to Hg exposure." Data were obtained from 187 participants in

2017 and analyzed using a “simple method for estimating MeHg exposure.” Among women, there was a significant correlation between the total hair Hg concentration and the MeHg amount estimated from questionnaires on seafood intake frequency. In addition, we have analyzed the data of residents’ health survey conducted by Minamata City in the 1970’s. Our results indicate that the “high intake group”, in which mothers ate a lot of seafood in the past, showed a significant increase in the odds ratio of “Do you think your child is slow in wisdom?” for boys, “Do you think your child is not growing well?” for girls, and “Did your child have an abnormal birth?” for both boys and girls, compared with the “low intake group”, in which mothers ate seafood less than once a week in the past. However, there are many limitations in this study, such as uncertainties of blood relationship between mother and child and lack of objective biomarkers of Hg exposure and infant health status. Thus, further research is needed.

[Activity theme and summary]

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

Rie Harada

(Department of International Affairs and Research)

In the exhibition facilities, we installed a brochure case at the entrance in 2018 and an information counter in the open space on the 2nd floor, and this year, we decorated these places each season to create spaces where visitors can get familiar with.

In the small exhibition room on the 1st floor, the exhibition on the Minamata Convention on Mercury has been continuing. The auditorium is used not only by NIMD but also by other organizations for lectures and environmental programs throughout the year.

“The Minamata Convention on Mercury Commemoration Forum 2019” was held at the Minamata Disease Archives on December 21st, and it contributed to the dissemination of knowledge on Hg.

As an information dispatch, we held a lobby exhibition in

Kumamoto City jointly with the Minamata Disease Municipal Museum and the Environmental Education and Intelligence Center and introduced our research (8/27–9/10).

As an executive committee member, we organized two subcommittees for the 7th Pollution Museum Joint Forum in Kurashiki, which was held from December 13th to 15th.

Regarding document maintenance, we continued to work on cataloging MD-related documents, digitizing documents, and releasing the catalog.

In cooperation with the Minamata Disease Health Care Affairs Division of Kumamoto Prefecture, the Minamata Disease Municipal Museum of Minamata City, and the Minamata Disease Museum of NPO Soshinsha, we partially integrated catalog data of materials stored at each organization. The trial operation of the database, which commenced last year, is going on.

In addition, based on the agreement for Comprehensive Cooperation with Minamata City, we conducted a survey to implement the policy recommendations submitted to Minamata City.

(2) Information service using hair mercury analysis

Masaaki Nagano

(Department of Basic Medical Science)

In 2019, 372 hair samples were collected from visitors to National Institute for Minamata Disease, Minamata Disease Archives, and from other organizations. The total Hg levels of the samples were measured. The analytical results were sent to each individual. Hair Hg levels were analyzed in a total of 3,064 individuals between 2015 and 2019.

5. Nature Environment Group

The aim of our research group was to understand the Hg cycle and the methylation of inorganic Hg in various natural environments, including land, water, and atmosphere at the local (the Minamata Bay area), regional, and global scale. Therefore, our group implemented integrated studies, including field survey, monitoring, and laboratory experiments for environmental Hg. In addition, a MC-ICPMS (Multi collector - Inductively coupled plasma mass spectrometry) for measuring Hg isotopes with high precision was installed to obtain more detailed information on emission sources and chemical reaction processes of Hg. Moreover, we worked towards obtaining valuable data for the effective implementation of the Minamata Convention by collaborating with other domestic and foreign research scientists.

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

[Research theme and summary]

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

Kohji Marumoto

(Department of Environment and Public Health)

The observation of Hg in the air and wet depositions has been continuing for several years at several sites, including Minamata, Hirado, Fukuoka, Minami-Awaji, Omaezaki, and Kashiwazaki. However, the monitoring at Kashiwazaki and Minami-Awaji was terminated in March 2018 and August 2019, respectively. The continuous observation of the atmospheric gaseous elemental Hg (GEM) at Fukuoka and the total gaseous Hg (TGM) at Minamata revealed that their concentrations showed a year-to-year decrease. The concentrations of particle-bound Hg in airborne particles less than 2.5 μm ($\text{PBM}_{2.5}$) at Fukuoka also had a year-to-year decreasing trend. However, gaseous oxidized Hg (GOM) concentrations

increased in the last 2 years. Further continuous observation is necessary for understanding their long-term trends. Using the observation data, atmospheric Hg deposition flux was calculated as approximately $2 \text{ ng m}^{-2} \text{ h}^{-1}$ at Fukuoka. Hg emission fluxes from the sea surfaces of the Seto Inland Sea (SIS), Kuroshio Current region of the East China Sea (ECS), and sub-Arctic region of the North Pacific Ocean (SANPO) were also estimated at $2\text{--}3 \text{ ng m}^{-2} \text{ h}^{-1}$ on an average. Although the estimations had high uncertainty, it was indicated that atmospheric Hg deposition and Hg emission from the sea are at almost the same level as those in the sea surrounding the Japanese Islands. In addition, according to the results of our studies and those from previous literature, dissolved Hg concentrations in the SIS and ECS had a yearly decreasing trend. This is one of the reasons for the decreasing trend in atmospheric GEM or TGM concentrations owing to the air-sea interaction.

Considering Hg outflow from rivers to coastal areas, Hg is transported and accumulated into a marine environment. T-Hg and MeHg in seawater were also evaluated in this study. The analysis of T-Hg and MeHg in all seawater samples obtained from the SIS, ECS, and SANPO was completed this year, and their vertical and horizontal distributions were obtained. T-Hg concentrations gradually increased from the surface to the mesopelagic layers. MeHg concentrations were also higher in the mesopelagic zone than in the euphotic zone, except for the subsurface chlorophyll-a maximum layers in the SANPO. Higher concentrations of MeHg and proportion of MeHg to the total Hg(%MeHg) were observed in the western part of the SANPO, where the North Pacific Intermediate Water is formed. In the ECS, they were also higher at the depths where seawater properties such as temperature and salinity are similar to those in the North Pacific Intermediate Water.

In this study, valuable knowledge on the factors affecting the vertical profile of MeHg and the air-sea-marine

organism interactions of Hg was also obtained from the laboratory experiments relevant to the exposure of phytoplankton to MeHg and MeHg elution from planktons under the water pressure at 1000 m below sea level. However, more elaborate studies on MeHg behavior in the marine environment are needed in the future.

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

Akito Matsuyama

(Department of International Affairs and Research)

1) Annual average values for 2019 (three measurements/year) of dissolved total mercury (diss-THg) and dissolved Methylmercury Hg (diss-MeHg) from the Hg monitoring in Minamata Bay were 0.29 ± 0.08 ng/L and 0.03 ± 0.03 ng/L, respectively. In addition, the analysis for each nutrient, such as $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, for Minamata Bay seawater that was stored for 5 years since 2014 were almost completed. Based on the results, multiple regression analysis was performed by using all the analytical data of 2017 Autumn as the high diss-MeHg concentration level; then, the concentrations of diss-MeHg and each nutrient were set as the dependent and independent variables, respectively. We obtained a relatively high multiple correlation coefficient ($R^2 = 0.7$) from three types of nutrient combinations as independent variables (Total organic carbon (TOC), available phosphorus, and available ammonia).

2) To artificially simulate Hg methylation in Minamata Bay seawater, an incubation experiment was restarted this year. The basic condition of the incubation experiment was as follows, based on the analytical data of the incubation experiment that was performed previously: seawater temperature, 19–20 °C; salinity, 30–33‰ (3–3.3%); dissolved oxygen (DO), 7–7.5; pH, 8.1–8.3; TOC, 1–1.5 ppm; available ammonium < 0.02 ppm; and available phosphorus < 0.01 ppm. Currently, a preliminary incubation experiment is going on.

3) To confirm the accuracy of the 3D simulation by obtaining the critical shear stress of Minamata Bay sediment, the 1st observation was performed in Minamata Bay. The general value range of critical shear stress of sediments was almost 0.1–5.0 Pa (mud to sand). Through measurement of the 1st observation in Minamata Bay, the average value of critical shear stress of sediment was determined as 0.02 Pa. Our results indicated that the liquidity of Minamata Bay sediment is higher than that of other sediments. In addition, fluid mud that contains small fine particles was distributed in the fluid Minamata Bay sediment.

(3) 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 Environment and Public Health)

We developed a simple screening method for measuring Hg using a quartz crystal microbalance for the detection of Hg (QCM-Hg system) in collaboration with the National Institute of Advanced Industrial Science and Technology. The QCM-Hg system is small and easy to transport. Therefore, it is suitable for Hg observation in inaccessible field sites, such as volcanic areas. At first, we tested the QCM-Hg system for measuring air Hg in volcanic and geothermal areas. The frequency shift of the QCM-Hg system was changed in response to air Hg concentrations at the vicinity of the fumarole, which was measured by a conventional Hg monitor based on gold amalgamation and cold-vapor atomic absorption spectrometry. At the time, air Hg concentrations were in the tens of thousands ng m^{-3} . However, apart from that in the fumarole, air Hg concentrations became extremely low at several dozen ng m^{-3} . Hg concentrations in the air space above the Aso Nakadake 1st crater were also lower than 5 ng m^{-3} . As the detection limit of Hg using the QCM-Hg is approximately 500 ng m^{-3} , it is difficult to measure lower Hg concentrations.

As a substitute for the QCM-Hg system, we set up a sampling system for Hg and SO₂ in volcanic gas using a drone. SO₂ is one of the dominant components in volcanic gas. The system consists of a portable air pump with a mass flow controller, an Hg amalgamation trap, a filter unit for SO₂ gas, and the QCM-Hg system. The QCM-Hg system was used for recording meteorological parameters, including air temperature, relative humidity, and air pressure, as it has the relevant sensors and a data logger. After sampling, Hg and SO₂ were analyzed in our laboratory. At the ground level of the geothermal areas, air Hg concentrations were strongly correlated with SO₂ concentrations. However, the Hg/SO₂ ratios varied on a day-to-day basis. In addition, their correlation was not observed at a height of several meters in the air. Condensed water is generated at the upper air because of a lower temperature and higher relative humidity. Thus, the lack of correlation between Hg and SO₂ can be attributed to the difference in their water solubility.

Second, we also investigated the application scope of the QCM-Hg system as a continuous Hg monitor and/or personal Hg exposure monitor in gold mining activity, which is one of the sources most responsible for the worldwide emission of atmospheric Hg. The frequency shift of the QCM-Hg system was changed in response to air Hg concentrations at the working environment of the gold mining areas and gold trading shops, in which air Hg concentrations ranged from 1000 to tens of thousands ng m⁻³. In addition, it is clear that the QCM-Hg system is applicable as a real-time monitor for air Hg by continuously calculating the slope of the frequency shift. However, more detailed inspection on several parameters is required for its practical application in the future.

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

Satoshi Irei
(Department of Environment and Public Health)

If methylmercury (MeHg) is formed in the atmospheric reactions of gaseous elemental mercury (GEM), it is foreseen that gaseous oxidized mercury (GOM) would be the precursor. Therefore, the establishment of GEM and GOM standards is very important for fundamental studies of the Hg cycle in the natural environment. The aims of this project were (1) to fabricate a commercially available volatile organic compound permeation device for the generation of a standard gas for GEM and GOM, (2) to apply the device for the investigation of homogeneous and heterogeneous chemical reactions of GEM and GOM under simulated atmospheric conditions, and (3) to also evaluate the precision and accuracy of ongoing field measurements of atmospheric Hg by Dr. Marumoto using Tekran 2537/1130. The following update for this project in the fiscal year of 2019 is presented.

First, based on the advice from the research evaluation in the last year, sub-project (3) was removed because its achievement during the project period was not feasible.

For sub-project (1), the generation of GOM standard gas was successful using a home-made permeation tube, which was made of perfluoroaroxy polymer (PFA) tubing enclosing mercuric (II) chloride. The concentration of the gas, however, was too high for use in the laboratory studies planned in sub-project (2). Thus, this permeation tube is currently under modification. GEM standard gas has been made using a commercially available GEM permeation tube (VICI Inc., Schenkon, Switzerland).

As part of sub-project (2), the heterogeneous uptake of GEM by acidified water was explored. In this experiment, 0.5 L of pure water, acidified by either nitric or sulfuric acid (adjusted to an approximate pH of 3), was left in a 2.5 L rectangular-shaped glass container, at the both ends of which a half inch o.d. glass tubing was attached. From one end of the container, a defined concentration of GEM generated from the permeation device (40–50 ng m⁻³, approximately 25 times the concentration of atmospheric GEM) was introduced into the space above the solution in the container at a flow rate of 0.5 L min⁻¹. During the experiment, the other end of the container was opened to

the atmosphere, and the temperature and relative humidity of the outflowing gas were monitored at this end. After 2.5 – 6 h of exposure, 290 mL of the solution was quickly siphoned into a 375 mL PFA impinger (AS ONE, Osaka, Japan), followed by the conventional quantitative Hg analysis.

The experimental results obtained in the previous year showed a large variation in GEM uptake; therefore, we investigated the source(s) of this variation. One of the possibilities was the instability of the instrument (a cold-vapor atomic absorption spectrometer) for the analysis of low-concentration samples. To investigate this issue, we used a new instrument with a higher sensitivity, which is a cold-vapor atomic fluorescent spectrometer or CV-AFS (WA-5F, Nippon Instruments, Corp., Tokyo, Japan). Surprisingly, even when the CV-AFS was used, the calibrations made in the morning and afternoon showed a large variation. After the investigation, we identified that the large variation was attributed to the instability of the 100-V electric power. We do not know why the electric power of this facility was so unstable, but we could avoid this problem by installing an UPS.

Blank values were another issue slowing down progress. The total concentrations of Hg contained in the Milli-Q water produced by water devices (Direct 3–UV and Integral 3, Merck KGaA, Darmstadt, Germany) in different laboratories increased five times the initial values since the end of the last fiscal year. The initial low blank values suddenly returned during the November–March period of this fiscal year, but the high blank values returned back again after this period. The amount was comparable to the concentrations found in the uptake experiments, thus, we needed to identify and fix the problem. Replacement of the resin cartridges and routine maintenance did not improve the blank values, therefore, a change in the quality of tap water was suspected. Indeed, analysis of tap water showed a high content of Hg. Further investigation for the source of this problem in detail would have required substantial amounts of time, budget, and effort. Therefore, we ceased further investigation and,

instead, decided to use commercially available pure water (Ultrapure water, Kanto Chemical Co. Inc., Tokyo, Japan). Owing to these problems, we have not yet successfully gained reproducible results, and the experiments are still ongoing.

(5) Source apportionment of mercury in the air and marine sediment based on stable isotopic composition (Fundamental research)

Satoshi Irei

(Department of Environment and Public Health)

For assessing the effectiveness of the Minamata Convention on Mercury, which regulates the use of Hg worldwide, stable Hg isotope measurements are expected to provide insight into the contributions of its sources in the natural environment. The aims of this project were to (1) apportion the sources of the total Hg found in the marine sediment of Yatsushiro Sea (run under the RS-19-11 project), (2) characterize stable isotopic composition of gaseous Hg emitted from emission sources, and (3) evaluate the fractionation in stable Hg isotopic compositions of atmospheric GEM under the conditions of simulated atmospheric oxidation.

In this fiscal year, we focused on the identification and solving of the instrumental problems with the multicollector-ICP-MS or MC-ICP-MS (Thermo Fisher Scientific K.K., Tokyo, Japan), which substantially delayed the progress of the project. Some of the problems observed, for example, were that the signals for thallium, an internal standard, suddenly became unstable, the signals for Hg isotopes periodically fluctuated suddenly, the signals for Hg and thallium suddenly became noisy, and the ICP was suddenly blown off. All these problems appeared and disappeared inconsistently. We devoted substantial amount of time and effort to identify the source of these problems and found that the unstable 200 V and 100 V electric power supply and argon gas pressure supplied from the center of the facility partly explained these problems. Specifically, it was found that the 200 V supply powering the MC-ICP-MS was divided at the

circuit breaker in the electrical room and supplied to the neighboring machine room. Owing to such an unusual connection, sudden voltage decrease and/or interference of different electric frequency, confirmed by an electricity quality checker, have likely occurred for this power supply and caused some of the problems mentioned above. Such instability was likely generated by the use of some devices downstream of the branched electric current. The signal noises disappeared after re-wiring the cable and installing a 200 V UPS. Similarly, the instability of the 100 V power supply, which operates a vacuum fan to maintain the optimum pressure inside the ICP housing, was also confirmed. This problem disappeared by installing a 100 V UPS. Moreover, argon gas for the MC-ICP-MS supplied from the next room was branched at the upstream and the branching flow was supplied to other laboratories. This branching supply caused a change in the pressure of argon gas, resulting in another occasional blown off of the ICP and the fluctuation in thallium signals introduced by the argon carrier gas. The problem was fixed by separating the two gas lines.

Meanwhile, we proceeded with the development of fast sampling and novel extraction technique of gaseous Hg for its stable isotopic composition, which is required for sub-project (2). Based on a conventional gold-coated sand trap (4 mm i.d. × 160 mm length), a new sampling trap, namely a big gold-coated sand trap (BAuT, 45 mm i.d. × 300 mm length), was designed for faster sampling. Sampling tests performed using double BAuTs connected in series showed 99.9% of collection efficiency for the front BAuT, which is sufficient for ambient sampling. The novel extraction method of GEM using 5 mL of 20–40% reversed aqua regia in a 2 L Tedler bag exhibited that 95% of standard gaseous Hg (SRM 8610) was captured as Hg²⁺ by 5 mL of 40% reversed aqua regia solution. The stable isotope measurements of the trapped Hg for six isotopes showed only 0.2‰ or less of offset from the reference values, which is acceptable for application in the analysis of authentic samples.

(6) Metagenomic study of microbial functional genes involved in mercury methylation in the coastal environments (Fundamental research)

Yuya Tada

(Department of Environment and Public Health)

In this study, we surveyed diss-T-Hg and MeHg concentrations as well as the functional genes involved in microbial Hg methylation (*hgcA* and *hgcB* and the reductive acetyl-CoA pathway) using a metagenomic approach in the Oyashio Current (OC) and Seto Inland Sea (SIS). The T-Hg concentrations were 0.09–0.28 ng/L and 0.10–0.16 ng/L in the OC and SIS, respectively. The MeHg concentration accounted for 1.0–69% and 0–1.0% of T-Hg in the OC and SIS, respectively. In the OC, the maximum abundance of *hgcA* and *hgcB* relative to the total predicted genes was observed in the deeper layers (approximately 450 m of depth) and accounted for 2.0 genes/million (M) genes and 2.3 seqs/M genes, respectively. As for the SIS, *hgcA* was relatively abundant in the bottom water of Osaka Bay, and accounted for 1.3 genes/M genes. The complete module related to the reductive acetyl-CoA pathway was observed at a station of the SIS. Phylogenetic analysis revealed that the *hgcA* sequences detected in the OC and SIS are closely related to the *Nitrospina*-like and *Desulfobacteraceae*-like lineages, respectively. These data suggest that distinct microbes can be involved in microbial Hg methylation in the OC and SIS.

(7) Study of food web structures of fish as top predators and bioaccumulation of mercury in Minamata Bay (Fundamental research)

Kenji Yoshino

(Department of Environment and Public Health)

The total Hg content (hereafter referred to as T-Hg) and carbon and nitrogen stable isotope ratios were measured in fish in Minamata Bay to investigate the Hg bioaccumulation pathway to the top predator of the food web of the bay. However, there is little information on subtidal macrobenthos, which play a significant role in

transferring the Hg bioconcentrated by primary producers to fish. This year, I analyzed winter and spring samples, including subtidal macrobenthos and fish, continuing the analysis of summer and autumn samples of the previous year. Isotope analysis showed that food web structures in the four seasons were overall fueled by microphytobenthos, and the contribution of pelagic phytoplankton was extremely low to that of both macrobenthos and fish. The benthic community was very poor in abundance and species richness, and small annelids such as lumbrinerid, sigalionid, and glycerid worms constituted the community all year round. Although fish preyed on organisms of many taxa, including fish (mainly Japanese anchovy), crabs, shrimps, copepods, and annelids, fish preying on benthic crustaceans tended to accumulate high T-Hg. T-Hg was high in both fish and macrobenthos strongly fueled by microphytobenthos. These results suggest that the main pathway of Hg bioaccumulation in fish in Minamata Bay is the benthic food chain linked to crustaceans fueled by microphytobenthos.

6. International Contribution Group

Environmental pollution due to Hg is spreading, and environmental pollution is particularly serious in developing countries. To decrease the risk of Hg pollution to the human body and environment, the Minamata Convention was adopted by 140 participating nations in October 2013, and came into effect in August 2017. Based on this background, international contribution groups in NIMD have planned mutual cooperation with foreign researchers for studies on Hg and the exchange of information via the NIMD forum. Moreover, based on the needs of the developing countries facing Hg pollution, we use our experience, knowledge, and latest technology by obtaining the mutual cooperation of Japan International Cooperation Agency (JICA). In addition, we developed a new simple Hg analysis methodology that is focused on MeHg, and it is positioned in the Minamata Convention as a commitment to the MOYAI initiative. A summary of the studies and duties of the international contribution group in 2019 is reported.

[Research theme and summary]

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

Koichi Haraguchi

(Department of International Affairs and Research)

The Minamata Convention on Mercury was launched, and it draws attention to Hg exposure through fish consumption and gold mining. Our research project consists of two major parts: 1) the development of a simple method for the determination of Hg compounds; and 2) the development of reference materials to evaluate the accuracy of Hg analysis for human biomonitoring. These are a part of the MOYAI initiatives providing technical assistance to promote the effective implementation of the convention.

Guidelines for water intended for human consumption have been proposed by various countries and organizations.

The commonly used methods for the determination of Hg contamination in drinking water require several steps for extraction. One of the main problems in drinking water examination 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, and this method can extract and quantify inorganic Hg in drinking water. This method involves only a few steps: Hg extraction with gold-coated silica and its separation. The method was validated using the spiked test analyzing various drinking water samples (commercial bottled water, spring water, river water, and tap water). The performance of the method suggested that sub-ng amounts of inorganic Hg present in a few milliliters of drinking water samples can be measured. This new method, therefore, represents a simplified tool for monitoring Hg in drinking water. Furthermore, the method requires few reagents, making it particularly attractive for the examination of drinking water in developing countries and remote sites.

A certified reference material (CRM), NIMD-01, was developed for the analysis of Hg speciation in human hair. Unlike other hair CRMs, this minimum quantity is small enough to be adequately homogeneous for the current standard analytical techniques. We also developed two candidate human urine reference materials (NIMD-02 and 3), in collaboration with multiple laboratories, and a standard operating procedure to prepare the CRM for testing the levels of total Hg and Cd. NIMD-02 and 3 will be certified and distributed in 2020.

- (2) Assessment of methylmercury exposure in Vietnam (Fundamental research)

Megumi Yamamoto

(Department of Environment and Public Health)

For risk management of fetuses, which are sensitive to MeHg exposure, assessing MeHg exposure via fish intake in pregnant women is an important public health issue

worldwide and is particularly important in countries and regions with high seafood consumption. Despite the increased fish consumption in Vietnam in recent years, the assessment system for MeHg exposure in pregnant women through fish consumption has hardly been established.

In this fiscal year, for a research project on prenatal exposure to MeHg in Vietnam, we negotiated with Bach Mai Hospital for biological sample collection and concluded a joint research contract. In addition, we have set up a biological sample collection system (training for sample collection personnel and sample processing personnel, dietary survey, compilation/translation of food frequency questionnaire, and construction of a sample transportation/transportation system) with Vietnamese counterpart organizations (Hanoi University of Public Health, National Institute of Occupational and Environmental Health, and National Hospital of Dermatology and Venereology). Samples are currently being collected at Bach Mai Hospital.

In addition, as a collaborative research project with the National Institute of Occupational and Environmental Health, we are preparing a research proposal for a preliminary study on the presence or absence of Hg pollution in the gold mining area of northern Vietnam.

[Activity theme and summary]

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

Masatake Fujimura

(Department of Basic Medical Science)

We performed Hg analysis of hair samples and assessed the survey information including gender, age, occupation and residence in areas of the Philippine (Batangas area) associated with Hg pollution (48 samples). Hg hair levels data indicated that Hg pollution was very low in this area. This year, we presented our findings of previous research at a conference.

- (2) Cooperation of research in the international organization

Akito Matsuyama
(Department of International Affairs and Research)
(WHO Collaboration Center)

NIMD researchers (twelve persons) travelled abroad to countries such as Poland, Vietnam, and Indonesia. The purpose was to participate in the International Conference on Mercury as a Global Pollutant (ICMGP steering committee) and survey on Hg pollution or Hg exposure in developing countries. Furthermore, researchers belonging to universities in Indonesia were invited, and the analytical method for Hg in biological samples was shared. Then, two researchers from the USA and Canada were invited in September 2019, to present their research at the NIMD forum. Furthermore, to study Hg methylation in the Minamata Bay sediment, a researcher from the USA was invited in November 2019. In addition, visits and training were conducted actively. Some examples are a training course on Hg pollution and the history of MD to JICA and Tsukuba University or the multi-media course on Hg monitoring capacity improvement related to the Minamata Convention. Furthermore, not only visits and training but also various lectures on the issue of Hg were delivered. Thus, we accepted, on a total of 8 occasions, 109 trainees from 27 countries.

A researcher from NIMD was delegated to Korea to participate in the first meeting of the Western Pacific Regional Network of WHO Collaboration Centers for Environmental & Occupational Health related to WHO work. Our activities as a WHO collaboration center was introduced in the meeting of the Society for Environmental Epidemiology and the International Society of Exposure Science -Asia Chapter in October, 2019 in Korea. Finally, to investigate Hg pollution in a factory that had a fire in Hanoi, Vietnam, an NIMD researcher was delegated on the request of the Vietnamese government from October 10, 2019 to October 16, 2019.

- (3) NIMD Forum and International Workshop

Akito Matsuyama
(Department of International Affairs and Research)

The NIMD Forum 2019 was held at the Education and Cultural Center of Kyoto on September 19, 2019. The NIMD Forum 2019 was held as a special session in the 18th International Conference of the Pacific Basin Consortium for Environment and Health. The theme of this forum was “Recent Progress on Toxicology and Environmental Health of Mercury.” To present and discuss in this forum, two researchers from the USA and Canada were invited and four researchers from NIMD (one was a post-doctoral fellow in NIMD) participated. Meaningful discussions were conducted by the six researchers focusing on the human health impact owing to Hg exposure from the view of Hg toxicology, environmental health, and medical science.

7. Publications and Scientific meetings

[International Journals]

Matsuyama A, Yano S, Matsunoshita K, Kindaichi M, Tada A, Akagi H: The spatial distribution of total mercury in sediments in the Yatsushiro Sea, Japan. *Mar. Pollut. Bullet.*, 2019; 49: 110539.

Usuki F*, Fujimura M*, Nakamura A, Nakano J, Okita M, Higuchi I: Local vibration stimuli induce mechanical stress-induced factors and facilitate recovery from immobilization-induced skeletal muscle atrophy in rats. *Front. Physiol.*, 2019; 10: 759. *Co-first author.

Sakamoto M, Itai T, Marumoto K, Marumoto M, Kodamatani H, Takashi Tomiyasu, Nagasaka H, Mori K, Alexandre J.Poulain, José L, Doming, Milena Horvat, Matsuyama A: Mercury speciation in preserved historical sludge: Potential risk from sludge contained within reclaimed land of Minamata Bay, Japan. *Environ. Res.*, 2020; 180 :108668.

Nakamura M, Bekki M, Miura Y, Itatani M, Jie LX: Cerebellar Transcranial Magnetic Stimulation Improves Ataxia in Minamata Disease. *Case Rep. Neurol.*, 2019; 11: 167–172.

Unoki T, Akiyama M, Kumagai Y: Nrf2 activation and its coordination with the protective defense systems in response to electrophilic stress. *Int. J. Mol. Sci.*, 2020; 21: 545.

Akiyama M*, Unoki T*, Shinkai Y*, Ishii I, Ida T, Akaike T, Yamamoto M, Kumagai Y: Environmental electrophile-mediated toxicity in mice lacking Nrf2, CSE, or both. *Environ. Health Perspect.*, 2019; 127: 67002. *Co-first author.

Yoshino K, Kimura K, Fujii N, Orita R, Katano T, Ito Y, Yamada K: Biophysical interactions on the succession of subtidal benthic community in the inner part of Ariake Sea. *Proceedings of CWMD International Conference 2019*, Kumamoto University: 496-504.

Marumoto K, Suzuki N, Shibata Y, Takeuchi A, Takami A, Fukuzaki N, Kawamoto K, Mizohata A, Kato S, Yamamoto T, Chen J, Hattori, Nagasaka H, Saito M: Long-Term Observation of Atmospheric Speciated Mercury during 2007–2018 at Cape Hedo, Okinawa, Japan. *Atmosphere*, 2019, 10, 362.

Tada Y, Marumoto K: Uptake of methylmercury by marine microalgae and its bioaccumulation in them. *J. Oceanogr.*, 2020; 76: 63-70.

Kataoka C, Sugiyama T, Kitagawa H, Takeshima A, Kagami Y, Tatsuta H, Kashiwada S: Temperature-dependent toxicity of acetaminophen in Japanese medaka larvae. *Environ. Pollut.*, 2019; 254: 113092.

Zhou C, Tada A, Yano S, Matsuyama A: Mercury dynamic simulation of Minamata Bay by using a three-dimensional numerical model, *Ocean Dynam.*, 2019; 69: 175–186.

Takahashi T, Kim MS, Iwai-Shimada M, Fujimura M, Toyama T, Fujiwara Y, Naganuma A, Hwang GW: Induction of chemokine CCL3 by NF- κ B reduces methylmercury toxicity in C17.2 mouse neural stem cells. *Environ. Toxicol. Pharmacol.*, 2019; 71: 103216.

Griebel G, Stemmelin J, Lopez-Grancha M, Boulay D,

Boquet G, Slowinski F, Pichat P, Beeské S, Tanaka S, Mori A, Fujimura M, Eguchi J: The selective GSK3 inhibitor, SAR502250, displays neuroprotective activity and attenuates behavioral impairments in models of neuropsychiatric symptoms of Alzheimer's disease in rodents. *Sci. Rep.*, 2019; 9: 18045.

Kumagai Y, Akiyama M, Unoki T: Adaptive responses to electrophilic stress and reactive sulfur species as their regulator molecules. *Toxicol. Res.*, 2019; 35: 303-310.

David J, Muniroh M, Nandakumar A, Tsuji M, Koriyama C, Yamamoto M: Inorganic mercury-induced MIP-2 expression is suppressed by N-acetyl-L-cysteine in RAW264.7 macrophages. *Biomed. Rep.*, 2020; 12: 39-45.

Drennan R, Wlkund H, Rouse GW, Georgieva MN, Wu X, Kobayashi G, Yoshino K, Glover AG. Taxonomy and phylogeny of mud owls (Annelida: Sternaspidae), including a new synonymy and new records from the Southern Ocean, Northeast Atlantic Ocean and Pacific Ocean: challenges in morphological delimitation. *Marine Biodiversity* 2019; 49: 2659-2697.

Kobayashi S, Yamada K, Yoshino K, Henmi Y: Longitudinal distribution patterns of brachyuran crab (Crustacea, Decapoda) community along tidal rivers flowing into the inner area of Ariake Sea. *Proceedings of CWMD International Conference 2019*, Kumamoto University: 523-532.

[International meetings]

Fujimura M, Usuki F: Site-specific neural hyperactivity via the activation of MAPK and PKA/CREB pathways triggers neuronal degeneration in methylmercury-intoxicated mice. 35th Korean Society of Toxicology, Jeju, 2019. 11.(Invited lecture)

Fujimura M, Usuki F, Nakamura A: Fasudil, a ROCK

inhibitor, recovers methylmercury-induced axonal degeneration by changing microglial phenotype in rats. 14th International Conference on Mercury as a Global Pollutant, Krakow, 2019. 9.

Fujimura M, Usuki F, Unoki T: Thiol antioxidant capacity in blood is a proper biomarker for individual sensitivity for methylmercury neurotoxicity in rats. 59th Society of Toxicology annual meeting, Anaheim, 2020.3.

Sakamoto M, Itai T, Marumoto K, Matsuyama A: Mercury speciation of preserved historical sludge to estimate risks from sludge entrapped under the reclaimed area of Minamata Bay, Japan. *EUROTOX 2019*, Helshinki, 2019. 9.

Sakamoto M: Brain methylmercury uptake in fetal, neonate, weanling, and adult rats as risks at different life stages. 18th International Conference of the Pacific Basin Consortium for Environment and Health, Kyoto, 2019. 9.

Haraguchi K, Sakamoto M, Matsuyama A, Yamamoto M, Nagasaka H, Yoshinaga J: Development of human urine and hair reference materials for human biomonitoring of mercury. 14th ICMGP, Krakow, 2019.9.

Nakamura M, Hachiya N, Tatsuta N, Nakai K, Murata K, Koriyama C, Yamamoto M, Sakamoto M: Cross sectional study on MeHg exposure and health survey in Kinan region, Japan. 18th Pacific Basin Consortium International Conference. Kyoto. 2019.9.

Nagano M, Fujimura M: Nondigestible oligosaccharide decrease tissue concentration of mercury after methylmercury exposure in mice. 14th International Conference on Mercury as a Global Pollutant, Krakow, 2019.9.

Unoki T, Akiyama M, Shinkai Y, Kumagai Y: Nrf2 and CSE as critical molecules in parallel pathways for repression of xenobiotic-mediated electrophilic stress in mice. 59th Society of Toxicology annual meeting, Anaheim, 2020.3.

Yamamoto M, Hien TTD, Agusa T, Ishibashi Y, Sakamoto M, Koiyama C: Exposure assessment of methylmercury among Vietnamese in Hanoi. 18th International Conference of the Pacific Basin Consortium for Environment and Health, Kyoto, 2019.9.

Yamamoto M, Hien TTD, Agusa T, Sakamoto M, Koriyama C: Assessment of exposure to methylmercury among Vietnamese people in Hanoi. Joint Meeting of Asian Chapters of International Society for Environmental Epidemiology and the International Society of Exposure Science 2019, Daegu, 2019.10.

Yamamoto M, Yanagisawa R, Mogi M, Shudo M, Nakamura M, Kashiwagi H, Kudo M, Sakai A, Shuto S: Comparison of Toxicokinetics of Methylmercury in Diabetic KK-Ay Mice and C57BL/6 Mice. 59th Society of Toxicology annual meeting, Anaheim, 2020.3.

Marumoto K, Takeuchi A, Tada Y, Obata H: Vertical profile on total, methyl and gaseous mercury in dissolved phase of the seawaters collected in a subarctic region of the North Pacific Ocean. 14th International Conference on Mercury as a Global Pollutant, Krakow, 2019.9.

Marumoto K, Noda K, Paulo Góes J, De Oliveira LM, De Jesus IM, Marumoto M, Akagi H: Availability of a simple and small mercury measuring device, QCM-Hg for mercury measurement in an artisanal and small gold mining area and gold dealing shop. 14th International Conference on Mercury as a Global Pollutant, Krakow, 2019.9.

Irei, S: A novel method for stable isotope measurement of gaseous elemental mercury. International Conference on Chemistry and Nanosciences, Rome, 2020. 3.

Izumo K, Yamamoto M, Komatsu M, Koriyama C, Horiuchi M, Sakamoto M: Possible estimation of methylmercury concentration in the muscle by total mercury concentration in the gill in Marbled rockfish. 18th International Conference of the Pacific Basin Consortium for Environment and Health, Kyoto, 2019.9.

Kataoka C, Kato Y, Tomiyama H, Kagami Y, Higashibata H, Umehara M, and Kashiwada S: Dose disruption of gut microbiota by silver nanocolloid cause bacterial infection? A case study using medaka model. The 18th Pacific Basin Consortium International Conference, Kyoto, 2019.9.

Batista HCS, Ignacio ARA, Fujimura M, Lazaro WL: Evaluating risk to human health: mercury concentration in hair enced bake the fish intake in Mato Grosso, Brazil. 14th International Conference on Mercury as a Global Pollutant, (2019). Krakow, 2019.9.

Bekki M, Nakamura M, Miura Y, Itatani M, Jie LX: Repetitive transcranial magnetic stimulation method (rTMS) for patients with chronic Minamata disease: two case reports. 13th International Society of Physical and Rehabilitation Medicine World Congress, Kobe. 2019.6.

Hirai T, Abe O, Nakamura M, Azuma M, Kadota Y, Hattori Y: Human Brain Structural Change Related to Chronic Methylmercury Poisoning. 25th Annual Meeting of the Organization for Human Brain Mapping, Roma. 2019.6.

Inui S, Nomura Y, Nakamura M, Hirai T, Abe O: Application of Machine Learning to multiparametric MRI in Organic Mercury Poisoning (Minamata disease). American Society of Functional Neuroradiology 13th Annual Meeting, San Francisco. 2019.11.

Muniroh M, Gumay AR, Indraswari DA, Bakhtiar Y, Hardian H, Bakri S, Maharani N, Karlowee V, Koriyama C, Yamamoto M: Activation of MIP-2 and MCP-5 expression in methylmercury-exposed mice and their suppression by N-Acetyl-L-Cysteine. 18th International Conference of the Pacific Basin Consortium for Environment and Health, Kyoto, 2019.9.

Noda K, Aizawa H, Marumoto K: Characteristics of a simple detection logging system for mercury using quartz crystal microbalance. 18th International Symposium on Olfaction and Electronics Nose, Fukuoka, 2019.5.