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National Institute for Minamata Disease
Ministry of the Environment
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Report on Research and Other Activities in the Fiscal Year 2022

1. Pathomechanism Group

Our research group aims to understand the molecular mechanisms underlying methylmercury (MeHg) toxicity in humans. Our specific goals were to understand the initial signs of MeHg poisoning, evaluate MeHg toxicity, and develop methods to protect against MeHg-induced disorders and new treatment options for MeHg poisoning. To achieve these aims, we employed advanced techniques in the fields of biochemistry, molecular biology, and pathology, using cell cultures and animal models.

During the fiscal year (FY) 2022, our group conducted the following research.

[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

A comprehensive gene expression analysis paper was submitted for peer review and accepted for publication. Next, we conducted a detailed functional analysis of factors (transthyretin and BDNF) specifically expressed in hippocampal neurons to determine their role in MeHg toxicity and presented our results at conferences and research meetings. We also presented a symposium on the vulnerability of excitatory cells in the deep cerebral cortex to MeHg toxicity, which we had previously conducted.

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

In this study, the polythiolation of blood proteins was not proven to be a predictive marker of MeHg toxicity. However, we found that miRNAs that are effective in protecting against toxicity in the nervous system, were expressed at high levels in the blood exosomes of rats resistant to MeHg toxicity.

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

The results of our study on the inhibitory effects of ROCK inhibitors on neuropathic pain in a rat model of MeHg intoxication have been accepted for publication in an international journal. We also analyzed the therapeutic effects of gabapentin and found that these effects were attributable to the suppression of neural activity and neural circuit rewiring in the somatosensory cortex.

(4) Joint research with external research institutes

We conducted a joint study with external research institutions on MeHg toxicity (endoplasmic reticulum stress), which was not conducted at our research center, and demonstrated that MeHg exposure causes neuronal cell death via endoplasmic reticulum stress at the animal experimental level. These findings have been presented at academic conferences and published in international journals.

(5) Others

A review article was compiled based on 17 original papers on the “cellular conditions responsible for methylmercury-mediated neurotoxicity,” which have been researched in this project for about 17 years and have been accepted and published in an international academic journal.

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

(Fundamental research)

Masaaki Nagano

(Department of Basic Medical Science)

In this study, we aim to reduce the health risks of MeHg by utilizing the functions of food ingredients. Thus far, we have demonstrated that the daily intake of wheat bran (bran) or fructooligosaccharides significantly reduced tissue mercury concentration in mice exposed to MeHg.

In FY 2022, to demonstrate the effect of bran on cerebral cortical dysfunction after MeHg exposure, we performed immunohistochemical analyses on mouse brains. The results showed that bran intake suppressed MeHg-induced neuronal degeneration in the deep layers of the cerebral cortex. These pathological changes were consistent with the results of behavioral tests, suggesting that bran intake may reduce MeHg toxicity.

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

Takamitsu Unoki
(Department of Basic Medical Sciences).

Redox (reduction-oxidation) 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 (RSS), which are low-molecular-weight molecules with releasable sulfane sulfur. Sulfane sulfur is easily transferred to protein thiols to form the respective persulfide/polysulfide species (i.e., protein polysulfidation). MeHg can covalently modify protein thiols, causing their dysfunction and thereby exerting deleterious effects. In our study, we aimed to assess the physiological role of the sulfane sulfur-mediated modification of protein thiols in the nervous system during MeHg exposure.

In FY 2022, we isolated polysulfidated proteins from primary cultured rat neurons exposed to MeHg using an alkylating agent-based pulldown method. The identification of the isolated proteins and analysis of the effects of MeHg on these proteins are ongoing. The accumulation of excess RSS in cells leads to toxicity. As a mechanism to prevent this adverse effect, we found that cystine-dependent antiporters on the plasma membrane are responsible for the efflux of RSS, such as cysteine persulfide, into the extracellular space.

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

Akio Sumioka
(Department of Basic Science).

Methylmercury (MeHg) induces oxidative stress in the brain, causing 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. As such, we developed a new sensor vector, Krab-U/Luc, to detect MeHg toxicity and studied its molecular mechanisms.

(1) Development of a sensor for methylmercury toxicity

In FY 2022, we attempted to improve the sensor vector for application in different situations. We identified causal factors and modified the gene transfection procedure to reduce the variance between samples in individual experiments. In addition, we investigated the sensitivity of shrimp and coral luciferases to MeHg. Coral luciferase showed a firefly luciferase-like response at the CMV-driven expression and was selected as the reference signal.

We also developed a single-vector sensor for use in stably expressing cells and transgenic mice. Unfortunately, the single-vector sensor lost sensitivity to MeHg. We addressed the cause of this incompetence and identified the non-specific suppression of Krab. Finally, we solved a transcriptional problem, but could not overcome an unusual translation problem.

(2) Research on the mechanism of the methylmercury neurotoxicity

This year, the specificities of MeHg and other electrophiles for target proteins and nucleophiles were investigated. In Cos-7 cells, MeHg-induced cell death

was not blocked by Trolox (a vitamin E derivative) or ebselen (organoselenium). On the other hand, in primary cultured mouse cerebellar granule cells, MeHg-induced cell death can be blocked by Trolox and ebselen. These results imply that the target proteins differed between Cos-7 cells and cerebellar granule cells. The classical balanced electrophilic stress model cannot explain this finding, suggesting the need for a new target-specific model.

During the development of sensor vectors, selenocysteines are rarely incorporated into selenoproteins in cultured cells. Therefore, we examined different conditions of selenoprotein expression. Among the selenoproteins, GPx1 and DIO3 showed relatively high expression of the full-length form rather than one of the truncated forms, specifically in Cos-7 cells. This mechanism is expected to protect against MeHg, suggesting that a molecular mechanism underlies the cellular specificity of MeHg toxicity.

Group Research

Minamata disease (MD) is an intoxication of the central nervous system caused by the ingestion of contaminated 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 to diagnose in mild cases. Thus, 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 they affect the quality of life of patients with MD. We initiated a clinical study on repetitive transcranial magnetic stimulation (rTMS) therapy for neuropathic pain.

To create a detailed plan for the regional revitalization of MeHg pollution victims, we conducted a multidirectional analysis of issues related to these victims.

The research conducted by our group during FY 2022 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 the pathophysiology of Minamata disease (MD), we used magnetoencephalography (MEG) and magnetic resonance imaging (MRI) to compare the findings of certified patients with MD and cases from the Kumamoto area, a control area.

In the analysis of the primary sensory cortex (SI) by MEG, we found that the N20m amplitude of somatosensory evoked magnetic fields (SEF), reproducibility of N20m waveforms, and γ activity in SI are useful for discriminating between MD and controls. This year, by classifying the N20m waveforms of individual responses, we examined the response of the SI to median nerve stimulation and found that it was useful for distinguishing patients with MD from controls. Furthermore, we are developing a script to evaluate the SI network in patients with certified MD, as the γ activity of SEF is reduced in these patients, suggesting an impairment in the connectivity of the sensory cortices.

In the imaging analysis of brain structures in patients with MD, we used an MRI to analyze brain volumes and nerve fibers and found that MRI findings in the cerebellum and thalamus were useful in distinguishing between patients with MD and controls. Therefore, we calculated the Harmonized Z-scores of the lateral cerebellum and thalamus, compared them with those of patients with MD and controls and found that they were useful for distinguishing between the two. By integrating MEG and MRI data, the sensitivity was generally 80% and the specificity was 90%, suggesting that MEG and MRI are useful for discriminating patients with MD from controls.

In a treatment study on MD, a reference value for sensory gating (paired pulse stimulation with magnetoencephalography), which is considered useful for determining the effectiveness of magnetic stimulation in pain treatment, was established. To clarify the mechanism of action of magnetic stimulation therapy on the motor cortex (M1), we compared functional changes in the SI caused by magnetic stimulation of M1 in healthy subjects using MEG. In the future, we plan to establish optimal magnetic stimulation conditions for neuropathic pain and

objective evaluation methods for the therapeutic effects.

[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 project is being conducted from multiple perspectives: (1) to understand the actual situation of Minamata disease damage; (2) to uncover local welfare issues; (3) to conduct local studies and regional research as know-how for regional revitalization to solve these issues; and (4) to examine regional development through art as a pioneering and experimental approach.

This year, the theme of issue (4) was presented at a conference. It was then transformed into a paper, submitted with me as a co-author and accepted.

The paper presents a case study documenting a participatory art process.

In May 2019, a network of artists, researchers, students, residents, and participants from outside the region was created and titled the Minamata Dance Collective. The Collective conducted dance sessions and workshops with patients with MD at various locations in Minamata, with 180 participants over 10 days.

This case showed that art in Minamata could be experienced as "MOYAI." Multiple conflicts over MD have created a deep divide among the people. However, the artistic exchange of listening to the voice of the land through the use of the body showed the possibility of bridging the divide between conflicts.

The data necessary for writing a paper on the interview survey of pregnant and nursing mothers in MD-affected areas (1) were almost completely obtained.

Research on issue (2) is ongoing. A field survey was conducted in the town of Shiraoi, Hokkaido (3). The survey will be conducted in the next fiscal year or later.

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 (B-SES) 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 the 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 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 2022.

[Activity theme and summary]

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

Masaaki Nakamura
(Department of Clinical Medicine)

To examine how to support aging patients with Minamata disease (MD) and their families, including rehabilitation to improve ADLs, the "Home support model study, including care prevention" was implemented from FY 2006 to FY 2009, the "Community development project for home care support, including healthcare practice " from FY 2009 TO FY 2012, and the "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 been implementing the "Community Welfare Promotion Business to support MD victims." Based on these achievements, further activities have been undertaken to help care prevention projects take root in areas where MD occurs.

The Minamata City Council of Social Welfare supported the community center activities of the Minamata City Council of Social Welfare by providing "brain training" through handicrafts. As for Izumi City, we supported the "Fureai Iki-iki Salon activities" of the Izumi City Council of Social Welfare, Takaono Branch Office and Noda Branch Office. This year, a new handicraft class was held in Tsunagi Town. The class was so well received that it would be held again in the following fiscal year and beyond. To provide better handicrafts, each district conducted a questionnaire survey of community-dwelling participants and created a handicraft program that resulted in higher levels of satisfaction.

Measures should be taken to prevent frailty and sarcopenia and to extend healthy life expectancy. After close discussions with the Minamata City Council of Social Welfare, it was decided a project to prevent frailty and sarcopenia in Minamata City in the next fiscal year will be initiated.

We participated in the Minamata/Ashikita Regional Health and Welfare Network for Minamata Disease Victims, etc., however, the executive committee meeting was not held because the "Moyai Music Festival" was postponed indefinitely.

To encourage local residents to participate in the care prevention project, publicity activities were conducted through the website of the National Institute of Minamata Disease, Facebook (activity reports of handicraft classes held each time and a notice of the next one), and the local living room.

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

Atsushi Nakamura
(Department of Clinical Medicine)

We continue to provide rehabilitation to patients with Minamata disease (MD) to improve their quality of life (QOL). Rehabilitation focusing mainly on gait disorders was continuously implemented 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 (ALC). Furthermore, since the last fiscal year, we have been practicing neuromuscular electrical stimulation (NMES) interventions as rehabilitation for swallowing dysfunction in Minamata disease patients, and this fiscal year we conducted it on one new patient and found that the repeated salivary swallow test (RSST) and some of the speech functions were improved. Since the results vary depending on the degree of dysfunction for which the intervention is performed, we plan to increase the number of cases and investigate the optimal conditions for neuromuscular electrical stimulation therapy for dysphagia in fetal Minamata disease in the future.

The annual workshop on rehabilitation and welfare techniques, which had been postponed due to the

COVID-19 pandemic, was held with the addition of a live webcast, in addition to face-to-face sessions held in the past.

As efforts related to information dissemination to local residents and care prevention, we visited the Orange Hall, which is used as a base for health and welfare in the southern part of Minamata City, two times a month. At the Orange Hall, we conducted physical and cognitive function evaluations, ADL evaluations, and health exercise classes for local elderly people, including MD patients.

[5] Appropriate management and information provision of pathological specimens related to Minamata disease

Masumi Marumoto
(Department of Basic Medical Sciences).

Pathological tissue specimens of autopsy cases of Minamata disease are extremely valuable. Our institute permanently preserves many pathological tissue specimens from patients with Minamata disease. However, it is difficult to permanently preserve pathological tissue slides until they fade over time. Therefore, our objectives were to digitize these pathological slides for permanent preservation and to publish digitized pathological tissue slides on a website for students and doctors learning pathology. Simultaneously, we will create a pamphlet explaining Minamata disease pathology for the public and children. In addition to histopathological specimens, our center holds many valuable pathological samples that we will continue to organize and preserve.

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

Rie Harada
(Department of International Affairs and Research).

The Regional Policy Section acts as a coordinator between citizens and the local government and has established a cooperative system to address the problem of vacant houses and support newcomers to Minamata.

We held a "Study Group on utilization of vacant houses in Minamata City and measures to support immigrants." The members of the study group consisted of key people for local revitalization such as people who moved to Minamata for work, people who have returned to their hometowns, owners of vacant houses, shop owners, and specialists such as architects. The Minamata City Planning Division was used as the observer.

Minamata City is planning to formulate the "Second Phase Minamata City Draft Plan for Measures against Vacant Houses," which focuses on measures for specific vacant houses that are abandoned or difficult to live in.

However, we decided to study the use of vacant houses and support for immigrants as a set of measures.

The study group inspected vacant, renovated houses, and proposed their use and utilization.

Specifically, a "co-working space" utilizing an old private house was attempted. In addition, a list of host families was compiled to provide a place to stay for a short period of time for those who were considering moving to Minamata and to show them around town. Therefore, a support system is established.

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

Sawako Horai
(Department of Environment and Health)

In this study, we aimed to clarify the changes in ADL in patients with chronic Minamata disease and the differences in ADL decline over time depending on the disease type.

To obtain sustainable and reliable data in the future, it is essential to apply a highly objective evaluation method that can be compared with previous studies. In this fiscal year, we developed an evaluation method suitable for this study and clarified its work design. To evaluate methods for the ability to perform activities of

daily living (ADL), an instrumental ability to perform activities of daily living (IADL), N-type ADL rating scale for the elderly (N-ADL), and N-type mental status scale for the elderly (NM) were used to conduct a questionnaire survey on subjective symptoms. Based on this, we created a work design, applied for ethical review of the subjective symptom epidemiology research plan and obtained approval. Since 2021, there was a total of 11 patients who agreed to participate in the survey, including one adult-type patient, two pediatric-type patients, seven fetal-type patients, and one unknown. On the other hand, we visited four newly certified Minamata disease patients. Therefore, we would like to continue building a better relationship of trust and ask for cooperation in the survey. We believe that aroma therapy is an effective means of building a relationship of trust in patients when conducting ADL research. In May 2022, I obtained sports aroma trainer qualifications from the Japan Sports Aroma Trainer Association.

3.Risk Assessment Group

High-risk groups for methylmercury (MeHg) exposure include populations exposed to high concentrations of mercury and those with a high sensitivity to mercury. 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 theme 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)

(1) Reanalysis of mercury and selenium in Minamata disease-related samples (JSPS19K12353)

Selenium (Se) works protectively against methylmercury (MeHg) toxicity; however, its functional role in human MeHg intoxication remains unclear. We reanalyzed Minamata disease-related preserved samples, including patient organs, Minamata Bay sediments, seafood, and residual properties in the factory. Increased Se levels were observed in all specimens compared to controls. This is the first report of Se leakage from a factory and seafood/patients being exposed to Se simultaneously with high MeHg. However, the Hg/Se molar ratios in shellfish and the patient's brain were as high as 2–3, indicating that MeHg exposure exceeded the increased Se levels. In rat experiments with simultaneous exposure to MeHg and selenomethionine (SeMet) at a Hg/Se molar ratio of 3, brain Se levels increased, as was seen in patients, but the defensive effects of Se were limited. Sufficient effects were demonstrated at the same molar ratio as SeMet. Our study provides insights into the role of Se in protecting humans against MeHg toxicity.

(2) The Tapajós River basin in the Amazon region of Brazil is one of the most active gold mining areas in the world. In this study, we evaluated the fish consumption habits and Hg exposure in 110 pregnant women in Itaituba by measuring their total hair Hg concentration. Published in Arh Hig Rada Toksikol. 2022 (2):131-142.(IF＝2.078)

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

Megumi Yamamoto
(Department of International Affairs and Research).

(1) In our previous studies, we developed a Diet-Induced Obesity (DIO) mouse model based on 12 weeks of HFD feeding and evaluated markers of abnormal glucose metabolism (such as OGTT). However, because of the low pregnancy rate and difficulty in processing fetal samples, we were unable to obtain the necessary samples for analysis. We attempted to improve the specimen processing by mating after estimating the sex cycle using impedance measurements. We are currently measuring the mercury concentration in the tissues of mothers and pups obtained after MeHg administration.

(2) In collaboration with the University of Occupational and Environmental Health, we are conducting research on mercury in biological samples (hair, blood, placental tissue, and umbilical cord tissue) from mothers and infants with abnormal glucose metabolism (gestational diabetes mellitus and diabetic complicated pregnancy). Although sample collection was difficult due to the spread of COVID-19, 24

samples were obtained this year. Analysis of T-Hg in the obtained samples is currently underway.

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

Megumi Yamamoto
(Department of International Affairs and Research).

(1) Regarding the study on prenatal exposure to MeHg in Vietnam, we collaborated with counterpart researchers from Vietnam to translate the FFQs of 47 mothers recruited at Bach Mai Hospital by the end of FY 2019.
(2) Hair samples from mothers in Vietnam were obtained and analyzed for mercury.
(3) Fecal and nail samples stored at a collaborating research institute in Vietnam were obtained this year.
(4) Commercial fish and shellfish (9 species x 5 fish) from Hanoi were obtained and pretreated for mercury analysis.
(5) We conducted the training on mercury analysis for a Vietnamese counterpart researcher (National Institute of Occupational and Environmental Health).
(6) We conducted the training on mercury analysis for Indonesian counterpart researchers (Diponegoro University).
(7) We published a paper on an exposure assessment of MeHg among pregnant women (general population) in Semarang, Indonesia, conducted as international collaborative research (Muniroh et. al., Int. J. Environ. Res. Public Health, 2022).

[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 methylmercury toxicity, we investigated (1) the transfer of mercury and essential trace elements between dams and fetuses and (2) the concentration dependence of mercury and essential trace elements using the small Indian mongoose, which is a mercury-accumulating species. (3) The relationship between selenium compounds (selenoproteins and selenium-containing enzymes) and mercury concentrations in the blood of mongooses, cetaceans, and Taiji residents to characterize the dose-response relationships of mercury, selenium, and other essential trace elements in methylmercury-tolerant species.

Considering (1), the liver, kidney, brain, lungs, whole blood, and urine from 12 mothers, and the liver, kidney, brain, heart, lung, whole blood, and tail tip from 29 fetuses were collected. Mercury analysis was performed on all organ tissues except the heart, urine, and fetal tail. A comparison of total mercury and organic mercury concentrations between dams and fetuses revealed that total mercury concentrations in the liver and kidney, and renal organic mercury concentrations were significantly higher in the mother, whereas there were no significant differences in organic mercury concentrations in the liver, brain, lung, and whole blood. Next, to examine whether maternal mercury exposure affects mercury concentrations in fetal organs, we analyzed the relationship between organic mercury concentrations in the maternal blood and fetal organs (liver, kidney, lung, and brain). A significant and strong positive correlation was observed between these two variables. It has been suggested that organic mercury accumulates in the maternal blood, especially in the fetal liver. Analysis of the relationship between maternal blood and fetal brain organic Hg concentrations revealed a significant and strong positive correlation. In addition, the fetal brain to maternal blood ratios in the two groups were approximately 1 and 2, respectively. A comparison of the levels of mercury and trace elements in the liver and brain between the two groups showed a significant difference only in the total mercury and organic

mercury concentrations in the brain. In contrast, in addition to total and organic mercury concentrations, the Hg/Se molar ratio and arsenic concentration were significantly different in the liver. The findings of the analysis suggested that arsenic might be involved in brain organomercury levels and the hepatic mineralization of organomercury.

Regarding point (2), we could not clearly observe the distribution of Hg in the brain because of the sensitivity of the BL39 detector. In the future, we plan to employ histochemical observation techniques.

Regarding (3), it was possible to measure urinary selenium metabolites in the small Indian mongoose. Furthermore, as a result of measuring the urinary selenium metabolites of individuals with a relatively high total mercury concentration in the adult cerebrum and an Hg/Se molar ratio > 1 and those with an Hg/Se molar ratio of less than 1, there were differences in the levels of selenium metabolites, and some unknown substances were detected, suggesting the existence of species-specific selenium metabolism.

Measurement of blood selenium metabolites will be a topic for next year.

[5] Evaluation of methylmercury-induced neurological symptoms and the association of selenium compounds with toxicity in common marmosets (Fundamental research)

Chisato Kataoka
(Department of Environment and Public Health)

This study aimed to establish a basis for research on the improvement of motor function in patients with Minamata disease using an experimental non-human primate animal model (common marmosets).

In FY 2021, a preliminary study based on the experimental conditions established in a previous study (Yamamoto et. al., J. Toxicol. Sci. 2012), MeHg (1.5 mg Hg/kg BW) was administered to marmosets for 2 weeks, followed by 2 to 3 weeks without MeHg administration), and motor function (spontaneous locomotion and gait) was recorded by using video cameras. In FY 2022, we completed video analysis and showed that the marmosets experienced a decrease in spontaneous locomotion and a change in posture during gait from 2 to 3 weeks after the start of MeHg administration.

[Activity theme and summary]

[6] Information service using hair mercury analysis

Masaaki Nagano
(Department of Basic Medical Science)

In 2022, 133 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, the pamphlet on mercury was revised.

4.Nature Environment Group

The group research consists of one project, six fundamental studies, and two working activities. We conducted comprehensive research, including field studies, laboratory experiments, and various types of analyses, aiming to elucidate the environmental cycle of Hg at the global and regional levels, as well as in the Minamata Bay area. This research targets a wide range of media, including air, water, soil, sediment, and living organisms, and, in particular, a wide range of research topics covering low-level producers, such as phytoplankton, to large-scale consumers, such as swordfish. With regards to the field research, we monitored Hg levels in the atmosphere and precipitation in various regions of the Japanese islands, and marine observations were conducted in a wide range of areas from coastal areas such as Minamata Bay and the Seto Inland Sea to the open ocean in the northern Pacific Ocean. We also attempted to elucidate Hg emission sources and chemical reaction processes using stable Hg isotope ratios. In addition, our group is working with domestic and international research institutions to obtain comparable environmental Hg data, which will contribute to the evaluation of the effectiveness of the Minamata Convention. Simultaneously, we are actively engaged in outreach activities targeting students in elementary, junior high, and high schools for the purpose of education and dissemination of Hg research and other sciences and technologies.

The research outlines of each research and working activity for FY2022 are as follows.

[Research theme and summary]

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

(Project research)

Kohji Marumoto

(Department of Environment and Public Health)

The objective of this study is to understand the factors controlling the vertical distribution of Hg in the ocean, mainly in the North Pacific Ocean, and to obtain data that will contribute to formulation as much as possible for application to mathematical models. Therefore, we examined Hg fluxes between the atmosphere and ocean, and Hg uptake rates in phytoplankton, which are thought to influence variations in Hg concentrations in the surface and subsurface layers. In addition, factors that produce the relatively high concentrations of MeHg observed in the mesopelagic layer and Hg leaching from particles in the deep ocean will also be investigated.

In this fiscal year, we re-examined the pretreatment method for the analysis of speciated Hg in seawater and investigated the effects of different pretreatment methods on the analytical values. We also analyzed the Hg in seawater collected from the sea area near the Ryukyu Trench in February 2022 to determine the percentages of the four main forms of Hg: elemental Hg (Hg(0)), inorganic Hg compounds (Hg(II)), monomethylmercury (MMHg), and dimethylmercury (DMHg). Furthermore, water quality data were analyzed, and it was found that the level of anthropogenic Hg contamination in the area was not high.

Next, we obtained MMHg concentrations in plankton by size and seawater-plankton partition coefficients in the Oyashio region during the May 2021 voyage of the Hokko Maru but were unable to obtain information on seasonal variations as originally planned because of the contamination of equipment used during the voyage in October of the same year. In contrast, MMHg uptake experiments were conducted using green algae and cryptophytes as target strains for laboratory experiments, and the findings regarding uptake rates were obtained. Interesting results were obtained regarding the effects of water temperature on the

MMHg uptake rate.

For the air-sea exchange flux of Hg, data were obtained from observations in February 2022, July-August 2022, and December 2022 in the sea area near the Ryukyu Trench, 155°E meridian in the western North Pacific, and near the Izu and Ogasawara Islands, respectively. The data are currently being analyzed. In addition, a preliminary survey of Hg flux was conducted in Minamata Bay and the Yatsushiro Sea in May 2022 to compare it with open ocean areas; surveys have been conducted each season since November of this fiscal year.

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

(Fundamental research)

Akito Matsuyama

(Department of International Affairs and Research).

The outcomes of three study activities in 2022 are outlined as follows.

1) Investigation of Hg methylation in seawater.

As a result of the incubation experiments using Minamata Bay seawater, it was understood that mercury methylation occurred because of differences in seawater temperature. In contrast, Hg methylation did not occur at a seawater temperature of 17 °C with no relationship to the additional concentration of the carbon source.

2) Investigation of the distribution tendency of Hg in sediments accumulated in the Yatsushiro Sea and the origin of Hg in sediments.

The results of the Hg isotope analysis using MC-ICP-MS (multi-collector) analysis showed that there was no recognized difference in Hg isotopes from surface sediments and sediments 12 cm below the surface layer in the Yatsushiro Sea and the Amakusa Sea.

3) Understanding the changing characteristics of Minamata Bay sediments by seawater characteristics variation.

To understand the behavior of Hg in seawater, incubation experiments were carried out with N_2 bubbling and air bubbling, and glucose was added 10 times and 50 times the amount of carbon in Minamata Bay seawater. As a result of N_2 bubbling, the dissolved total Hg concentration increased with decreasing ORP, which was also related to the decreasing curve of the carbon concentration in seawater. At 50 times the carbon concentration, the ratio of the MMHg concentration to the total Hg concentration exceeded 90%, which is more than 10000 times the average value of the dissolved MMHg concentration in Minamata Bay.

[3] Bio-protection 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. Mineralization ability varies among animal species. Selenium (Se), an essential metal, plays a major role in mineralization; however, its full extent 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 that aggregate with Hg and Se. We utilized the unique ability of EPMA to determine the tissue distribution of Hg and Se in large marine organisms, particularly in toothed whales, sharks, and sailfish. This study focused on the relationship between the total Hg and Se concentrations in the organs of the giant silvertip shark (*Carcharhinus albimarginatus*).

[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 are the main producers in coastal

ecosystems. However, microphytobenthos on the sediment surface mainly contribute to the subtidal benthic production in Minamata Bay. Sediment contains a higher amount of total Hg (THg) than surface water, and microphytobenthos-derived Hg may cause relatively high Hg bioaccumulation in demersal fish. In this study, focusing on both phytoplankton and microphytobenthos, the seasonal dynamics, stable isotope values, THg content, and environmental factors influencing these properties were investigated. In this study, the seasonal dynamics of surface phytoplankton, microphytobenthos, and their carbon-transformed biomass were evaluated. Biomass was fundamentally higher in water column phytoplankton than in surface sediment microphytobenthos. However, the contribution of microphytobenthos was often comparable to that of phytoplankton during periods when the biomass was low. Moreover, the microphytobenthos biomass was seasonally stable, contrary to that of phytoplankton, which may make a high contribution of microphytobenthos to consumers in the food web of Minamata Bay. I also pursued a method of high-purity microphytobenthos collection from sediments using phototaxis. Although the purity of the collected samples was still unstable, analyses of high-purity samples showed that the THg content of microphytobenthos was not always higher than that of phytoplankton, varying depending on the season and year.

[5] Characterization of stable isotopic composition of mercury at the emission source

(Fundamental research)

Satoshi Irei

(Department of Environment and Public Health)

The objective of this research project was to characterize and report the initial stable isotopic compositions of Hg at the emission and discharge sources. This achievement contributes to a better understanding of the global Hg cycle using stable isotope ratio measurements.

In this fiscal year, we focused on open grass field burning (Noyaki) studies, and our goals were set to continuously conduct total gaseous Hg (TGM) sampling from open grass field burning, analyze Hg concentrations and its stable isotope ratios, as well as inorganic ions containing PM from field burning, and publish results from these studies as original research articles in internationally well-established scientific journals. We also re-evaluated and re-designed the experiments for the open grass field burning studies.

The analytical results demonstrated that the concentrations increased by a factor of three or more compared with the background atmospheric concentrations of Hg. These observations indicated the emission of gaseous Hg from Noyaki into the atmosphere. The stable Hg isotope ratios also implied that the isotopic compositions were consistent with the reported isotope ratios found in other plant species. Based on these findings, we posed the following questions: 1. What is the origin of observed Hg? Was atmospheric Hg deposited on the ground or was Hg actively taken up by plant species and fixed in them? 2. How much Hg is emitted from the atmosphere? To answer these questions, a research team, including scientists from other research institutes, was organized in the middle of this fiscal year, and we updated our objectives to estimate the burnt area of Noyaki, which will be delivered by the analysis of land images obtained before and after Noyaki, and by measuring carbon monoxide and dioxide to gain the emission factors of Hg. The project with updated objectives started in February of this fiscal year, and the results we gained will contribute to a better understanding of the Hg cycle in open grass fields and the Earth and provide good examples of stable isotope research in the geoscience field.

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

(Fundamental research)

Yuya Tada

(Department of Environment and Public Health)

The objective of this study was to evaluate microbial Hg speciation (methylation, demethylation, and reduction) in the ocean using metagenomic and molecular biology methods. This year, I have performed [1] a metagenomic analysis of Hg speciation genes (*hgcAB*: Hg methylation, *merB*: MeHg demethylation, and *merA*: Hg(II) reduction) as well as estimation of THg and MeHg concentrations for samples collected in the subtropical gyre in the Western North Pacific (WNP), [2] qPCR analysis to determine the Nitrospina-*hgcA* copy number in seawater collected from the Oyashio region in 2018. The metagenomic analysis revealed that the *hgcAB*, *merB*, and *merA* genes were abundant at depths of 1000–1500 m, suggesting that microbial Hg methylation, MeHg demethylation, and Hg(II) reduction could occur simultaneously in the mesopelagic layers in this region. Phylogenetic analyses of these genes showed that the Nitrospina-like *hgcA* was dominant in this region. Alphaproteobacteria and Gammaproteobacteria were abundant in *merB* and *merA*, respectively. These data suggest that distinct phylogenetic lineages may contribute to Hg speciation in the subtropical gyre of the WNP. The qPCR analysis revealed that Nitrospina-*hgcA* was abundant in the mesopelagic layers of the Oyashio region. These results supported our previous metagenomic data.

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

(Fundamental research)

Kohji Marumoto

(Department of Environment and Public Health)

Continuous monitoring of Hg in the air was conducted in Minamata City and Fukuoka City to obtain continuous data on Hg concentrations in the air and precipitation, which will contribute to the evaluation of the effectiveness of the Minamata Convention. Continuous monitoring of speciated Hg in the air was also conducted at Sensuikyo, located north of the first crater of the Aso Nakadake volcano, to investigate the spreading and deposition processes of Hg emitted from the volcano, as in the previous year.

Observations at the Aso Sensuikyo showed that the concentrations of gaseous elemental Hg (GEM), gaseous oxidized Hg (GOM), and Hg in atmospheric particles less than 2.5 μm of diameter ($PBM_{2.5}$) tended to be higher during periods when volcano was considered to be active. The increase in $PBM_{2.5}$ was particularly pronounced, with high concentrations observed one–two weeks before large-amplitude volcanic microtremors were observed. Therefore, this may be an important parameter for understanding volcanic activity, and we will continue to observe and accumulate such data.

Monitoring of Hg in precipitation was continued at six sites including Minamata, Hirado, Fukuoka, Matsue, Omaezaki, and Tsukuba. To monitor Hg concentrations in precipitation, we participated in an international comparison program conducted by the U.S. Geological Survey in response to the Minamata Convention. In addition, we conducted joint research with the National Central University of Taiwan on comparative experiments using the precipitation samplers used in Japan and Taiwan to ensure data reliability.

[Activity theme and summary]

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

Akito Matsuyama

(Department of International Affairs and Research).

The outcomes of 2 activities of CT in 2022 are outlined below.

1) Mercury monitoring in Minamata Bay

Mercury monitoring was performed twice (in June

and October) in Minamata Bay in 2022. The seawater sampling points were at Hadakase, Wanoh, and Koijishima. Mercury monitoring at Shinsui Park was performed twice (June and September). Seawater sampling was performed at five points between the Minamata Disease Information Center and the ferry stop. The annual average value of dissolved total mercury concentration in Minamata Bay seawater was 0.41± 0.03 ng/L (2022). The concentration of dissolved methylmercury concentration was 0.16 ± 0.02 ng/L. The annual average dissolved total mercury concentration in Shinsui park is 1.52 ± 0.64 ng/L.

2) Oyster cultivation experiment using aquaculture raft in Fukuro Bay in support of various activities in Minamata area

To conduct this experiment, we prepared three cultivation backgrounds, which were set at the surface(0m), -3m, and-5m as the seawater depth. The experimental period was initiated in the beginning of April, and the number of oyster shells in each aquaculture basket was 700. The weight, length, width, and thickness of 65 randomly selected oyster shells from each aquaculture basket were measured once a month. Red tides occurred in the Yatsushiro Sea from July 2022 until head of September. Many oyster shells died due to the effect of the red tide in comparison with the previous year. This affection was largest at seawater depth -3m in Fukuro Bay, then almost more than 80% of the oyster shell of -3m aquaculture basket dead. The minimum influence of the red tide was on the surface layer(0m). Although the number of dead oysters in the surface layer increased compared with that in the previous year, the oyster growth level was almost the same as that in the previous year.

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

Masumi Marumoto
(Department of Basic Medical Sciences).

Research scientists and institutions are expected to disseminate scientific knowledge and results to the public. Kumamoto Prefecture, where Minamata City is located, is known both domestically and internationally as the area where the Minamata disease occurred, but elementary and junior high school students in Kumamoto have limited knowledge of Hg itself. Therefore, we will provide correct knowledge about Hg by conducting classes at elementary and junior high schools in and out of Minamata City. To emphasize interactivity rather than one-way transmission from the researcher, a question box was set up before the delivery class, and a questionnaire was administered to answer questions in advance of the presentation. Contributing to the community by responding proactively to cases other than delivery classes on Hg. This year, we provided guidance not only to elementary and junior high schools but also to high school students in on-site classes and exploratory activities. Additionally, we conducted classes in collaboration with researchers affiliated with the Shiretoko Foundation and the UNEP.

5.International Contribution · Information Group

Mercury causes environmental pollution, which is a serious problem in developing countries. This pollution can harm both humans and the environment. To address this issue, the Minamata Convention was created in October 2013 by delegates from 140 countries, which came into effect in August 2017. NIMD's International Contribution Information Group works with foreign researchers to study mercury and shares information through the NIMD forum. They also collaborated with the Japan International Cooperation Agency (JICA) to share knowledge, experiences, and the latest technology with developing countries facing mercury pollution. The NIMD collaborated with UNEP and WHO and also provide reference materials, training programs, and proficiency testing to improve mercury-related research and monitoring. This collaboration aimed to continually improve the quality of data collection and analysis. The MOYAI Initiative, a simple, robust, cost-effective mercury analysis method, was developed as part of the Minamata Convention. This overview summarizes the research and activities of our group during FY 2022.

[Research theme and summary]

[1] Development of a simple method for the determination of mercury and reference materials
(Project research)

Koichi Haraguchi
(Department of International Affairs and Research).

Our research project consists of the following three major parts:

1) Development of a simple method for the determination of mercury compounds
2) Development of reference materials to evaluate the accuracy of mercury analysis
3) Providing proficiency testing to help improve quality data collection and analysis

The MOYAI Initiative provides technical assistance to ensure the effective implementation of the Minamata Convention. For resource-limited countries, it is important to have an affordable and straightforward method to analyze mercury levels in individuals exposed to mercury through gold mining, skin-lightening creams, and other means. Urinary mercury concentration is a suitable biomarker for assessing mercury exposure. However, it can be affected by bacterial growth during storage and transportation, causing loss of metallic mercury. To address this issue, we investigated the conditions that affect mercury adsorption onto porous gold particles to efficiently amalgamate divalent mercury. By studying the use of elemental mercury in traditional Buddha statues, we developed a simple inorganic mercury biosampling kit and compared it to the conventional method of directly measuring urinary mercury concentrations.

We are also developing a blood reference standard to assess prenatal mercury exposure, as part of an evaluation of the effectiveness of the Minamata Convention. We collaborated with testing laboratories to determine the values for certification, and the testing will be completed the following year. We monitored the characteristic values of the standard materials that we developed and provided existing users with information about the guarantee period through our website.

Each country must enhance its analytical capabilities to effectively evaluate the Minamata Convention. We collaborated with the United Nations Environment Programme (UNEP) and provided an evaluation report on the total mercury analysis capabilities of 26 hair testing laboratories in the Asia-Pacific region. We identified any problems with the participating laboratories. We plan to release a training video on analytical precision management by the end of the fiscal year. At the International Mercury Conference, we organized a UNEP-NIMD workshop on international mercury management methods. We

prepared a sample using sediment as the base material for testing the third proficiency.

[Activity theme and summary]

[2] Examination of hair mercury in areas concerned with mercury pollution around the world

Masatake Fujimura
(Department of Basic Medical Science)

In this fiscal year, we measured mercury levels in the hair of residents living near e-waste dumping sites in Bangladesh, and one paper was accepted for publication as a co-author. Consequently, we were able to dispel the concerns of the local residents by showing that internal exposure to mercury in the vicinity of the e-waste dumping site was almost nonexistent.

In addition, as a member of the steering committee of the International Conference on Mercury as a Global Pollutant (ICMGP), we organized a special session on mercury in hair and storytelling at the ICMGP2022 conference held this year and presented one paper as a co-presenter. Consequently, we were able to inform the world of the current health risks associated with mercury contamination at a global level.

[3] NIMD forum and international workshop

Megumi Yamamoto
(Department of International Affairs and Research).

The NIMD Forum in FY2022 was conducted by the Pathomechanism Group as follows:

Theme: Toward Prevention of Methylmercury Poisoning. Presenters: Three NIMD staff members, four domestic researchers, and overseas researchers (USA,2; Brazil,1; France,1). Spain: 1). Venue: Minamata Disease Archives.

Subjects: Protective Effect of Oleanolic Acid-3-Glucoside on Methylmercury Toxicity; Bacteria as Dietary Supplements to Mitigate Damage Associated to Mercury Exposure; Usefulness of the Functional Food Ingredients on Reducing Methylmercury Burden: Wheat Bran and Fructooligosaccharides; Microbial Derived Factors in Methylmercury Demethylation and Reduction of Toxicity; Roles of DHA and Its Metabolites in Protection against Methylmercury-Induced Neurotoxicity; The Human LRRK2 Modulates the Age-Dependent Effects of Developmental Methylmercury Exposure in Caenorhabditis elegans; Development of a Sensor for the methylmercury Toxicity; Demethylation of Methylmercury in Bird, Fish, and Earthworm; The importance of Rabenstein's Reaction in the Toxicity of Methylmercury: What is missing? capture of methylmercury by supersulfide species; protective function of sulfides against methylmercury toxicity.

Participants November 29 (actual attendance:46. Online audiences: up to 39 (average 28). Media: 3 persons. November 30 (actual attendance:27; online listening: maximum. 20 (average 28); media:3) November 30 (actual attendance:27; online listening: max. 20 (average 13))

[4] Promotion of international collaborations

Megumi Yamamoto
(Department of International Affairs and Research).

Based on lessons learned from Minamata disease and Japan's mercury countermeasures, 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 related to mercury worldwide.

This year, several studies and tasks were conducted. (1) Exposure assessment of methylmercury for pregnant women in Hanoi, Vietnam; (2) Training of mercury analysis for researchers at the National Institute of Occupational and Environmental Health, Vietnam, and Diponegoro University, Indonesia; (3) Exposure assessment of metallic mercury to gold plating factory workers in Nepal; (4) Continuous monitoring of mercury in the air at the APMMN (Asia-

Pacific Mercury Monitoring Network), and presentation at the online meeting (11th APMMN annual meeting, Nov. 2022); and (5) Training of mercury analysis in the Republic of Nicaragua for five Central American countries (Uruguay, Belize, Honduras, Guatemala, and Panama) as a JICA cooperative project.

In addition to online training and participation in meetings, we plan to continue promoting international collaboration by visiting countries with environmental problems related to mercury and inviting researchers and technicians.

[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 was the second year in which three public museums in Manabi-no-oka introduced VR. In addition to these museums, NIMD approached the private museum, Minamata Disease Museum at Soshisha to introduce VR, and this was accomplished.

The introduction of VR strengthened the appeal of not only MDA but also of all Minamata disease-related facilities located in Minamata City.

Since last year's special exhibition was well received, we have renewed it. The title was "Regional and International Contributions by NIMD - As the Only institute in the World to research about Minamata Disease and Mercury. We established a system to smoothly change the exhibitions.

The MDA devised ways to provide user-oriented services, including the creation of an MDA logo, updating pamphlets and videos, and the creation of interpretive panels and VR corners.

Furthermore, we took the initiative to expand the scope of cooperation with other institutions related to Minamata Disease and achieved success by increasing awareness of the MDA, improving convenience, and significantly increasing the number of visitors (especially users of the reference room), even though COVID-19 had not yet converged.

[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 mercury. During this fiscal year, we performed the following tasks:

(1) Attendance to "The Fourth Regional Forum of the WHO Collaborating Centres in the Western Pacific" (November 28-29, 2022, Cambodia), and gave a poster presentation on "the transfer of mercury analysis technology to the National Institute of Occupational and Environmental Health, Vietnam (VTN-3)," "Supports for the fire at a fluorescent lamp manufacturing plant in Vietnam," and "the preparation of certified reference materials for hair and urine at the NIMD."

(2) At the above forum, meetings were held with the following WHO Collaborating Centers to discuss collaboration: (1) National Institute of Occupational and Environmental Health, Vietnam (VTN-3) for cooperation on the transfer of mercury analysis technology; (2) Niigata University (JPN-75) for disposal of dental amalgam; (3) Tokyo Medical and Dental University (JPN-73) for cooperation on the transfer of mercury analysis technology to the Tanzanian graduate student; (4) National Center for Global Health and Medicine (JPN-94) for an epidemiological study on mercury exposure under COVID-19 infestation; 5. Singapore Food Agency (SIN-17) for participating in proficiency tests on mercury analysis: Collaboration between NIMD and UNEP.

(3) The WHO CC annual report on the activities of the WHO CC from January to December 2022 was prepared, and the procedure was performed.

6. publications and Scientific meetings

［International Journals］

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

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［International meetings］

Underline: NIMD staff,

Haraguchi K. Laboratory proficiency testing for mercury‐strengthening mercury analytical capacity meeting international standards. Minamata Online, side event COP4, Web meeting, 2022. 6. 招待講演

Haraguchi K. Laboratory proficiency testing for mercury. 15th International Conference on Mercury as a Global Pollutant, Web meeting, 2022. 7.

Haraguchi K: Laboratory proficiency testing. UNEP Workshop for Promoting Minamata Convention on Mercury by making the most of Japan's knowledge and experiences, Minamata, 2023. 2. 招待講演

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