

NIMD Annual Report
2013
(April 2013 to March 2014)

National Institute for Minamata Disease
Ministry of the Environment
Japan

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Report on research and other activities during the 2013
fiscal year

1. Mechanism Group

The aim of this study group is to understand the molecular mechanisms underlying mercury toxicity in humans. To that end, the group focused on the biological effects of mercury. Our goals are as follows: (1) to understand the initial signs of methylmercury (MeHg) poisoning (2) to evaluate MeHg toxicity (3) to develop protecting methods against MeHg-induced disorders and (4) to develop new treatments to repair the damage caused by MeHg poisoning. In this study, we used biochemical, molecular biological, and pathological techniques in cell cultures and animal models. To determine the differences in MeHg sensitivity among organs and individuals, we analyzed stress responses and changes in the activation of cellular signal transduction caused by MeHg exposure. In addition, to elucidate the mechanisms underlying MeHg toxicity, we investigated cell death and regeneration in nerve cells damaged by MeHg, and the effect of dietary fiber on mercury excretion after MeHg exposure. We are also in the process of identifying drugs that suppress MeHg toxicity and promote nerve regeneration.

This group conducted the following research during 2013 fiscal year:

Research

[Research theme and summary]

(1) Research on selective cytotoxicity and sensitivity of individuals toward methylmercury (Project research)

Masatake Fujimura
(Department of Basic Medical Science)

We analyzed mRNA expression levels of hydrosulfuric enzymes (CBS and CSE) for each neuronal layer by using a microdissection system. We observed reduced CBS and CSE mRNA expressions in the cerebellar granule layer vulnerable to MeHg toxicity, than in the

other neuronal layers (e.g. in the Purkinje cells and in the molecular layer of nerve cells). These findings have been presented at a scientific conference, and the results published in peer-review article. Additionally, we used small interfering RNAs (siRNA) to identify EF-1 as a causative factor of synaptic dysplasia due to prenatal MeHg exposure. These findings were also presented at a scientific conference. In collaboration with external research institutes, we have presented our findings at 4 conferences and published one peer-reviewed article.

(2) Study on the molecular, genetic, and biochemical factors that cause differences in stress responses to methylmercury (Fundamental research)

Fusako Usuki
(Department of Clinical Medicine)

The outcome of this year's research was published in Scientific Reports. In that article, we uncovered using the preconditioning endoplasmic reticulum stress (ER) technique, that the ER-resident 78-kDa chaperon glucose-regulated protein (Grp78) plays a critical role in the protection of cells against MeHg toxicity.

The level of thiol antioxidant barrier (sHp) in the blood plasma of MeHg-exposed rats significantly decreased 2 weeks after MeHg exposure; however, no reactive oxygen metabolite (dROM) and biological anti-oxidant potential (BAP) in the plasma, and no histopathological changes in cerebellum were observed at this stage. To determine whether the early decrease in plasma sHp in MeHg-exposed rats is attributable to specific MeHg toxicity mechanisms, and whether this effect could be used as a relevant biomarker, we examined the plasma oxidative stress markers in rats exposed to 20 ppm MeHg, 15 ppm cadmium (Cd), or 0.2% lead acetate (Pb) in drinking water for 4 weeks. We observed an increase in dROM and a decrease in

sHp in rats exposed to 20 ppm MeHg, but not in Cd- or Pb-exposed rats. We further examined selenoprotein P1 levels in these rats. The results demonstrated that the expression of selenoprotein P1 significantly decreased in MeHg-exposed rats whereas it significantly increased in Pb- or Cd-exposed rats. The results indicated that the MeHg-induced relative selenium-deficient condition was caused by the high affinity of MeHg for the selenohydril group and selenide. This selenium-deficient condition blocked the synthesis of selenoprotein P1 through a posttranscriptional defect caused by the execution of nonsense-mediated mRNA decay. The decrease in selenoprotein P1 and the decrease in sHp seem to be specific signs of MeHg toxicity.

Our recent study showed that glial cell line-derived neurotrophic factor (GDNF) and IL-6 mRNAs were upregulated after MeHg exposure. They are related to neuronal survival and repair. Therefore, we investigated the effect of GDNF and IL-6 on MeHg-exposed cerebral neuronal cultured cells. Recombinant GDNF or IL-6 was administered to cerebral neuronal cultured cells exposed to MeHg. The results showed that GDNF significantly protected neuronal cells against MeHg toxicity compared to non-treated cells; however, IL-6 showed some tendency to protect neuronal cells, but not significantly. Further studies would be needed to understand the effects of exposure time and dose in the IL-6 treatment.

(3) Experimental research on relief of methylmercury-induced neurotoxicity (Fundamental research)

Masatake Fujimura

(Department of Basic Medical Science)

By using neuron cultures and a MeHg-intoxicated model, we confirmed that the neurotrophic-factor activator MCC-257 prevents MeHg toxicity by activating TrkA. These findings were presented at a

scientific conference. Next, we successfully obtained NPC from rat embryonic brains. Using cultured NPC, we showed that cyclin E is involved in the inhibition of cell proliferation in neural stem cells following MeHg exposure, and lithium antagonized this effect through the inhibition of GSK-3 β .

(4) Research on effect of dietary fibers on mercury excretion after methylmercury exposure (Fundamental research)

Masaaki Nagano

(Department of Basic Medical Science)

We investigated the effect of wheat bran on Hg accumulation after a single administration of methylmercury chloride (5 mg MeHgCl/kg). Six weeks after MeHg administration, a diet containing 30% bran significantly decreased total Hg concentration in the kidney, but not in the liver. Four weeks after MeHg administration, the cumulative amount of Hg in urine increased markedly in the bran group, whereas fecal Hg amount increased slightly. This result indicates that the effect of bran on Hg excretion mainly relates to an increase in urinary Hg. The effect of bran on Hg excretion was observed up to 14 days after MeHg administration. In addition, most of the Hg found in both urine and feces was in the form of MeHg. One of the main factors leading to the increase in fecal Hg excretion may be the acceleration of MeHg demethylation in the liver.

2. Clinical Group

Research

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

This research group conducted experiments to evaluate the neurological functions of patients with Minamata disease, using magneto encephalography (MEG) and MRI.

The research conducted by this group during FY2013 is outlined as follows:

[Research theme and summary]

(1) Clinical research on Minamata disease, with focus on the development of an objective assessment technique using MEG (Project research)

Masaaki Nakamura
(Department of Clinical Medicine)

The aim of this study was to develop an objective evaluation protocol using MEG to assess brain function following MeHg poisoning, and MRI to evaluate brain atrophy and biochemical changes.

Within this year, we tested the MEG method in 143 people, including 65 subjects from the Kumamoto district, an area that is not polluted with MeHg. Then, we weighed the abnormality frequency of two-point discrimination and somatosensory evoked magnetic fields (SEF) in Minamata City and Kumamoto District.

Presence of chronic pain is one of the main symptoms disturbing the quality of life of Minamata disease patients. We measured pain-related magnetic

fields and found that it changed after inhalation of an aroma, suggesting that this method could be used to evaluate the effect of therapy for chronic pain.

We also performed the following analyses by using MRI: (1) Evaluation of brain atrophy and (2) Evaluation of neuronal loss in the cerebellum and gyrus postcentralis periphery (motor and sensory areas). This latter evaluation was performed using MRI spectroscopy, a non-invasive diagnostic test for measuring the biochemical changes in the brain.

Activities

In recent years, the compensation claims from Minamata disease patients has moved toward a political resolution. The Department of Clinical Medicine actively conducts events on Minamata disease in cooperation with related organizations. In addition to undertaking conventional rehabilitation activities, including providing daycare for congenital and infantile Minamata disease patients, we organize rehabilitation technical schools, and care technical schools. We also examined the usefulness of vibration therapy for the relief of pain and spasticity associated with various chronic nervous diseases, including Minamata disease.

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

The following section includes an outline of the clinical practice conducted by this group in FY2013:

[Activities theme and summary]

(1) Rehabilitation programs for patients with Minamata disease and care and rehabilitation outreach(Other activities)

Fusako Usuki
(Department of Clinical Medicine)

We continued to provide outpatients with rehabilitation, in the form of daycare, twice a week. The principal objective is to improve their quality of life. Continuous vibration therapy relieved the severe pain and spasticity of patients with fetal-type Minamata disease, and improved their activities of daily living (ADL). These findings were published in a peer-reviewed occupational therapy journal in 2013. Electrophysiological examination revealed that the vibration of the plantar fascia decreased the amplitude of the H wave, which reflects excitability of spinal motor neuron, suggesting that vibration therapy works for pre-synaptic inhibition of the spinal cord. Vibration therapy was also used to relieve spasticity in 7 chronic patients with cerebral vascular disorders in cooperation with another facility. In all cases, spasticity of lower extremities and dorsiflexion power of the foot improved with the therapy. These results suggested that vibration therapy is effective to relieve spasticity in patients with chronic neurological disorders, as well as in patients with fetal-type Minamata disease. We have plans to finalize the best vibration therapy method to relieve spasticity.

In addition, we provided two wheelchair patients with training for standing and seating movements, which are known to effectively strengthen muscle power in the back, the abdomen, the lower extremities, and the neck. We also have a program of training activities for basic ADL, including an active introduction to the care equipment. These activities

enhanced their ADL abilities.

Annual workshops on rehabilitation and assistance techniques are held in order to improve techniques used by regional specialized staffs, to be applied to the patients. This year, the themes of the 6th workshops organized included “Pathophysiology of sarcopenia-knowledge and techniques for rehabilitation” as a rehabilitation technique and “ Well-chosen gardening therapy for care— an effective use of a way of thinking in occupational therapy” as a care technique. According to the results of a questionnaire given to attendees, the workshops were well received. We believe these workshops are useful outreach forums, to share information on care and rehabilitation techniques that can be applied in the community.

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

Masaaki Nakamura
(Department of Clinical Medicine)

We carried out a study entitled “Home support model study, including care prevention” (FY2006–2008) to identify support methods, including rehabilitation, that lead to the improvement of ADL for aging Minamata disease patients and their families. Following this project, we performed two other projects entitled “Community development project for home care support including healthcare practice” (FY2009–2011) and “Community welfare promotion business for supporting Minamata disease victims” (FY2012), to develop methods to apply these concepts into the community. After these projects, we continued carrying out educational activities in Minamata and Izumi, to improve occupational therapy in this area. Through this support, we strengthened the connection between our institute and the local community.

3. Risk Recognition and Information Services Group

Intake of trace methylmercury (MeHg) because of eating fish is of increasing concern worldwide; however, the information available is often difficult to interpret or mostly biased. This research group aims to identify the exact level of risk and the current state of MeHg intake in the diet, through the transmission of accurate and precise information.

The research carried out by this group in FY2013 is outlined as follows:

Research

[Research theme and summary]

(1) Study on information and perception of the health risk of low-level exposures to methylmercury. (Fundamental research)

Noriyuki Hachiya
(Department of Environmental Science and
Epidemiology)

A risk communication survey was conducted in Fukuoka, Fukuoka Prefecture, including hair mercury analysis program. The results obtained in this survey confirmed the conclusion drawn from a previous survey carried out in Shirakawa, Fukushima Prefecture. Both surveys showed that the participants were relatively tolerant to the health risk of low-level exposure to methylmercury through consumption of fishery products ordinary purchased in the market. The tendency contrasts with the attitude toward health risk associated with the radiation derived from the Fukushima nuclear power plant accident. More than half of the participants agreed that the knowledge about risk of methylmercury provided by the hair mercury analysis program is also helpful to understand the health effects of radiation. These results suggested that the risk communication program is useful to promote understanding the risk of not only methylmercury itself

but also of different hazardous environmental agents.

On the basis of an affect heuristic model, there is a tendency, markedly for Japanese consumers, to consider fish as good and healthy food and then having a low-risk, after the trade-off between high benefits and low risks in the risk perception on the daily consumption of fishery products.

Activities

[Activities theme and summary]

(1) Organization of documents and materials on Minamata Disease, and dispatch of related information in Minamata Disease Archives (Other activities)

Noriyuki Hachiya
(Department of Environmental Science and
Epidemiology)

The operations of the Minamata Disease Archives has been effectively maintained including the utilization of the lecture hall, periodical updates of the contents of the exhibition room, and management of the materials and documents on Minamata disease. As an administrative institution that holds historical materials and documents on Minamata disease, the Minamata Disease Archives includes the consolidated contents lists of more than 13,000 titles and search systems in conformity to applicable rules and regulations. The general policy for inspection and copy was defined based on the principles of the public use of the collection and of personal information protection. The annual number of visitors was 48,561 in 2013, and a total of 491,171 visitors have been welcomed to the archives since their establishment in 2001.

(2) Examination of hair Hg in areas concerned with Hg pollution around the world (Other activities)

Masatake Fujimura
(Department of Basic Medical Science)

This year, we analyzed Hg concentration in hair samples and gathered survey information regarding gender, age, occupation, and residence in areas of the Philippines, China, and the French Guiana concerned with Hg pollution. We presented this information at the National Institute for Minamata Disease, and distributed information leaflets at an international conference and on the home page. As a result, we received inquiries from the Philippines (from a different region), Bolivia, and Sri Lanka and the research has grown in scope. In addition, for the ninth consecutive year, we monitored hair in the French Guiana and for the fourth year in the Philippines.

(3) Information service using hair Hg analysis (Other activities)

Masaaki Nagano
(Department of Basic Medical Science)

In 2013, 1894 hair samples were collected from visitors at National Institute for Minamata Disease, the Minamata Disease Archives, and from other organizations, were analyzed for total Hg. The analytical results were sent to each individual. The geometric mean of total Hg concentration was 1.29 $\mu\text{g/g}$ for females ($n = 881$) and 1.65 $\mu\text{g/g}$ for males ($n = 570$) in Japan. On the other hand, mean concentrations of 0.64 $\mu\text{g/g}$ and 0.51 $\mu\text{g/g}$ were obtained from females ($n = 67$) and males ($n = 123$) in other countries, respectively.

4. Social and Epidemiological Group

It was more than half a century ago that first patient of Minamata disease was officially acknowledged. While the significant levels of environmental pollution with methylmercury are now only a historical episode in the coastal areas, the social problems of the Minamata disease epidemics remains to be settled in the local communities. This research group is carrying out historical verification on risk management, surveying present health problems in Minamata disease patients, and studying the mechanisms of social restoration of the affected areas.

Research

[Research theme and summary]

(1) Historical study on risk management in Minamata disease episode (Fundamental research)

Noriyuki Hachiya
(Department of Environmental Science and
Epidemiology)

Risk assessment of methylmercury includes quantification of the health impact and exposure assessment in a battery of risk management process on adverse health effects of the environmental chemical. The evaluation of health effect is based on the epidemiological evidence that is obtained from human population exposed with varied level of the chemical. Nonspecific or sub-clinical symptom may also be important outcome as possible health effects in general population. It usually takes time to confirm any proposed epidemiological hypothesis as a consistent theory. Therefore, the epidemiological evidence on the moderate health effect may be not useful in quick measures at the earliest stages of first epidemics of Minamata disease, for example, during 1950's. When serious health damages are appearing in local community, on the other hand, the countermeasure should be set as a crisis management to prevent

ongoing damages. Demonstrative identification, or detailed risk assessment, of the suspected causative agent is not necessary in a quick response to this type of hazardous events. It is important to consider how crisis management and/or risk management should be effectively accomplished at the stage when evidence is insufficient or hardly available. This problem is one of the most challenging issues in the study of historical problems in risk management of Minamata disease. In the history of Minamata disease, the company was not considered responsible unless the causal relationship was demonstrated between the outbreak of Minamata disease and the factory effluent. The lack of responsibility caused the delay of measures and resulted in irreparable expansion of damages.

(2) Research on regeneration in Minamata disease area (Fundamental research)

Tazusa Arakaki
(Department of International Affairs and Research)

This research aimed to analyze the regeneration process in Minamata disease areas, especially regarding measures and efforts for local restoration and their evolution. A survey was conducted in 2014 for 1060 citizens of Minamata, randomly selected from the qualified voter list, using an anonymous questionnaire distributed and collected by the postal mail with a valid response rate of 40.5%. The survey results showed a need for restoration, via "Moyainaoshi," of the social scars created by the incident. They also showed that citizens are still uncomfortable speaking to outsiders about their hometown or the Minamata disease, because of the association between the disease and geographical location, which could often lead to misunderstandings.

5. Local and Global Environment Group

Our research group carries out heuristic research focused on the mercury cycle, including several chemical forms, in the environment, based on data from the Minamata disease, which was caused by exposure to environmental methylmercury. Mercury circulates through land, water, and the atmosphere, and its chemical form varies depending on the domain where it is found. That is, mercury released as a metal is transformed to methylmercury, which bioaccumulates in the food web to eventually reach humans. Set against this background, our research group investigates the movement of methylmercury in the atmosphere, rain, seawater, sediment, soil, and life forms. This is a multidisciplinary group, where each primary researcher exchanges information with other researchers, establishing joint ownership of the results, promoting individual research projects. The research carried out by this group during 2013 is outlined as follows:

Research

[Research theme and summary]

(1) Marine ecosystem and mercury behavior in Yatsushiro Sea—Quantitative survey of the marine benthic community in Yatsushiro Sea and mercury concentration in different fish species in the food web—

Keisuke Mori

(Department of Environmental Science and Epidemiology)

In and around Minamata Bay, fish and benthic sampling for mercury, stable isotopes, and genetic analysis has advanced smoothly throughout the year. We have also made significant progress in the total mercury analysis, and the prospect of following it up with methyl mercury analysis is positive.

The collaborative project, including genetic analysis of prey species found in fish guts and stable isotope

analysis, have also progressed significantly and we are expecting that these results will largely contribute to our understanding of the food web. Our data have been gathered in one peer-review article and we have given two conference presentations.

(2) Research on the influence and behaviors of mercury in the aquatic environment of Minamata Bay

Akito Matsuyama

(Department of Environmental Science and Epidemiology)

1) Dissolved methylmercury concentration (diss-MeHg) in Minamata Bay has not experienced some increasing as well as the previous year (2012). Total concentration of dissolved mercury (diss-THg) has also remained stable throughout the year. We also extracted microbial DNA from Minamata Bay seawater samples, but the data analysis is still pending. We also carried out experiments based on microbial behavior in seawater. Our results showed a possible microbial route for mercury methylation in Minamata Bay.

2) Multiple regression analysis (performed using the statistical software SPSS) was applied to some of the data from Minamata Bay collected between 2006 and 2010. This analysis showed a relation between dissolved methylmercury and dissolved oxygen (DO), non-purgeable organic carbon (NPOC), and salinity in Minamata Bay ($R = 0.702$, $R^2 = 0.484$). Currently, we are carrying out an aquarium-based experiment, based on these results, at Kyushu University.

3) Dissolved mercury in pore water in sediments from Minamata Bay was analyzed by identifying the chemical form and through adsorption strength. The results showed that dissolved MeHg in pore water was easily adsorbed to sediment particles.

4) Engineering works to improve Minamata Bay

seawall were initiated last year (2013). As a result, a seasonal variation in dissolved THg, between May and September, might have occurred due to disturbances created by the engineering works.

5) Project that was requested by JICA initiated a field survey on mercury pollution in Nicaragua from end of March 2014.

6) Chronological variations in total mercury content in seaweed (i.e., Wakame and Konbu) cultivated in Minamata Bay were continually evaluated between March and May 2013. We observed a small fluctuation in mercury concentration in Wakame; however, this variation was below 10% of the regulatory standard value for mercury in fish and shellfish in Japan.

(3) Research on chemical reactions, transport, and deposition of atmospheric Hg (Fundamental research) —Factors influencing the variation in wet deposition of methylmercury and evidence of long range transport of atmospheric mercury obtained by simultaneous observation at 2 sites—

Kohji Marumoto

(Department of Environmental Science and Epidemiology)

Mono-methyl Hg (MMHg) was measured in rain samples at Minamata. The concentrations of MMHg varied seasonally and are higher in winter and spring than in summer and fall. We investigated the factors influencing this seasonal variation. MMHg concentrations significantly correlated with UV-A radiation, indicating that MMHg is break down by strong solar radiation in summer. In addition, the back trajectory analysis showed that substances transported from the Asian continent drive the high concentration of MMHg observed in winter and spring. MMHg concentrations were also measured weekly through continuous sampling, for 2 years, at Hirado, in the northwest of the Kyushu islands. These samples showed a similar seasonal pattern. We started

monitoring the total Hg in precipitation at Fukuoka in June 2013 and at Omaezaki (Shizuoka Prefecture) in December 2013.

Simultaneous observations on atmospheric gaseous Hg at Minamata and Fukuoka were carried out since June 2012. The frequency at which high concentration of gaseous Hg was concurrently observed at the 2 sites increased between late autumn and early spring. Based on the analysis of atmospheric pressure distribution and back trajectory analysis, the observed episodes of high concentration of atmospheric Hg could relate to the passage of cold front including polluted air transported from the Asian continent.

(4) Study on natural emission sources of atmospheric mercury (Fundamental research)—Estimation of the rate of mercury exchange across the air-sea interface in the Minamata Bay—

Kohji Marumoto

(Department of Environmental Science and Epidemiology)

Dissolved gaseous Hg (DGM) concentration and Hg emission flux were measured in the Minamata Bay from June 2012 to May 2013. The annual Hg emission flux from the sea surface of the Minamata Bay (Surface area: 3.82 km²) was 180 ± 210 g, corresponding to almost twice the input from atmospheric Hg deposition. This suggests that mercury emissions from the sea surface contribute to Hg clean-up and the Hg cycle in the Minamata Bay, which suffered serious Hg pollution incident in the past. In addition, DGM concentration and Hg fluxes were also measured in Osaka Bay and the eastern part of Seto Inland Sea in June 2011 and June 2013. The average DGM concentration and the Hg flux in these sea areas were one-fourth lower than those observed in summer in the Minamata Bay. Total Hg and MMHg concentrations in seawater and sediments were also lower than those recorded from the Minamata Bay,

indicating that the differences in the amounts of Hg in the seawater and sediments affect the Hg flux from the sea surface. Further research is still needed to understand the mechanism of DGM production in different regions.

(5) Study on biomagnification of mercury from sediment to demersal fish

Shoko Imai
(Department of Environmental Science and
Epidemiology)

We carried out an experiment on uptake and elimination of mercury, using marine demersal fish, to understand the mechanism of bioaccumulation in marine food webs. The redspotted grouper (*Epinephelus akaara*) and the scorpionfish (*Inimicus japonicus*) were used as test fishes. The fish were fed food contaminated with mercury, and subsequently, we observed the uptake of mercury by the fishes. The total concentration of mercury in fish increased with the concentration of mercury in the food and the uptake period. We plan to estimate the biomagnification factor after the elimination period.

(6) Research on the relation between methylmercury levels and marine bacteria in the water of Minamata Bay (Fundamental research)

Masaaki Nagano
(Department of Basic Medical Science)

Dissolved methylmercury (MeHg) concentration in Minamata Bay has been reported to be considerably higher during summer than at any other time of the year (March 2006 to March 2008). MeHg is known to be produced by anaerobic sulfate- and iron-reducing bacteria in freshwaters; therefore we studied the relationship between microbial activity and MeHg concentration in seawater in Minamata Bay. Using polymerase chain reaction, we found that sulfate- and

iron-reducing bacteria were present in seawater samples; however, it remains unclear whether sulfate- and iron-reducing bacteria are involved or not in the increase of MeHg concentration in water columns, because the presence of these bacteria was not consistent with the increase in MeHg concentration observed. To confirm whether marine bacteria are involved in the methylation of mercury, we added inorganic mercury to seawater samples collected from Minamata Bay. MeHg formed from the methylation of inorganic mercury in non-filtered seawater, but not in filtered seawater, suggesting a role of marine bacteria in the water of Minamata Bay in mercury methylation.

(7) Mercury contamination by many small-scale gold ore smelters in Talawaan River, North Sulawesi, Indonesia.

Keisuke Mori
(Department of Environmental Science and
Epidemiology)

We finished analyzing the mercury content in sediment, fish, benthic organism, and resident hair samples from three villages along the Talawaan River, that were collected during 2011–2012. We also performed particle size analysis of the sediment samples. We also have done significant progress in the data processing. Some fishes showed considerably high mercury contents and, therefore, we need to inform the local community.

(8) Study on alkyl derivatization technique for speciation of mercury in biological and human samples (Fundamental research)

Koichi Haraguchi
(Department of International Affairs and
Environmental Sciences)

Cold vapor-atomic fluorescence spectrophotometry (CV-AFS) has the adequate sensitivity to measure the

low levels of inorganic- and methylmercury often found in biological and human samples. Here, we introduced a different dithizone extraction method to substitute the purge and trap followed by CV-AFS method, for a gas chromatography with an electron capture detector. This method is based on the extraction of mercuric dithizonates onto an organic solvent, followed by a back-extraction into a Na₂S solution and a subsequent ethylation with NaBEt₄. Volatile mercury was then collected on a Tenax trap and introduced into an isothermal gas chromatograph connected to pyrolysis and CV-AFS. We expect that this method will significantly contribute to our laboratory quality control and to the evaluation of mercury exposure in future studies.

Activities

[Activities theme and summary]

(1) Cooperation of research in the international organization (Other activities)

Mineshi Sakamoto

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

We conducted the animal experiment using rats with two researchers from the Brazil. In total, 13 researchers were dispatched for the international conference (UK, Brazil and Indonesia) and field research (Indonesia). We conducted the training and education the health and environmental effects of mercury compounds to 12 times JICA Trainings (In total 110 participants). In addition, two weeks training on the “Project for Strengthening the Health-related Mercury Monitoring in Acre State, Brazil” was conducted to 12 trainees.

(2) NIMD Forum and International Workshop (Other activities)

Mineshi Sakamoto

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

The NIMD Forum 2013 “Mercury in marine mammals and human health risk” was held during 28th July to 2nd August in Scotland as a special session in “International Conference Mercury as a Global Pollutant 2013”. Eight researchers invited to the session were from USA, Canada, and Japan. The Symposium entitled “Managing Mercury Pollution in the 21st century: bridging science and policy” was held on 6th October in Kumamoto Prefectural University, which was co-sponsored by Society of Environmental Toxicology and Chemistry (SETAC) in the Asia /Pacific region and NIMD. This Symposium was held just before the Minamata Convention. (The Minamata Convention on Mercury is a legally binding instrument to protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds which is promoted by the United Nations Environment Program (UNEP). The conference was held from 9 to 11 October 2013 in Kumamoto and Minamata, with more than 1,000 participants from approximately 140 countries).

6. Environmental Health Sciences Group

The Environmental Health Sciences group is conducting epidemiological surveys in Taiji-cho, Wakayama Prefecture, where the population has been exposed to high concentrations of methylmercury (MeHg). The group's main research also concerns the population particularly sensitive to MeHg exposure, such as fetuses or people suffering from various diseases, to contribute to appropriate risk assessment of MeHg exposure.

Research

[Research theme and summary]

(1) Studies on MeHg exposure in a whale-eating district (Project research)

Masaaki Nakamura
(Department of Clinical Medicine)

We have presented our results from the neurological examination (194 residents: 117 males and 77 females), carried out in 2010 and 2011 in Taiji, in the 11th International Conference on Mercury as a Global Pollutant) and in a peer-review article (Environment International).

Magnetic resonance spectroscopy, a noninvasive diagnostic test for measuring biochemical changes in the brain, was used in 11 subjects with hair mercury concentrations higher than 50 µg/g, to evaluate neuronal loss in cerebellum and gyrus postcentralis periphery (motor and sensory areas). None of the subjects presented any signs of neuronal loss in these areas.

We obtained serum samples from 155 subjects to determine why no apparent health effect was found.

We made the final preparations for the study on the effect of MeHg exposure on child development, in Tokyo on June 3, 2013, and performed pediatric examinations at the Taiji-cho multi-purpose center from August 10 to 11, 2013. The examinations were

carried out in cooperation with Doshisha University, Tohoku University, Akita University, and Jin-ai Women's College.

(2) Evaluation of the effect of exposure to methylmercury and other elements during gestation and breast feeding (Fundamental research)

Mineshi Sakamoto
(Department of Environmental Science and
Epidemiology)

Developing brains are highly susceptible to MeHg damage. MeHg can cross the placenta and accumulates at higher concentrations in the umbilical cord blood than in the maternal blood. Consequently, the effect of dietary seafood intake in pregnant women remains an important issue, especially in populations that consume large quantities of fish. In this study, we investigated the relation between mercury concentrations measured in fingernails and toenails and those measured in maternal blood and hair in pregnant women, at early gestation stages and around parturition.

(3) Protective effect of selenium against methylmercury toxicity and co-existence of mercury and selenium in human and sea-mammals (Fundamental research)

Mineshi Sakamoto
(Department of Environmental Science and
Epidemiology)

Selenium (Se) is an essential element and may play a role in the protection against MeHg toxicity. In a previous study, we reported the co-existence of Se and MeHg in pregnant women (Ecotoxicology and Environmental Safety, 2010). Here, we confirmed the protective effect of Se against neuronal degeneration in

developing rat brains (Environmental Science and Technology, 2013). We also conducted a study on co-existence of Se and Hg in the blood in inhabitants of a whaling town in Japan and in red meat of tooth whales. This year, we reanalyzed historic samples of Minamata disease, such as HI solution, Hosokawa cats, and preserved sediments.

(4) Evaluation factors affecting sensitivity to MeHg exposure—Application of disease model animal and knock-out animal (Fundamental research)

Megumi Yamamoto

(Department of Basic Medical Science)

1) In this study, we showed that body fat gain in T2DM and low mercury accumulation in adipose tissue increased the MeHg concentrations in organs, and enhanced MeHg toxicity in KK-Ay mice, at the same dose of MeHg per BW. This research has been accepted for publication in the Journal of Applied Toxicology. We evaluated peripheral neuropathy associated with MeHg using behavioral tests (von Frey test, DWB test) and pathological analysis; however, no significant differences were observed between the control group and the MeHg-treated group in KK-Ay mice using the von Frey test. On the other hand, the DWB test might be useful for evaluating behavioral changes due to MeHg exposure in this model. Peripheral neuropathy associated with MeHg could be evaluated by Masson trichrome staining and macrophage marker expressions in KK-Ay mice.

2) We examined inflammatory responses against MeHg exposure in U937 macrophages. In addition, we have succeeded in achieving appropriate experimental condition for a knock down experiment of NF- κ B in U937 macrophages.

Publications and Conference Presentations

[International Publications]

Sakamoto M, Yasutake A, Kakita A, and et.al.: Selenomethionine protects against neuronal degeneration by methylmercury in the developing rat cerebrum. *Environ Sci Technol*, 2013; 47: 2862-2868.

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Sakamoto M, Kakita A, Chan HM: Changes in methylmercury uptake into the brain at various growth phases from fetus to adult rats. The VIII International Congress of Toxicology 2013, Seoul, Korea, 2013. 6.

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