

NIMD Annual Report
2012
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National Institute for Minamata Disease
Ministry of the Environment
Japan

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Report on research and other activities in
fiscal year 2012

1. Mechanism group

The aim of this study group is to understand the molecular mechanisms underlying mercury toxicity in humans by studying the biological effects of mercury. Our goal is to develop the research results into the following: (1) understanding the initial disease state of methylmercury (MeHg) poisoning; (2) evaluating MeHg toxicity; (3) protecting against disorders; and (4) developing new treatments to repair damage caused by MeHg poisoning. In this study, we used biochemical, molecular biological, and pathological techniques in cultured cell systems 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. Further, to elucidate the mechanism underlying MeHg toxicity, we investigated cell death and regeneration in nerve cells damaged by MeHg, and the effect of dietary fiber on the mercury excretion after MeHg exposure. In addition, we are in the process of identifying drugs that suppress MeHg toxicity and promote nerve regeneration.

An outline of the research conducted by this group in fiscal year 2012 is as follows:

[Research theme and summary]

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

Masatake Fujimura

(Department of Basic Medical Science)

We analyzed the mRNA expression levels of various antioxidant enzymes for each neuronal layer by using a microdissection system. We observed that mRNA expression of Mn-SOD, GPx1, and TRxR1 is less in

the cerebellar granule layer, which is vulnerable MeHg toxicity, than in other neuronal layers (Purkinje cells and molecular layer nerve cells). Similar expression patterns of these antioxidant enzymes were also observed in the immunohistochemical analyses, suggesting that decreased expression of Mn-SOD, GPx1, and TRxR1 may be involved in determining the vulnerability of cerebellar granule cells exposed to MeHg. These findings were presented at a conference, and we are papering a paper for publication. We also identified Maspine, BLNK, and EF-1 as candidate causative factors of synaptic dysplasia without neuronal cell death due to prenatal MeHg exposure (5 ppm in drinking water). These findings were presented at a conference and published. In collaboration with an external research institute, we have presented our findings at 3 conferences and published 2 papers.

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

Fusako Usuki

(Department of Clinical Medicine)

Our findings clearly show that oxidative stress is important in the pathogenesis of MeHg cytotoxicity. To understand *in vivo* MeHg-induced pathological conditions, we investigated whether the early decrease in thiol anti-oxidant barrier (sHp) in the plasma of MeHg-exposed rats is attributable to MeHg toxicity and whether this effect could be used as an important biomarker. We examined sHp, reactive oxygen metabolite (dROM), and biological anti-oxidant potential (BAP) in the plasma of rats exposed to 15 ppm MeHg, cadmium (Cd), or lead (Pb). Cd and Pb are known to induce oxidative stress. Increase in dROM and decrease in sHp were observed in rats exposed to 15 ppm MeHg for 4 weeks, but not in Cd- or

Pb-exposed rats. We further made plans to examine whether the doses of Cd and Pb were sufficient to cause toxicity and oxidative stress.

To understand differences in cell susceptibilities to MeHg, we examined the involvement of the stress-related proteins, metallothionein 1 (Mt1) and the endoplasmic reticulum (ER)-resident chaperon 78 kDa glucose-regulated protein (Grp78). Both Mt1 and Grp78 were upregulated after exposure to MeHg. To validate these findings, siRNA-mediated knockdown study and ER stress preconditioning approach were carried out. The results suggested that Grp78 plays a critical role in the protection of cells against MeHg toxicity. Increased expression of Grp78 in a MeHg-non-susceptible cell line supported this finding.

Furthermore, we investigated specific responses to MeHg in astrocytes. The changes in glial cell line-derived neurotrophic factor (GDNF) and IL-6 were examined. Both GDNF and IL-6 mRNAs were upregulated after exposure to MeHg, and GDNF mRNA level showed remarkable increase. Since GDNF and IL-6 are related to neuronal survival and repair, these results suggested that astrocytes function in the protection of neurons during the early phase of MeHg toxicity.

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

Masatake Fujimura

(Department of Basic Medical Science)

We confirmed that MCC-257, a neurotrophic-factor activator, prevents MeHg toxicity through the activation of TrkA by using cultured neurons from a MeHg-intoxicated model. Next, we clarified that Cyclin E is involved in the inhibition of cell proliferation in neural stem cells following MeHg exposure, and lithium antagonized this inhibitory effect. We presented our findings at a conference and published a paper.

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

Masaaki Nagano

(Department of Basic Medical Science)

Experiment 1: Effect of wheat bran on mercury concentration in tissues after MeHg exposure.

We investigated the effect of wheat bran on Hg accumulation after a single administration of MeHg chloride (5 mg MeHgCl /kg). Female BALB/c mice were housed in metabolic cages and fed diets containing 0% or 30% wheat bran in a basal diet (AIN-76); urine and feces were collected for 4 weeks. At the end of the experiment, mice fed the 30% bran diet showed lower total Hg levels in blood and brain than animals fed the basal diet. Two weeks after MeHg administration, the cumulative Hg excretion in urine was increased markedly by incorporation of 30% bran in the diet of the mice, whereas fecal Hg excretion increased slightly. These results suggest that dietary bran accelerates urinary Hg excretion and subsequently decreases tissue Hg levels in mice.

Experiment 2: Metabolism of MeHgCl by *Bifidobacterium* and *Lactobacillus* strains

The presence of *merA* genes, encoding mercuric reductase, the key enzyme in detoxification of mercury in bacteria, was examined using PCR in *Lactobacillus* strains. *Lactobacillus* strains were obtained from the American Type Culture Collection. Putative *merA* sequences were amplified from DNA extracts of *Lactobacillus* strains, using a PCR primer set designed to capture the known diversity of *merA*. Amplified PCR products were then sequenced, and similarity to *merA* genes was examined by *Blastn* search. The results of *Blastn* analysis suggested that the amplified PCR products may be putative *merA*.

2. Clinical group

§ Research

In Minamata disease victims, with increasing age, symptoms due to cervical spondylosis or metabolic syndrome occur along with Minamata disease. It becomes difficult to diagnose Minamata disease solely on the basis of clinical neurological data. Therefore, establishment of an objective method for accurate identification of MeHg poisoning including inamata disease is necessary.

This research group conducted experiments for the objective evaluation of the neurological functions of patients with Minamata disease by using magneto encephalography (MEG).

The following is an outline of the research conducted by this group in fiscal year 2012:

[Research theme and summary]

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

(Project research)

Masaaki Nakamura

(Department of Clinical Medicine)

The aim of this study was establishment of an objective evaluation protocol using MEG for assessing brain function of MeHg poisoning. We focused on the responses in the sensory cortex and the candidate MEG findings specific to the Minamata area, which had been detected during past testing. We plan to examine the sensitivity and the specificity of these findings by comparing with the results from subjects in Kumamoto city, a MeHg non-polluted area.

The tremor is one of the important symptoms of Minamata disease, but little is known about the pathophysiologic mechanisms underlying this symptom. In the corticokinetic coherence analysis, coherence spectra showed peaks at less than 10 Hz and the sources of the coherent MEG signals were located in the rolandic sulcus that was contralateral to the hand movement in control subjects. We plan to collect data on cases of Parkinson's disease, spinocerebellar degeneration, and essential tremor to establish a tremor evaluation system using MEG.

§ Activities

In recent years, the issue of compensation of Minamata disease has begun to move toward a political resolution. 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 perfume rehabilitation technical schools, and care technical schools. We also examined the usefulness of vibration therapy for 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 the project entitled "Home support model study including care prevention" (FY 2006–2008). After this project, we conducted projects entitled "Community development project for home care support including healthcare practice" (FY2009-2011) and "Community welfare promotion business for

supporting Minamata disease victims” (FY: 2012) so that these concepts could be applied to the community. Furthermore, through health seminars, we are working to improve the health of residents in MeHg polluted areas.

Activities theme and summary

(2) Practice of rehabilitation for patients with Minamata disease and dissemination of information on care and rehabilitation

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 quality of life (QOL). The continuous vibration therapy undertaken to relieve the severe pain and spasticity caused patients with fetal-type Minamata disease the improvement of activities of daily living (ADL). The findings were reported in an academic research journal. Further study on the usefulness of the vibration therapy to relieve the pain and spasticity in chronic patients with neurological disorders was performed in cooperation with another facility. We have acquired the good data for all cases. Repetitive facilitation exercise for neuronal circuit strengthening (the KAWAHIRA method) was performed in cases with spasticity or deep sensory disturbance; it also was useful for improvement of motor function. In addition, the training programs for the practice of basic ADL, i.e., eating and swallowing were performed. An active introduction of care equipment enhanced their ADL abilities.

Annual workshops are held for rehabilitation and specialized staffs to improve their techniques that can be brought back to patients. This year the 5th workshops were held with themes such as “Regional

medical cooperation for patients with chronic obstructive pulmonary disease (COPD)—exercise therapy and lifestyle habit” as a rehabilitation technique and “Dietary modification to prevent poor nutrition in the elderly or persons with difficulty in swallowing” 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 to share information on techniques for care and rehabilitation that can be applied to communities.

(3) 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” (FY 2006-2008) to examine methods of support including rehabilitation that lead to improvement of ADL for aging Minamata disease patients and their families. Following this project, we performed 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) so that these concepts could be applied to the community.

In addition, we conducted educational activities in Minamata city and Izumi city for improving occupational therapy in this area. Through this support, we strengthened the connection between our institute and the local community.

(4) Health seminars (Other activities)

Koji Murao

(Department of Clinical Medicine)

We have provided information on various illnesses through health seminars conducted in cooperation with the Minamata-shi Ashikita-gun Medical Association since FY 2003. In FY 2012, we held seminars on the following topics: “Exercise and physical activity to prevent metabolic syndrome and locomotive syndrome,” “Lecture on home medical care,” and “Correct usage of health food and supplements.” These seminars generated considerable interest among citizens.

3. Risk recognition and information services group

Interest in trace methylmercury (MeHg) intake due to eating fish has been increasing all over the world. A variety of information including biased interpretation has been presented. This research group intends to recognize the exact level of risk and the current state of MeHg intake through the transmission of accurate and precise information.

The research outline of this group in fiscal year 2012 is as follows:

§ Research

[Research theme and summary]

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

Masaaki Nakamura

(Department of Clinical Medicine)

We analyzed data carried out by neurological examination (194 residents: 117 males and 77 females) in 2010 and 2011 and held a briefing session about the health effect of mercury on Taiji residents at Taiji-cho public hall on May 30, 2012. Magnetoencephalography (MEG) was performed on 15 subjects with hair mercury concentrations higher than 50 µg/g. An abnormal waveshape was found in five of 15 subjects. We will evaluate the significance of this finding in a future study.

To the survey on the effect of MeHg exposure on child development, we made final preparations in Tokyo on June 11, 2012 and performed pediatric examinations at Taiji-cho multi-purpose center from August 12 to August 6. The examinations were carried out in cooperation with Doshisha University, Tohoku University, Akita University, Osaka City University, Jin-ai women's college, Kagawa University, and

Nachikatsuura spa hospital.

(2) Exposure assessment of MeHg and other heavy metals in pregnant women and fetuses (Fundamental research)

Mineshi Sakamoto

(Department of International Affairs and Environmental Sciences)

Chorionic tissue of the placenta, cord tissue, and maternal and cord red blood cells (RBCs) samples were collected from 48 Japanese mother-child pairs after birth. The profiles of MeHg, inorganic mercury (I-Hg), lead (Pb), cadmium (Cd), selenium (Se), zinc (Zn), and copper (Cu) were assessed in freeze-dried chorionic tissue of the placenta and umbilical cord tissue to determine their concentrations in maternal and cord RBCs. Concentration of Pb, Cd, I-Hg, Se, Zn and Cu in placenta were significantly higher than in cord tissue. Among the investigated elements, Cd concentration in placenta was significantly higher (66 times) than cord tissue, suggesting that the placental barrier appears to function strongest against Cd. On the other hand, MeHg concentration in cord tissue was only 1.6 times higher than observed in the placenta, indicating exceptionally higher placental transfer of MeHg compared to other elements. Total mercury (T-Hg) showed significant and strong correlations among placenta, cord tissue, maternal RBCs, and cord RBCs. MeHg in placenta and cord tissue also showed significant and strong correlations ($r = 0.84$ and 0.94 , respectively) with T-Hg in cord RBCs. Therefore, T-Hg or MeHg in placenta and cord tissue can be used as equally good biomarkers for prenatal MeHg exposure in new-borns. Se among the examined tissues showed significant and moderate correlations. Pb, Cd, Zn, and Cu levels in cord and placenta tissues showed no significant correlation with cord RBCs. Exceptionally,

Cd in placenta showed a significant correlation ($r = 0.63$) with maternal RBCs, indicating that Cd in placenta may be a useful predictor of maternal exposure to Cd during gestation.

(3) Protective effect of Se against MeHg toxicity and co-existence of Hg and Se in humans and sea-mammals (Fundamental research)

Mineshi Sakamoto
(Department of International Affairs and
Environmental Sciences)

The effects of MeHg in the developing mice brain and the protective effect of Se were studied.

Pregnant mice (ICR) were administered diets containing MeHg (8 ppm as Hg), Se-yeast (2 ppm as Se) alone or co-exposure to MeHg and Se-yeast during the entire gestation period. After the birth, normal diet (CE-2) was fed to the maternal mice during the breast-feeding period. Mice were sacrificed at postnatal day (PD) 22. Increases in oxidative stress markers of malondialdehyde (MDA), 4-hydroxy hexenal (4-HHE) and hexanoyl-lysine (HEL) were observed in the cerebrum and hippocampus in the MeHg groups, but these oxidative stresses were negated by co-exposure to Se-yeast. These results indicated that MeHg exposure during gestation caused the oxidative stress in the cerebrum and hippocampus of pups at PD 20, but Se could prevent the symptoms.

(4) Evaluation of sensitivity factors to MeHg exposure – Application of pathological animal model and knock out animal (Fundamental research)

Megumi Yamamoto
(Department of Basic Medical Science)

We examined the toxic effects of MeHg exposure in KK-Ay type 2 diabetic mice to clarify how metabolic changes associated with type 2 diabetes mellitus (T2DM) affect MeHg toxicity. MeHg (5 mg Hg/kg/day p.o.) was given to male KK-Ay and C57BL/6J (BL/6) mice 3 times per week for 6 weeks beginning at 4

weeks of age. The average body weights (BW) of vehicle-treated BL/6 and KK-Ay mice were 16.3 g and 16.4 g, respectively, on the first day and 24.8 g and 42.3 g, respectively, on the last day of the experiment. MeHg-treated KK-Ay mice began to lose weight around 5 weeks after MeHg administration. Six of 7 MeHg-treated KK-Ay mice showed hind-limb claspings in the final stage of the experiment. The mean blood Hg level of MeHg-treated KK-Ay mice reached a maximum of 9.8 $\mu\text{g/ml}$, whereas that of the MeHg-treated BL/6 mice was 2.8 $\mu\text{g/ml}$ after 10 days of MeHg treatment. The average T-Hg concentrations in the cerebrum and epididymal fat pad were 7.4 $\mu\text{g/g}$ and 0.57 $\mu\text{g/g}$, respectively, for BL/6 mice and 27 $\mu\text{g/g}$ and 1.6 $\mu\text{g/g}$, respectively, for KK-Ay mice. In MeHg-treated KK-Ay mice with neurological symptoms, CD204-positive macrophages were observed in the brain, kidney and spleen, indicating CD204 could be a marker for injured tissues. BW loss and significant pathological changes were not observed in other groups of mice. These results indicate that body fat gain in T2DM and low Hg accumulation in adipose tissue increased MeHg concentrations in organs and enhanced toxicity in KK-Ay mice at the same dose of MeHg per BW.

(5) Study of information and perception on health risk of low-level exposures to MeHg (Fundamental research)

Noriyuki Hachiya
(Department of Epidemiology)

The cognitive implications were discussed on results obtained from a series of experience-based risk communication surveys, on participants of hair Hg analysis, for MeHg exposure through consumption of fishery products, and the results were also compared to findings on radiation risk awareness observed in another survey recently conducted in Shirakawa, Fukushima prefecture. In the risk communication survey, it was observed that health risk of the low-level

MeHg exposure was perceptible without emotional rejection, and demand for information should be met for their sufficient understanding and for anxiety reduction of their concerns over the risk problem. The observed risk perception of MeHg was contrasted with that of radiation risk derived from the large nuclear power plant accident after the earthquake and tsunami. On the other hand, it should be pointed out, on the basis of an affect heuristic model, that there is a tendency, markedly among Japanese consumers, to consider “fish is a good and healthy food, and then the risk of fish consumption is small” after a risk trade off in the perception of damages and benefits on daily consumption of fish having not only health benefits but health risks. The affect heuristic model can also explain irrational risk perception found in the risk communication survey and some inconsistency with consumers in some other countries.

§ Activities

[Activities theme and summary]

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

Noriyuki Hachiya

(Department of Epidemiology)

The effective operation of the Minamata Disease Archives has been processed on applications of the hall, utilization of the exhibition room including periodical update of the contents, and managements of materials and documents on Minamata Disease. As an administrative institution that holds documents and materials on Minamata Disease for academic uses, the Minamata Disease Archives consolidates the contents lists and search systems in conformity to applicable rules and regulations. The general policy for inspection and copy was clarified supposing the principles of the public use of the collection and of personal information protection. The system-construction was pursued for the effective operation under limited man power, and

an information searching and inspection corner was setting up in the first floor lobby. The advisory panel for the Minamata Disease Archives was held, in November 2012, to report and discuss the current summary of the visitor, updating of the collection and the exhibition contents, and annual activities plan.

(7) 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 in hair samples and assessed survey information including gender, age, occupation and residence in areas of the Philippines concerned with Hg pollution. Furthermore, we presented information on hair Hg measurements at the National Institute for Minamata Disease by distributing pamphlets at an international conference and on the home page. As a result, we received inquiries from the Philippines (different from the current report), Italy and Sri Lanka as the research has grown in scope. In addition, we have continued monitoring of hair Hg for 8 years in French Guiana and 3 years in the Philippines.

(8) Digitization of histopathological slides of Minamata Disease patients for permanent reservation (Other activities)

Masumi Marumoto

(Department of Basic Medical Science)

Digitization of 18 cases of Minamata Disease pathological slides was carried out for permanent reservation. Moreover, HTML files for public presentation and to open to the public after the following fiscal year are due to be created. Hg location analysis, which was not carried out at the time of dissection, was carried out using formalin fixed materials in paraffin blocks. To show

distribution of MeHg, X-ray analytical microscope (XRF) and electron probe microanalyzer (EPMA) analysis were carried out. Although T-Hg in formalin fixed materials by XRF was detected in the organ, which has thickness, detailed analysis of localization could not be specified. The localization of Hg and Se was analyzed by EPMA using paraffin embedded slices of Minamata Disease patients. As a result, T-Hg (MeHg and I-Hg) and Se were detected shown to co-exist in a Minamata Disease patient's organs (liver, Kidney and cerebrum). In some areas of the cerebrum, Se and Hg existed independently. It has been suggested that Hg which exists independently without co-existing Se is possibly MeHg.

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

Masaaki Nagano

(Department of Basic Medical Science)

In 2012, 1697 hair samples were analyzed for Hg; the samples were collected from the applicant among visitors at National Institute for Minamata Disease, Minamata Disease Archives, and from other organizations. The analytical results were sent to each individual. The geometric mean of T-Hg concentration was 1.23 $\mu\text{g/g}$ for females (n=937) and 1.64 $\mu\text{g/g}$ for males (n=620) in Japan. On the other hand, mean concentrations of 0.66 $\mu\text{g/g}$ and 0.78 $\mu\text{g/g}$ were obtained from females (n=66) and males (n=74) in other countries, respectively.

4. Social and epidemiological group

More than half a century has passed since the official acknowledgement of Minamata disease. While the significant environmental pollution of methylmercury has become a historical episode in its fishing sea areas, the social impacts of the Minamata disease epidemics can still be found in the local communities with the past pollution. This research group carried out historical validations of risk management problems for environmental and health destruction by MeHg, and social restoration was analysed on the affected areas.

The outline of this group's research in fiscal year 2012 is as follows:

[Research theme and summary]

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

Noriyuki Hachiya

(Department of Epidemiology)

Literature research was conducted on two issues of risk evaluation and management during Minamata disease history. The first issue focused on the problem of epidemiological evidence with respect to threshold of the onset of sensory disturbance caused by MeHg. A non-observable adverse effect level (NOAEL) of MeHg is 50-125 ppm in hair for sensory disturbance among the adult (WHO 1990), and was derived with consideration of the Swedish report (1971), which evaluated the data on hair Hg concentrations of 31 Minamata disease patients in Niigata. Some problems were discussed on the evaluation from an epidemiological viewpoint with additional consideration of findings obtained from studies carried out afterwards. The second issue was on the risk

management problem in Japan for prevention of health effects associated with MeHg contained in fishery products. The provisional regulatory standard, 0.4 ppm of total Hg and 0.3 ppm on MeHg in fish, was determined in 1973 as a measurement to control so-called "mercury panic" that had appeared after a concern to possible epidemics of Minamata disease in areas other than Minamata and Niigata during the year. Tuna and some other fish categories have been known to accumulate MeHg at relatively high concentrations. These fishes were, however, excluded from subjects to the regulation, because of limitation and imperfection in setting a unified regulatory standard for fishes with different feeding habitats. Furthermore, no dietary guidance was reported to be conducted for frequent consumer of tuna, even though the guidance was specified to be necessary in the official announcement of the regulatory standard. Although the regulatory standard was also cited in countrywide investigations on mercury pollution of water environment in Japan during the mid 1970's, the practical implications of the standard have now mostly diminished. On the other hand, fish advisories have been announced since 2003, for pregnant or may be pregnant woman to suppress the consumption of specific kinds of fishery products, including tuna, with the aim of preventing adverse health effects on development of the fetus as the most susceptible sub-population.

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

Tazusa Arakaki

(Department of International Affairs
and Environmental Sciences)

Social issues were clarified on the local regeneration of areas affected by Minamata disease. One of the main subject was changes in the emotional confrontation degenerated in the local community after the outbreak of Minamata disease.

1) Questionary survey in Minamata

A questionnaire was distributed and collected from residents' association officers in Minamata City, via postal mail, to investigate a situation for activity of Moyainaoshi (the re-establishment of emotional ties or reconciliation). Results indicated that current facilities, including Moyainaoshi Community Center, were well known, and different efforts of the city were mostly supported among the officers. There was small need to make any specific effort in the local area they inhabited.

2) Hearing study in Niigata

A supporter of Minamata disease patient in Niigata, an officer of Niigata local government, and researchers of river engineering and sociology in Niigata were interviewed on concerned issues of the current situation of local regeneration in Niigata.

Local governments in Niigata have been providing few original measures against the social opposition appeared because of the outbreak of Minamata disease. However, Niigata prefectural government are now conducting projects including a field museum planning and original measures for patient welfare under a co-operation with a patient supporting group.

(3) Research on the Revitalization of the Central City Area and the Job Creation in Minamata (Fundamental research)

Rie Harada
(Department of International Affairs
and Environmental Sciences)

The result of Minamata City's shopping street conducted by Minamata City with Rie Harada's support in 2011 showed typically 1) aging owners, and 2) low liquidities of shops.

Since 60% of store buildings were designed for shopping and residential space occupied by shop owners, this makes "Shuttered Street" shopping malls (deserted mall) easier in the case of owners closing their doors.

Another issue is that only 40% of store-owners have a designated successor, which also seems to have contributed to mall decline.

Moreover, the survey indicated other issues with Minamata City, which may be hurdles toward future revivals. They include:

- 1) Difficulties such as financial, depopulation and aged society, commonly faced by Japanese regional cities in general.
- 2) Victims and victimizer of Minamata diseases living together in the same region.
- 3) National government and Kumamoto prefecture government strongly stepping-up and engaging regional governmental conducts in Minamata City.
- 4) Many public projects initiated by the national government (Ministry of Environment) may provide rich regional economies, however this makes regional characteristics inactive.

5. Local and global environment group

Our research group implemented investigations that focused on the mercury (Hg) cycle or chemical change of Hg in the environment based on Minamata disease caused by exposure of methylmercury (MeHg) in the environment. Hg circulates through land, water, and the atmosphere, and its chemical form changes in each environment, i.e., Hg is released as a metal that becomes MeHg and so on and circulates to arrive in the human body via the food chain. Based on this fact, our research group investigated the movement of MeHg in the atmosphere, rain, seawater, sediment, soil and life. Also, each primary researcher exchanged information with other researchers, establishing joint ownership of the results. Because all of the aims are mutually related this sharing of information assisted the aims of individual research projects.

The fiscal year 2012 outline of this group's research is as follows:

§ Research

[Research theme and summary]

(1) Marine ecosystem and mercury behavior in Yatsushiro Sea.-Quantitative survey of marine benthic community in Yatsushiro Sea and mercury concentration of several fishes through food web- (Fundamental research)

Keisuke Mori

(Department of Epidemiology)

In the five year study plan, the sample collection that becomes the basic data was roughly finished in the first three years. In Minamata Bay, sediments, fishes and benthic organisms for mercury analysis were sampled in intertidal area and bottom area. The total mercury analysis of them advanced well, and the prospect followed to a methyl mercury analysis. The frame and the research plan were able to be fixed to a joint research concerning the gene analysis and the stable isotope analysis.

It is thought that the mercury contamination investigation of Indonesia advances according to schedule by the collection of the bottom sediment, fish and benthos, and human hair along the river. Because some fishes showed a considerably high mercury value, the announcement to the resident is necessary.

(2) Research on its influences and behaviors of mercury in an aquatic environment of Minamata Bay (Fundamental research)

Akito Matsuyama

(Department of Epidemiology)

- 1) Mercury monitoring in Minamata Bay was carried out. As a result, dissolved methylmercury concentration did not increase via year as well as 1 behavior of dissolved methylmercury concentration in 2012.
- 2) Investigation of time course change of microorganisms activity in Minamata Bay was carried out by using seawater samples of 2012 in this year. As a result, species of microorganisms in Minamata Bay obviously changed among winter to summer.
- 3) Investigation of Hg concentration in Minamata bay sediment was carried out at the request of the Minamata fisheries cooperative. As a result, 717 cut samples were obtained from sediment samples that were taken at Minamata Bay. An average Hg concentration derived from all sediment samples was 6.2 ppm; an average value of surface in Minamata Bay was only 3.2 ppm. A previous average value of total mercury concentration in surface sediment carried out 25 years ago was 4.7 ppm.
- 4) Mercury monitoring was carried out at 5 points of seawall of reclamation area in Minamata Bay in 2012. Also, especially, the mercury monitoring was carried out in rainy season (Jun to July) 1time per every week. As

a result, large differences of dissolved total mercury concentrations were not recognized in this year as well as last year.

- 5) In order to get reliability of dissolved methylmercury analysis as for seawater, intercomparison exercise was carried out by using ethylation method and Dithizon method(Akagi method). As a result, since a large difference in both analytical data was not recognized, reliability of Dithizon method for methylmercury analysis in interstitial-water was kept.
- 6) Experimental indoor system that was based on water tanks is building at department of engineering- Kyushu University as a response of scientific research grant now. Basically, this system will be operated from April in this year. Also, continuous experimental condition as for nutrients and DOC analysis etc. in seawater was adjusted.

(3) Research on chemical reactions, transport and deposition of atmospheric Hg (Fundamental research)

Koji Marumoto
(Department of International Affairs
and Environmental Sciences)

To investigate transport and deposition processes of atmospheric Hg in the East Asian region, weekly sampling of wet depositions and air for Hg analysis have continued at Minamata and Hirado since June, 2011. MeHg in the wet deposition samples was also measured. In addition, manual sampling of gaseous elemental Hg, gaseous oxidized Hg and particle bound Hg was carried out at intervals of 12 hours, 6 or 7 days a month at Minamata. Then, the same sampling was also carried out at Hirado in 6 or 7 days in each season. The atmospheric Hg has been continuously monitored at Fukuoka city since March, 2012. Moreover, in March, 2012, simultaneous Hg monitoring in the air at the several sites in northern Kyushu islands was achieved for 2 weeks in

collaboration with other research organizations. Based on this monitoring data, we investigated factors affecting seasonal variations in atmospheric concentrations of Hg on Kyushu, which is the nearest region from the Asian continent.

(4) Study on natural emission sources of atmospheric mercury (Fundamental research)

Koji Marumoto
(Department of International Affairs
and Environmental Sciences)

To better understanding on Hg evasion from sea surfaces, dissolved gaseous Hg (DGM) in seawater was investigated in the Minamata Bay. First of all, a new sampler for the determination of DGM in seawater was developed to prevent volatile loss of DGM from samples. Using this sampler, more accurate measurement of DGM in seawater was achieved. Next, the concentrations of DGM in seawater and atmospheric Hg, water characteristics and meteorological parameters which are needed for calculating Hg flux from sea surfaces by a gas exchange model were observed at 6 sites of Minamata bay 8 times from July to December, 2012. Based on multiple regression analysis using the observed datasets, it was clear that the DGM concentrations are controlled by water surface temperature, salinity, redox potential (ORP) and total Hg concentration. Then, the Hg fluxes from the sea surface of Minamata Bay were calculated. The Hg fluxes ranged between 0.1 and 33 ng/m²/h, with average ($\pm 1\sigma$) of 5.4 ± 6.8 ng/m²/h. The observation will continue to calculate annual flux of Hg from sea surface of Minamata Bay.

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

Syoko Imai
(Department of Epidemiology)

The purpose of this study was to explain bioaccumulation in the food web from sediment to demersal fish. We carried out exposure tests of the sandworms as benthic organisms using the sediment from Minamata Bay. As a result, total Hg concentration in sandworms increased depending on total mercury concentration in the sediment. We plan to experiment exposure test of demersal fish fed sandworm including mercury compound. From these exposure test results, we will study the biomagnification of Hg compound from sediment to demersal fish.

total of about 120 people took part in the Forum from 15 countries.

§ Activities

[Activities theme and summary]

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

Mineshi Sakamoto

(Department of International Affairs and Environmental
Sciences)

We conducted Hg analysis of the samples with two researchers from the Mongolia and one researcher from Indonesia. A cooperative study on mercury analysis was conducted with a researcher from USA. NIMD Forum was held in September 2012 by 10 researchers from 7 countries (Canada, Denmark, Hongkong, Taiwan, USA, Brazil and Japan). In total, 18 researchers were dispatched for the international conference and field research.

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

Mineshi Sakamoto

(Department of International Affairs and Environmental
Sciences)

The NIMD Forum 2014 “Recent Advance in Mercury Toxicology and Environmental Health” was held during 27-28th September 2014 with 13 researchers from Hong Kong, Taiwan, Brazil, Denmark, USA, Canada, and Japan. A

6. Publications and Scientific meetings

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