

NIMD Annual Report
2021
(April 2021 to March 2022)

National Institute for Minamata Disease
Ministry of the Environment
Japan

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Report on Research and Other Activities
in the Fiscal Year 2021

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 2021.

[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 conducted a comprehensive analysis of gene expression of "cerebrocortical neurons" and "hippocampal neurons" isolated and cultured from rat brain, and found factors specifically expressed in hippocampal neurons that are resistant to MeHg toxicity (Transthyretin, BDNF etc.). We also analyzed the vulnerability/resistance of these neurons to toxic substances other than MeHg (such as glutamic acid), and found hippocampal neurons to be vulnerable to glutamate toxicity. The above research results were presented at meetings of academic societies, and have been submitted to a peer-reviewed journal.

- (2) Research on individual susceptibility to MeHg neurotoxicity and related biomarkers
In order to search for more sensitive predictors of MeHg toxicity, we aimed to develop a method for

measuring polythiolization of blood proteins in MeHg-intoxicated model rats.

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

Previous studies on the pathogenic mechanisms of neuropathic pain in MeHg-intoxicated model rats were accepted/published in a peer-reviewed journal,

- (4) Joint research with external research institutes

The results of joint research with external research institutes were presented at meetings of academic societies, and were accepted/published in peer-reviewed journals.

- (5) Others

The requested writing of the "MeHg Neurotoxicity Chapter" was accepted/published in an academic book (3rd Edition of Handbook of Neurotoxicity).

2. Study on reducing the health risk of methylmercury by food ingredients
(Fundamental research)
Masaaki Nagano
(Department of Basic Medical Science)

The aim of this study is to reduce the health risk of MeHg by utilizing the function of food ingredients. So far, we demonstrated that daily intake of wheat bran or fructooligosaccharides significantly reduced mercury concentration in tissue of mice exposed to MeHg.

In FY 2021, in order to evaluate the effect of food ingredients on the health risk of MeHg, we established experimental method in mice. In addition, the binding of MeHg with tea and wheat bran components was examined *in vitro*, no binding of MeHg with either green tea or black tea components was observed at the concentrations that we drink. On the other hand, 10% of the added MeHg was found to be combined with or adsorbed to lignin, a wheat bran component.

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 mechanism underlying the toxic effect of MeHg on the nervous system. In our previous study, we had elucidated the mechanism of maintenance of redox homeostasis by certain reactive sulfur species (RSS), low molecular weight molecules with releasable sulfane sulfur. Sulfane sulfur easily transfers to protein thiols to form the respective persulfide/polysulfide species. MeHg can covalently modify protein thiols, causing their dysfunction and thereby exerting deleterious effects. Our research aims to assess the physiological role of sulfane sulfur-mediated modification of protein thiols in the nervous system during MeHg exposure.

In FY 2021, we investigated the transition of sulfane sulfur in cellular proteins by alkylating agent-based pull-down assay during RSS and MeHg exposure. Incubation of SH-SY5Y cell lysate with model polysulfide Na_2S_3 increased the level of protein polysulfidation, whereas further incubation with MeHg decreased the level in vitro. Exposure of SH-SY5Y cells to Na_2S_3 increased polysulfidated proteins while exposure to MeHg decreased polysulfidation of proteins, such as glyceraldehyde-3-phosphate dehydrogenase. The results suggested that sulfane sulfur in low-molecular compounds readily transfers to protein thiols and the protein-bound sulfane sulfur then acts as a bait to form a sulfur adduct, such as $(\text{MeHg})_2\text{S}$, protecting cysteine residues from modification by MeHg.

4. Development of a sensor for the methylmercury toxicity and research on the mechanism of the

methylmercury neurotoxicity

(Fundamental research)

Akio Sumioka

(Department of Basic Science)

Exposure to methylmercury (MeHg) induces oxidative stress in the brain and consequently causes neurodegeneration. However, mechanisms underlying cell specificity and stage-dependency of MeHg toxicity remain unclear. Therefore, it is necessary to monitor at an appropriate stage for investigating neurotoxicity with translocation and exertion MeHg in the brain. Consequently, we developed a new sensor vector Krab-U/Luc for detecting MeHg toxicity and studying MeHg-mediated dysfunction of synapse and axon.

This year, we validated Krab-U/Luc as a MeHg sensor vector. Luciferase activities mediated by Krab-U/Luc were stronger than the signals by ER stress sensor ERAI-Luc and the oxidative stress sensor Nrf2-Luc. Krab-U/Luc showed signals for MeHg specifically among other oxidants, including hydrogen peroxide, DEM, and metal ions. Krab-U/Luc also responded to MeHg in a dose-dependent manner.

For generating the Krab-U/Luc sensor mouse, each cDNA was combined to a vector with a selection marker gene. Unfortunately, one vector sensor lost its sensitivity to MeHg. We addressed the cause of this incompetency, and identified a non-specific suppression mediated by Krab.

Previously, we had found that MeHg abnormally accumulates extra-synaptic AMPA-type glutamate receptors (AMPA) at the synapse. This year, we investigated the molecular mechanisms underlying this accumulation. Based on experiments with glutamate receptor inhibitors, glutamate signals, including NMDA-type glutamate receptors, were concluded to not induce AMPAR accumulation. However, synaptic scaffolding proteins were found to have increased during the AMPAR accumulation.

2. Medical practice • Welfare • Society Group Research

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

Moreover, effective therapies are required for intractable chronic pain, tremor, ataxia, and spasticity, since they affect the quality of life of patients with MD. We have started a clinical study on repetitive transcranial magnetic stimulation (rTMS) therapy for neuropathic pain.

To create a detailed plan for regional revitalization in the area for MeHg pollution victims, we have started a multidirectional analysis of issues related to MeHg pollution victims.

The research conducted by our group during FY 2021 is outlined below.

[Research theme and summary]

1. Research on health effects of methylmercury exposure in humans and therapeutic development

(Project research)

Masaaki Nakamura

(Department of Clinical Medicine)

To develop an objective evaluation protocol using MEG and MRI, the somatosensory-evoked fields and morphological features of patients with MD were compared to those of control subjects from the Kumamoto district (not polluted with MeHg).

Using MEG, we found that the early cortical somatosensory processing, indexed by N20m

amplitude, the reproducibility of N20m in single-trial responses, and the induced gamma-band oscillations in the primary somatosensory cortex (SI) were useful for discriminating between MD and controls. This year, we were able to discriminate MD from controls with a sensitivity of approximately 70% by improving the analysis method of the induced gamma-band oscillations. Since connectivity in the SI may be impaired in patients with MD due to reduced induced gamma-band oscillations in SI, we are currently developing a script to evaluate the network of SI.

In MRI of brain structures in patients with MD, voxel-based morphometry analysis showed atrophy of the cerebellum, margin of the calcarine sulcus, and the thalamus. The areas with particularly significant atrophy included margin of the calcarine sulcus in the adult type, the cerebellum and margin of the calcarine sulcus in the child type, and the thalamus in the fetal type.

In the treatment study (single comparative study confirming the effectiveness of magnetic stimulation for neuropathic pain, including in patients with MD), we observed that repetitive transcranial magnetic stimulation (rTMS) was effective for pain in the right side of the body after left capsular hemorrhage, and that analysis of sensory gating could be useful in evaluating the therapeutic effect. In future, we plan to establish optimal rTMS and objective evaluation methods for the therapeutic effect.

2. Area studies regarding regional revitalization of Minamata City and neighboring cities

(Fundamental research)

Rie Harada

(Department of International Affairs and Research)

The areas of interest included in this research are as follows: (1) understanding the actual situation of

damage caused by Minamata disease, (2) digging up regional welfare issues, (3) conducting local studies and area studies to understand how regional revitalization can solve the issues, and (4) to ensure community development via art, as an advanced and experimental approach. In other words, this research is conducted from multiple perspectives.

Regarding (1), the paper "A study on the social environment of patients with fetal-type Minamata disease: an analysis of past interview data" was published in "Journal of Environmental Sociology Vol.27, 2021".

The abnormal pregnancy survey was conducted about one-third of the planned hearing

Regarding (2), I published two conference reports with my collaborators at Japanese Research Association for Community Development. The titles were "Verification of Initiatives for Community-based Welfare Issues in Minamata City: from Subregional Networks to New Developments" and "Issues for Carer Support in Minamata City: from the Minamata City Community-based Welfare Needs Survey". After a historical overview of Minamata City's community-based welfare policy, the latest issues were considered from the data of the "Community Welfare Needs Survey in Minamata City", conducted for central and mountainous areas. Thereafter, the survey target was expanded to the coastal areas and additional survey was conducted accordingly.

The JIMOTOGAKU survey in Shiraoi-cho, Hokkaido, as mentioned in (3), was postponed twice due to COVID-19, and was eventually canceled this year.

Regarding (4), we are cooperating with the Tsunagi Art Museum project and continuing to observe the participants. With Philippe Chéhère, Julie Salgues (Kachashi Dance Association), Aya Kasai (Miyazaki International College), Nonoko Kameyama (Freelance photographer), and others, "Case Study of

La danse du détour: A collaborative arts performance with people touched by Minamata disease" is being drafted and formatted for submitting to 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 application of these concepts in the community. After completing the projects, we continued supporting welfare activities in the cities of Minamata and Izumi.

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) to maintain and increase muscle strength in the lower limbs, and neuromuscular electrical stimulation (NMES) for dysphagia and dysarthria in patients with MD.

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

Based on the comprehensive cooperation agreement with Minamata City, we supported the survey related to regional revitalization, including "Moyainaoshi", in collaboration with Minamata City. This year, to obtain basic data that would contribute

to the improvement of ADL in chronic MD, we started a survey to clarify the 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 provides an outline of the clinical and social practice conducted by this group in FY 2021.

[Activity theme and summary]

1. Local welfare support service

Masaaki Nakamura
(Department of Clinical Medicine)

We conducted a study titled “Home support model study, including care prevention” (FY 2006–2008) to identify support methods, such as rehabilitation and improvement of ADL for aging patients with MD and their families. Following this project, we undertook two other projects titled “Community development project for home care support, including healthcare practice” (FY 2009–2011) and “Community welfare promotion business for supporting MD victims” (FY 2012) to develop methods for implementing the concepts in the community. Subsequently, we continued implementing educational activities in the cities of Minamata and Izumi to provide an opportunity for occupational therapy. Through this support, we strengthened the connection between our institute and the local community.

We improved the craft class program based on responses to a questionnaire. As a result, we achieved high satisfaction levels of the participants in a subsequent survey. However, the participation increased only slightly due to the COVID-19 pandemic.

After negotiations between the Ashikita Town and Tsunagi Town, activities in the latter are set to begin in the next fiscal year.

We participated in the "Minamata-Ashikita

Regional Health and Welfare Network for Minamata Disease Victims" to deepen cooperation with the local community; however, activities of the "Moyai Music Festival Executive Committee" were cancelled due to COVID-19.

Furthermore, we created pamphlets about the craft class and publicized its activities and schedule on the homepage and Facebook page of our institute to encourage many local residents to participate in the care prevention project. We also explained the care prevention project to local residents at the NIMD forum.

2. 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 patients with Minamata disease (MD) in order to improve their quality of life (QOL). The robot suit HAL that had been implemented in the past was reviewed and changed to the HAL single-joint type (for medical use), which enabled rehabilitation for the elbow, knee, and ankle joints. Rehabilitation focusing on gait disorders was continued by combining peripheral magnetic stimulation, belt electrode skeletal muscle electrical stimulation, and non-powered walking aids, which were introduced in the previous fiscal year. In addition, an intervention using neuromuscular electrical stimulation (NMES) was conducted for a patient with MD as rehabilitation for dysarthria.

The annual workshop on rehabilitation and welfare techniques was postponed due to the COVID-19 pandemic, and alternative methods for holding the workshop were discussed.

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, twice a month. At the Orange hall, we held physical and cognitive function evaluations, ADL evaluations, and health exercise classes for local elderly people, including patients with MD.

3. Information dissemination using Minamata disease pathology specimens

Masumi Marumoto
(Department of Basic Medical Sciences)

Pathological tissue specimens from autopsy of patients with Minamata disease are extremely valuable. Our institute has preserved a lot of pathological tissue specimens of Minamata disease permanently. However, pathological tissue slides are difficult to preserve permanently owing to their tendency to fade with the passage of years. Therefore, we aim to digitize the pathology slides for permanent preservation and to publish the digitized pathology tissue slides in the website for the benefit of students and doctors learning pathology. At the same time, we will create a pamphlet explaining Minamata disease pathology for the general public and children. In addition to histopathological specimens, our center holds a large number of valuable pathological samples, and we will continue to organize and preserve them.

4. Needs survey of the agreement for comprehensive cooperation with Minamata City

Rie Harada
(Department of International Affairs and Research)

This year, we have planned to clarify the activity related to the agreement and conduct a needs survey, in order to get the basic data for making policies for Minamata City administration; we changed the activity name according to the activity content.

Continuing from last year, we conducted inspections and hearings on measures for vacant houses and support for migrants, which were the

priority issues of the Minamata City Planning Division Regional Promotion Section.

In addition, we hosted the "Study group on utilization of vacant houses in Minamata City and measures to support immigrants". Minamata City Planning Division participated as an observer. We gathered opinions on increasing the number of immigrants to Minamata from community leaders and key persons, such as migrants, transferees, U-turners, vacant house owners, architects, and shop owners.

Accordingly, we decided to continue conducting public participation-type inspections and hearings, and holding study groups, rather than waiting for steps by the local government alone.

In response to requests from migrants, a bulletin board for migrants will be set up in a store in the central city area, in future, and in response to voices from teleworkers, a coworking space and HUB center, utilizing an old private house, will be started as a pilot case.

As a measure against vacant houses, conducting public relation activities there was considered in cooperation with the city.

5. Temporal trend analysis of activities of daily living (ADL) in patients with Minamata disease based on the disease-type

Sawako Horai
(Department of Environment and Public Health)

The purpose of this study is to clarify the difference in ADL changes in patients with chronic Minamata disease (the features of differences in ADL decrease depending on the disease type), and to evaluate the effect of aromatherapy on pain in the patients.

In order to obtain long-term, sustainable, and reliable data, it is essential to apply a highly objective evaluation method that is comparable to many previous studies.

In this fiscal year, we selected the evaluation

method suitable for this work and clarified the work design. As an evaluation method for ADL (Activities of Daily Living), IADL (Instrumental Activities of Daily Living), N-ADL (ADL Evaluation Scale for N-type Elderly), and NM Scale (Mental State Scale for N-type Elderly) were selected, and for an objective evaluation method for pain, the degree of pain (VAS), properties (SF-MPQ-2), and psychological state evaluation (BDI) were selected. Scale for the Assessment and Rating of Ataxia (SARA) was also selected to evaluate ADL deterioration. We plan to conduct a questionnaire survey on subjective symptoms. This research plan has been approved by the Research Ethics Committee as an epidemiological research plan. We have applied for ethics review of clinical research plan for a study on verifying the effectiveness of aroma treatments for pain relief. The number of patients who consented to the survey included 1 adult-type, 2 pediatric-types, 7 fetal-types, and 1 unknown-type patient, making a total of 11 patients. In order to qualify as a sports aroma trainer, I have passed the on-the-job and written exams held in May 2022 and will obtain a diploma in August.

3. Risk Assessment Group

High-risk groups for methylmercury (MeHg) exposure include populations exposed to high concentrations of Hg and groups with high sensitivity to Hg. The risk assessment group aims to comprehensively study the assessment of human exposure to Hg and the effects of environmental pollution on health. We will conduct both epidemiological and experimental studies, assessing 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 in vulnerable populations, such as fetuses, children, and those with diseases.

[Research theme and summary]

1. Studies on coexistence of mercury and selenium in Minamata disease and effects of methylmercury on 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)

Increased selenium concentrations were confirmed in all historical samples, such as the preserved sludge near the outlet to Minamata Bay, organs of cat No. 717 (a historical cat that showed Minamata disease-like symptoms by administering acetaldehyde distillation residue solution in 1961), the muscle of a red sea bream, and shellfish meat. The Hg/Se molar ratio exceeded 1 in all samples, suggesting substantial MeHg pollution in Minamata Bay. Therefore, patients might have been exposed to considerable amount of MeHg and high Se at the time of pollution. Increased Se was observed in all organs of patients; the Hg/Se molar ratio in the brain of patients was higher than 1, suggesting that they

were exposed to extremely elevated levels of MeHg. Increased Se in brain was lower than that in other organs, indicating that the increase in Se content was insufficient to protect the brain against MeHg.

(2) Plasma and red blood cell distribution of total mercury, inorganic mercury, and selenium in maternal and cord blood collected from a group of Japanese women

Fifty-five pairs of maternal and cord blood samples, obtained at parturition, were collected from pregnant women in Fukuoka, Japan. The study evaluated the background factors that might modify a fetus's susceptibility to methylmercury (MeHg) exposure in a fish-eating population by comparing the profiles of total Hg (THg), inorganic Hg (IHg), and Se in plasma/red blood cells (RBCs) between maternal and cord blood. Furthermore, correlation of THg in RBCs, which is a biomarker of MeHg exposure, with THg in plasma, which is a biomarker for IHg exposure, was examined in maternal and cord blood. Environ Res. 2021 May;196. (IF: 6.498)

(3) Contribution of breast milk to mercury levels in the tissues of rat pups examined by cross-fostering at birth

The developing perinatal brain is vulnerable to methylmercury (MeHg) exposure. Contribution of breast milk to tissue MeHg levels in offspring is a significant public health concern, since breast milk contains a certain amount of MeHg. In this study, the contribution of MeHg, transferred via breast milk, to the Hg levels in the tissues of pups (Wistar rats) was investigated. mated female rats were fed a MeHg (2 ppm)-supplemented or a control diet during pregnancy. Following parturition, male neonates from each group were cross-fostered between exposed or control dams, and further raised by dams fed a MeHg-supplemented diet or a control diet

during lactation. Our results suggested that if the MeHg exposure level during pregnancy is not high enough to cause neuronal development defects in the fetus, the exposure via breast milk is not a significant concern. Environ Res. 2022 Jan 18; (IF:6.498)

2. Improvement of the exposure assessment system for groups at high risk of methylmercury exposure
(Fundamental research)

Megumi Yamamoto

(Department of Environment and Public Health)

(1) In FY2020, a diet-induced obesity (DIO) mouse model was obtained by feeding a high-fat diet for 12 weeks and evaluating the markers of abnormal glucose metabolism (such as HbA1c). In FY2021, DIO model mice were created under the same conditions with FY2020, and experiments related to mating, pregnancy, and delivery conditions were also conducted (“Experiment-1”). However, due to low pregnancy and delivery rates and abandonment of offspring, we were unable to obtain the necessary samples for analysis. Therefore, in “Experiment-2”, we mated DIO model mice after estimating the sexual cycle (optimal timing for mating) by measuring intravaginal impedance. Since improvement was observed from Experiment-1, we are now measuring the concentration of total Hg in the tissues of both the mother and fetuses after the administration of MeHg.

(2) Research on Hg levels in biological samples (hair, blood, placental tissue, and umbilical cord tissue) of mothers and infants with glucose metabolism disorder (gestational diabetes and pregnancy complicated by diabetes) is being conducted in collaboration with the University of Occupational and Environmental Health. The spread of COVID-19 made sample collection extremely difficult. The total Hg content in these samples is currently being analyzed (in FY 2021, 17 samples

were collected).

3. Exposure assessment of mercury and technology transfer in developing countries

(Fundamental research)

Megumi Yamamoto

(Department of Environment and Public Health)

- (1) For the survey of pregnant women in Hanoi, Vietnam, we proceeded with analysis of the 48 food frequency questionnaires (FFQs) obtained from respondents recruited by the end of FY 2019.
- (2) Hair samples from 48 mothers were obtained and total Hg levels were measured.

4. Relationship between 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)

The purpose of this study is to elucidate the biological defense mechanism against methylmercury toxicity in small Indian mongoose, which accumulates high levels of mercury, under the following three topics: (1) transfer of mercury and essential trace elements between dam and fetus, (2) changes in mercury distribution and selenoprotein expression in brain with dependent mercury concentration, (3) analysis of the relationship between selenium compounds (selenoprotein and selenium-containing enzyme) and mercury concentrations using mongoose, whale, and human blood samples.

For (1), liver, kidney, brain, blood, and urine samples (liver, kidney, brain, and tail only from fetus) were collected from 9 pairs of mother and fetus. THg concentrations in liver and kidney were significantly higher in dam than in fetus, whereas no significant difference in MeHg concentration was seen in liver, kidney, and brain between dam and

fetus.

The distribution ratio (DR) of MeHg from dam to fetus (concentration in fetus tissue/concentration in dam blood) was higher in liver, followed by that in kidney and in brain. DR of MeHg in brain was approximately 5, which was much higher than that of arsenic, lead, and cadmium. DR of selenium in brain was less than 1. The results suggested that maternal MeHg crossed the placenta and was transferred to the fetus of marmoset as well as humans. Relationship between the concentrations in maternal blood and in the brain of fetus showed significantly positive correlation ($p < 0.001$). Therefore, exposure level of the mother was revealed to be clearly reflected in the fetus. The relationship between THg and Se concentrations in the liver of fetus showed a significant positive correlation ($p < 0.05$), whereas it was not so in the fetus brain. Based on these facts, it was inferred that the protective effect of selenium against mercury toxicity was not observed in fetal brain.

5. Evaluation of neurological symptoms induced by methylmercury and association of selenium compounds with toxicity in the common marmoset

(Fundamental research)

Chisato Kataoka and Megumi Yamamoto

(Department of Environment and Public Health)

The purpose of this study is 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).

(1) Establishment of a quantitative evaluation system for neurobehavioral disorders in marmosets exposed to MeHg

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 evaluated by analyzing video recordings. Semi-quantitative preliminary data were obtained, which indicated that the marmosets had a decrease in spontaneous locomotion and change in posture during gait from 2 to 3 weeks after the start of MeHg administration.

(2) Elucidation of the foci responsible for neurobehavioral disorders associated with MeHg exposure

Immunohistochemical analysis of marmoset brains under the experimental conditions showed that the expression of inflammatory markers (GFAP, Iba1) was activated in brains of the MeHg-exposed group compared to that in the control group, and nerve fiber atrophy was observed in the peripheral nerve (sciatic nerve).

[Activity theme and summary]

1. Information service using hair mercury analysis

Masaaki Nagano

(Department of Basic Medical Science)

In 2021, 55 hair samples were collected from visitors to the National Institute for Minamata Disease, Minamata Disease Archives, and from other organizations. Total Hg levels of the samples were measured. The analysis results were sent to each individual.

4. Nature Environment Group

This group includes one project research, six fundamental researches, and two working activities, and conducts 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. The research targets a wide range of media, including air, water, soil, sediment, and organisms, and in particular, a wide range of biota, from low-level producers, such as phytoplankton, to large-scale consumers, such as swordfish. In the field research, Hg levels were monitored in the atmosphere and in 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 currently aim to explore Hg emission sources and elucidate the chemical reaction processes using Hg stable isotope ratios. In addition, our group is working with domestic and international research institutions to obtain comparable environmental Hg data that can contribute to the effectiveness evaluation of the Minamata Convention. At the same time, we are actively engaged in outreach activities targeting elementary, junior high, and high school students for the purpose of education and dissemination regarding Hg research and other scientific topics.

The outline of each research and activity for FY2021 is 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 formulate their relationships as much as possible with application to mathematical models. To this end, we have conducted studies on Hg fluxes at the air-sea interfaces and partition coefficients between seawater and phytoplankton, which are thought to influence the speciated Hg concentrations in the surface and sub-surface layers. In addition, we analyzed Hg species, such as elemental mercury (Hg(0)), inorganic mercury compounds (Hg(II)), monomethylmercury (MMHg), and dimethylmercury (DMHg) in seawater samples collected in the Izu-Ogasawara Sea area in the previous fiscal year and clarified the percentage of the four main forms of Hg.

First, to obtain more accurate data for the Hg fluxes, we improved a gas-liquid equilibrator system, which would allow for continuous data acquisition. Since Hg concentration in the air is low, it was necessary to increase the flow rate of air introduced into the equilibrator. However, high flow rate condition made it difficult to maintain a constant water level in the equilibrator. This problem could be circumvented by the installation of valves for regulation of water level in the equilibrator. The improved system was operated during the KM22-02 cruise from late January to mid-February 2022, and was found to be capable of unmanned operation even at night.

Next, the MMHg concentration in planktons by their size and the partitioning coefficient between seawater and planktons were measured at five stations in the Oyashio region during the Hokko Maru cruise in May 2021. The MMHg concentrations in planktons ranged from 0.1 to 0.55 pmol/g wet weight, with no increase in concentration with increasing size. Large variations were found

across sizes and stations. The partitioning coefficients ranged from 0.42 to 2.87×10^4 L/kg, with the largest values in the Oyashio-Kuroshio transition zone. This suggested that the variation depended on characteristics of the ocean area.

On the other hand, we completed the analysis of Hg species in seawater samples collected in the Izu-Ogasawara region in October of the previous year, and obtained the vertical distribution of the four main species. Total Hg concentrations were low in the surface layer and high in the deep layer, although values in deep water (below 1,000 m depth) were lower than those observed in other cases in the Pacific Ocean. Hg(II) was dominant in the deeper ocean layers while the proportion of Hg(0) was higher in the surface layers; MMHg and DMHg were higher in the middle and deeper layers, though DMHg was higher in the surface layers as well. The proportion of DMHg to methylated Hg (MeHg = MMHg + DMHg) was higher than that in previous studies, indicating that the analytical method would need to be validated further.

2. Research on the behavior of mercury in soil, water and sediment

(Fundamental research)

Akito Matsuyama

(Department of International Affairs and Research)

The outcomes of 3 study activities of 2021 are outlined as follows.

1) Study on mercury methylation in seawater

This study was conducted in 2021 using filtered seawater (filtered through 0.8 μ m membrane filter) and non-filtered seawater based on the results of mercury monitoring in Minamata Bay conducted between 2014 and 2018. Especially, incubation experiments that used glucose as a carbon source with three-step of seawater temperature were conducted in this study. Results showed methylmercury concentration of non-filtered

seawater (seawater temp 20 °C) to be the highest; the results of mercury monitoring in Minamata bay were published.

2) Study on the origin of mercury in sediments that were distributed in Yatsushiro Sea, and characteristics of mercury distribution in the sediments of Yatsushiro Sea

3) Till date, almost the same between sediments moving tendency of computer simulation and distribution of relatively higher total mercury concentration (≥ 0.5 ppm) at the bottom surface in Yatsushiro sea. Furthermore, to verify this result and to obtain more analytical data, 10 sediment samples that were distributed between Minamata Bay and Yatsushiro Sea were taken from the core sample that had been collected 3 years ago. The total mercury concentration was analyzed for each sediment particle that was dispersed using a special technique for dispersing sediment particles. There were obvious differences in total mercury concentration at each depth of sediment in vertical direction of 10 sediment samples. Mercury isotope analysis was performed with sediment samples, taken from Kagoshima Bay and Ariake Sea, using MC-ICP-MS. Results of the analysis, showing the characteristics of mercury isotope ($\delta^{199}\text{Hg}$) in sediments that were taken from Kagoshima Bay and Ariake Sea, were obviously different compared to those of the sediments that were taken from Yatsushiro Sea and Amakusa sea areas.

4) Understanding the changes in characteristics of Minamata Bay sediment due to changes in seawater quality in Minamata Bay

To understand the characteristics of dissolved mercury in the Minamata Bay sediment, an experimental model was prepared using sediments that were taken from Fukuro Bay in 2021 with focus on surface of the sediment at the bottom. To exert some influence on the sediment in seawater, glucose was added as a carbon source, and nitrogen bubbling

and air bubbling were performed. As a result of bubbling, according to the change in ORP (reduction condition), dissolved mercury concentration in seawater increased.

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

(Fundamental research)

Masumi Marumoto

(Department of Basic Medical Sciences)

Methylmercury taken into the body through food is gradually converted to inorganic mercury in the body. The ability to mineralize methylmercury varies across animal species. Selenium, an essential metal, is known to play a major role in mineralization, although mechanisms are not known in detail. The distribution of mercury and selenium in organs has been studied pathologically using an X-ray probe microanalyzer (EPMA), which can reveal which cells in various organs aggregate mercury and selenium. We will utilize the unique ability of EPMA to determine the tissue distribution of mercury and selenium in large marine animals, especially toothed whales and sailfish. Since most reports till date have been on cetaceans of the family Delphinidae, we will focus our research on cetaceans of the family Phocoenidae, which have not been reported yet, focusing on the relationship across sexual maturity, body length, and total mercury concentrations in various organs.

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 is the main primary producer of coastal ecosystems in general. It fuels not only surface production but also benthic productions due

to deposition from the surface. However, microphytobenthos on the sediment surface contribute highly to subtidal benthic productions in Minamata Bay, and might cause relatively high Hg bioaccumulation that is often found in demersal fish. In this study, we focus on both phytoplankton and microphytobenthos to understand the seasonal dynamics, stable isotope values, total Hg contents, and the environmental factors influencing the properties. This year, I continued to investigate seasonal dynamics of surface phytoplankton and the structure of water mass, and collected microphytobenthos at high purity from the sediment using phototaxis. Phytoplankton density was highest in July, after the rainy season, as was the case last year, and the reason was salinity stratification and improvement in surface photic environment. Nutrient levels were generally low compared to the average levels of Yatsushiro Sea, which suggested low primary production in Minamata Bay. The amount of microphytobenthos in the field was high from spring to early summer, but was low thereafter. In the low-amount period, it is difficult to collect microphytobenthos at high purity; therefore, the collection method would need further improvement.

5. Characterization of stable isotopic composition of mercury at the emission sources

(Fundamental research)

Satoshi Irei

(Department of Environment and Public Health)

The objective of this research project is to characterize and report the initial stable isotopic compositions of mercury (Hg) at emission and discharge for the progress of studies on global Hg cycle using stable isotope ratio measurements. In this fiscal year, we aimed to continuously conduct total gaseous mercury (TGM) sampling from the Aso open field burning, to establish an analytical method for Hg-containing commercially available goods,

and to publish results from these studies as original research articles in internationally well-established scientific journals.

Measurement results of sampled TGM from the Aso open field burning showed higher atmospheric TGM concentrations than the concentrations collected during the background periods (i.e., the periods when the open field burning was not conducted), indicating that the open field burning events emitted TGM. Analysis of isotope ratios for the TGM collected during the open field burning showed the isotopic composition of mass-independent fractionation, and the compositions were similar to those fractionated during the Hg uptake by plant species, implying emission of Hg in plant species.

The analysis of Hg enclosed in thermometers showed isotopic compositions with very high precision. However, comparison with literature values of isotopic compositions of Hg from cinnabars exhibited insignificant differences. This possibly indicated a limitation since stable isotopic compositions could not distinguish the geographical origins of Hg.

The Hg isotope measurements from three parts of fluorescent tubes, namely electrode, glass wall, and gas-phase inside the tube, showed remarkably fractionated isotopic compositions, and the differences between observed isotopic compositions and literature values for different sources, such as coal combustion and natural gas production, were significant. The results indicated a natural phenomenon accompanying large isotope fractionation inside fluorescent tubes, which is possibly electric discharge in the low-pressure environment. Releasable Hg enclosed in fluorescent tubes have such unique isotopic compositions; thus, our study demonstrated that isotope measurements have potential for fingerprinting Hg emitted or discharged from fluorescent tubes.

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 the mercury (Hg) speciation (methylation, demethylation, and reduction) by microorganisms in the ocean. This year, I focused on the microbial Hg speciation process in Minamata Bay, Japan. Seawater samples were collected from 2-m and 10-m depths in the bay (in April and June 2021). For these samples, we tried to detect the Hg speciation genes (*hgcAB*, *merB*, and *merA*) using metagenome analysis and estimated THg and MeHg concentrations in the dissolved and particulate (small particles: 0.2–3 μm, large particles: > 3.0 μm) fractions.

The Spirochaetes-like *hgcA* (mercury methylation gene) was detected in the large particle fraction from a 2-m depth sample in April, suggesting that microbial Hg methylation could occur in the particulate matter in Minamata Bay. We detected the sequences of *merB* and *merA* encoding organomercury lyase and mercuric reductase, respectively, in particulate fractions of both April and June samples. Phylogenetic analyses revealed that Alphaproteobacteria and Actinobacteria (both aerobic microbes) were dominant in the *merA* and *merB* genes detected in the bay, respectively. However, there was no correlation between the gene numbers and Hg concentrations. These data suggested that microorganisms can contribute to MeHg degradation and Hg reduction in seawater column in Minamata Bay.

7. Study on factors affecting long-term trend of atmospheric mercury concentrations in Asian-Pacific regions

(Fundamental research)

Kohji Marumoto

(Department of Environment and Public Health)

Continuous monitoring of Hg in the air was conducted at Minamata and Fukuoka in order to obtain continuous data on Hg concentrations in the air and in precipitation, which will contribute to the effectiveness evaluation of the Minamata Convention. In this study, we analyzed the data to clarify the seasonal and interannual variations and their underlying factors, as well as the influences of emission sources. We also conducted correlation analysis with respect to air pollutants and investigated metal concentrations in air particulates at Fukuoka. In order to investigate the influence of Hg emissions from volcanoes, continuous atmospheric monitoring was conducted at Aso-Sensuikyo, located north of the first crater of Aso Nakadake volcano, as in the previous year. However, the atmospheric Hg monitoring at Aso showed a high probability of obtaining peculiar daily fluctuation data due to malfunction of the monitoring instrument. Therefore, the instrument was replaced by another one from October 2021. Since the volcano may be emitting a large amount of gaseous oxidized mercury, we are currently monitoring the concentration of gaseous elemental Hg, gaseous oxidized Hg, and particle-bound Hg.

Meanwhile, monitoring of rainwater Hg was continued at six sites, namely Minamata, Hirado, Fukuoka, Matsue, Omaezaki, and Tsukuba. In response to the Minamata Convention, we participated in an international accuracy control program led by the U.S. Geological Survey and conducted joint research with the Central University of Taiwan to ensure reliability of the data.

[Activity theme and summary]

1. Implementation of seawater quality monitoring in Minamata Bay and support of various regional activities around the Minamata area

Akito Matsuyama

(Department of International Affairs and Research)

The outcomes of 2 activities of CT in 2021 are outlined below.

1) Mercury monitoring in Minamata Bay

Mercury monitoring was performed three times (May, August, and December) at Minamata Bay in 2021. Seawater sampling points were Hadakase, Wanoh, and Koijishima. Mercury monitoring at Shinsui park was also performed three times (April, August, and December). Seawater sampling was performed at 5 points, between Minamata disease information center and ferry stop. The annual average value of dissolved total mercury concentration in Minamata Bay seawater was 0.30 ± 0.07 ng/L (2021). Dissolved methylmercury concentration was 0.06 ± 0.02 ng/L. An annual average value of dissolved total mercury concentration of Shinsui park was 1.64 ± 0.97 ng/L.

2) Support of various activities in Minamata area

Not only last year (2020), but this year also (2021) we conducted oyster cultivation experiment using raw raft at 3 setting points (Fukuro Bay, Marushima harbor, and Minamata river). Based on the experience of destruction of the raw raft by typhoon last year, a subsidence-type raw raft and an aquaculture basket were utilized for the experiment. Although a big typhoon came to Minamata area again, it was not destroyed this year. Results of the experiment that was started in April 2021 suggested Fukuro Bay as the best place for experimental oyster growth. Moreover, effectiveness of the aquaculture basket for oyster growth was very good. More than half the volume of oyster pieces that was set at starting point (700 pieces were set in aquaculture basket) survived. Moreover, chlorophyll concentration in Fukuro Bay seawater was comparatively higher than in another place (Marushima harbor, and Minamata river).

2. 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 research results to the public. Minamata City, where the NIMD is located, is known both domestically and internationally as the town where Minamata disease occurred, but elementary and junior high school students' knowledge of mercury itself is limited. Therefore, we plan to provide correct knowledge about mercury by giving classes on mercury at elementary and junior high schools in Minamata City. In order to emphasize on interaction, a question box will be set up after the classes to receive feedback from the children, rather than having a one-way communication from the researchers. In FY2021, we arranged for a class at an elementary school in Minamata City, set up a question box, and prepared answers to the questions.

5. International Contribution • Information Group

Environmental pollution due to Hg, which is the causative substance of Minamata disease, is spreading worldwide, and is quite severe in developing countries. To decrease the risk of Hg pollution for humans and the environment, the Minamata Convention was adopted by delegates from 140 participating nations in October 2013 in Minamata. It came into effect in August 2017. The international contribution group at the NIMD has promoted cooperation with foreign researchers to participate in studies on Hg and exchange information via the NIMD forum. Moreover, based on the needs of the developing countries facing Hg pollution, we share our knowledge, experiences, and the latest technology by establishing cooperation via the Japan International Cooperation Agency (JICA). In collaboration with UNEP and WHO, we provide reference materials, training programs, and proficiency testing to improve mercury-related research development and monitoring. The collaboration is a part of the continual improvement of quality data collection and analysis. Furthermore, a simple, robust, and cost-effective Hg analysis method was developed, which has been positioned as the MOYAI Initiative in the Minamata Convention. The following is an overview of the research and activities of the International Contributions Group during FY 2021.

[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 Hg compounds
- 2) Development of reference materials to evaluate the accuracy of Hg analysis
- 3) Providing proficiency testing to help improve quality data collection and analysis

The tasks represent a part of the MOYAI Initiative by providing technical assistance to promote effective implementation of the Minamata Convention.

A simple and inexpensive method for speciating MeHg is required for human biomonitoring in artisanal and gold-mining communities or in skin-lightening cream users in resource-limited countries. This study focused on improving a thin-layer chromatography (TLC) method based on dithizone extraction using less-toxic solvents than those employed in the existing methods, modern TLC plates, and thermal decomposition atomic absorption spectrophotometry (TDA AAS). The proposed method was an optimized dithizone extraction and application procedure. It successfully detected MeHg in hair samples, reaching a LOD of 0.18 ng of MeHg as Hg (0.018 mg MeHg/kg of hair from a 10 mg sample). A solvent-free method using gold nanoparticles, adsorbing Hg⁰ and Hg²⁺, has also been investigated, and MeHg was confirmed to separate in the residues. With few treatment steps and zero solvent emission, the analytical method could be a candidate for mercury analysis.

Blood is one of the human biological media reflecting mid-term exposure to MeHg. Certified reference materials (CRMs) of blood have to be transported frozen or refrigerated, and a cold chain is essential for transport. To circumvent this problem,

we began developing a blood CRM that can be transported at room temperature. Blood samples were collected from 60 volunteers using methods approved by the Epidemiological Ethics Review Committee. After confirming negative antigen-antibody tests, the blood was sterilized, mixed, and lyophilized to produce candidate CRM. Further, we optimized the EDTA concentration as an anticoagulant, the duration of gamma rays, and the lyophilization temperature to ensure MeHg stability of the candidate.

UNEP ROAP and we contacted laboratories in the Asia-Pacific region that can undertake mercury analysis or will do so in the near future, for monitoring, survey, or research purposes, to participate in the proficiency testing for assessing their analytical capacity. The test scheme concerning total mercury determination in hair involved 28 laboratories as participants, who used their standard analytical methods. The results will be analyzed and published in 2022.

[Activity theme and summary]

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

Masatake Fujimura

(Department of Basic Medical Science)

In response to an advertisement in an English website about the measurement of hair mercury in our institute, a research institute in Bangladesh (icddr, b) inquired in that regard. Bangladesh has a large electronic waste dumping area, and possible exposure of humans to mercury outflow from fluorescent lamps is a great concern. Amidst the spread of the new coronavirus infection, we succeeded in collecting hair from 267 people around the dumped area and measured their hair mercury levels. The mean hair mercury (total mercury) was

0.5 ppm (197 men) and 2.7 ppm (70 women). In addition, when the MeHg concentration was measured in the hair of 5 women who showed high concentration (10 ppm or more), the ratio to total mercury was 10% or less. Therefore, it was clear that human exposure (internal exposure) due to mercury outflow in this area was almost zero. Since there was no gold mine worker among the hair providers, the high concentration of mercury in hair was considered to be caused by mercury-containing cosmetics.

In addition, as a steering committee member of the International Mercury Conference (ICMGP), I have set up a special session plan for hair mercury at the International Conference (ICMGP2022) to be held next year, and have given a lecture on the Minamata Convention website.

2. NIMD forum and international workshop

Akito Matsuyama

(Department of International Affairs and Research)

Although NIMD forum was not held due to the influence of COVID-19 last year, it was held at Minamata Disease Information Center this year (November 6, 2021). Main title of the NIMD forum was Minamata Regional Revitalization-Partnership between Citizens, Companies, and Government. As a result of the event, in combination with the online event, we were able to get a total of 148 participants, 95 at the time of the actual event and 53 at the online event. In addition, by uploading the forum scene on YouTube channel of Ministry of the Environment and conducting post-publication of this even immediately after the event, it could be broadcasted across a wide area and to the people who could not visit on that day and those in the area near Minamata. The number of views was almost 75,000.

3. Research cooperation in the international

organization

Akito Matsuyama

(Department of International Affairs and Research)

There was no dispatch and acceptance of overseas researchers due to the influence of COVID-19 this year, which was the second year in mid-term plan. On the other hand, an online training program or participation in international congress was actively conducted via a website using a personal computer. Specific examples are provided as follows.

- 1) Participation in pre-conference meetings for holding ICMGP (International Conference on Mercury as a Global Pollutant). One among the NIMD researchers participated in the pre-conference meeting via the website (May, 2021 – October, 2021; total 6 times). Therefore, it was decided that ICMGP2022 will be held as a web conference.
 - 2) One NIMD researcher participated in the 10th Annual Asia Pacific Mercury Monitoring Network Partners Meeting (October 6, 2021), and he presented NIMD activities related to this category.
 - 3) One NIMD researcher participated in UNEP/ROAP Project to promote the Minamata Convention on Mercury: Asia-Pacific Annual Webinar on Mercury Science 2021 on-line, and he delivered a lecture to the participants.
 - 4) We actively responded to the videos, such as Science view (NHK program for foreign people) or documentary program, which were promoted from overseas and were related to mercury (Video title, Amazon, the new Minamata).
4. Transmission of information on Minamata Disease, and organization of documents and materials in the “Minamata Disease Archives”

Rie Harada

(Department of International Affairs and Research)

In this fiscal year, in order to strengthen the information dissemination ability despite the COVID-19 pandemic, we aimed to (1) strengthen the information dissemination ability to get more people to know about the Minamata Disease Information Center (2) improve the appeal power by reviewing the exhibition contents, and (3) focus on three points of the approach to develop new target groups. First, the information center led the introduction of VR for the three facilities of "Hill of Learning" in Eco Park Minamata. This alleviated COVID-19 and geographic disadvantages. Next, a special exhibition was held, and the exhibition video was updated. As a result of active public relations, it was featured in many media and attracted new visitors.

Use of the auditorium was significantly reduced. However, the number of users was increased by taking measures against infection and promoting general use. The document maintenance work completed the catalog preparation for publication of the catalog. In order to increase the number of users in the reference room, we have initiated work towards renewing the official website.

5. Activities as a WHO Collaborating Center

Megumi Yamamoto

(Department of Environment and Public Health)

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 activities on the health effects of mercury since then. In this fiscal year, we mainly performed the following tasks:

- (1) Renewal documents for the next quadrennial (January 2021-December 2024) concerning the

WHO CC designation of NIMD, which were processed in FY2020, was reviewed by WHO and WPRO, resulting in its re-designation as a WHO CC.

- (2) A contributed article on the role and activities of NIMD, which was submitted to the official journal of the Friends of WHO Japan “Medemiru WHO” in FY2020, was published in the Spring 2021 issue.
- (3) The NIMD staff participated in a round-table discussion on the Capacity of Poison Control Centers in the Western Pacific Region for Chemical Safety Management: Chemical Incidents/Emergencies and Toxicovigilance, organized by WPRO, HAE Unit (web conferences were held on November 18 and December 2, 2021). Results of the meeting were compiled into proceedings.
- (4) The WHO CC annual report on the activities of the WHO CC from January to December 2021 was prepared.

6. Publications and Scientific meetings

[International Journals]

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[International meetings]

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