NIMD Annual report
2023
（April 2023 to March 2024）

National Institute for Minamata Disease
Ministry of the Environment
Japan

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Report on Research and Other Activities in the Fiscal Year 2023

1. Pathomechanism Group

Aim of the study group is to understand the molecular mechanisms underlying methylmercury (MeHg) toxicity in humans. The 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. Techniques from the fields of biochemistry, molecular biology, and pathology are used in cell cultures and animal models in order to achieve the above objectives.

The group conducted the following research during the fiscal year 2023.

[Research theme and summary]

1. Fundamental research on neurotoxic mechanism of methylmercury and its prevention and treatment (Project research)

Masatake Fujimura

(Department of Basic Medical Sciences)

(1) Research on selective cytotoxicity of MeHg

We found that p44/42 MAPK, a downstream pathway of BDNF specifically expressed in hippocampal neurons, is involved in the anti-neuronal cell death effect. One paper was accepted by an international journal as the first author. Furthermore, we initiated experiments on an exercise-loaded model and confirmed that exercise load increases BDNF in the brain.

(2) Research on individual susceptibility to MeHg neurotoxicity and related biomarkers

As research for biomarker development, we investigated measurement methods using LC-Mass, etc. for discriminating polythiolation of proteins in blood and analyzed proteins in target tissues with measurement of miRNAs in extracellular vesicles. As a result, candidate miRNAs for biomarkers were discovered. In addition, we presented one abstract as a representative researcher at a conference.

(3) Research on the onset of neuropathic pain caused by MeHg and its drug effect.

As a representative researcher, we presented an abstract on the therapeutic effects of gabapentin on neuropathic pain in a rat model of MeHg intoxication and submitted it to an international academic journal.

(4) Joint research with external research institutes

We conducted joint research with external institutions on MeHg toxicity (endoplasmic reticulum stress, epigenome), which is not conducted at our research center, and presented three abstracts on cellular damage in the olfactory nerve caused by MeHg exposure and the involvement of the CHOP pathway in endoplasmic reticulum stress. Two papers were accepted for publication in international journals. In addition, the “Department of Biometallurgy” was established as an extramural course in the Graduate School of Medical, Dental and Pharmaceutical Sciences, Okayama University, and one student was accepted.

2. Study on reducing the health risk of methylmercury by food ingredients

(Fundamental research)

Masaaki Nagano

(Department of Basic Medical Science)

This study aims to reduce the health risks of MeHg by utilizing the function of food ingredients. So far, we have demonstrated that daily intake of wheat bran (bran) or fructooligosaccharides significantly reduces tissue mercury concentration in mice exposed to MeHg.

In FY 2023, we re-examined the combined effects of fructooligosaccharides and bran on tissue mercury concentrations. The tissue mercury concentration was significantly lower in both the fructooligosaccharide and bran groups than that in the control group; however, no additive effect was observed under their combined administration.

3. Research on the methylmercury-induced alteration of protein function and its protective factors
(Fundamental research)

Takamitsu Unoki
(Department of Basic Medical Sciences)

Redox balance is an essential concept for examining the mechanisms underlying the toxic effects of MeHg on the nervous system. In our previous study, we elucidated the mechanism of redox homeostasis maintenance by certain reactive sulfur species, which are low-molecular-weight molecules with releasable sulfane sulfur. Sulfane sulfur is transferred to protein thiols to form the respective persulfide/polysulfide species (i.e., protein supersulfidation). MeHg can covalently modify protein thiols, causing their dysfunction and exerting deleterious effects. Our study aimed to assess the physiological role of the sulfane sulfur-mediated modification of protein thiols in the nervous system during MeHg exposure. In FY 2023, we isolated supersulfidated proteins from primary cultured rat neurons exposed to MeHg using an alkylating agent-based pulldown method. Subsequent DIA proteomics and biochemical analyses identified several proteins that showed reduced supersulfidation after MeHg exposure. This indicates that MeHg may disrupt the redox regulation of specific proteins, thereby altering their functions.

4. Development of sensor for methylmercury toxicity and research on the mechanism of methylmercury neurotoxicity
(Fundamental research)

Akio Sumioka
(Department of Basic Science)

Notably, MeHg induces oxidative stress in the brain, leading to neurodegeneration. However, the mechanisms underlying the cell specificity and age dependency of MeHg toxicity remain unclear. Therefore, monitoring at an appropriate time is necessary to investigate the neurotoxicity associated with translocation and exposure to MeHg in the brain. Therefore, we developed a new sensor vector, Krab-U/Luc, to detect MeHg toxicity and studied its molecular mechanisms.

To elucidate the mechanism of MeHg toxicity, in addition to the traditional balance model, we proposed a target-specific model that involves specific interactions among electrophiles, nucleophiles, and proteins. Based on this hypothesis, expression profiles of target proteins in each cell may contribute to the cell-specific toxicity of MeHg, and we investigated to verify this model.

(1) Research on the underlying mechanisms of MeHg toxicity in neuronal cells

In the previous year, the specificity of MeHg and other electrophiles for target proteins was confirmed. In FY 2023, we have investigated to determine the specificity of nucleophiles for MeHg toxicity. We examined the inhibitory effects of ebselen and trolox, which have been reported to inhibit MeHg toxicity, in primary cultured cerebellar granule, Cos-7, HEK293, SH-SY5Y, and N2a cells. The results identified three patterns: in cerebellar granule cells, two types of cell death were identified, one sensitive to ebselen and one not; in Cos-7 cells, cell death was not sensitive to ebselen; and in HEK293, SH-SY5Y, and N2a cells, cell death was

sensitive to ebselen, demonstrating the cell specificity of the inhibitors.

(2) Development of sensor vectors for MeHg toxicity

In the previous year, we developed and improved an MeHg toxicity sensor and evaluated its effectiveness. In FY 2023, before generating sensor mice, we established a cell line that constitutively expressed the sensor vector. We obtained a clone that was responsive to MeHg, and no issues such as toxicity due to the sensor vector itself were observed. Furthermore, we are currently generating sensor mice with the reporter component pCT-Luc of the sensor vector and have produced four F0 mice carrying pCT-Luc.

Next, we validated the target-specific model using various toxicity sensors and inhibitors. We used the endoplasmic reticulum stress sensor ERAI-Luc, electrophilic stress sensor Nrf2-Luc/Keap1, and MeHg toxicity sensor Luc-491Sec. Our results showed that ERAI-Luc exhibited cell specificity to MeHg toxicity and Nrf2-Luc/Keap1 was inhibited by ebselen; however, no ebselen sensitivity was observed in Luc-491Sec. Combined with the results from (1), these results suggest that the MeHg-induced cell death observed in cerebellar granule and Cos-7 cells may be due to selenoproteins.

(3) Analysis of the underlying mechanisms of selenoprotein toxicity

The results of (1-2), indicate that selenoproteins are one of the targets of MeHg toxicity. However, the mechanisms underlying selenoprotein toxicity, such as loss-of-function or gain-of-function, remain unclear. Therefore, we established experimental platforms for assessing selenoprotein toxicity. The first system utilizes H1-drived short hairpin RNA and luciferase as reporters (shRNA-Luc). We confirmed a reduction in MeHg toxicity by suppressing selenoprotein DIO3 using RNA interference. The second method is a long-term assessment selenoprotein toxicity using transposon-mediated gene manipulation, and to date, we have identified that selenoprotein GPx2 exhibits particularly strong toxicity.

In FY 2023, We have addressed the cell specificity of MeHg, validated the target-specific model and the specificity of inhibitors, and successfully classified MeHg toxicity using inhibitors. Furthermore, from the inhibitor sensitivity experiments using the sensor vector, we focused on selenoproteins as targets. Consequently, significant progress has been made in establishing experimental platforms to investigate the underlying mechanisms of selenoprotein toxicity.

Group Research

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

In addition, effective therapies are needed for intractable chronic pain, tremors, ataxia, and spasticity as these affect the quality of life (QOL) of patients with MD. We conducted a clinical study on repetitive transcranial magnetic stimulation therapy for neuropathic pain.

We conducted a multidirectional analysis of the issues associated with the victims of MeHg pollution to create a detailed plan for their regional revitalization.

The research conducted by our group during FY 2023 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 establish an objective evaluation method for MD pathophysiology, we used MEG and MRI to compare the findings of certified patients with MD and cases in control areas.

This year, we examined the accuracy of combined MEG and MRI data of 34 certified patients with MD and 290 control individuals with both MEG and 3-tesla MRI data. Brain Anatomical Analysis using Diffeomorphic deformation software has been useful in the evaluation of cerebellar atrophy. Next, we examined the performance of MEG and MRI in discriminating certified patients with MD from control individuals. Machine learning with regularized (Ridge and Lasso) logistic regression models, random forests, and support vector machines was performed, and all areas under the curve values were >0.8, indicating good discriminative ability.

Resting MEG has been recently suggested to be an indicator of neuropathic pain. Therefore, we investigated whether resting MEG could be used as an indicator of treatment efficacy for neuropathic pain. This year, we compared the results of resting MEG in the sensory cortex of healthy individuals with those of patients with pain, suggesting that resting MEG may be an indicator of neuropathic pain.

As fetal and childhood exposure to MeHg is considered to lead to its most severe effects, we examined the association between neurodevelopmental indicators and prenatal and postnatal MeHg exposure. The results showed that the latencies of the auditory and visual pathways were significantly prolonged in boys with increased prenatal exposure to MeHg. This suggests that male fetuses may be more susceptible to the effects of MeHg exposure.

[2] Area studies about the regional revitalization in Minamata City and neighboring cities

(Fundamental research)

Rie Harada

(Department of International Affairs and Research).

This research is being conducted from a multifaceted perspective: (i) understanding the actual situation of MD damage, (ii) uncovering local welfare issues, (iii) conducting local studies and regional research as know-how for regional revitalization to solve problems, and

(iv) examining regional development through art as a pioneering and experimental approach.

This year, regarding theme (i), a case study on abnormal pregnancies in areas affected by MD will be submitted. In addition, a study on the life histories of women of the fetal generation with MD has been submitted for publication. Regarding (ii), a statistical analysis of data from the Community Welfare Needs Survey was conducted. Participatory observational fieldwork for (iii) is ongoing. A study on (iv), "La danse du détour: A collaborative arts performance with people affected by Minamata disease" has been published in the Journal of Applied Arts & Health.

Activities

To examine the effectiveness of home care support for patients with MD and their families, we initially 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 completing these projects, we continued to support the welfare activities in Minamata, Izumi, and Tsunagi.

In addition, we organized conventional rehabilitation activities, including daycare facilities for fetal-type patients with MD, along with annual workshops on rehabilitation and assistance techniques. We also examined the usefulness of lumbar nerve root magnetic stimulation for spasticity, belt electrode skeletal muscle electrical stimulation for maintaining and increasing lower-limb muscle strength, and neuromuscular electrical stimulation (NMES) for dysphagia and dysarthria in patients with MD.

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

Based on the comprehensive cooperation agreement with Minamata City, we also supported the survey related to regional revitalization, including "Moyainaoshi" in collaboration with Minamata City.

To obtain basic data that contribute to the improvement of activities of daily living (ADL) in patients with chronic MD, we conducted a survey to clarify ADL changes in patients with chronic MD and the differences in sequential ADL decline depending on the clinical entity of chronic MD.

The following section outlines the clinical and social practices conducted by this group in FY 2023.

[Activity theme and summary]

[3] Community development project for home care support, including health care practice

Masaaki Nakamura
(Department of Clinical Medicine)

To examine methods to support aging patients with MD and their families, including rehabilitation to improve ADL, the following projects were implemented: "Home support model study, including care prevention" from FY 2006 to FY 2009, "Community development project for home care support, including healthcare practice" from FY 2009 to FY 2012, and "Project for Promotion of Community Welfare for Support of Minamata Disease Victims" from FY 2012 to FY 2013. In addition, for one year since FY 2012, we have implemented the "Community Welfare Promotion Business to support MD victims." On the basis of these achievements, further activities have been undertaken to help care prevention projects take root in areas with MD occurrence.

In Minamata City, we supported the community center activities of the Minamata City Council of Social Welfare by providing "brain training through handicrafts." In Izumi City, we supported the "Fureai

Iki-iki Salon" activities of the Izumi City Council of Social Welfare, Takaono and Noda Branch Offices. In Tsunaki Town, we held handicraft classes at the "Itte-miyu-kai" project for preventing bone fractures from falls and at the "Tatshaka Juku" program conducted by the Tsunaki Town Office. To provide better handicrafts, we surveyed the participants in each district and developed a handicraft program that resulted in higher participant satisfaction. In addition, the use of a dialogue support speaker (comuoon) to facilitate participation by people with hearing loss was found to improve the ease of hearing for people with hearing loss.

Measures should be taken against frailty and sarcopenia to extend the healthy life expectancy. In cooperation with the Minamata City Council of Social Welfare, a project to prevent frailty and sarcopenia in Minamata City began this fiscal year to collect basic data and conduct exercise classes.

To encourage local residents to participate in the care prevention program, we actively conducted publicity activities for local living through the website and Facebook page (activity reports of handicraft classes held each time and a notice for the next class) of the National Institute for Minamata Disease.

[4] Rehabilitation programs for patients with Minamata disease and dissemination of information on care and rehabilitation

Atsushi Nakamura
(Department of Clinical Medicine)

We continued to provide rehabilitation to improve the QOL of patients with MD. Rehabilitation focusing mainly on gait disturbance was continuously implemented for affected individuals by combining equipment such as HAL medical single-joint type, peripheral magnetic stimulation, belt electrode-type skeletal muscle electrical stimulation, and non-powered walking aids. Furthermore, development of a neuromuscular electrical stimulation intervention is underway for the rehabilitation of swallowing dysfunction in patients with MD, which has recently become a contributing factor to a decline in ADL. This year, we summarized the results of a study conducted in patients with MD and mild dysphagia and presented a report at an academic conference. Because the results of rehabilitation for dysphagia vary depending on the degree of dysfunction, we plan to increase the number of cases and investigate the optimal conditions for neuromuscular electrical stimulation therapy for dysphagia in fetal-type MD.

Face-to-face workshops were held to promote rehabilitation and welfare techniques in the MD outbreak areas. The workshop was recorded and made available on the website of the center for those who were unable to attend the workshop and for those who attended the workshop to learn afterwards.

As efforts for information dissemination to local residents and care prevention, we visited the Orange hall, which was established as part of the "Moyai-naoshi project," twice a month. At the Orange hall, we conducted physical and cognitive function evaluations, ADL evaluations, and health exercise classes for local older individuals, including patients with MD.

[5] Appropriate management and information provision of pathological specimens associated with Minamata disease

Masumi Marumoto
(Department of Basic Medical Sciences).

Unlike other diseases, autopsy histopathological specimens of MD are extremely valuable because these cannot be obtained again. However, these specimen are difficult to store permanently because they fade over time. We are continuously digitizing the histopathological specimens of MD cases with the aim of permanently preserving them. We also intend to create a website that utilizes the digitized histopathological specimens as educational materials for pathology students and researchers. Simultaneously, a pamphlet explaining the pathology to the general

public and children will be prepared. In addition to histopathological specimens, the center contains a large number of valuable pathology-related samples, and we will continue to organize and preserve these samples.

This year, we enhanced the Japanese version of our website and created an English version. In addition, we created a pamphlet for the public to deepen their understanding of the central nervous system (transverse temporal gyrus) and completed a series on the mechanisms of the brain and MD.

[6] Needs survey on the agreement for comprehensive cooperation with Minamata City

Rie Harada

(Department of International Affairs and Research).

A study group on the utilization of vacant houses etc. in Minamata City and support measures for newcomers was held under the theme "On Innovations to Increase the Number of Newcomers - Utilization and Design of the Web," and four visits to vacant houses and renovation cases were conducted.

As part of the support for newcomers, a pilot migration community "Tomattekanne" was established with the help of ideas etc. from citizens, and the "Tomattekanne" website was created.

As part of the utilization of vacant houses, the self-renovation of properties matched by the study group has progressed, and these properties were being used as meeting places for newcomers and visitors.

More advanced cases in different regions and initiatives in the city will be followed up in the next year.

[7] Temporal trend analysis of activities of daily living (ADL) in patients with Minamata Disease

Sawako Horai

(Department of Environment and Health)

This study aims to clarify the changes in ADL in patients with chronic Minamata disease patients (MD) and the differences in ADL decline over time depending on the disease type.

Evaluation methods for ADL, Instrumental Ability to perform ADL (IADL), N-type ADL rating scale for older individuals (N-ADL), and N-type Mental status scale for older individuals (NM Scale) are being used. A questionnaire survey regarding the symptoms was conducted. To characterize the actual ADLs of patients with MD (nine patients), 33 residents of Kagoshima City and Makurazaki City, Kagoshima Prefecture, were used as controls and compared with patients with MD. Our results showed that the N-ADL score (50-point scale) was significantly lower in patients with MD ($p < 0.001$). Furthermore, patients with MD showed a marked decline, especially in walking and sitting up. These results suggest that patients with MD are more likely to have difficulty walking. In addition, in the evaluation of the residential area, all the control individuals were normal, whereas three of the patients with MD were in the "nearby." This finding suggests that walking difficulties result in smaller residential areas. In the assessment of I-ADL, no significant differences were observed between the control and patients with MD groups in men; however, a significant difference was observed in women with MD ($p < 0.01$). This could be attributed to the fact that only women included the items "moving around" and "shopping" and both items were significantly lower in the patient group than in the control group ($p < 0.001$). Taken together, our results suggest that patients with MD are prone to walking difficulties, which forced them to restrict their living space and hindered their mobility and shopping. Furthermore, the "frequency of pain feeling" was significantly higher in the patients with MD group than in the control group ($p < 0.01$). In the next year, our goal is to evaluate frail sarcopenia and conducting the Hasegawa Cognitive Function Test.

3. Risk Assessment Group

High-risk groups for MeHg exposure include populations exposed to high concentrations of Hg and those with a high sensitivity to Hg. The risk assessment group will comprehensively assess human exposure to Hg and its effects on health caused by environmental pollution. We will conduct both epidemiological and experimental studies to assess various confounding factors, such as selenium (Se), to elucidate the exposure, risk assessment, and health effects of MeHg, especially in populations exposed to high concentrations of MeHg and vulnerable populations, such as fetuses, children, and those with diseases.

[Research themes and summary]

[1] Studies on coexisting of mercury and selenium in Minamata disease and effects of methylmercury to fetus and breast-feeding infants
(Fundamental research)

Mineshi Sakamoto
(Special advisor to the Director-General)

Protective role of Se in MeHg toxicity: Insights from MD reanalysis and a rat model

Exposure to MeHg, a neurotoxicant, from fish consumption poses global health risks. The MD outbreak due to MeHg intoxication in Japan in the 1950s revealed elevated Se levels in the liver and kidneys of patients, which were initially mistaken for Se intoxication. However, the mechanism of action of Se in MeHg toxicity remains unclear. Therefore, we reassessed MD-related samples to elucidate the role of Se in MeHg toxicity and its prevention. Patients with MD were co-exposed to MeHg and Se via contaminated fish and shellfish, resulting in organ-specific Se accumulation and a notably elevated Hg/Se molar ratio in the brain. Rat experiments with MeHg and selenomethionine demonstrated that MeHg increased the liver and kidney Se levels and notably elevated the brain Hg/Se molar ratio. Furthermore, MeHg and selenomethionine administration at a Hg/Se molar ratio of 3.15, similar to that observed in Minamata Bay shellfish, did not protect against MeHg toxicity. However, their administration at a ratio of 1 significantly reduced brain Hg/Se, protecting rats despite brain Hg levels reaching the onset threshold. Therefore, the Hg/Se molar ratio in seafood may be crucial for preventing seafood-derived MeHg toxicity in humans. (JSPS19K12353)

[2] Enhancement of the exposure assessment system for groups at high risk of methylmercury exposure
(Fundamental Research)

Megumi Yamamoto
(Department of International Affairs and Research).

(1) After completing the experiments (feeding, mating, dissection, tissue processing, and Hg analysis) using a diet-induced obesity mouse model in females fed a high-fat diet (HFD), the collected samples (blood and tissue) were analyzed for total Hg. Furthermore, MeHg transfer was evaluated using K_p values (total Hg in each tissue of the mother or fetus/total Hg in the maternal plasma). Under the pathophysiology of mild abnormal glucose metabolism, maternal K_p values were Ctrl group≧HFD group in the brain, Ctrl group≒HFD group in the kidney, and Ctrl group＞HFD group in the liver. Fetal K_p values were Ctrl group≧HFD in the brain, Ctrl group＞HFD in the kidney, Ctrl group＞HFD in the liver, and Ctrl group≧HFD in the placenta. The statistical analysis and writing of the manuscript are in progress.

(2) A study on Hg in biological samples (hair, blood, placental tissue, and umbilical cord tissue) from mothers and infants with abnormal glucose metabolism (gestational diabetes mellitus and complicated diabetic pregnancy) is being conducted in collaboration with the University of Occupational and Environmental Health. This year, specimen collection was completed, and the

obtained samples were pretreated and analyzed for total Hg.

[3] Exposure assessment of mercury in developing countries and technology transfer
(Fundamental research)

Megumi Yamamoto
(Department of International Affairs and Research).

(1) To understand the actual situation of MeHg exposure among pregnant women in Vietnam, we conducted an exposure assessment among general pregnant women in Hanoi, Vietnam. The mean value of Hg in the mother's hair was 0.47 μg/g, the mean value of total Hg in fetal feces was 20.8 ng/g dw, and the correlation coefficient between hair and fecal Hg values was 0.46. In cases where cord blood and tissue were difficult to obtain, fecal feces were considered a possible indicator of prenatal exposure to MeHg. Total Hg and MeHg concentrations in fish and shellfish (nine species; n=5) were measured, with total Hg values ranging from 2 to 60 ng/g, MeHg values ranging from 1 to 56 ng/g, and MeHg/total Hg ranging from 91.2 to 98.4%. The fish and shellfish mainly consumed in Hanoi are freshwater fish with relatively low Hg levels. (2) A preliminary investigation of the Hg contamination associated with ASGM in the Jember region of Indonesia was conducted. We visited the study area and assessed the exposure of gold mining workers to Hg during their work. Masks (three workers and six survey staff members), hair (two workers), and nails (one worker) were collected and measured for total Hg.

(3) Transfer of Hg analysis technology to developing countries and a preliminary study on the actual situation concerning Hg pollution in Dodoma University, Tanzania. Graduate students from Tokyo Medical and Dental University (World Health Organization [WHO] Collaborating Center: JPN-73); Diponegoro University, Indonesia; Jember University, Indonesia; and Rajshahi University, Bangladesh.

[4] Relationship between the exposure level and dose-response of methylmercury and essential trace elements in mammals with high mercury levels
(Fundamental research)

Sawako Horai
(Department of Environment and Public Health)

To elucidate the biological defense mechanism against MeHg toxicity, we investigated (1) the transfer of Hg and essential trace elements between dams and fetuses, (2) concentration dependence of Hg and essential trace elements using the small Indian mongoose, which is a Hg-accumulating species, and (3) relationship between Se compounds (selenoproteins and Se-containing enzymes) and Hg concentrations in the blood of mongooses, cetaceans, and Taiji residents to characterize the dose-response relationships of Hg, Se, and other essential trace elements in MeHg-tolerant species.

Regarding (1), we reanalyzed data on maternal whole blood, fetal liver, cerebrum, and whole blood of 27 Okinawa small Indian mongoose mother-fetus pairs collected last year and measured trace elements, including Hg. In the fetal liver, cerebrum, and whole blood, Hg concentrations did not change significantly with increasing fetal body weight. In contrast, in the liver, O/total Hg (THg) (%) decreased significantly with increasing fetal body weight ($p < 0.05$), indicating that mineralization of organic Hg in the liver occurs from the fetal period. Next, we analyzed the relationship between the fetal blood/maternal blood (FB/MB) ratio of Hg, which is considered an indicator of maternal-to-fetal Hg exposure, and fetal organ concentrations, and found significant negative correlations in the liver ($p < 0.05$) and cerebrum ($p < 0.01$). The results suggest that a higher FB/MB ratio will decrease the Hg concentration in fetal organs. Next, to elucidate the cause of the variation in the FB/MB ratio, we analyzed the relationship between the FB/MB ratio and Hg concentrations in maternal and fetal whole blood, and found a significant negative correlation ($p <$

0.0001) between Hg concentrations in maternal whole blood and the FB/MB ratio. The slope between fetal whole-blood Hg concentration and the FB/MB ratio differed depending on the degree of maternal Hg exposure. These results suggest that lower maternal Hg exposure correlates with greater variation in the FB/MB ratio.

Regarding (2), Hg co-localized with Se mainly in the choroid plexus and lateral ventricular wall astrocytes; however, it may not be HgSe. The results of this study are summarized in Devabathini, P., Fischer, D. L., Chen, S., Pattammattel, A., Bury, G., Antipova, O., Huang, X., Yong S., Chu, Y., Horai, S., and Pushkar, Y. (2023): Wild high-resolution imaging of Hg/Se aggregates in the brain of a terrestrial species (small Indian mongoose)–insights into intracellular Hg detoxification, summarized and published in Environ. Chem. Lett.

Regarding (3), the urinary Se metabolites in small Indian mongooses and mice were compared. The urinary Se metabolite of mice was SeSugarB, and trimethylselenonium ion (TMSe) was detected, whereas the Se metabolites of mongooses included a low proportion of SeSugarB, and TMSe was hardly detected. In contrast, the ratio of SeSugarA to unknown substances was higher in mongooses than in mice. These results suggest that the physiological requirement for Se in mongooses was higher than that in mice, which was influenced by Hg exposure. Next year, we intend to analyze the relationship between body Hg levels and urinary Se metabolites.

[5] Evaluation of methylmercury-induced neurological symptoms and the association between selenium compounds and toxicity in common marmosets

(Fundamental research)
Chisato Kataoka
(Department of Environment and Public Health)

(1) Establishment of a quantitative evaluation system for neurobehavioral deficits in MeHg-exposed marmosets. Based on the exposure conditions in a previous study (Yamamoto et. al., *J. Toxicol. Sci.* 2012) that reported that MeHg administration induces motor dysfunction in marmosets, MeHg was administered (MeHg 1.5 mg Hg/kg BW for 2 weeks followed by 2 to 3 weeks of non-administration), and locomotion (spontaneous locomotion and walking) was captured using a video camera. The results of video analysis showed a decrease in spontaneous locomotion and movement speed in the marmosets from 2 to 3 weeks after initiation of MeHg administration, and a decrease in stride length and an increase in stride time from 3 to 4 weeks after initiation of MeHg administration.

(2) Elucidation of the foci responsible for neurobehavioral disorders associated with MeHg exposure. Immunostaining of marmoset brains under experimental conditions revealed that Iba1 expression in the cerebellum and thalamus was increased in individuals with markedly impaired motor function, indicating cell injury. In contrast, the Iba1 expression levels in MeHg-exposed individuals, whose motor function was almost unchanged, were similar to those in controls, indicating that cellular injury in the cerebellum and thalamus would be associated with changes in motor function.

[Activity theme and summary]

[6] Information service using hair mercury analysis

Masaaki Nagano
(Department of Basic Medical Science)

In 2023, 278 hair samples were collected from visitors to the National Institute for Minamata Disease and the Minamata Disease Archives. The total mercury concentrations in the samples were measured. The analytical results were sent to each individual. In addition, lectures on hair mercury concentrations were given.

4. Nature Environment Group

This group consists of eight research projects and two work projects, and conducts comprehensive research, including field studies, laboratory experiments, and various types of analyses, with the aim of elucidating the environmental Hg cycle from the global to the regional level and around Minamata Bay and the Yatsushiro Sea. The research of this group targets a wide range of media, including air, water, soil, sediment, and living organisms, and, in particular, a wide range of research topics covering from primary producers, such as phytoplankton, to top predators, such as swordfish. In field studies, Hg in air and precipitation has been monitored in various parts of the Japanese islands, and marine Hg observations have been conducted in a wide range of areas, from coastal areas such as Minamata Bay and the Seto Inland Sea to the open North Pacific Ocean. We are also attempting to elucidate Hg emission sources and chemical reaction processes using stable Hg isotope ratios and to observe Hg fluxes from forests. Furthermore, in cooperation with domestic and international research institutions, we are working to obtain comparable data that will contribute to the assessment of the validity of the Minamata Convention since it has been entered into force in 2017. Simultaneously, we are actively engaged in outreach activities targeting elementary, junior high, and high school students for the purpose of education and dissemination of Hg research and the whole field of science.

The research outlines of each research and working activity for FY 2023 are as follows:

［Research theme and summary］

[1] Study of factors influencing the vertical distribution in speciated mercury in the ocean

(Project research)

Kohji Marumoto

(Department of Environment and Public Health)

This study aims to understand the factors controlling the vertical structure of Hg in the ocean, mainly in the North Pacific Ocean, and to investigate the Hg flux between the atmosphere and ocean and Hg uptake by phytoplankton, which are thought to affect the variation in Hg concentrations in the surface and sub-surface layers. We also aim to investigate the formation of relatively high concentrations of MeHg in the mesopelagic layer and the leaching of Hg from particulate matter obtained from deep seawater.

We mainly focused on field observations and laboratory experiments on Hg flux and Hg uptake by plankton during this year. Dissolved gaseous Hg (DGHg) was semi-continuously observed using a gas-liquid equilibrator on the Hakuho Maru KH-22-07 cruise in Minamata Bay and the Yatsushiro Sea. The DGHg concentrations at 30° to 45°N of the 155°E transect ranged from 10 to 50 pg/L (0.05–0.25 pmol/L), and were higher in the southern region than in the northern region. Because wind speeds were higher around 30°N during this season, Hg emission fluxes were also greater in this area. Observations of Hg flux from the sea surface using the relaxed eddy accumulation method have also been conducted, and data analysis is currently underway to compare the results with those obtained using the equilibrator. Whereas, the DGHg concentrations in the Yatsushiro Sea and Minamata Bay ranged from 40 to 150 pg/L (0.20–0.75 pmol/L), which were higher than those in the open ocean. The concentrations were higher during spring and summer, possibly because of the generation and release of Hg(0) via photochemical reactions. It also tended to be slightly higher south of the Kuma River estuary in the Yatsushiro Sea and Minamata Bay. However, the reasons for this are still unknown and will be investigated in more detail in the future.

On the KH-22-07 cruise, plankton were collected by size fractionation and their THg and MeHg were

analyzed. The results showed that the MeHg concentration in plankton based on wet weight tended to increase with increasing size. Furthermore, MeHg concentration and %MeHg were higher at high latitudes than at low latitudes, which is consistent with the results of previous studies on the distribution of THg concentrations in skipjack tuna at sea.

In contrast, a monomethylmercury (MMHg) uptake experiment was conducted using cryptophytes as the target strain in a laboratory experiment, and the results were compared with those of diatom and green alga experiments conducted in the previous year. The results showed that green algae and cryptophytes showed a smaller change in MMHg uptake in response to temperature than diatoms and the amount of MMHg uptake differed among phytoplankton phylogenetic groups in response to changes in water temperature. The uptake rate constant calculated from the experimental results was also higher in green algae with smaller cell sizes.

[2] Researches on the behaviors of mercury in soil, water and sediment

(Fundamental research)

Akito Matsuyama

(Department of International Affairs and Research)

Each outline of outcomes in theme is described as follows:

① Study of characteristics on Hg methylation in seawater

Results of incubation experiments using Minamata Bay seawater for 2 years, reasonable temperature of incubation experiments was 20–23℃ , and salinity was 32–34‰. A positive correlation was observed between the dissolved carbon and MeHg concentrations.

② Consideration of points of attention in the MeHg analysis of soil and sediment using the Akagi method

A dithizone-toluene solution was used to extract Hg from the soil and sediment. The most effective pH range for this solution was 2.5–3.5. Furthermore, if the pH was outside of this range, the efficacy of Hg extraction decreased significantly.

③ Understanding of changes in characteristics of Minamata bay sediments because of changes in characteristics of Minamata bay seawater

Incubation experiments were performed using batch models adjusted to 1–2 and 3–4 mg/L as dissolved oxygen (DO) concentrations in seawater. In the case of 3–4 mg/L DO, there dissolved total Hg concentration in seawater scarcely increased. Therefore, the threshold value for Hg methylation in seawater using DO as a reaction indicator is thought to be approximately 2–3 mg/L.

[3] Bioprotection of methylmercury toxicity by complex formation with selenium in large marine mammals

(Fundamental research)

Masumi Marumoto

(Department of Basic Medical Sciences)

MeHg ingested through food is gradually converted into inorganic Hg. This mineralization ability varies among animal species. Notably, Se, an essential metal, plays a major role in mineralization; however, the full extent of this role is unknown. The distribution of Hg and Se in organs has been studied pathologically using an X-ray probe microanalyzer (EPMA), which can reveal the cells in various organs where Hg and Se aggregate. We will use EPMA to determine which cells in various organs aggregate Hg and Se to clarify the tissue distribution of Hg and Se in large marine organisms, especially in toothed whales, sharks, and sailfish. This year's research focused on the relationship between THg and Se concentrations in various organs of dolphins and their fetuses. A simple method for measuring MeHg concentrations was investigated, and the results agreed well with the guaranteed values within the margin of error of the standard samples.

[4] Research on primary producer dynamics and mercury bioaccumulation to fish through food chains (Fundamental research)

Kenji Yoshino
(Department of Environment and Public Health)

Phytoplankton and microphytobenthos are important primary producers in shallow coastal ecosystems, and their contributions can differ depending on the region, year, and season. We study the seasonal dynamics of their biomass, stable isotope values ($\delta^{13}C$ and $\delta^{15}N$), THg contents, and the environmental factors influencing those properties in Minamata Bay. This year, we also analyzed stable isotope values and THgs of macrobenthos and fishes sampled in 2022 to determine if a recent food web structure differs from that studied from 2018 to 2019, where a microphytobenthos-based food chain is the main pathway of Hg bioaccumulation throughout the year. Notably, $\delta^{13}C$ of fish and macrobenthos in 2022 did not differ from that of previous results. However, $\delta^{13}C$ of phytoplankton and microphytobenthos greatly varied seasonally; $\delta^{13}C$ of phytoplankton often enriched close to that of macrobenthos and fish, whereas $\delta^{13}C$ of microphytobenthos greatly depleted from them except spring seasons. Moreover THg was usually higher in phytoplankton than microphytobenthos, and THgs of macrobenthos and fish increased with their $\delta^{13}C$. These results suggest that food web structures in recent Minamata Bay are mainly fueled by phytoplankton and that the phytoplankton-based food chain is the main Hg bioaccumulation pathway for organisms at higher trophic levels.

[5] Characterization of stable isotopic composition of mercury at the emission sources (Fundamental research)

Satoshi Irei
(Department of Environment and Public Health)

This research project aims to characterize and report the initial stable isotopic compositions of Hg at emitting and discharging sources. Our results will contribute to a better understanding of the global Hg cycle through stable isotope ratio measurements.

At the beginning of the FY 2023, we set our goals to continuously study the emissions of total gaseous Hg (TGM), carbon monoxide, carbon dioxide, and inorganic ions contained in airborne particulate matter (PM) from prescribed open grassland burning; determine the burned areas and their fuel loads; and publish the results in internationally well-established scientific journals. We had also planned to conduct another sub-theme for TGM emissions from coal - combustion/cement production facilities.

As the project progressed, we found that more thorough and systematic studies on the Hg cycle in grasslands, such as long-term monitoring of Hg concentrations and isotope ratios in the air, uptake study of TGM by the plant species in the grasslands, and increasing the number of case studies, were needed to answer the intriguing questions of the total TGM emissions from Japanese grassland fires and their origin(s). These studies were placed as the top priority because the novelty of the grassland study was very high compared to the man-made emission studies we originally had set previously and even to other Hg cycle studies performed worldwide. In addition, it was also because the research resources were limited. Considering this priority and limitation, we decided to withdraw man-made emission studies from this 5-year project.

With respect to the progress of the grassland burning studies, we, in this fiscal year, successfully determined the burned area estimation for the Aso region in March 2023 using the analysis of satellite images and found a decrease of approximately 6% compared to the previously reported value. For the fuel load analysis, we found that the fuel loads in the studied fields varied from 0.6 to 1.6 kg m^{-2}. Currently, these data are being analyzed. In this fiscal year part of these results and the overview of our study have been organized and

published as an original article "*Emission of Particulate Inorganic Substances from Prescribed Open Grassland Burning in Hirado, Akiyoshidai, and Aso, Japan*" in the journal *Air*, a book chapter "*Mercury Emission from Prescribed Open Grassland Burning in the Aso Region, Japan*" in the book "*Grasslands - Conservation and Development*" edited by Dr. Muhammad Aamir, and an editorial for the special issue "*Sources, Processing, Transport, Health and Climate Impacts of Air Pollutant*" in the journal *Applied Sciences*.

[6] Study of microbes involved in mercury speciation in the ocean

(Fundamental research)

Yuya Tada

(Department of Environment and Public Health)

This study aims to evaluate microbial Hg speciation (methylation, demethylation, and reduction) in the ocean using metagenomic and molecular biology methods. This year, our study revealed the presence of Hg methylation genes (hgcA and hgcB) in the particle-attached bacterial fraction within the seawater column of Minamata Bay. This implies that microbial Hg methylation may occur within the suspended particles in seawater. Phylogenetic analysis showed that the Desulfobacterota lineage, commonly detected in coastal sediments, was dominant in all hgcA sequences, suggesting a strong influence of sediment resuspension on suspended particles in seawater columns. However, no Hg-related genes were detected in the metatranscriptome analysis, possibly because of insufficient sequence reads. Quantitative PCR analysis revealed that Nitrospina-hgcA was abundant at depths of 400–1,500 m in the western North Pacific (WNP). In addition, a significant positive correlation between Nitrospina-hgcA abundance and MMHg concentration was observed in the upper depth of 2,000 m, indicating a potentially significant effect of the Nitrospina lineage on MeHg dynamics in the mesopelagic zone of the WNP.

[7] Study on factors influencing long-term trend of atmospheric mercury concentrations in Asia-Pacific regions

(Fundamental research)

Kohji Marumoto

(Department of Environment and Public Health)

Continuous monitoring of Hg in air and precipitation was performed in the cities of Minamata and Fukuoka to obtain continuous data, which will contribute to the evaluation of the effectiveness of the Minamata Convention and to clarify its seasonal and inter-annual variations and their factors. Monitoring of Hg in the precipitation was also conducted at six sites in Minamata, Hirado, Fukuoka, Matsue, Omaezaki, and Tsukuba. To investigate the effects of Hg emissions from volcanoes, atmospheric-speciated Hg was continuously monitored at Senankyo, located north of the Aso Nakadake 1st crater. However, as the volcano was not active during this fiscal year, the frequency of high-concentration events was also low.

We examined the interannual variation in atmospheric gaseous elemental Hg (GEM) concentrations in the cities of Minamata and Fukuoka over the past 10 years and found that concentrations gradually decreased at both locations. The reasons for these decreases are considered to be the slowdown of economic growth in the Asian continent, the shift of the center of economic activity from coastal areas to inland areas, and the decrease in atmospheric-oceanic circulation in the Northern hemisphere as a result of the decrease in Hg emissions in the United States and Europe.

As for Hg concentrations in precipitation, we examined inter-annual variations in the cities of Minamata, Hirado, Fukuoka, and Omaezaki, where approximately 10 years of data have been accumulated. We found that only the THg concentration in precipitation in Omaezaki showed a downward trend, whereas the concentrations at other sites remained almost unchanged.

To ensure the reliability of the data for Hg monitoring

in precipitation in response to the Minamata Convention, we participated in an inter-calibration program led by the U.S. Geological Survey. In addition, we collaborated with the Central University of Taiwan to compare the rainwater sampler used in Japan and that used at the Asian-Pacific Mercury Monitoring Network.

[8] Evaluation of air-terrestrial flux and exchange of elemental mercury using relaxed eddy accumulation technique

(Fundamental research)

Yuichi Maruo

(Department of Environment and Public Health)

The flux of GEM was measured in forests that cover approximately 66% of the Japanese land area. This study aimed to evaluate the diurnal and seasonal variations in GEM fluxes and estimate the annual GEM exchange in forests.

In FY 2023, we developed a relaxed eddy accumulation (REA) system to measure the GEM flux between the atmosphere and forests. The system was installed at the Fuji-Hokuroku Flux Observation Site (Yamanashi, Japan) of the National Institute for Environmental Studies on December 13, 2023, and the GEM flux between the atmosphere and the larch forests was continuously measured. The REA system separately collects the GEM in the upward and downward winds and measures the GEM concentration in each air mass. The GEM flux was calculated from the difference in GEM concentration between the upward and downward winds. From December 13 to 26, 2023, the average GEM concentrations in upward and downward winds were 1.36 ± 0.47 ng/m^3 and 1.26 ± 0.42 ng/m^3, respectively. The average GEM flux during the analyzed period was 58.5 ± 66.4 ng/m^2/h, indicating the GEM emitted from larch forests to the atmosphere. A higher GEM flux was observed during the day than at night. We will continue the observations to evaluate the seasonal variation in the GEM flux and estimate the annual GEM exchange between the atmosphere and larch forests.

[Activity theme and summary]

[9] Implementing seawater quality monitoring in Minamata Bay and supporting various regional activities around Minamata area

Akito Matsuyama

(Department of International Affairs and Research)

Each outline of outcomes in theme is described as follows:

① Hg monitoring in Minamata Bay

Notably, Hg monitoring was performed twice in 2023 (June and October) in Minamata Bay. The seawater sampling station in Minamata Bay has three locations (Hadakase, Wanoh, and Koijishima). Moreover, Hg monitoring was performed three times at five points in the hydrophilic revetment of the reclamation area in Minamata Bay (June, September, and December). Our results revealed that the average value of dissolved THg concentration in Minamata Bay seawater (2023) was 0.29 ± 0.04 ng/L and dissolved MeHg concentration was 0.06 ± 0.05ng/L. In contrast, the average dissolved THg concentration in the hydrophilic revetment of the reclamation area was 4.55 ± 0.63 ng/L.

② Support for various activities in the Minamata area (Subtitle: Development of efficient oyster farming techniques in Minamata Bay)

Based on the results of the 2022 study, new oyster farming cages were set up on oyster culture rafts in Fukuro Bay at four depths (surface, -2m, -3m, and -5m), and approximately 800 young oysters entered each oyster farming cage at the beginning of April. Once a month, 65 oysters were randomly selected based on statistics and measured weight and so on. In 2023, compared to the previous year, Minamata Bay was not affected by the summer red tide and was doing well. Consequently, oyster growth was optimal in cages placed at a depth of -2 m (as of November 2023). However, the summer seawater temperature was about 1℃ higher in 2023 than last year, and the number of dead oysters cultivated at the surface and at -2 m in the

summer was higher than last year. Finally, oyster growth of this year (2023) was better than last year (2022). Temperature of surface seawater in February 2024 was 2.5℃ higher than last year in February 2022. Therefore, we hypothesized that this affects oyster growth.

[10] Outreach activities related to science and technology research for elementary and junior high school students

Masumi Marumoto
(Department of Basic Medical Sciences)

Researchers and research institutes are expected to disseminate scientific knowledge and results to the public.

Kumamoto Prefecture, where NIMD is located, is known both domestically and internationally as the area where MD occurred. However, the knowledge of elementary and junior high school students regarding Hg is limited. Therefore, we intend to provide correct information about Hg issues by conducting classes on Hg issues at elementary and junior high schools in Minamata City and beyond. To emphasize interactivity rather than one-way transmission from the researcher, a question box will be set up before the delivery class, and a questionnaire will be administered to answer questions before the presentation. Furthermore, we intend to contribute to the community by proactively responding to requests for cases other than delivery classes on Hg issues. This year, a third-year student from Minamata High School, whom we taught last year, gave a presentation at an environmental science conference and won a prize for the best presentation. In addition, as new initiatives, we conducted a work experience program for second-year junior high school students in Minamata City and a “Minamata” calligraphy contest.

5. International Contribution · Information Group

In alignment with the goals of the Minamata Convention on Mercury, which was enacted in 2017 to mitigate the environmental and health effects of Hg usage, our International Contribution Information Group has continued to enhance collaboration with international bodies such as the WHO and the United Nations Environment Programme (UNEP). We supported the management of the International Conference on Mercury as a Global Pollutant (ICMGP) and organized events under the NIMD Forum to foster networking among Hg researchers. In partnership with the Japan International Cooperation Agency (JICA), we have offered knowledge, technology, and experience to address the Hg pollution challenges faced by developing countries. Our efforts also extend to the MOYAI Initiative of the Japanese government, under which we have developed simple Hg measurement techniques, analytical training, proficiency tests, and quality control materials for analysis. We provide biomonitoring analysis and evaluation techniques for regions lacking adequate analytical capabilities, and our findings are disseminated through scholarly articles and conferences.

This year, we evaluated the health risks associated with the use of elemental Hg in traditional gold-plating crafts in Nepal. We identified significant worker exposure risks when Hg vapor concentrations exceeded permissible workplace standards. In response, we developed Hg exposure monitoring technologies, including portable devices, activated carbon masks for exposure monitoring, and porous gold particles for Hg extraction. Furthermore, as part of our Human Biomonitoring Project, we have developed blood certification reference materials, conducted proficiency tests to enhance Hg analysis capabilities, assisted treaty countries in accurately assessing Hg pollution, and implemented measures to mitigate health risks.

Our investigations into hair Hg levels in Hg pollution-concerned regions of Bangladesh, Brazil, and Vietnam have clarified the MeHg exposure status of the residents, helping to alleviate health concerns. Moreover, by organizing special sessions for ICMGP 2024 and publishing review articles on human exposure to Hg, we have contributed to raising international awareness and strengthening countermeasures.

At the Minamata Disease Information Center, a complete renewal of exhibitions has been undertaken, incorporating digital signage to facilitate future updates. These initiatives are expected to contribute to the prevention of health damages caused by Hg exposure, effectively assess the impact of the Minamata Convention, and enhance global analytical capabilities. This report outlines the research and operational achievements of FY 2023.

[Research theme and summary]

[1] Development of a simple method for the determination of Hg and reference materials

(Project research)

Koichi Haraguchi

(Department of International Affairs and Research).

This research aimed to evaluate the health risks of using elemental Hg in traditional gold-plating crafts in Nepal and propose technical measures to mitigate these risks. Environmental assessments of Nepalese gold-gilding facilities have revealed that Hg vapor concentrations exceed permissible workplace limits, posing direct health risks to workers. Therefore, we focused on developing Hg exposure-monitoring technologies, including portable direct Hg analyzers, activated carbon masks for Hg exposure monitoring, and Hg extraction using porous gold particles. Additionally, as part of our Human Biomonitoring project, we advanced the development of blood-certified reference materials and conducted proficiency tests to enhance the Hg analysis capabilities. These

efforts provide technical support to treaty countries to accurately assess Hg pollution and implement measures to avoid health risks. This research is expected to contribute to reducing the health risks associated with Hg exposure and enhance international analytical capabilities that support the effectiveness of the Minamata Convention.

[Activity theme and summary]

[2] Examination of hair Hg in areas at risk of Hg pollution around the world

Masatake Fujimura

(Department of Basic Medical Science)

This year, we measured Hg in the hair of residents living near e-waste dumping siites in Bangladesh and submitted a paper as a co-author. We also measured hair Hg levels in urban residents of Ho Chi Minh City, Vietnam. The results revealed that internal exposure to MeHg in the residents of Ho Chi Minh City, Vietnam was almost negligible, thus contributing to the elimination of their health concerns.

As a member of the steering committee of the ICMGP, we prepared a special session on hair Hg and a storyteller's lecture at the next international conference (ICMGP 2024). In addition, we prepared a review on current global human exposure to Hg, and a study was accepted for publication as a co-author. As the above-mentioned results, we proposed that in the fish-eating regions of Asia (especially Japan), relatively high MeHg exposure is not directly associated with neurological symptoms.

[3] NIMD forum and international workshop

Megumi Yamamoto

(Department of International Affairs and Research).

Our work aims to publicize the results of research and tasks in the NIMD through the organization of international forums and to exchange opinions with domestic and overseas researchers in the field and general participants to help solve future environmental problems associated with Hg.

The Nature Environment Group was in charge of the NIMD Forum in FY 2023 and conducted it as follows:

Theme: Current Status and Challenges of Environmental Mercury Monitoring in the Asia-Pacific and African Regions for Contributing the Effectiveness Evaluation of the Minamata Convention.

Presenters: Three NIMD staff members, six domestic researchers, one domestic high school student, one college of technology student, and overseas researchers (USA: 2, South Africa: 1, Taiwan: 1, and Korea: 1).

Place: Minamata Disease Archives.

Subjects:

- Global Mercury Concentrations in Biota: Their Use as a Basis for a Biomonitoring Framework in Asia
- Mercury Concentrations in Sediment, Water, and Fish in Freshwater Ecosystems: Summary of the National Mercury Monitoring Network of South Korea
- Indispensable Methylmercury (MeHg) Analysis for Reconstructing Historical Bird Hg Burden Using Museum Specimens
- Keynote Speech Mercury Observations in Africa: How Monitoring Can Fill in the Gaps to Determine the Effectiveness of the Minamata Convention
- New Observations in the National Atmospheric Deposition Network (NADP) in North America
- Overview of the Asia Pacific Mercury Monitoring Network (APMMN)
- Long-term Monitoring of Mercury in Air and Rainwater in Taiwan
- Long-term Trends of Mercury in Air and Wet Depositions in Japan
- Mercury Contents in Rainwater in Matsue and Minamata, Japan during 2020 to 2023
- Pathway of the Air Mass Causing Mercury Deposition in Matsue, Japan
- Comparative Study of the Different Methods of Atmospheric Mercury Monitoring - Manual and Automated Active Sampling
- Analysis of the Variation in the Annual Mean

Estimate Obtained from Intermittent Measurements of Atmospheric Mercury
- How is a Concentration of Atmospheric Mercury in My High School?
- Observation in the Atmospheric Concentrations of Speciated Hg in Volcanic Area
- Spatial Distribution of Total Mercury and Its Stable Isotope Ratio in Skipjack Tuna from Northwest Pacific Ocean
- Spatial Variation in Methylmercury Accumulation within Plankton Communities across the Western North Pacific
- Global-scale Prediction of Medium-term Variation of Methylmercury in Seafood

Participants: - November 18 (actual attendance: 69. including three press members). November 19 (actual attendance: 60)

The NIMD Forum in 2024 will be organized by the International Contribution and Information Group and will be held at the ICMGP 2024 (Cape Town, South Africa) in the session "Strengthening Research Capacity on Mercury in Low- and Middle-Income Countries with a Focus on Vulnerable Populations."

[4] Promotion of international collaborations

Megumi Yamamoto

(Department of International Affairs and Research).

Based on lessons learned from MD and the Hg countermeasures implemented by Japan, the NIMD has been promoting international collaborations with international organizations, such as the WHO and UNEP, as well as with JICA, and through networks among researchers, with the aim of contributing to solving environmental problems associated with Hg worldwide.

This year, we conducted the following research and tasks as examples. We will continue to promote international joint research and tasks through online training and participation in meetings, in addition to surveying countries with environmental problems associated with Hg and the transfer of technology to researchers and technicians in Hg analysis.

1) Exposure assessment of methylmercury for pregnant women in Hanoi, Vietnam.
2) Exposure assessment of metallic mercury to gold plating factory workers in Nepal.
3) Exposure assessment of mercury contamination from small-scale gold mining (ASGM) in Jember, Indonesia.
4) Preliminary exposure assessment of mercury contamination from ASGM in Banten, Indonesia.
5) Joint work with UNEP: Proficiency testing of mercury analysis (total mercury and methylmercury levels in fish meat).
6) Supply of blood-certified reference materials (total mercury and methylmercury levels in whole blood).
7) Asia-Pacific Mercury Monitoring Network (APMMN) activities: Continuous monitoring of mercury in air in Japan and presentation on this activity at the APMMN annual meeting: July 12–14).
8) Transfer of mercury analysis technology to developing countries and a preliminary study on the actual situation concerning mercury pollution: Dodoma University, Tanzania. Graduate students from Tokyo Medical and Dental University (WHO Collaborating Center: JPN-73); Diponegoro University, Indonesia; Jember University, Indonesia; and Rajshahi University, Bangladesh.
9) Hair mercury surveys around the world: Vietnam and Bangladesh.
10) Face-to-face training: *Minamata Environmental Academia Sakura Science Plan Minamata training; *Nagasaki University Graduate School of Tropical Medicine and Global Health, Short-term field training; *UNEP "Minamata Convention on Mercury" Executive Director; *Keio University: Minamata Fieldwork; *Nichietsu (Japan-Vietnam) University: Minamata Environmental Academia Public Policy Program; Jember University: International Seminar.

11) Online training: UNEP-Asia and Pacific Regional Office; Diponegoro University.
12) Joint work with JICA: Discussion on the continuation of the mercury analysis training program for six Central American countries (Uruguay, Belize, Honduras, Guatemala, Panama, and Costa Rica) for the following fiscal year.

[5] 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).

This fiscal year, the permanent exhibition was completely renewed. In the permanent exhibition, the introduction of digital signage has made it easier to update data. For special exhibitions, scientific displays have been strengthened in cooperation with the Basic Research Department, making them more attractive and easier for the general public to understand. In addition to the renewal of the permanent and special exhibitions, the facility was upgraded to cope with the increased number of visitors after COVID19, using VR and other technologies. In addition, materials for the 70th anniversary of the official confirmation of MD was successfully collected.

Cooperation with the three museums in Manabino-Oka, Network of Pollution Reference Centers, and other related organizations is ongoing.

Research funded by Grants-in-Aid for Scientific Research on the Maintenance and Use of Materials has commenced, and the results of the study group are being used in the workings of the Minamata Disease Archives and the Minamata Disease History Preservation Project.

[6] Activities as a WHO Collaborating Center

Megumi Yamamoto
(Department of International Affairs and Research).

The NIMD was designated as a WHO Collaborating Center (CC) for "Studies on the Health Effects of Mercury Compounds" (World Health Organization Western Pacific Regional Office: WPRO) in September 1986 (Ref. # JPN-49) and has been conducting surveys and research on the health effects of Hg. During this fiscal year, we performed the following tasks:

1) Exposure assessment of MeHg for pregnant women in Hanoi, Vietnam.
2) Exposure assessment of metallic Hg to gold-plating factory workers in Nepal.
3) Exposure assessment of Hg contamination from small-scale gold mining (ASGM) in Jember, Indonesia.
4) Preliminary exposure assessment of Hg contamination from ASGM in Banten, Indonesia.
5) Joint work with UNEP: Proficiency testing of Hg analysis (total Hg and MeHg levels in fish meat).
6) Supply of blood-certified reference materials (total Hg and MeHg levels in whole blood).
7) APMMN activities: Continuous monitoring of Hg in the air in Japan and presentation on this activity at the APMMN annual meeting: July 12–14).
8) Transfer of Hg analysis technology to developing countries and a preliminary study on the actual situation concerning mercury pollution: Dodoma University, Tanzania. Graduate students from Tokyo Medical and Dental University (WHO Collaborating Center: JPN-73); Diponegoro University, Indonesia; Jember University, Indonesia; and Rajshahi University, Bangladesh.
9) Online training: UNEP-Asia and Pacific Regional Office; Diponegoro University.
10) Preparation and processing of annual reports (WHO CC activities from January to December 2023).

6. publications and scientific meetings

［International Journals］

Underline: NIMD staff, *: first author, #:corresponding author

Fujimura M*#, Unoki T: BDNF specifically expressed in hippocampal neurons is involved in methylmercury neurotoxicity resistance. Environ Toxicol, (https://doi.org/10.1002/tox.24174). Online first.

Nakamura M*#, Taulu S, Tachimori H, Tomo Y, Kawashima T, Miura Y, Itatani M, Tobimatsu S: Single-trial neuromagnetic analysis reveals somatosensory dysfunction in chronic Minamata disease. Neuroimage Clin, 2023; 38: 103422.

Nakamura M*#, Tatsuta N, Murata K, Nakai K, Iwata T, Otobe T, Sakamoto M, Yamamoto M, Itatani M, Miura Y, Koriyama C: Neurodevelopmental associations of prenatal and postnatal methylmercury exposure among first-grade children in the Kinan region, Japan. Environ Res, 2023; 235: 116688.

Yoshino K*#, Yamada K, Kanaya G, Komorita T, Okamoto K, Tanaka M, Tada Y, Henmi Y, Yamamoto M: Food web structures and mercury exposure pathway to fish in Minamata Bay. Arch Environ Contam Toxicol, 2023; 85: 360-373.

Irei, S*#, Kameyama S, Shimazaki H, Sakuma A, Yonemura S: Book Chapter Mercury emission from prescribed open grassland burning in the Aso region, Japan. In Muhammad Aamir (Ed) Grasslands - Conservation and Development. (https://doi.org/10.5772/intechopen.113293). Online first.

Irei S*#, Tak W: Chan Sources, Processing, Transport, Health and Climate Impacts of Air Pollutants. Appl Sci, (https://doi.org/10.3390/app14041361). Online first.

Chi B, Yano S, Matsuyama A, Hao L: Vertical distribution of mercury in bottom sediments and particle size effect on migration of mercury-containing sediments in the Yatsushiro sea. J JSCE, 2023; 11(2): 23-17139.

Chi B, Matsuyama A#, Yano S, kindaichi M, Taniguchi Y: A novel particle size classification-based method for analyzing total mercury concentration distribution of sediment in the Yatsushiro sea. J JSCE, 2023; 11(2): 23-18037.

Iijima Y, Miki R, Fujimura M, Oyadomari S, Uehara T: Methylmercury-induced brain neuronal death in CHOP-knockout mice. J Toxicol Sci, 2024; 49(2): 55-60.

Basu N, Bastiansz A, Dorea J, Fujimura M, Horvat M, Shroff E, Weihe P, Zastenskaya I: Our evolved understanding of the human health risks of mercury. Ambio, 2023; 52: 877-896.

Oguro A, Fujiyama T, Ishihara Y, Kataoka C, Yamamoto M, Eto K, Komohara Y, Imaoka S, Sakuragi T, Tsuji M, Shibata E, Kotake Y, Yamazaki T: Maternal DHA intake in mice increased DHA metabolites in the pup brain and ameliorated MeHg-induced behavioral disorder. J Lipid Res, 2023; 64: 100458.

Ishitsuka K, Tsuji M, Yamamoto M, Tanaka R, Suga R, Kuwamura M, Sakuragi T, Shimono M, Kusuhara K: Association between maternal fish consumption during pregnancy and preterm births: the Japan Environment and Children's Study. Environ Health Prev Med, 2023; 28: 47.

Kasai A, Chéhère P, Harada R#, Kameyama N, Salgues J: La danse du détour: A collaborative arts performance with people touched by Minamata disease. J Appl Arts Health, 2023; 14(2): 207-225.

Yamamoto Y, Yoshino K#, Koga T: Anti-predator responses to chemical, visual, and combined cues by an estuarine hermit crab from sandy tidal flats. Behav Ecol Sociobio, 2023; 77: 60.

Yoshinaka Y, Liu Y, Katano T, Yoshino K, Nishiyama K, Yasui-Tamura S, Hashihama F: Temporal niche partitioning of *Skeletonema*: Seasonal succession of the community composition in surface water of Tokyo Bay. Aquat Microb Ecol, 2023; 89, 87-98.

Ueno Y, Takano M, Katano T, Yoshino K, Miyazaki-Horimoto N, Yasui-Tamura S, Sukigara C, Hamana I, Hashihama F, Kanda J, Ishimaru T: Succession of three dominant diatoms, *Skeletonema*, *Thalassiosira*, and *Pseudo-nitzschia* in the inner area of Tokyo Bay from 2003 to 2017. J Ocearnogr, 2023; 79, 265–279.

Devabathini P, Fischer DL, Chen S, Pattammattel A, Bury G, Antipova O, Huang X, Yong S, Chu Y, Horai S, Pushkar Y: High-resolution imaging of Hg/Se aggregates in the brain of small Indian mongoose, a wild terrestrial species: insight intracellular Hg detoxification. Environ Chem Lett, (https://doi.org/10.1007/s10311-023-01666-3). Online first.

［International meetings］

Underline: NIMD staff,

Sakamoto M, Marumoto M, Haraguchi K, Toyama T, Saito Y, Balogh S, Tohyama C, Nakamura M: Reevaluation of Minamata disease provides insights into the protective role of selenium against methylmercury toxicity. International Society of Exposure Science 2023, Chicago. 2023. 8.

Yamamoto M: Current situation and issues surrounding the health effects of mercury. University of Jember: International Seminar on Heavy Metal Contamination. A Crucial Health Issue in Agromedicine, Jember. 2023.12. (Invited lecture)

Yamamoto M, Kataoka C,Yanagisawa R, Tsuji M, Shibata E, Yokooji T, Mori N, Horiuchi M, Shuto S, Nakamura M, Sakamoto M: Maternal transfer of methylmercury in high-fat diet-induced obese mice with glucose metabolism disorder. 63rd Society of Toxicology, Salt Lake City. 2024. 3.

Haraguchi K: Laboratory proficiency testing–Results and future plan – Enhancing collaboration and communication among mercury. UNEP Networking Webinar, Web meeting. 2023. 8. (Invited lecture)

Irei S, Kameyama S, Shimazaki H, Sakuma A, Yonemura S: Mercury and carbon cycles in the Japanese grasslands (Virtual oral presentation). Renewablemeet 2024. Lisbon. 2024. 3.

Iijima Y, Iwawaki T, Kumagai Y, Fujimura M, Uehatra T: Methylmercury induces ER stress and subsequent activation of apoptosis pathway leading to neuronal cell death in the mouse brain. 12th International Congress of ASIATOX, Taipei. 2023. 7.