Preventive Measures against Environmental Mercury Pollution and Its Health Effects

JAPAN PUBLIC HEALTH ASSOCIATION

October 2001

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Contents

Preface

There have been two outbreaks of Minamata disease in postwar Japan, responsible for a huge number of victims, representing the great methylmercury poisoning disaster due to environmental pollution. This manual was prepared based on the lessons learned from the Minamata example in order to prevent such disasters from ever occurring again.

Human use of mercury dates back several thousand years and continues to this day. Mercury pollution and subsequent poisoning have been experienced repeatedly. The major cases of mercury pollution reported since the 1960s are listed below.

1. In the early 1960s, mercury-based pesticides caused a rapid decline in the number of wild birds in Sweden.

   In the mid-1960s, methylmercury contamination in fish and other foods was then reported.
2. In 1969, the methylation of inorganic mercury in the bottom sediment of fish farms and other such locations was clarified and attention was again focused on environmental pollution with methylmercury.
3. Large-scale methylmercury poisoning occurred in the spring of 1971 in Iraq. The number afflicted reached 6,530 (including 459 deaths) in only 2 months. The poisoning was caused by ingestion of wheat seed that had been disinfected with methylmercury.
4. In the Amazon River area, metallic mercury from gold mining activities has been discharged into the environment. Methylmercury contamination in fish in the area was revealed in the 1990s and attracted world attention. The same type of contamination is becoming a problem in Tanzania, the Philippines, Indonesia, Vietnam and China.
5. Improvements in mercury analysis technology are making it possible to detect much smaller quantities of the element. These advances continue to shed light on the extent of mercury pollution throughout the world.

Environmental contamination by mercury is clearly a growing problem even today. As shown by the Minamata disease experience, countermeasures adopted after such an outbreak has occurred are too late. In order to prevent damage in the earliest possible stages, the state of contamination must be constantly monitored and prevention measures must be taken at the earliest possible time.

This manual was drawn up for practical application to prevent the current increase in the extent of mercury contamination, primarily in the countries mentioned above. The manual, containing knowledge and countermeasures for preventing environmental mercury contamination and the accompanying health effects, is intended for persons working in environmental protection, public health and medical treatment. The manual is therefore useful for NGO, such as environmental protection groups. In addition to providing action guidelines to combat mercury contamination, this manual acts as a study text for use in preparation for contamination.

Although great efforts were made to use the latest data for standards, there was a degree of unevenness left in the data used. Therefore, be aware that not all data is in complete agreement. The most recent data, from an international standpoint, must be collected and used. There are currently no universal standards or legal restrictions related to mercury and mercury compounds. The example of Japan is therefore often used as a reference in the manual.

October 2001

Chapter 1　Introduction

Section 1 Chemical Properties of Mercury

Item 1 Inorganic mercury

1. Definition of inorganic mercury

Metallic mercury is a liquid at room temperature. Mercury is the only metal with this property. Mercury also evaporates to create a vapor (thought to be in elemental form, called mercury vapor (Hg)) at room temperature.

Inorganic mercury compounds are compounds of mercury and can be divided into monovalent and divalent compound groups. Monovalent compounds include mercury(I) oxide (mercurous oxide) and mercury(I) chloride (mercurous chloride).

Common commercially-available divalent compounds include mercury (II) chloride and mercury (II) oxide. Mercury (I) oxide is unstable and easily decomposes into metallic mercury and divalent mercury. Other monovalent compounds can also be decomposed by heat to create metallic mercury and divalent mercury.

Mercury (II) fulminate is explosive and was previously used in detonators.

Mercury in metallic state and these various inorganic compounds is generally called inorganic mercury. Some of the properties of inorganic mercury are shown below.

2. Physical and chemical properties and toxicity of some inorganic mercury compounds

2-1 Metallic mercury

Atomic weight: 200.59 (Hg^0)

Melting point: -38.89～-38.87°C

Boiling point: 356.58～356.9°C

Specific gravity: 13.59 (0°C)

Vapor saturation concentration: 13.2 mg/m^3 (20°C)

2-2 Mercury (I) chloride (Mercurous chloride)

Molecular weight: 472.09 (Hg_2Cl_2)

Virtually insoluble in water, the compound is a white, tasteless, odorless, powder. The compound is decomposed by sunlight into metallic mercury and mercury (II) chloride.

2-3 Mercury (II) chloride (Mercuric chloride)

Molecular weight: 271.52 ($HgCl_2$)

Melting point: 277°C

Specific gravity: 5.44

Soluble in alcohols, ethers, and other solvents in addition to water.

The compound is a colorless crystal, a white granule, or a white powder. The compound acts as an irritant to skin and mucous membranes.

2-4 Mercury (I) oxide (Mercurous oxide)

Molecular weight: 417.22 (Hg_2O)

The compound is a black powder that is insoluble in water. The compound is easily decomposed by light or heating into metallic mercury and mercury (II) oxide.

2-5 Mercury (II) oxide (Mercuric oxide, mercuric oxide red, mercuric oxide yellow)

The compound exists as an irregularly shaped, orange-yellow powder (yellow precipitate) or an orange-red powder (red precipitate) with high lustre.

Molecular weight: 216.61 (HgO)

Specific gravity: 11.03 (yellow precipitate), 11.00～11.29 (red precipitate)

The compound decomposes without displaying a specific melting point.

Slightly soluble in water; 5.2 mg/100ml (yellow precipitate) and 4.87 mg/100ml (red precipitate) at 25°C.

Item 2 Organic mercury

1. Definition of organic mercury

Organic mercury describes mercury atoms chemically bonded to carbon atoms. The behavior of organic mercury in the environment and the organism differs from that of inorganic mercury. The toxicological characteristics of organic mercury are also different.

While various organic mercury compounds exist, alkyl mercury compounds (represented by methylmercury) are the most important from the standpoint of environmental pollution and toxicology. Other compounds include the aryl mercury (represented by phenyl mercury) and alkoxyalkyl mercury (represented by methoxyethyl mercury) used in pesticides.

Methylmercury is generated naturally, primarily by microorganisms, in addition to synthetic processes.

2. Physical and chemical Properties and toxicity of some organic mercury compounds

2-1 Methylmercury compounds

These compounds are expressed using the CH_3HgX chemical formula and molecular weights vary depending on X. Compounds with chlorine are called chloromethyl mercury (methylmercuric chloride). When X is a halogen the vapor pressure is high. In particular, when X is chlorine or bromine, the vapor saturation concentration at 20°C is very high at 94 mg/m^3. However, halogen compounds do not dissolve easily in water but are soluble in organic solvents.

Other compounds of this type include ethyl mercury (C_2H_5HgX) and propyl mercury (C_3H_7HgX) with the methyl group substituted by some other alkyl groups.

2-2 Phenyl mercury compounds

These compounds are expressed using the C_6H_5HgX chemical formula and molecular weights vary depending on X. Examples include compounds where X is chlorine, nitrate, and acetate. The vapor

saturation concentration of these compounds is very low when compared to methylmercury compounds. The compounds are also easily dissolved in water.

Chlorophenyl mercury (Phenyl mercuric chloride)

Hydroxyphenyl mercury (Phenyl mercuric hydroxide)

2-3 Methoxyethyl mercury compounds

These compounds are expressed using the $CH_3OC_2H_4HgX$ chemical formula and molecular weights vary depending on X. The vapor saturation concentrations of these compounds vary, probably due to mixing of metallic mercury.

Chloro(2-methoxyethyl) mercury (Methyoxyethyl mercuric chloride)

Acetato(2-methoxyethyl) mercury (Methoxyethyl mercuric acetate)

2-4 Dialkyl mercury

Dimethyl mercury compound is expressed using the $(CH_3)_2Hg$ chemical formula. The compound is in the form of volatile liquid. Dialkyl mercury compounds include diethyl- mercury and dinorpropyl mercury. These are all volatile liquids.

Section 2 Biochemical Characteristics and Toxicities of Mercury and its Compounds

Item 1 Inorganic mercury

1. Metallic mercury

Poisoning occurs through inhalation due to the easy vaporization and high vapor saturation concentration of metallic mercury. The vapor has a high absorption rate in the airway (80% or more in humans). After being absorbed into the body, mercury is oxidized into the divalent mercury ion. However, since a certain amount of time is required before oxidation, some of the unoxidized mercury vapor exists in the blood stream. Mercury vapor has no charge and easily passes through the blood-brain barrier. Therefore, even though metallic mercury is classified as inorganic, mercury poisoning with primarily central nervous system symptoms occurs.

With high concentration exposures chemical pneumonitis occurs. At lower concentrations mercury poisoning with primarily central nervous system symptoms occurs. Biological effects still occur when the concentration of the exposure is even lower (Refer to "Section 7 Signs and Symptoms, and Treatment for Poisoning" for details). In addition to inhalation, mercury can be absorbed through ingestion and contact with the skin. However, the quantities absorbed through these routes are small. Toxicites reported are following:

Human, inhalation, 150 $\mu g/m^3$ / 46 days (insomnia, lack of appetite, restlessness, diarrhea) <Archives of Environmental Health 33, 186, 1978)

Human, oral ingestion, 43 mg/kg (tremors, jaundice, etc.) <Journal of Toxicology, Clinical Toxicology 31, 487, 1993>

2. Inorganic mercury compounds

The problematic poisonous characteristic of inorganic mercury compounds is corrosion. When solutions with high concentration of these compounds are ingested, corrosion occurs inside the oral cavity and in the upper digestive tract. Pain is felt in the oral cavity and pharynx and is accompanied by continuous vomiting, chest pain, abdominal pain, and bloody diarrhea. When the corrosion is severe dehydration and shock occur.

The absorption rate in the digestive tract is approximately 10% at the most. In contrast to metallic mercury, distribution to the central nervous system is low and kidney damage is the primary result. Renal insufficiency occurs due to the degeneration of renal tubules. The poisonous characteristics of various materials are shown below.

2-1 Mercury (I) chloride

A poisonous characteristic of mercury (I) chloride is acrodynia (pink disease). This was seen in children exposed to tooth pastes, lotions, and ointments containing the compound. Other poisonous characteristics are derived from decomposition into metallic mercury and divalent mercury.

2-2 Mercury (II) chloride

Some of the information related to poisonous characteristics is shown below.

Human, oral ingestion, 50 μg/kg (miscarriage at 10 weeks of pregnancy) <American Journal of Obstetrics and Gynecology 80, 145, 1960>

Human, oral ingestion, 57 mg/kg (gastritis, lung function damage) <Japanese Journal of Toxicology 8, 157, 1995>

Human, oral ingestion, 86 mg/kg (blood plasma volume changes, bleeding from the stomach) <Journal of Toxicology, Clinical Toxicology 26, 189, 1988>

Human (female), oral ingestion, 18 mg/kg (urine volume reduction～anuria) <Human & Experimental Toxicology 11, 53, 1992>

Rat, oral ingestion, LD_{50} 1 mg/kg <Pesticide Manual 9, 550, 1991>

Rat, subcataneous, LD_{50} 14 mg/kg <Japanese Journal of Experimental Medicine 39, 47, 1969>

Rat, intra-venous, LD_{50} 1272 μg/kg <Archives of Toxicology 58, 243, 1986>

Mouse, abdominal, LD_{50}, 3900 μg/kg <Pharmaceutical Chemistry Journal (English Translation) 25, 891, 1991>

Mouse, subcataneous, LD_{50} 4500 μg/kg <Nippon Eiseigaku Zasshik. Japanese Journal of Hygiene 34, 193, 1979>

Mouse, intra-venous LD_{50} 4992 μg/kg <Quarterly Journal of Pharmacy & Pharmacology 21, 364, 1948>

2-3 Mercury (II) oxide

Some of the information related to poisonous characteristics is shown below.

Rat, oral ingestion, LD_{50} 18 mg/kg <National Technical Information Service. Springfield, VA22161 Formerly U.S. Clearinghouse for Scientific & Technical Information. PB214-270>

Rat, muscular, LD_{50} 22 mg/kg <Progress Report for Contract No. PH-43-64-886, Submitted to the National Cancer Institute by The Institute of Chemical Biology, University of San Francisco>

Mouse, oral ingestion, LD_{50} 16 mg/kg <Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases 25(7), 27, 1981>

Mouse, abdominal, LD_{50} 4500 μg/kg <Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases 25(7), 27, 1981>

Item 2 Organic mercury

1. Methylmercury compounds

In contrast to inorganic mercury compounds (excluding mercury vapor), these compounds are distributed in greater quantities in the central nervous system. The toxicity of the compounds is based on this characteristic.

In addition, alkyl mercury compounds also include ethyl mercury (C_2H_5HgX) and propyl-mercury (C_3H_7HgX). Although these other compounds are thought to behave within and have the same effects on living organisms as methylmercury, these other compounds are more easily decomposed.

Rat, oral ingestion, LD_{50} 29915 μg/kg <Bulletin of Environmental Contamination and Toxicology 14, 140, 1975>

Rat, abdominal, LD_{50} 11 mg/kg <Toxicology and Applied Pharmacology 22, 313, 1972>

Rat, muscular, LD_{50} 23 mg/kg <Progress Report for Contract No. PH-43-64-886, Submitted to the National Cancer Institute by The Institute of Chemical Biology, University of San Francisco. San Francisco, CA 941171, U.S.>

Mouse, oral ingestion, LD_{50} 57,600 μg/kg <Acta Anatomica 104, 356, 1979>

Mouse, abdominal, LD_{50} 10 mg/kg <Toxicology and Applied Pharmacology 42, 445, 1977>

2.Phenyl mercury compounds

Phenyl mercury decomposes quickly in the body. The toxicology is therefore nearly identical to that of inorganic mercury.

- **Chlorophenyl mercury (Phenylmercuric chloride)**

Rat, oral ingestion, LD_{50} 60 mg/kg <Pharmaceutical Journal 185, 361, 1960>

Rat, abdominal, LD_{50} 50 mg/kg <National Academy of Sciences, National Research Council, Chemical-Biological Coordination Center, Review. Washington, DC 5, 30, 1953>

Rat, subcataneous, LD_{50} 47 mg/kg <Japanese Journal of Experimental Medicine. 39, 47, 1969>

- **Hydroxyphenyl mercury (Phenylmercuric hydroxide)**

Mouse, intra-venous, LD_{50} 18 mg/kg <U.S. Army Armament Research & Development Command, Chemical Systems Laboratory, NIOSH Exchange Chemicals. Aberdeen Proving Ground, MD 21010 NX#03648>

3. Methoxyethyl mercury compounds

Methoxyethyl mercury decomposes quickly in the body. The toxicity is therefore similar to that of inorganic mercury.

• **Chloro(2-methoxyethyl) mercury (Methoxyethyl mercuric chloride)**

Human, oral ingestion, TDLo 114 mg/kg (Sleepiness, nausea, vomiting) <Journal of Toxicology, Clinical Toxicology JTCTDCW 19, 391, 1982>

Rat, oral ingestion, LD_{50} 22 mg/kg <Farm Chemicals Handbook. Meister Pub., 37841 Euclid Ave., Willoughy, OH 44094 -, C194, 1991>

Mouse, oral ingestion, LD_{50} 47 mg/kg <Prehled Prumyslove Toxikologie; Organicke Latky, Marhold, J., Prague, Czechoslovakia, Avicenum, -, 1200, 1986>

• **Acetato(2-methoxyethyl) mercury (Methoxyethyl mercuric acetate)**

Rat, oral ingestion, LD_{50} 25 mg/kg <Occupational Health Review. 15, 5, 1963>

Mouse, oral ingestion, LD_{50} 45 mg/kg <Eisei Kagaku. Hygienic Chemistry. Nippon Yakugakkai, 18, 248, 1972>

4. Dimethyl mercury

Dimethyl mercury becomes monomethyl mercury within the body and then becomes toxic.
The toxicity is therefore the same as that of methyl mercury. However, dimethyl mercury is volatile and therefore easily inhaled. The compound is also easily absorbed through the skin.

Section 3 Use of Mercury

Mercury has been used in dry batteries, mercury compounds, fluorescent lamps, thermometers, meters, electronic equipment, amalgam (for dentistry and alloys), and other areas.
The use of mercury is rapidly declining. The domestic demand for mercury in Japan was 1,187t in 1970. Of this, 75% was for caustic soda production. In 1973, due to the third outbreak of Minamata disease, the caustic soda production method was changed to the diaphragm method and the quantity of mercury used rapidly declined. The total domestic demand in 1975 was much reduced 220 t. The quantity of mercury used in amalgam was 24 t in 1975 and 32 t in 1980.

Methylmercury, phenyl mercury, and methoxyethyl mercury were all used in antimold agents, pesticides, medical supplies and other items.

Section 4 Pollution Sources

Item 1 Man-made pollution

Pollution sources for metallic mercury and inorganic mercury include waste processing plants and factories producing metering equipment (p.g. thermometers), fluorescent lamps, batteries, and other products. In the manufacture of pesticides containing mercury, cosmetics containing mercury, and other products, various inorganic and organic mercury compounds are handled. These locations can

therefore also be sources of mercury pollution. Mercury may be mixed in the water drainage from caustic soda production facilities and chlorine manufacturing industries using chlor-alkali processing. When the mercury amalgam method is used in metal refining, mercury vapor will be released into the atmosphere during combustion. Mercury vapor is also released into the atmosphere from the burning of fossil fuels and the incineration of waste containing mercury. Since methylmercury, phenyl mercury, and methoxyethyl mercury have all been used in antimold agents, pesticides, medical supplies and other items, pollution can also occur through the spreading of pesticides, disinfection of seeds, and use or discarding of medical supplies.

The above types of pollution occur under the following conditions or accidents.

1. Pollution may occur due to accidents caused by errant operations at work sites where mercury is used. In these situations workers maybe acutely exposed to relatively high concentrations of mercury.
2. Continuous pollution at low levels can occur at work sites using mercury on a daily basis. In this situation workers may suffer chronic exposure.
3. Pollution may come from discharge into water drainage routes. Waste water containing mercury is held in a reservoir and processed until the level is below the standard (e.g. 0.0005 mg/L in Japan). This water is then released into rivers and streams. However, environmental pollution may still occur from long-term discharge of low mercury levels in processed waste fluid or escape of waste fluid still containing high mercury concentrations from reservoirs that has not yet been processed. In these cases, the mercury is methylated in the environment and accumulates in fish and other organisms. Chronic general public exposure then may occur.
4. When materials containing mercury are discarded, the soil and water surrounding the disposal site may become contaminated.
5. Discharge into the general atmosphere can also occur in cases 1 and 2 above.

Item 2 Pollution from natural sources

In the natural environment mercury is discharged into the atmosphere through volcanic activity. Hot water derived from mercury mines and volcanos must also be considered as a pollution source. In addition, mercury is thought to spread from the land surface and the bottom of the ocean. Although dimethyl mercury is probably the form spread from ocean water and other bodies of water, this becomes mercury vapor in the air (refer to "Section 5 Transport and Methylation in the Environment").

Section 5 Transport and Methylation in the Environment

Item 1 Mercury in the environment

Mercury in the atmosphere is broadly divided into gas form and particulate form.

Most of the mercury in the general atmosphere (95% or more) is in gas form. Gaseous mercury includes mercury vapor, inorganic compounds (chlorides and oxides), and alkyl mercury (primarily methylmercury). However, 90～95% or more of the gaseous mercury is mercury vapor. The total mercury concentration (not distinguishing between chemical forms) in the atmosphere is often several ng/m^3.

Little is known about the chemical forms of mercury in water. Inorganic mercury dissolved in ocean water is thought to exist in the form of dissociated $[HgCl_4]^{2-}$ ions. With fresh water, however, there are few chlorine ions and mercury is thought to exist in the form of $Hg(OH)_2$. In both ocean and fresh water methylmercury is thought only to exist as a complex. This complex consists of methylmercury bonded to SH groups. Therefore, if the environment provides abundant organic particulate matter, most of the methylmercury present will exist in a form bonded to other matter.

The environmental standard in water areas in Japan is 0.0005 mg/l of total mercury. In a survey conducted in 1996, 1 out of approximately 5000 survey points for ocean water had a value that exceeded the environmental standard.

Mercury is also present in food. The food with the highest concentration of mercury is fish, particularly large fish. In these cases over 90% is in methylmercury form and there is little inorganic mercury. The provisional standard for fish in Japan is 0.4 ppm for total mercury.

However, some samples do exceed this amount. It has also been reported that marine mammals exhibit high levels of mercury, particularly in the liver.

Item 2 Methylation in the environment

Inorganic mercury may become methylated in the environment (particularly in soil). It is not rare to find bacteria and other microorganisms in the environment (soil), such as methane gas producing bacteria, which contain methylcobalamine (methyl B_{12}) in their bodies. When this methylcobalamine meets inorganic mercury ions, methylmercury is easily generated through chemical processes. The methylmercury thus generated then decomposes (by UV light, etc.).

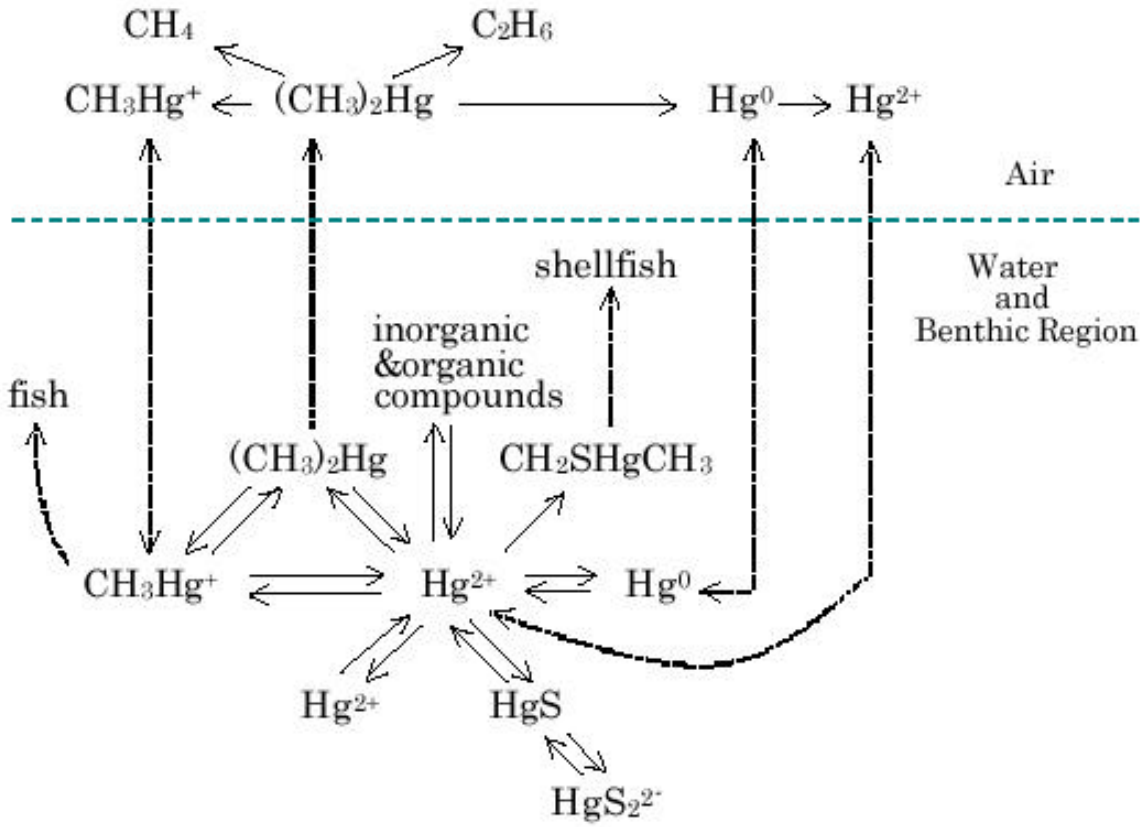

Fig.1　Conversion of Mecury in the Environment
(Beijer and Jernelöv, 1979)

In this way an equilibrium is eventually created between inorganic mercury and methylmercury. The time required to reach this equilibrium in soil is approximately 2 months when the mercury concentration is 1 mg/kg, 0.5 years when the concentration is 0.1 mg/kg, and 1.5 years when the concentration is 0.01 mg/kg. Although only a maximum of 5% of the inorganic mercury is methylated under normal conditions, this rate increases under certain conditions such as contamination by organic materials, soil contamination by hydrochloric acid, and contamination by high concentrations of inorganic mercury.

Methylation of mercury in the environment has an important meaning when considering effects on humans. If the mercury methylated in the soil and other locations reaches water systems, biological concentration occurs through the food chain. Even if the methylmercury generated is in very small amounts, concentration to high levels can occur in fish and other foods. When these foods are ingested, chronic human exposure becomes possible.

Section 6 Routes of Exposure

Item 1 Exposure to and intake of inorganic mercury

Exposure to inorganic mercury may occur through absorption in the airway, ingestion into the digestive tract, and absorption through the skin. There are some rare examples of suicide attempts involving subcutaneous and i.v. (intra venous) injections of metallic mercury.

Mercury vapor is absorbed at high rates of 75～85% in the airway during inhalation. Persons with dental fillings that use mercury amalgam are exposed to the mercury vapor generated. Although there is no quantitative evaluation for the exposure volume, there are estimates of 2.5～17.5 μg per day. Mercury is also taken in through smoking. The amount involved is thought to be directly proportional to the mercury content of the tobacco leaf. Since use of mercury pesticides in the cultivation of tobacco in Japan has been stopped, the quantity, on the 1～10 ng order, is not very large.

The gastro-intestinal absorption of mercury, as a metal, taken orally is very low, on the order of 0.01% or less. Although metallic mercury thus taken may vaporize then be absorbed, vaporization within the digestive tract is limited due to covering of the surface by sulfides. When inorganic mercury compounds are ingested, although absorption rates vary depending on the water solubility of the compound, the highest absorption rates are 5～10% or less.

Absorption of mercury vapor through the skin is, on average, 0.024 ngHg/cm^2 when the concentration in the air is 1 mg/m^3. Although absorption through the skin is possible from medications and chemicals containing mercury (ointments, germicides, pesticides, etc. (not currently used in Japan)), the level is not expected to be high.

Item 2 Exposure to and intake of organic mercury

Exposure to organic mercury may also occur through absorption in the airway, ingestion into the digestive tract, and absorption through the skin. However, exposure to methylmercury, which has a high absorption rate of 90% or more in the digestive tract, occurs primarily through food. In particular, fish tend to accumulate high levels of methylmercury through biological concentration. This exposure route is of major importance. Although methylmercury has a high vapor saturation pressure, there are no cases of poisoning occurring through inhalation of the vapor. The findings in the Hunter-Russell report discussed later <Hunter D, Russell D.1954>, are thought to have occurred due to a methylmercury powder.

Dimethyl mercury is thought to be poisonous even as a vapor. In addition, absorption through the skin may cause systemic toxicity.

While other organic mercury compounds will be absorbed in the digestive tract if orally ingested, the absorption rates are thought to be between the rate for inorganic mercury and methylmercury.

Section 7 Signs and Symptoms, and Treatment for Poisoning

Item 1 Inorganic mercury poisoning

The signs and symptoms of poisoning are different, even for the same chemical, depending on the concentration and route of exposure. The exposures described here are divided into acute exposure to mercury vapor at high concentration, repeated exposure to lower concentrations of mercury vapor, and exposure to inorganic mercury compounds.

1. Exposure to high concentrations of mercury vapor

Exposure to high concentrations of mercury vapor (10 mg/m^3 or more) is known to cause respiratory distress (bronchitis, bronchiolitis, interstitial pneumonitis, difficulty breathing, and coughing) and renal tubule injury and other problems. In severe cases the respiratory and kidney problems (and failure) may lead to death. On May 7th of 1993 in Fukushima, workers inhaled mercury vapor during the cutting of a heat exchanger with a gas burner. The above exposure is thought to have occurred in this accident. A total of 36 people were poisoned and 4 died. <Kurisaki E, Sato M, Asano S, et al. 1999> However, although people were exposed to mercury vapor at concentrations in the air of 44 mg/m^3 (exposure time did not exceed 8 hours), neurological symptoms, such as tremors and irritability (sleeplessness, emotional instability, etc.), were reported to be reversible. <WHO (1991) Environmental Health Criteria 118. Inorganic Mercury>

2. Repeated exposure to mercury vapor

With repeated or long-term exposure to mercury vapor, the central nervous system is the target organ. When the concentrations are relatively high (on the mg/m^3 order), tremors and personality changes called "mercury erethism" are observed. Symptoms within the oral cavity are important and include subjective symptoms such as a sensation that the individuals teeth are floating, tooth pain, gingivitis, and excessive salivation occur. Although proteinuria is also observed, it is not clear whether severe kidney dysfunction occurs. At lower concentrations, symptoms characterized by a feeling of weakness, easy fatigue, loss of appetite, weight loss, and digestive dysfunction are observed, called "asthenic-vegitative syndrome".

Even when the concentration of exposure is lower (0.1 mg/m^3 or less, average concentration sometimes even below 0.05 mg/m^3), increases in abnormal enzyme occurrences in urine (β-galactosidase and N-acetyl-β-glucosaminidase) <Langworth S. et al., 1991> and increases in subjective symptoms <Piikivi L. et al., 1989> and complaints in questionnaire surveys <Langworth S. et al., 1991> are seen. Fluctuations in the parameters of tremor <Fawer RF. et al., 1983> <Verberk MM. et al., 1986> determined by quantitative equipment, and electroencephalographic changes <Piikivi L. et al., 1989>, determined by computer analysis, are also observed.

3. Exposure to inorganic mercury compounds

Poisoning by inorganic mercury compounds most often occurs through the accidental drinking of

mercury (II) chloride or ingestion with the intent of suicide. With high concentration ingestion, the corrosive effects first damage the digestive tract, cause vomiting and stomach pain, and, in severe cases, may result in shock. Finally, renal tubule degeneration, kidney dysfunction, and nephrotic syndrome may be seen. Mercurochrome, which was previously used, also contains mercury and may cause poisoning when spread in large quantities, as with abdominal wall hernia treatment.

Item 2 Organic mercury poisoning

Among organic mercury poisoning the most important problem is methylmercury poisoning through exposure at the workplace and from environmental pollution. Exposure in the fetal period results in a set of signs and symptoms different from exposure in adults. In this section poisoning by methylmercury and poisoning by other organic mercury compounds will be described separately.

1. Poisoning by methylmercury

Methylmercury, absorbed at high rates in the digestive tract, passes through the blood-brain barrier and enters the central nervous system. As a result, nerve cell degeneration and neuron loss occur, although the mechanism for these changes remains unclear. Methylmercury also passes through the placenta and will affect a fetus during growth. During growth of the central nervous system the fetus is more susceptible, neuron migration is affected, and the structure of the central nervous system itself is altered. Therefore, exposure before birth causes more serious conditions than exposure in adulthood.

Typical methylmercury poisoning results in what is called Hunter-Russell syndrome. Three characteristics of this syndrome are; sensory nerve dysfunction, ataxia, and constriction of visual field. However, various other symptoms may be caused depending on the degree of exposure. Sensory symptoms, including paresthesia and numbness in the extremities, tongue, and lips, occur in the initial stages of mild poisoning. At moderate levels of poisoning, examples of symptoms include ataxia, constriction of visual field, hearing impairment, and extrapyramidal signs. In severe cases intermittent convulsions and death may occur.

Histopathological changes include widespread neuronal degeneration in the cerebral cortex, resulting in atrophy of the cortex. In the cerebellar cortex as well, although the changes are less severe than those in the cerebral cortex, changes such as loss of granular cells do occur.

Clinical findings in fetal methylmercury poisoning (also called fetal Minamata disease due to the cases that occurred in Minamata), caused by exposure to methylmercury prior to birth, show non-specific cerebral palsy-like features, including ataxia and various mental dysfunctions. These symptoms become more obvious during growth and development. In autopsy findings the cerebrum and cerebellum both have low structure levels and show symmetric atrophy. Histological findings show reduced numbers of cortex neurons and distorted cell structures. In less severe cases, delayed motor and mental function development are seen. Fetal methylmercury poisoning also occurred in the Iraq cases and the growth and development of 84 sets of mothers and infants were investigated in detail. “A prudent interpretation of the Iraqi date implies that a 5% risk may be associated with a peak

mercury level of 10～20 μg/g in the maternal hair. (Environmental Health Criteria.101 Methylmercury, WHO/IPCS,1990)“ (refer to "Section 9 Risk evaluation").

2. Poisoning by organic mercury other than methylmercury

The first report of organic mercury poisoning was in 1866 and involved a laboratory assistant who fell ill from dimethyl mercury poisoning. In 1877 diethyl mercury was tried as a treatment for syphilis in Germany. The treatment was quickly abandoned due to the strong toxicity of the compound. Ethyl mercury poisoning causes symptoms similar to methylmercury poisoning. While dimethyl mercury poisoning also causes symptoms similar to methylmercury poisoning, the time from exposure to the appearance of symptoms is thought to be fairly long, as in the poisoning case of a researcher that occurred in the US in 1997. <Byard RW, Couper R, Lockwood AH, et al. 1998>

Phenyl mercury and methoxyethyl mercury are easily decomposed in the body to create inorganic mercury. Distribution within the body after absorption and resulting symptoms are therefore similar to those of inorganic mercury.

Item3 Treatment

1. Inorganic mercury

1-1 Treatment at acute stage

With exposure to mercury vapor, the subject must first be removed from the exposure environment. In other words, the subject must be moved to a location with clean air. Next, care must be taken as various symptoms occur depending on the exposure concentration. If chemical pneumonitis occurs, respiration will become difficult and renal impairment will arise. Measures to deal with respiratory and renal failure must therefore be taken.

When a solution containing inorganic mercury compounds is ingested, milk should immediately be given and vomiting should be induced. Gastric lavage should also be done as required. Preventing corrosion of the digestive tract through administration of active charcoal and cathartics is very important. Corrosion of the digestive tract will cause shock and dehydration. When this occurs, water and electrolytes must be infused. Measures should also be taken in anticipation of possible kidney dysfunction.

1-2 Treatment at chronic stage:

Stimulation of mercury excretion from the body using chelating agents

Once the patient is through the acute period, treatment for mercury poisoning, in principle, involves stimulation of mercury excretion from the body. For this purpose a number of antidotal drugs have been used. These include chelating agents such as calcium disodium ethylenediaminotetraacetic acid (EDTA), penicillamine, and 2,3-dimercapto-1-propanol (dimercaprol or British anti-lewisite (BAL)). In recent years meso-2, 3-dimercaptosuccinic acid (DMSA, succimer or Chemet) and meso-2, 3-dimercapto-1-propanesulfonate (DMPS, unithiol or Dimaval) have been recognized as effective and come into wide use.

These chelating agents also have side-effects. For example, since penicillamine-mercury chelate is

discharged in the urine, there is a danger that renal failure can be increased. Since BAL is discharged in bile and urine the danger is not as great. However, side-effects include hypertension, tachycardia, and convulsions. Although DMSA is known to have low toxicity, it reportedly causes a slight and transient increase in transaminase. Side-effects of DMPS include skin erythema, nausea, and leukopenia.

With inorganic mercury poisoning, renal problems often occur. In these cases penicillamine and BAL cannot be used due to the possibility that they may increase the toxicity. In these cases DMSA or DMPS are effective. However, if histological damage is already significant, recovery from the damage cannot be expected even when these drugs are used.

2. Organic mercury

There are few reports of acute methylmercury poisoning and no effective treatment method has been definitively established. Blood transfusions to reduce the organic mercury concentration in the blood may be used in combination with the chelating agents described below.

With methyl (alkyl) mercury poisoning treatment, although penicillamine was previously used, the removal rates are extremely low. After administration, although mercury blood concentrations may temporarily increase, this is caused primarily by mercury shift from tissue. None of the chelating agents is expected to be effective in removing mercury from the central nervous system. Although BAL was once used for treatment, this treatment is currently contraindicated due to redistribution of mercury into brain tissues observed in experiments on animals.

3. Treatment protocol

Although no actual dosages have been established, the following method has been tried in the reports.

3-1 DMSA

Up to a maximum of 30mg/kg was administered with no side-effects.

a) 10 mg/day was administered for 3 days, followed by a 2 week period with no medicine, followed by another 3 day administration. This treatment cycle is repeated 5～10 times.

b) 500 mg/day is administered on an empty stomach on alternate days for a minimum of 5 weeks.

c) As a supplementary treatment 500 mg of N-acetylcystein is administered 3 times per day.

3-2 DMPS

300 mg/day was administered for a maximum of 6 months with no side-effects.

a) For outpatients 300 mg/day in 3 parts was administered for 5 days.

b) 600 mg/day in 3 parts was administered in a 6 day cycle.

Measurement of urinary mercury levels is a useful method for quantifying mercury excretion. First a 24 hour baseline mercury measurement is taken. Evaluation is then possible by measuring the quantity of mercury in the urine.

4. Evaluation of effectiveness for various chelating agents

Due to the small number of cases, there is no comparison of the efficacy of the agents using a large number of human subjects.

A comparison was done, using inorganic mercury in the kidney as the target organ, in an animal experiment using rabbits poisoned with mercury through the administration of $HgCl_2$. In this experiment DMPS was reported to be most effective, when compared to EDTA, penicillamine, and DMSA, in discharging mercury from the renal tissue. DMSA is most effective in mercury shift from tissue to the blood. However, in a experiment where DMPS or DMSA was given to mercury-poisoned mice, a significant increase in the storage of mercury (administered to the mice as $HgCl_2$) in the motor neuron was observed in comparison to mice that did not receive the chelating agents. This phenomenon is the result of the ability of the chelating agents to remove mercury from the tissue, thereby increasing the concentration in the blood. This increased concentration has the dangerous side-effect of causing further accumulation in the motor neurons.

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Section 8 Past Examples of Pollution

Item 1 Inorganic mercury

1. Workplace

Workplace contamination and exposure to mercury vapor have occurred for many years.

In Japan, the ability of mercury to create amalgams with other metals was employed in the application of a gold-mercury amalgam on a great image of Buddha built in Nara (in the 8th Century). A fire was then lit around the structure to leave only the gold leaf. A large number of poisonings occurred as a result. Similar contamination and exposure have occurred in recent years. Starting with the Amazon river region, gold mining in various areas around the world has involved creating amalgams with mercury that are then heated to drive off the mercury. As a result, workers are exposed to mercury vapor and the environment is contaminated (refer to "Item 2 Organic mercury" in this Section for details). Poisoning also often occurs at mercury mines. For example, at the Itomuka Mercury Mine in Hokkaido, Japan, typical poisoning cases occurred until the mine was closed in 1965.

Mercury is also used in the manufacture of thermometers and other precision equipment. This type of work is sometimes done at in-home factories and, since the work and living spaces are closely related or overlapped, some cases have involved the exposure of family members (including children) in addition to workers.

Mercury is the only metal that is liquid at room temperature. Due to this special property, large volumes of mercury were used by chemists in the Middle Ages and scientists in more recent times. Many of these people were therefore thought to have been afflicted with mercury poisoning.

The mercury (II) nitrate used in processing of felt hats is heated during the process. As a result, the compound decomposes into metallic mercury and divalent mercury. Poisoning of workers probably occurred through generation and evaporation of mercury vapor.

2. General Environment

In general living conditions, exposure of family members has occurred when workers return home with metallic mercury on their clothing. In addition, cases have involved students removing metallic mercury from school laboratories and playing with it at home, thereby exposing the student, friends, and family members. In cases where the gold refining work described above has been done in the home, family members have been afflicted with (chemical) bronchitis, pneumonitis, etc.

Airborne concentrations of mercury have been reported at 0.1～2 $\mu g/m^3$ in regions containing mercury ore deposits and within mine locations. Although this concentration is somewhat high, the levels return to background rapidly with distance from the mine. The effect is therefore very local. Although locations such as coal fired power plants, crematories, and garbage burial sites can be discharge sources for mercury, no differences in measurement values around these locations were found in comparison to the general environment. Moreover, the mercury concentration in the gas

discharged from waste incinerators has been reported at 0.02～0.45 mg/m^3 (0.29～3.88 g/hour as the discharge quantity).

One example of contamination by inorganic mercury compounds in the general environment involves detection of mercury concentrations over the environmental standard, in addition to arsenic and lead, at a cinder burial location. Some cases have also involved contamination of soil in former locations of pesticide factories.

Item 2 Organic mercury

Organic mercury, particularly methylmercury, has caused various contaminations. Among these cases are examples where inorganic mercury was discharged and methylated in the environment. As a result, conversion of inorganic mercury to methylmercury may result in exposure of biological systems and humans. A number of examples where poisoning occurred will be discussed here. In addition to these examples, contamination of rivers by mercury from pulp factories (Canada and Sweden) or acetoaldehyde factories (China) has been reported. In these cases the mercury accumulated in the fish then caused health problems in people who ate the fish.

1. Acetoaldehyde factory in Switzerland

Some workers handling sludge containing mercury at an acetoaldehyde plant were reported to have experienced poisoning, different from mercury vapor poisoning, in a report published in 1930. Since typical symptoms of mercury vapor poisoning, such as conditions in the oral cavity, gingivitis, and excessive saliva were not observed, the authors of this report suspected that organic mercury was being created as a by-product of acetoaldehyde production.

2. Report by Hunter and Russell

In 1940 Hunter, Bomford, and Russell reported 4 cases of methylmercury poisoning in workers at a factory manufacturing antifungal agents using unsealed equipment. Symptoms included ataxia, dysarthria, and constriction of visual field. Excluding tremors, symptoms of mercury vapor poisoning were not observed. One patient had symptoms, primarily ataxia, for 15 years after exposure ceased.

3. Minamata disease

Minamata disease occurred when methylmercury compounds were discharged into the sea and rivers from chemical factories. The compounds were directly absorbed by fish through the gills and from the digestive tract or indirectly taken into the body by consumption of food with concentrated mercury through the food chain. People living in the area then took in daily large amounts of mercury through fish and suffered from poisoning.

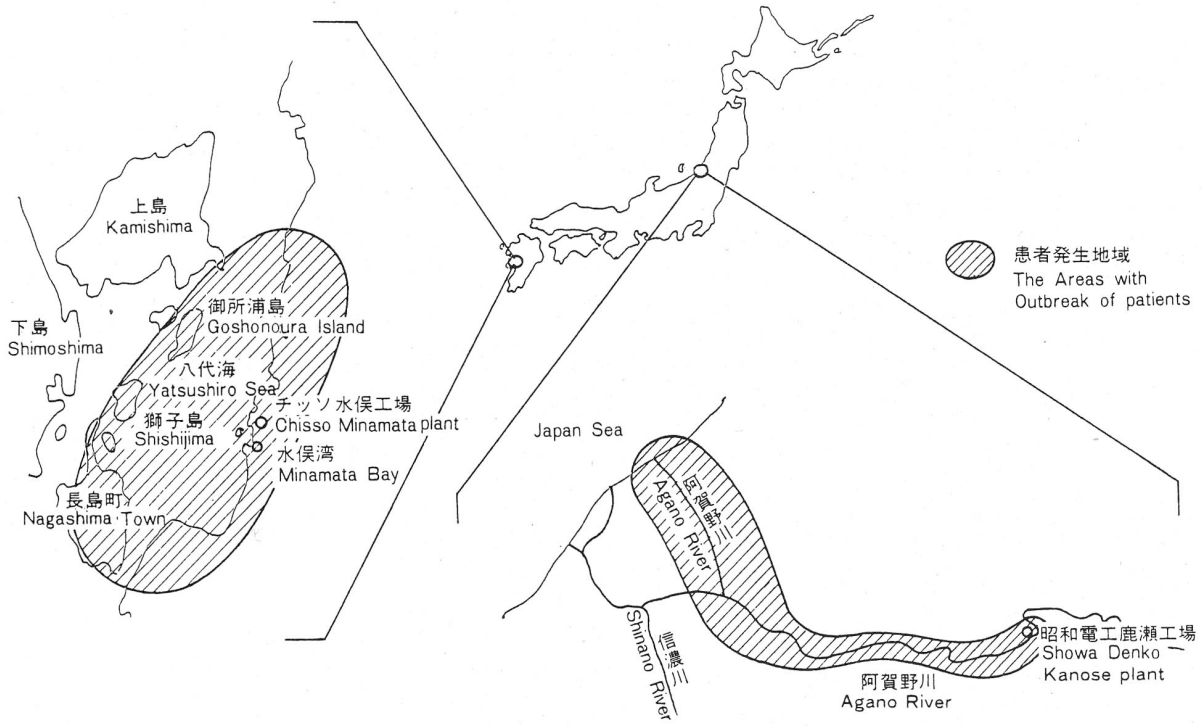

Fig.2　Map of the Areas with Outbreak of Minamata Disease

(Source: ”Our Intensive Effects to Overcome the Tragic History of Minamata Disease”)

The outbreak of Minamata disease in the area around Minamata bay in Kumamoto prefecture was first reported in May, 1956. Of the 54 patients afflicted with the disease from December, 1953 to the end of 1956, 17 were confirmed to have died. In addition to the people, floating fish, dancing cats, crows that could not fly, and other animals with abnormalities were observed. These were also demonstrated to be effects of methylmercury.

The first patients with Minamata disease in the Agano river region in Niigata prefecture were reported in May of 1965. By July of that year, 26 patients were confirmed, of which 5 died.

The number of officially recognized Minamata disease patients was 2,263 in the Kumamoto case and 690 in the Niigata case. These numbers included mothers who, although they only had mild symptoms, produced offspring having fetal Minamata disease analogous to cerebral palsy symptoms and delayed growth and development after birth. Although, typically, fetal Minamata disease patients are thought of as the 23 born between 1955 and 1959, a total of 72 fetal and infant Minamata disease patients actually exist when patients with the affliction born after 1955 are included.

The Kumamoto University Research Team charged with surveying and researching the Kumamoto Minamata disease outbreak reported pathological and clinical findings in July, 1959 along with the same symptoms as the previous organic mercury poisoning, based on the mercury values in contaminated sea sediment. In addition, methylmercury chloride (CH_3HgCl), thought to be a cause of

the disease, was extracted from the sludge of a reaction tower at an acetoaldehyde factory in 1962. Finally, in November of 1965, a model experiment succeeded in producing methylmercury compounds as a by-product of acetoaldehyde synthesis.

4. Methylmercury poisoning in Iraq

Organic mercury is often used in seed disinfection. As a result, poisoning from eating of seeds so disinfected has often occurred. The largest of these cases involved methylmercury poisoning between 1971 and 1972 in Iraq. This was a large scale poisoning with 6,530 afflicted subjects, including 459 deaths.

This poisoning was caused by wheat, supplied to help alleviate a famine in Iraq, which had been disinfected with methylmercury. Wheat seeds provided for the purpose of planting were dyed red. However, when the dye washed off after washing in water, the farmers thought the poison was washed off as well. The wheat was then ground into flour, baked into bread, and eaten. Due to the large number of victims, the relationship between intake and affect (dose-effect and dose-response relationship) was clarified through this case. In particular, the relationship between mercury concentration in hair and symptoms was clarified. In addition, the growth and development of children exposed before birth was followed in a tracking survey and the relationship to mercury concentration in the hair of mothers during pregnancy was investigated.

5. Amazon river area

In the Amazon river area mercury is used in order to more easily recover gold from bottom sediments. As a result, metallic mercury is discharged into the river and mercury vapor is driven off from amalgams into the atmosphere by heating. Although much gold has been recovered since the 1980's using these methods, approximately 3,000 tons of mercury has been released as a result.

This mercury is methylated in the river water, enters the biological system through the food chain, and becomes concentrated in living organisms. Some fish caught in the Amazon river have high concentrations of methylmercury and some subjects among groups eating fish have mercury concentrations in the hair of over 100 ppm. Probable mercury poisoning is now becoming a problem.

Section 9 Risk Evaluation

Mercury exists in various forms, including metallic mercury, inorganic mercury compounds, and organic mercury compounds. (See Section 1) The metabolism and toxicity of the various forms is different. Therefore, when evaluating risks to human health, each of the various forms must be considered separately. In addition, metabolism of mercury and mercury compounds and the resulting appearance of toxicity varywidely among different living organisms. A risk evaluation for humans should therefore be based on human data. Based on this data, if a dose-response relationship can be

established, mathematical models can be used to estimate threshold values and non-observable-effect level (NOEL). This would make risk evaluation more precise and countermeasures would be easier to take. A threshold value for the onset of disease is defined as the concentration of a harmful substance within a target organ at which point toxic effects can be observed. Although the target organ for methylmercury is the nervous system (the central nervous system in particular), since concentrations in the brains of subjects cannot be measured directly, representative values are often used from various biological samples. In addition, in the case of Minamata in Japan, children thought to be exposed in the uterus of mothers with minimal symptoms or no symptoms at all were later found to have serious brain damages. Fetuses are therefore known to be highly susceptible to methylmercury poisoning. As a result, risk evaluations with regard to methylmercury should be done separately between fetus and adults.

Item 1 Metallic mercury vapor

The primary target organ in mercury vapor exposures is the central nervous system. However, since dissolved mercury vapor is quickly oxidized to divalent mercury through the action of catalase in the red blood cells and organs, the kidneys can also be regarded as a target organ. Acute poisoning through mercury vapor exposure normally occurs as a result of accidents. Since the mercury concentration in the air under these circumstances is not known, normally risk evaluations are difficult. The relationship between dose and reaction in long-term exposures was reported for American and Canadian chloralkali factories. In this case, as the exposure of workers increased symptoms such as reduced appetite, weight loss, tremors, and insomnia also increased. In addition, continuous exposure to low levels of mercury vapor occurs through the release of mercury vapor from amalgam filings used in dentistry. IPCS criteria118 (Inorganic mercury, 1991) has summarized the risk evaluation for mercury vapor as follows.

a) When exposure is above 80 μg/m^3, corresponding to a urine mercury level of 100 μg/g creatinine, the probability of developing the classical neurological signs of mercury intoxication (tremors, erethism) and proteinuria is high.

b) Exposure in the rage of 25～80 μg/m^3 , corresponding to a level of 30 to 100 μg mercury per gram of creatinine, increases the incidence of certain less severe toxic effects that do not lead to overt clinical impairment. These subtle effects are defects in psychomotor performance, objectively detectable tremor, and evidence of impaired nerve conduction velocity, which are present only in particularly sensitive individuals. The occurrence of several subjective symptoms, such as fatigue, irritability, and loss of appetite, is also increased. In a few studies, tremor, recorded electrophysiologically, has been observed at low urine concentrations (down to 25～35 μg/g creatinine). Other studies did not show such an effect. Although the incidence of some signs was increased in this exposure range, most studies did not find a dose-response relationship. Some of the exposed people develop proteinuria (proteins of low relative molecular mass and microalbuminuria). The available studies are generally of small size and low statistical power.

c) Appropriate epidemiological data covering exposure levels corresponding to less than 30～50 μg

mercury per g creatinine are not available. Since a specific no-observed-effect level (NOEL) cannot be established and if larger populations are exposed to low concentrations of mercury, it cannot be excluded that mild adverse effects may occur in certain sensitive individuals.

Although miscarriage after mercury exposure at the workplace has been reported in some research (Goncharuk 1977, Gordon 1981), the effect could not be established in other research.

A WHO research group (1980) has emphasized that exposure to mercury vapor should be minimized as much as possible in women of child-bearing age.

Item 2 Inorganic mercury compounds

The kidney is the target organ when divalent inorganic mercury has been ingested. However, since human data on the dose-response and dose-effect relationships are insufficient, risk evaluations for inorganic mercury exposures are difficult. The IPCS criteria118 (Inorganic mercury, 1991) is also charging that research is required for the purpose of establishing a human risk evaluation for divalent inorganic mercury. When mercury (II) chloride is ingested in gram amounts due to an accident or attempted suicide, symptoms of severe renal tubule injury and of necrosis intestinal membrane may lead to death due to oliguria, anuria, and renal dysfunction. Even at smaller doses, although the effects are not as remarkable, renal tubule dysfunction are observed. These problems are reflected by such symptoms as amino acid in urine, increased urine, and discharge of enzymes into urine. When Suzuki (1983) summarized the mercury concentration in the kidneys and days to death in 9 subjects who died from acute divalent mercury poisoning, and the minimum mercury concentration was 16 μg/g.

However, since this minimum concentration in the human kidney was observed after death, when 6 days had elapsed since the actual mercury ingestion, the value is assumed to have been somewhat higher. In addition, the mercury concentration in kidney biopsy samples taken from people with nephrosis caused by mercury was 10, 25, or 15 μgHg/g.

Item 3 Methylmercury compounds

1. Adults

1-1 Threshold values

Large scale poisoning from methylmercury has occurred in Japan and Iraq. In the Japan cases, exposure indices in the period of highest concentration values were not obtained and finding the dose-response relationship was therefore not possible. On the other hand, a fairly detailed investigation was made into the dose-response relationship in the Iraq case. This case, however, involved subacute exposure and may still not provide sufficient answers for the long-term exposure like Japan cases.

With methylmercury, the most sensitive adults reportedly began to show neurological symptoms with, in the Niigata case, hair mercury concentrations of 52 ppm (Tsubaki, 1972; however, a result of 82.6 ppm was obtained when the same sample was measured again). In the analysis results for the Iraq case, Shahristan et al. (1976) found that mild methylmercury poisoning symptoms occurred with hair mercury concentrations of 120 mg/kg. Using analysis by a "hockey stick" model, Bakir et al.

(1973) calculated threshold values from the dose-response relationship between estimated methylmercury body-burden and incidence of signs and symptoms. The threshold values for body-burden that resulted in each condition were as follows. Approximately 25 mg resulted in parethesia, 50 mg resulted in ataxia, 90 mg resulted in dysarthria, 180 mg resulted in deafness, and 200 mg or more resulted in death. IPCS criteria 101 (Methyl mercury,1990) adds other information and summarizes various indices in a table showing values at which initial neurological symptoms appear in adults with the highest sensitivity.

1-2 Table. Various Indices showing the Threshold Value for Onset of Symptoms in Human Body

(Level at which nervous symptoms would appear in the most sensitive adults)

Average daily intake	3～7 μg/kg
Body burden	15～35 mg (50 kg weight)
Total mercury concentration in blood	20～50 μg/100ml
Total mercury concentration in hair	50～125 μg/g

(Source: “IPCS Environmental Health Criteria No.101 Methylmercury”,etc.)

2. Fetuses

When patients recognized to have Minamata disease born after 1955 in Minamata are assumed to be fetal cases, more than 50 overall fetal and infant Minamata cases occurred. Although 23 typical fetal Minamata disease cases with severe cerebral palsy-like symptoms occurred during the peak of the contamination, between 1955 and 1959, the mothers of these children had only mild symptoms or no symptoms at all. In the cases in Japan, as with adults, the dose-effect and dose-response relationships were impossible to obtain. Fetal type methylmercury poisoning also occurred in Iraq and the growth and development of 84 of the children were investigated in detail. As a result, a 5% of the risk of delayed walking or abnormal central nervous system function was implied when the peak mercury concentration in the hair of the mother during pregnancy was 10～20 μg/g(Environmental Health Criteria. 101 Methylmercury, WHO/IPCS,1990). However, since there were few mothers with hair mercury concentrations near the threshold level in the Iraq case, threshold values and maximum non-effective doses are difficult to estimate. The results are therefore not a sufficient answer to questions regarding the effect of low methylmercury concentrations.

In a survey conducted on the Faeroes Islands (Grandjean et al., 1997), the mercury exposure dose of mothers who frequently ate whale meat and other fish during pregnancy (geometric mean for hair concentration of mothers: 4.27 ppm) was investigated. The 917 children from the mothers were then tested at 7 years of age. A statistically significant negative association was found between the exposure and deficits in language, attention, memory and fine-motor function. Even when the mothers

with hair mercury values of 10 ppm or more were excluded, the association remained. The result suggests that even mercury concentrations of 10 ppm or less may have an effect. However, even in research showing a relationship with mercury exposure dose, most results were in the normal range and analysis of the dose-effect and dose-response relationship was not done. We have to consider seriously in evaluating the results. On the other hand, although the number of subjects in the Myers et al. (1995) survey on the Seychelles islands was increased to over 700 and the survey was repeated (Davidson et al., 1998), overall no test results showed a significant correlation between methylmercury exposure indicators before or after birth. Rather, in a number of tests, slightly better results were obtained for the exposed children. The same kind of survey is currently planned in Japan and Brazil.

The mercury concentration in umbilical blood (fetal blood) of newborn human infants is reported to be 1.3～2 times higher than the concentration in the mothers blood. The highly concentrated of mercury on the fetal side is a major problem in addition to sensitivity during the brain development period. In animal experiments conducted by the National Institute of Minimata Disease (NIMD), Japan, when methylmercury was continuously administered to pregnant rats, the mercury concentration in the brains of the offspring was approximately 1.4 times the concentration in the mothers. However, during the lactation period the quantity of mercury transpanted from the mother to the offspring was extremely low and the brain mercury concentration of the offspring rapidly decreased. Offspring exposure is therefore focused in the fetal period, whereas exposure in the lactating period is low.

An epidemiological survey on the birth sex ratio was also conducted for the residents of Minamata city and for certified patients (NIMD). The survey showed that the birth sex ratio was reversed and the number of male babies had decreased during the period between 1995 to 1959, when the methylmercury pollution was most severe. The result will be partially explained by the higher number of still-births for male babies during the time period. This type of high level methylmercury contamination is thought to increase still-birth and miscarriage rates.

3. Infants

USEPA has suggested that children 14 years and under may receive 2～3 times heavier exposure than adults due to their higher food intake per unit of body weight. Moreover, children may be thought of as a group with higher sensitivity to methylmercury than adults. However, data related to sensitivity in children is insufficient and future investigations are required.

Chapter 2　Pollution Prevention Measures

Section 1 Background Levels and Target Samples for Monitoring

In order to quickly identify mercury contamination, the background level of mercury in the environment must first be determined and periodic monitoring of environmental contamination is required. When monitoring, the sample to be analyzed must be determined according to the contamination to be monitored.

Environmental samples, such as soil, water, and air, and biological samples may be used for monitoring.

At work locations using mercury, inhalation is the primary exposure route. The concentration of mercury vapor and mercury compounds in the air as particles must therefore be measured.

Measurement of total mercury is normally sufficient for monitoring of environmental samples. However, measurement of methylmercury may sometimes be required in addition to total mercury.

Section 2 Monitoring

Item 1 Environment samples

1. Soil

Since soil accumulates mercury and is useful in elucidating contamination, it should be analyzed as a sample in any contamination case.

1-1 Collection points and method

Soil should be collected at the intersection points of a pre-determined grid. Collection from rock layers is not necessary. For soil, collect samples that do not contain gravel or wood chips.

For soil from the bottom of rivers, lakes, marshes, and ocean areas, use a mud collector, such as an Eckman Purge, and collect 10 cm of the surface layer from the bottom. Remove pebbles, shells, pieces of animals and plants, and other foreign objects from the sample. When the bottom soil is sand, most of the sinking suspension is not trapped by the sand layer. Rather, the sinking suspension accumulates on the surface of the clay layer under the sand. Samples that include this clay layer must therefore be collected.

All samples should be well mixed and passed through a 2 mm mesh sieve. The sample should then be placed in a stopped glass bottle or similar container and sealed for later analysis. In addition, a portion of the sample should be dried for weight loss measurement in order to determine the water content. Mercury concentration should then be calculated per unit of dry weight.

The date, location, general condition (appearance, color, smell, impurities, etc.) should be recorded.

1-2 Sample storage

Although containers of glass are best for storing collected samples, other containers that can be sealed may also be used. Wash the containers well beforehand using hydrochloric acid or other agents. Refrigerated storage is not necessary if a cool, dark place is used.

1-3 Evaluation of soil contamination

Generally the mercury content of soil per unit of dry weight is 0.15～0.2 mg/kg (ppm). When a measurement for total mercury content in soil exceeds 1 mg/kg, there is a risk of discharge from the soil into other environmental sectors. Mercury contamination in nearby water systems must therefore also be investigated in these cases.

2. Water

When the contamination source is directly connected to a river, lake, marsh, or ocean, or when contamination is forecast to spread from a river to a lake, marsh, or ocean, water samples from the various areas must be taken. In addition, when the total mercury per dry weight unit of soil around a contamination source exceeds 1 mg/kg (ppm), high concentration discharge into the water environment is a danger. Mercury concentrations in well water and other ground water must therefore be investigated. Even when the mercury in the water is at a fairly low level, this may accumulate at the bottom of lakes, marshes, and closed water areas. Continuous monitoring is therefore recommended. Samples from rapid water flows and from rivers after water increases, deluges, and other changes may not reflect actual contamination conditions and therefore are of little use.

2-1 Collection points and method

With lakes and marshes the collection points should center around the shore (or river entrance). At various distances water samples should be taken from the surface layer and the layer near the bottom. Surface water should be collected 20～30 cm below the actual surface if possible. Great care should be taken to prevent bottom soil from getting into water samples collected near the bottom. Remove any garbage or other waste materials. Samples should be collected after a period of relatively clear weather days with no rainy days.

Windy and rainy days should be avoided as much as possible for collection of ocean water samples and, in principle, collection should be done at high tide.

For lakes, marshes, and ocean regions, the collection date, location, general water quality, positional relationship to contamination source (if known), and other information should be recorded.

2-2 Sample storage

Water samples should be stored in glass containers that can be sealed and have been well washed beforehand with hydrochloric acid or other agents. For accurate measurement results, before transport and measurement the samples must be made acidic (pH 2 or lower) immediately after collection or EDTA must be added at a pH of 4.5 in order to stabilize the mercury. Analysis for mercury should be done as quickly as possible.

The reasoning for this treatment is as follows. The trace mercury in water samples is relatively stable at pH lower than 2. However, at pH 2～4 the mercury is easily absorbed by particles in the

water and the container wall. When the pH is 7 or more the mercury is even more easily absorbed by the container wall and other particles present. When complex ions, such as iodide and cyanide; perchloric acid; or acidic materials, such as permanganate, are present, absorption by the container wall and other particles is suppressed. On the other hand, when reducing materials such as tin are present, reduction of mercury and loss, through evaporation and scattering, is promoted.

2-3 Mercury contamination evaluation

Some representative total mercury values are 0.5～3 ng/l (ppt) for ocean water, 2～15 ng/l (ppt) for shore water, and 1～3 ng/l (ppt) for rivers and lakes.

In lakes, marshes, and other enclosed bodies of water, if the total mercury concentration in river water entering the body exceeds 2.5 ng/l, continuous monitoring is recommended.

The total mercury discharge standard based on the Water pollution control law in Japan is 0.0005 mg/l (ppb). The law also states that alkyl mercury should be undetectable at the detection limit. When a contamination accident causes a sudden release of waste water containing high levels of mercury, total mercury measurement values are evaluated based on this standard.

3. Atmosphere

Atmosphere samples are collected when release of mercury into the air is suspected. In cases involving incinerators, the stack height, weather conditions, land shape, and other factors greatly effect the scattering of the mercury released. Air samples must therefore be collected in concentric circles radiating out from the incinerator or other contamination source.

3-1 Collection points and method

Mercury concentrations in the atmosphere vary greatly. In particular, sampling points must be selected to clarify the mercury distribution by considering distance from the contamination source and prevalent wind direction. Considering possible exposure to humans, sampling points should be set at 1.5～2.0 m above ground, as this is the breathing zone of people.

For sampling, a pump is used and 100 L of air at each measurement point is pumped over 30 minutes through 100～200 ml of 0.05% potassium permanganate -1N sulfuric acid solution in a gas washing bottle. In addition to the sample, a second solution, through which air is not passed, is prepared as a reference.

Capture methods using gold and activated charcoal are also used.

3-2 Sample storage

Move the capture solution and the reference solution into sealable glass bottles and store in a refrigerator. Use a freezer when storing collected samples for 1 month or more.

3-3 Atmosphere contamination evaluation

When there is no particular contamination, atmospheric mercury levels are normally several ng/m^3 to 30 ng/m^3. Mercury concentration in the urban atmosphere in Japan is 0.005～0.1 μg/m^3 (5～100 ng/m^3). The value is lower outside of urban areas. Therefore, when a mercury value over 100 ng/m^3 is measured, a strong possibility of contamination exists.

However, values may also be high near volcanoes. For example, values of 1,000～20,000 ng/m^3,

2,000～4,000 ng/m^3, and 600 ng/m^3 have been reported in volcanic areas in Iceland, the northern-most part of Iceland, and in the capital city of Reykjavik, respectively. Mercury measurements of 120～20,000 ng/m^3 have been reported at mercury mines and precious metal mines. Therefore, high concentrations of mercury may be detected in the air in volcanic regions and mining regions.

Item 2 Biological samples

1. Significance of biological samples

Biological samples show an extent of environmental contamination by mercury. At the same time, fish in particular can be used to evaluate the exposure levels of human groups since methylmercury is accumulated through the food chain. This is done by monitoring the fish often eaten by people in the region. In addition, organisms living in the bottom sediment of the aquatic systems are useful in investigating the extent of mercury contamination in the bottom sediment.

2. Target organisms

Organisms that live in narrow habitats, in other words shellfish, particularly bivalves, are suitable as samples for determining the degree of mercury contamination. Bivalves filter and eat plankton and other organic materials through their gills. They therefore reflect the state of contamination in a relatively small area. In addition, since the gills of bivalves are relatively large compared to carnivorous shellfish, they are easier to use as samples. In ocean regions, suitable bivalves include mussels, and oysters. In land based water regions suitable bivalves include fresh-water clams and fresh-water mussels. Mussels adhere to various things and have particularly fixed habitats. In addition, they live in oceans over a broad latitude and are therefore often used in other investigations. Results for these organisms are therefore easily compared.

In carnivorous snails, the edible portion reflects the extent of contamination over a relatively long period of time.

In land based water bodies, particularly rivers, since the number of shellfish is small, there is no need to limit investigations to bivalves alone.

When considering exposure to humans through the food chain, fish types often eaten by people in the area must be selectively investigated.

2-1 Sample storage method

For fish, the collection date, location, type, gender, and, if possible, ages should be recorded. Weight, length, and other information should also be measured.

The gills, internal organs, digestive gland contents, and muscles of fish should be divided, placed in polyethylene bags, and stored in a freezer. For shellfish, the gills, digestive gland contents, and adductor muscle (edible portions of snails since they have no adductor muscle) are divided, placed in polyethylene bags, and stored in a freezer. Store the soft body parts (mantle, digestive glands, etc.) in case the adductor muscle is too small for analysis. Since bottom soil materials are often contained in the digestive glands of shellfish, remove these particles as much as possible before storage.

2-2 Contamination evaluation

The upper background limit for average (10 specimens or more) total mercury concentration in the adductor muscle or soft parts of bivalves is approximately 0.1 mg/kg (wet weight). The upper limit for meat portions of carnivorous shellfish is 0.4 mg/kg. Since the total mercury concentration in fish varies broadly between specimens, 10 or more specimens should be collected and the average value should be used. When the average value of meat portions exceeds 0.4 mg/kg (wet weight), contamination has occurred. However, when even a single specimen in a lake, marsh, or enclosed bay area exceeds 0.4 mg/kg, contamination must be suspected.

The upper background limit for average (10 specimens or more) total mercury concentration in the gills or digestive gland contents of fish is approximately 0.1 mg/kg.

Accumulation of mercury in the adductor muscle and soft body parts of bivalves and meat of fish is the result of biological concentration through the food chain. The concentrations therefore reflect contamination conditions of at least several months prior. The concentrations in gills and digestive gland contents reflect more recent contamination conditions.

The total mercury in edible meat portions of fish is nearly all methylmercury. The total mercury measurement value, which is relatively easy to obtain, is therefore sufficient.

However, since the mercury in the gills of fish is normally mostly inorganic mercury, a selective analysis of mercury is recommended when organic mercury contamination is suspected. When methylmercury in the gills exceeds 50% of the total mercury, methylmercury exposure is suspected.

3. Benthos (*Annelida*)

Annelida, including worms (on land) and clam worms (rivers, lakes, marshes, sea shores), pass soil directly through their digestive tracts to extract organic material as food. Since these worms can be used to directly investigate bottom contamination over time from the water environment, they are effective as monitoring samples.

3-1 Collection method

Soil is dug up and carefully checked to find and collect annelida. The organisms will move and be easy to find if a soil sample is placed in a polyethylene bucket then shaken onto a pad covered with water. On seashores and other places many of these organisms inhabit the backs of rocks and small stones where the sun does not shine. These can easily be found by simply turning the rocks over.

3-2 Storage method

Wash off any mud with water, remove any excess water, weigh, place in a polyethylene bag, and store in a freezer. These samples are often easily dried or, even when not dried, osmotic pressure changes cause the organism contents to ooze out. Therefore, when long-term storage is required, place the samples in a glass container for wet decomposition, weigh, and store in a freezer.

3-3 Contamination evaluation

The upper limit for background concentration of total mercury in annelida living in the soil is approximately 0.4 mg/kg (wet weight). However, the upper limit is 0.1 mg/kg (wet weight) for organisms adhering to rocks and gravel. If the mercury level in annelida does not reach the above

level even though the soil sample is contaminated, the contamination probably occurred within the last 3 months.

4. Plants

Soil contamination that has occurred over many years can be investigated using cross section samples of evergreen trees with more than several years of age. Mercury measurements for each annual ring can be compared to elucidate the contamination changes over time. In addition, blights and rotting unrelated to the seasons may show mercury contamination accompanying discharge of acid. The location and plant type are therefore recorded and a picture is taken. Mercury in plant samples may be absorbed through the roots or the air. Since these contamination paths cannot be distinguished, evaluation is difficult. Therefore, soil or animal samples are usually used when available.

4-1 Sample storage

Cross section samples of evergreen trees are placed in polyethylene bags for storage. Freezing is not required.

4-2 Plant contamination evaluation

Plants normally do not concentrate heavy metals and are therefore not suitable for contamination evaluations.

Item 3 Work places

1. Sample collection and simple measurement method

Samples for measuring mercury in the air at work places are collected using the method described (refer to Item 1 “3-2” in Section 2). Specifically, the air is passed through 100～200 ml of 0.05% potassium permanganate - 1N sulfuric acid solution. When mercury concentrations in the air are suspected to be high, the volume of air used is reduced.

Monitoring at work places is done either to determine the mercury concentration distribution and average concentration at the location or to determine the mercury concentration of air being inhaled by workers (individual exposure). When concentrations at the site are being determined a grid is created and used for sample collection within the location. When individual exposure is being determined, workers carry a pump and collection solution. Air samples near the nose and mouth are then collected. However, since carrying the pump and other equipment can be burdensome, passive samplers using gold or active charcoal may also be placed at the front of the neck for sample collection.

In work sites and other locations where high mercury vapor concentrations are suspected (such as work involving the driving off of mercury through heating an amalgam in metal refining), simple monitoring using gas detection tubes (GASTEC, Range 0.25～6 $mgHg/m^3$, Limit of detection 0.01 mg/m^3) is recommended. Mercury sniffers (Mercury/AM-2), capable of measuring mercury concentrations in the air in real time are also commercially available.

2. Contamination evaluation

2-1 Threshold limit value of mercury vapor in the air of work environments

The threshold limit value of mercury vapor in work environments in Japan, as recommended by the Japan Society for Occupational Health, is 0.025 mg/m^3. The control standards in directives from the Japan Labor Ministry are 0.05 mg/m^3 (as mercury and inorganic compounds) and 0.01 mg/m^3 (as alkyl mercury compounds*).

*Limited to compounds with methyl or ethyl as the alkyl radical.

Threshold Limit Value - Time Weighted Average (TLV-TWA) of American Conference of Governmental Industrial Hygienist (ACGIH) is 0.25 mg/m^3 (inorganic mercury including mercury vapor).

Item 4 Human exposure

The mercury in biological samples, including blood, urine, hair, nails, and breath, is measured in order to determine human exposure and body-burden. Biological samples and the significance of various measurement values are explained here for each type of mercury compound exposure.

1. Mercury vapor exposure

With mercury vapor exposure, mercury concentrations in the blood or urine are measured. Blood samples of several ml are collected as usual from a vein into an injection tube already containing an anticoagulant (heparin). The sample is then transferred into a sealed container. Freezing is used for storage and the container is filled to nearly full with only a small volume of air remaining. For urine, approximately 100 ml of sample is collected in a paper cup as with usual urinalysis. The sample is then stored refrigerated in a polyethylene bottle. Samples stored for more than 1 month are frozen.

Inhaled mercury vapor enters the red blood cells and then is oxidized. Since the oxidized mercury is then difficult to discharge from the red blood cells, concentrations in these cells are higher than concentrations in blood plasma or serum. In order to distinguish between exposure to mercury vapor and exposure to inorganic mercury compounds, measurements of the red blood cells and plasma or serum are done separately.

2. Inorganic mercury exposure

With inorganic mercury exposure, urine mercury concentrations are a good index. Samples are collected as explained above. Since this type of mercury is not readily absorbed by red blood cells, the ratio between values for red blood cells and blood serum is smaller compared to mercury vapor exposure.

3. Methylmercury exposure

In this case hair and whole blood are good samples for monitoring. Methylmercury is taken into the hair in concentrations that are 250 to 300 times the blood concentration. In addition, the mercury concentration in hair reflects the blood concentration at the time that part of the hair was formed. As a

result, if the hair is divided in the length direction and each segment is analyzed, the exposure history can be elucidated. Mercury does not accumulate in the hair in exposures to mercury compounds other than alkyl mercury. If high concentrations of mercury are detected from the hair in these other types of exposures, the mercury is probably adhering to the external part of the hair.

Hair samples should include 20 or more strands of hair (1 cm long, 10 mg), cut from the base, located behind the ear. Collect the strands so the base can be confirmed (tie bases together with thread, affix to adhesive tape, etc.). Place the sample in a polyethylene bag, close the opening, and store at room temperature.

Since methylmercury is easily taken into the red blood cells, the mercury concentration in red blood cells is higher than the concentration in serum (plasma).

4. Measurement value evaluation

ACGIH and the Japan Society for Occupational Health have established Biological Exposure Indices (BEI) for persons exposed to mercury vapor and inorganic mercury in the work environment. With ACGIH the urine value before work begins is set at 35 μg/g creatinine and the whole blood value after work at the weekend is set at 15 μg/L. With the Japan Society for Occupational Health, the spot urine value is set at 35 μg/g creatinine. As long as these values are not exceeded, nearly all people working 8 hours per day, or about 40 hours per week, will have no adverse health effects. These values cannot, therefore, be applied to groups exposed through the general environment.

In particular, groups not exposed to mercury have whole blood concentrations of 5 μg/l and urine concentrations of 10 μg/l or less. The concentration in hair for unexposed groups is 10 μg/g or less.

Section 3 Legal Regulations and Changes in Environmental Pollution Levels in Minamata

Item 1 Legal Regulations

1. Standards in Japan

The environmental standards and other legal regulations concerning mercury in Japan are as follows.

The environmental standards mentioned here are desired targets at present and do not guarantee that health effects will be certainly avoided if contamination is held below the levels.

2. Waste water

For factory waste water the general government rules determining discharge standards, based on the water quality corruption prevention law of 1974, state that the allowable level for total mercury is 0.005 mg/l and that methylmercury must not be detected (detection limit: 0.0005 mg/l).

3. Water quality

Although the regulations issued in 1971 stated that total mercury should not be detected (detection limit: 0.02 ppm) and methylmercury should not be detected (detection limit: 0.001 ppm), improved mercury measurement sensitivity later occurred. As a result, the actual environmental standard for mercury, issued in 1974, required a value of 0.0005 mg/l or less for total mercury and no detection for methylmercury (detection limit: 0.0005 mg/l).

4. Sediment quality

In a report issued by the Environment Agency in August of 1973, "Provisional Removal Standards for Sediments including Mercury (total mercury: 25 ppm (dry weight))" were shown.

5. Fish

Methylmercury concentrations in fish are known to become high due to biological concentration through the food chain. Standards for fish were set in the "Provisional Standards for Mercury in Fish" (notification for Ministry of Health and Welfare in July of 1973). In these standards wet weight is used and total mercury is set at 0.4 ppm and methylmercury is set at 0.3 ppm.

Item 2 Changes in environmental contamination levels in Minamata

With regard to Minamata Bay contamination, the Kumamoto Prefecture conducted an investigation based on the provisional removal standards of the Environment Agency notification. As a result, bottom sediment where total mercury exceeded 25 ppm (dry weight) was removed by dredging.

In this way, the bottom sediment of Minamata Bay was cleaned. In 1987, when this work was completed, the total mercury concentration in the sediment of the bay was confirmed to be below the above standard. In addition, the results of sediment quality surveys conducted in Minamata Bay and surrounding water areas in 1989 showed no locations where levels exceeded the standard.

The average value for total mercury in fish of Minamata Bay rapidly declined between 1966, when discharge from the factory became completely recycled, and 1968, when acetoaldehyde production plant was completely halted. Between that time and 1974 the levels hovered around the provisional standard of fish. However, after the sediment removal was completed, the values in fish dropped to below the standard.

Section 4 Countermeasures for Generation Sources [1)]

Item 1 Countermeasures in work processes

Countermeasures for discharge sources of mercury in principle involves halting the use of mercury, or if mercury is to be used, none of the mercury for the work process should be released outside. The following are possible countermeasures to take in work processes.

- Develop and implement processes that do not use mercury.
- Minimize mercury use.
- Set mercury concentration standards for managing water and air qualities within each work process. Also set countermeasures to take when the standard values are exceeded. Then, conduct periodic monitoring.

Consider an example using water quality, which is relatively easy to manage. Water that has touched mercury in the work process must be in a closed system that is absolutely not released to the outside. An outline is given in the below example.

- Water that contacts mercury in factories working with mercury should be completely separated from cooling water, washing water, rain water, and other water systems at the factory.
- The quantity of water used in the process of mercury using should be minimized.
- Water that has touched mercury should be reused after mercury removal processing, discussed later.
- Waste water should be stored then used as the mixing water in concrete solidification of waste materials.

Item 2 Processing countermeasures for discharge from factories, mining, etc.

Although realization of the above measures (process conversion, etc.) has a significant meaning from the standpoint of environmental protection, often the measures require some time and expense. Symptomatic countermeasures are therefore required at factories using and gold mining until removal and other measures can be realized within the work process. Processing countermeasures currently possible are discussed below for each processing target.

1. Discharged air

Metallic mercury evaporates at room temperature and has a high vapor pressure. When spilled on the factory floor, mercury generally breaks into small particles, thereby increasing its surface area by hundreds to thousands times. Vaporization therefore progresses rapidly. In addition, since the specific gravity of mercury is heavy, it is difficult to remove from small cracks and other minute locations. As a result, exposure to mercury vapor continues for a long time. Some representative gas discharge processes are discussed below.

1-1 Method using mercury absorbing solutions

The primary form of mercury in gas discharge is metallic mercury vapor. A fair amount of research

has been done into absorption solutions for removing mercury in the air using acids and oxidative agents. The solution with the greatest absorbing effect is sulfuric acid with potassium permanganate. Historically, this solution was called the Cameleon solution. The solution realizes its effect through the strong oxidizing power of potassium permanganate in the acidic environment of sulfuric acid. Regarding concentrations, potassium permanganate should be 0.01 mol/l and sulfuric acid should be 1.5 mol or greater. The purple color of the solution is lost when the effect is gone so replacement periods are easy to determine. The reaction formula is shown below.

$$2KMnO_4 + 3H_2SO_4 \rightleftarrows K_2SO_4 + 2MnSO_4 + 3H_2O + 5O$$

However, this oxidation reaction is not selective for mercury. Since all other coexisting components will also be oxidized, solutions must be prepared after considering the deterioration rate of the reaction solution.

1-2 Method using solid materials as absorbing agents

Many material types based on activated charcoal loaded with chemical compounds to increase absorption of mercury from exhaust gas have been reported. Some of the loaded compounds include $CaCl_2$, divalent iron compounds, and chelating agents. As an example, when activated charcoal specialized by chelating agents for the absorption of mercury is packed in an absorption tower, an inlet gas mercury concentration of 4.2 mg/m^3 can be reduced to 0.001～0.01 mg/m^3 at the outlet.

1-3 Purely physical method

Vapor pressures can be reduced by such activities as pressurization and cooling. The condensed mercury mist can then be recovered using sand filters, glass wool, or other materials. Since extremely high pressures and low temperatures are often required to sufficiently remove mercury in exhaust gas, this method easily becomes uneconomical. Normally this method is used in combination with other methods such as absorption.

1-4 Processing proposal: Gold mining using mercury amalgam

Capturing mercury scattered in air can require processing of a large volume of air. Therefore, this process is uneconomical. Processing at gold mining sites in developing countries is a hypothetical target. Metallic mercury is used in gold mining. Therefore, mercury evaporated using high temperatures is gathered in a local exhaust device. Reliable removal is possible using only the mercury vapor pressure difference when the entire collection container is then rapidly cooled using water. Currently, using this concept, simple processing equipment is being developed for mercury vapor countermeasures in gold mining.

2. Waste water

Excluding mercury compounds, such as $HgCl_2$, mercury is generally not very soluble in water. Solubilities are listed in 2-1.

In addition, the solubility of various compounds are greatly affected by solution pH. Normally, solubilities tend to increase as pH decreases. Since all of the mercury in a solution (including organic

mercury) may not be in ionic form, ionization of these materials beforehand is required for treatment. As an example, under the exsisting chloride in the solution, strong oxidative agent, such as hypochlorite, can be added to make oxidation state of solution and the mercury can therefore be converted to a more soluble form such as $HgCl_4^{2-}$.

2-1 Table. Solubilities of various mercury compounds

Mercury and Mercury Compounds	Solubility in gram per 100ml of water (20~25°C)
Hg	2×10^{-6}
Hg_2Br_2	4×10^{-6}
$HgBr_2$	6.1×10^{-1}
Hg_2Cl_2	2×10^{-4}
$HgCl_2$	6.9
HgI_2	Minimally soluble
HgI_2 (β-form)	Minimally soluble
Hg_2O	Insoluble
HgO	5.3×10^{-3}
α-HgS	1×10^{-6}
β-HgS	Insoluble
$Hg(SCN)_2$	6.9×10^{-12}
$HgNO_3 \cdot 1/2H_2O$	Soluble
Hg_2SO_4	6.0×10^{-12}
Hg_2CrO_4	Minimally soluble
$HgNO_3 \cdot H_2O$	Separable
C_2H_5HgCl	Insoluble
$C_6H_5HgOCOCH_3$	Slightly soluble
$Hg_2(CH_3COO)_2$	Soluble
C_8H_8-HgN_2O_4	5

2-2 Precipitation and separation method

Mercury ions in a solution can be converted to sulfides and separated through precipitation. Sodium sulfide (Na_2S) and other similar compounds are often used in agents as additives. For precipitation, a sodium sulfide concentration several times in excess of the amount required for the predetermined mercury concentration is added. However, care is required since complex compounds (multi-sulfides) with high solubilities can be created when too much sodium sulfide is added. The mercury removal effect using the sulfide method generally results in a final concentration, after processing of 0.1～0.5 ppm. The method is therefore often used in combination with other methods such as activated charcoal absorption.

2-3 Displacement method

Mercury easily bonds with metals to create amalgams (alloys of mercury and other metals). Using this property and the ionization tendency of metals, mercury in waste water can be removed. In practice, scraps and powders of metals, including copper and aluminum, are packed in a tower and waste water containing mercury is introduced from the bottom of the tower. Used metal scraps can be reused after heating and mercury recovery. With this method the mercury form in the solution can be non-valent metallic mercury or divalent mercury ions. Waste water containing 5〜10 ppm of mercury can be processed to a final concentration of 5〜10 ppb or less using this method.

As an example, waste water at a mercury refining plant was adjusted to a pH of 7.5, 0.5〜1 g/100ml of aluminum powder added, and the solution mixed for 20 minutes. The original mercury concentration of 200 ppm was reduced to 0.02 ppm or less. However, this method is ineffective for organic mercury. Therefore, in order to use the method, waste water containing organic mercury must first be processed to oxidize the organic mercury and generate divalent mercury ions as discussed earlier.

This method is easy to use due to its simplicity and large mercury removal effect.

2-4 Absorption method

Many absorption methods involve packing a mercury absorption material in a sealed tank or tower then passing waste water through in a directional or serial fashion. The equipment is simple and operations are easily automated. The number of containers can be increased as required and there is no gas generation. This method is generally used in a wide range in Japan. When the absorption method is used, the waste water flow rates, mercury concentrations, mercury forms (Hg^0, Hg^{2+}, $HgCl_4^{2-}$, etc.), absorption tower size, and other factors are limited by the capabilities and absorption principles of the absorption agent. Planning is therefore required and waste water characteristics must be elucidated beforehand. Here the method using active charcoal will be explained.

With activated charcoal, although both inorganic and organic mercury can be processed, the effect is better for acidic condition. As an example, when 5% activated charcoal was added, for absorption processing, to waste water from a vinylchloride factory in Minamata, the following concentrations were found.

Total mercury: 0.48 ppm → 0.053 ppm,　methylmercury chloride: 0.27 ppm → 0.0127 ppm

Mercury concentrations were therefore decreased to approximately 1/10 the original levels. Since there are many types of activated charcoal and most, when unaltered, have high removal limit concentrations and low absorption rate, this method is best used in combination with other processing.

2-5 Processing proposal

Considering processing of waste water containing mercury in countries other than advanced countries, the most important factors are low cost and simplicity of processing work. Therefore, since high cost reagents and facilities cannot be used, methods with easily understood principles and low processing costs are required. Of the processing methods described above, the displacement method is probably the most effective in this regard. Materials such as metal scrap (e.g. copper, aluminum) are more easily obtainable than other processing materials. In addition, in practical application, if the

metal scrap can be spread sufficiently throughout the waste water path instead of in a packing tower, mercury can be removed. Although the extent of concentration reductions is not clear, mercury in waste water can be reliably reduced.

Item 3 Waste processing

Waste materials containing mercury may include the following.

- Mercury can be found, in small quantities, in such items as electrode shavings from mercury plants and factories handling mercury, discarded electric tools, discarded meter parts, and discarded fluorescent lights. In everyday surroundings, mercury sources include mercury batteries and thermometers.
- Activated charcoal and waste chelation resins that have absorbed large quantities of mercury.
- Brine sludge generated by caustic soda production using mercury as a catalyst.
- General waste from mercury mining related activities (waste soil, ore scrap, etc.).

These waste materials are generally either processed to reduce the quantity of mercury or solidify the mercury and make it insoluble. In practice, before executing each process, pulverization, separation, waste water processing, and other forms of pretreatment are required. An outline of the process is shown below.

1. Processing to reduce content

One recovery method often used involves gathering waste materials, incinerating using high temperatures at the plant (600 ~ 900°C or higher), and recovering the evaporated mercury to prevent discharge to the outside. One often used mercury recover process involves absorption using solutions and other methods discussed above. In this process a reducing agent is added, the solution is boiled, and mercury is recovered in metallic form.

2. Insolubility processing

Insolubility processing is done using cement, chemical reagents, or both to mix the mercury with waste materials. When using the cement additive method, the quantity that can be added varies depending on the form of the waste material being processed and the mercury concentration. Normally, 150 kg or more of cement must be added and mixed well for every cubic meter of waste soil.

When the mercury concentration exceeds 100 ppm and in other circumstances, addition of sodium sulfide or other chemical agents should be done in order to provide chemical insolubility before using the cement additive method. Basically, regardless of which processing method is selected, waste material characteristics must be elucidated beforehand and laboratory scale test must be used to confirm the processing effectiveness. Care is required since re-elution of mercury from processed materials is possible under some circumstances.

3. Processing proposal

Processing to reduce mercury content is the most effective form of waste material processing. However, the cost is high and, when the heating temperature is low, generation of dioxin and other chemicals is possible. Use in developing countries is therefore difficult. When measures for these countries are considered, although long-term stability of the processing may be a problem, insolubility processing is suitable from a cost standpoint. If processing is only done for metallic mercury, mixture with sulfur alone, without the use of cement and expensive chemical reagents, can be used to achieve a certain level of insolubility in mercury through the following reaction.

$$Hg + S \rightarrow \beta\text{-HgS (insoluble)}$$

When considering the quantity to add, laboratory scale test with the target waste material is required beforehand. This method can be used at low cost.

4. Reference

1) The chemical society of Japan–mercury subcommittee ed.: Suigin, Maruzen, Tokyo, pp.123-171(1977).

Chapter 3　Countermeasures After Outbreak

Section 1 Emergency countermeasures

The emergency contamination countermeasures shown here are set with a priority on preventing health damage to humans from the contamination. Accidental cases involving suicide or accid4ental ingestion are not covered here.

1. Emergency actions

Environmental contamination resulting in methylmercury exposure and adverse human health effects can be expected through ingestion of fish from the contaminated water system. On the other hand, poisoning by mercury vapor can be caused through air contamination in the work environment. Mercury contamination cases are therefore broadly divided into methylmercury (A) and mercury vapor (B) and countermeasures against adverse health effects for each case are shown in the respective flow chart (fig.3-a and 3-b). In addition, when people have been or may have been exposed to methylmercury from an unknown source, countermeasures based on the mercury values in biological samples are shown directly in (a). When people have been or may have been exposed to mercury vapor or inorganic mercury, countermeasures based on the mercury values in human biological samples are shown directly in (b).

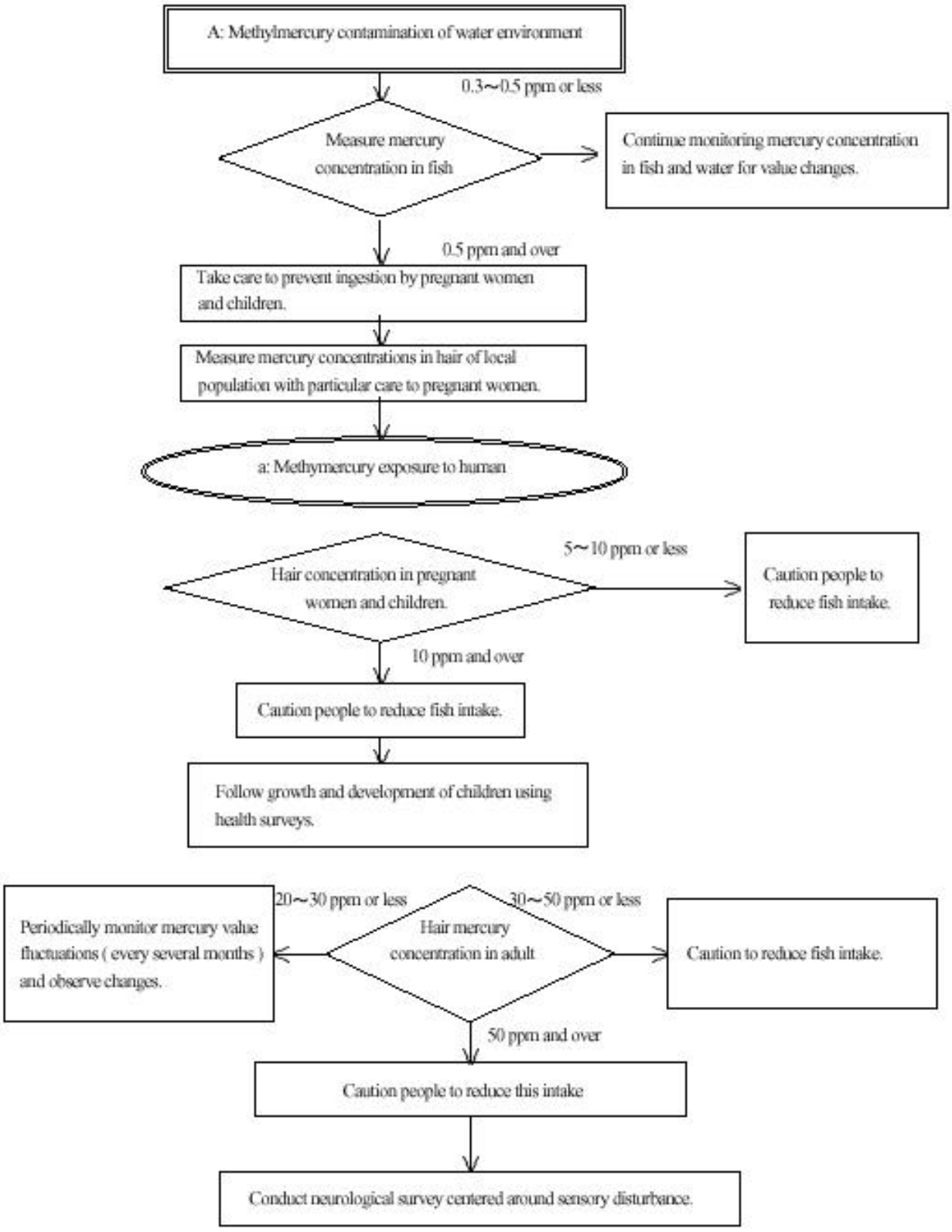

Fig. 3-a Emergency countermeasures for mercury contamination (methylmercury contamination of water environment and methylmercury exposure)

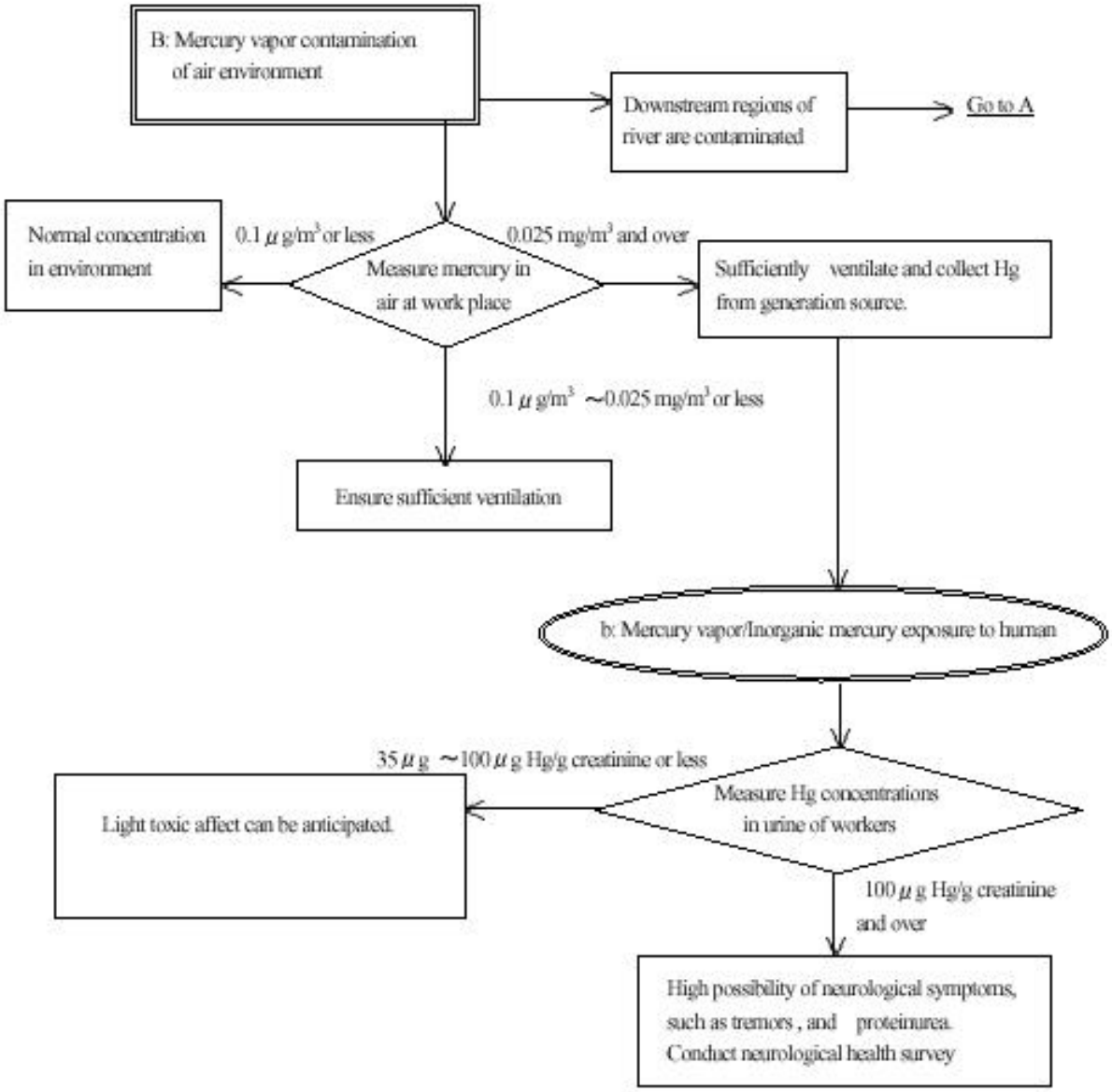

Fig. 3-b Emergency countermeasures for mercury contamination (mercury vapor at the work place , mercury vapor and inorganic mercury exposure)

2. Environment for possible problems, elucidation of workplace contamination, and countermeasures

A. Water contamination (suspected) caused by industrial waste water, mines, digging for metal, industrial waste materials, and nature through such phenomena as volcanoes Water system contamination (suspected) caused by unknown sources, measure mercury concentration in fish.

B. Contamination (suspected) caused by mercury vapor at factories, mines, or metal digging locations, measure the mercury concentration in the air.

3. Elucidation and countermeasures for anticipated human exposure to various mercury chemical forms

A. Methylmercury poisoning (suspected) of humans from unknown sources, measure mercury concentration in hair.

B. Mercury vapor/inorganic mercury poisoning (suspected) of humans from unknown sources, measure mercury concentration in urine.

Since environment and human exposure evaluations are done when executing countermeasures for emergency mercury contamination, measurement of methylmercury is not absolutely necessary. Measurement of total mercury alone is sufficient because nearly all of the mercury detected from edible (meat) parts of fish can be considered to be in methylmercury form. In addition, as long as there is no mercury vapor or inorganic mercury adhering to the sample from the outside, mercury in hair samples can also be considered as all methylmercury. Nearly all mercury in urine can be considered to be inorganic.

4. Support in emergency situations: Mercury measurement

When emergency surveys are required, if mercury cannot be measured domestically, National Institute for Minamata Disease (NIMD)* can be contacted. Suitable sample selection and transport methods will be suggested and, after analysis by NIMD, a quick response with the data will be made.

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Telephone: +81-966-63-3111 Facsimile: +81-966-61-1145 E-Mail: nimd@fsinet.or.jp

Section 2 Information Gathering and Analysis

1. Periodic monitoring

In order to prevent mercury contamination in the early stages and minimize damage, periodic monitoring of the environmental mercury levels (e.g. marine, river, soil, fish, etc.) and waste from factories, mines, and other possible contamination sources, is effective. When an increase in monitoring values is seen, the cause should be quickly clarified as soon as possible.

2. Unusual changes in the environment

When this type of quantitative data cannot be obtained, unusual changes in the environment must not be missed. Careful observation of these changes is very important. For example, in the methylmercury contamination that occurred in Minamata, fish catches were reduced, fish were floating, cats acted strangely, birds fell from the sky, and other biological abnormalities were observed before health effects in humans appeared. The local residents, particularly fishermen, are the first to recognize these types of changes. Agencies responsible for the environment and public health should visit the area in question and listen to people's complaints. These environmental abnormalities should be taken as precursors to possible health damage. Opinions of health and environment professionals should be sought and investigations of effects on the ecosystem should start in these situations.

3. Gathering information related to human health effects

When methylmercury contamination is suspected due to environmental abnormalities or monitoring results, it is very important to gather information related to human health effects from local hospitals, clinics, and public health centers. When these information should be gathered paying particular attention to the main symptoms already known for methylmercury
poisoning (sensory disturbance, ataxia, constriction of visual field, hearing impairment, etc.).

4. Knowledge on possible contamination sources

In addition, chemical materials and other substances that may affect human health should be evaluated for risk beforehand by government agencies. A system for determining and implementing countermeasure according to the nature and level of risk is needed. For example, since manufacturing processes, such as industrial processes and mines, and work conditions at possible contamination sources are normally not known by outside people, identification of a source may be difficult. However, in preparation for such situations, advance knowledge by government agencies is effective. This advance knowledge includes how much of various harmful chemicals have been discharged into the environment and how much of these chemicals were transported outside the location as waste (Pollutant Release and Transfer Register). This type of data should be used to prevent expansion of the health damage.

5. Gathering of a broad range of information

Rather than having just a response system for situations that arise, for clarifying causes, the gathering of a broad range of information across organizational lines and from various perspectives is of major importance in the early stages. Investigations from various angles, including government health agencies, clinical medicine, basic medicine, pharmacology, and engineering are required in order to gather and analyze all of the information. This type of rapid cooperation and information sharing among these specialists is indispensable in early clarification of the contamination cause. Holding periodic liaison committee to consider environmental abnormalities and accompanying health effects is an effective way to smoothly realize the necessary cooperation.

Section 3 Information Disclosure

1. Quick decision making

Countermeasures implemented after waiting for scientific clarification of the toxicity and mechanisms of polluting chemicals are too late. Therefore, even though some uncertainty may remain, it is important for agencies to decide countermeasures quickly.

2. Guidelines

In order to prevent environmental mercury pollution from worsening, government agencies must prepare legal guidelines for areas such as water quality (including both natural environments such as oceans and rivers, and waste water from factories, mines, and waste processing plants), soil, food (investigations into mercury contamination cases in many foreign countries have shown that health effects have occurred through the ingestion of contaminated food such as seafood), and workplace hygiene (at pesticide factories and mines) in order to devise appropriate countermeasures. Government agencies should use legal measures to the fullest extent possible. Government countermeasures must be set and quickly implemented with the focus on health, and necessary information should constantly be distributed.

3. Disclosure

However, before devising such countermeasures, it should be investigated whether samples are actually contaminated using samples of possible contaminated food and samples from oceans, rivers, and soil surrounding factories, mines, and other possible contamination sources, and then, it is required to determine whether or not health effects are possible. Detailed results of this investigation should be disclosed.

Moreover, constructive information sharing and cooperation is required from factories, mines, waste processing plants and other locations suspected to be contamination sources. Clear information should also be available regarding the preventative countermeasures conducted by these industries. If this type of information openness is achieved, more effective results are obtained because independent experts can evaluate and investigate the information to augment internal investigations.

4. Mass communication media

The role of mass communication media is highly useful in distributing such information. Constructive information distribution by government agencies is also necessary and effective in preventing the expansion of health effects. However, the local populations may become excessively concerned if experts do not confirm various points, including the level and extent of contamination and whether or not adverse health effects are possible. In order to allow the media to provide correct reports, impartial and unsensationalized information must be released with appropriate comments in a continuous and systematic fashion.

Section 4 Environmental Surveys

Item 1 Sample collection

1. Soil

The soil surrounding the contamination source should be sampled. As contamination is expected to migrate from the soil downward to the underground water system and then directly into rivers, lakes, marshes, and the ocean, deep soil from these sources is sampled. When collecting samples, the topography (including geological strata data if possible) should be recorded.

When the contamination source is less than 10 m above the ground and has a radius of 10 m, soil samples should be collected from the intersections of a 30 m grid centered on the contamination source area. When the contamination source is 20 ~ 40 m above the ground and spread over a radius that exceeds 100 m, topography and other factors should be considered and samples should be collected from the intersections of a 30 m grid orientated in the down-wind direction.

2. Water

When contamination of lakes and marshes is suspected, 2 water samples, one from near the surface and one from near the bottom, should be collected at varying distances centered around the suspected contaminated point on the shore (or river inlet). Surface water samples are best collected at 20 ~ 30 cm below the actual surface.

The collection of sea water, when ocean contamination is suspected, should be done on days with little wind or rain if possible. In principle, the tide should also be high. Ideally, the sample locations should be at the surface (0 ~ 50 cm below the actual surface) in the center of flow with respect to the investigation point.

For marsh, lake, and ocean samples, the collection date, location, general water quality, the distance to the contamination source (if known), and other information, should be recorded.

When a contamination source along a river flowing to a marsh, lake, or ocean area is suspected, water samples should be filtered through a glass filter (such as a Wattman GF/C) and the total mercury concentration in both the filtrate and the suspension should be measured. As the suspension will contain fine particles of both organic matter (mainly plankton, the abundance of which increases in certain seasons and may have significant daily fluctuations) and inorganic matter (bottom soil, etc.; may fluctuate depending on the river inlet point and collection time), repeatable measurement values are difficult to obtain. Therefore, if other biological or soil samples can be obtained, the suspension measurement may be omitted.

3. Biological samples

As biological samples, since mussels secure themselves to fixed habitats, they are good for elucidating contamination distribution. Refer to "Chapter 2 Section 2 Monitoring" for details regarding specific sample collection methods.

Item 2 Contamination source identification

1. Identification by mercury chemical form

1-1 Identification by bodily distribution

In the absence of reported health complaints, mapping the properties of various samples obtained in a survey of the local populace is helpful in identifying a contamination source.

Identification of the species of contaminating mercury as metallic mercury, inorganic mercury, or methylmercury is also helpful in identifying the contamination source. Normally, the total mercury concentration in blood is 1/250 of the concentration in scalp hair. When this ratio is less than 1/500, methylmercury exposure can be ruled out as a possible cause.

In addition, mercury accumulated in scalp hair due to mercury exposure through food is primarily methylmercury. When the proportion of inorganic mercury in scalp hair is more than 20% of the total mercury concentration, the adherence of metallic mercury is probably the cause.

The determination of mercury in blood serum and red blood cells is also useful in identifying the contamination source. In addition, with exposure to metallic and inorganic mercury, the total mercury concentration in urine increases immediately after the event. Normally, the ratio of total mercury concentration in serum compared to red blood cells is about 0.1. When this ratio exceeds 0.2, exposure to inorganic mercury is suspected. In the analysis of scalp hair, the sample is cut into 1 cm lengths from the base, and each section is analyzed individually. If there are no changes caused by the ingestion of fish and the mercury concentration is constant for all sections, inorganic mercury contamination, including metallic mercury, is highly probable. This type of direct mercury exposure to hair can occur as a result of using paints, skin whitening soaps, and creams that contain mercury, and from amalgams used in dental treatments, and work exposure to mercury in mines and in industry.

1-2 Epidemiological investigation

When groups of patients have already appeared, an epidemiological study can be helpful in identifying the contamination source. Although the entire group suspected of being exposed is the basis for the study,patient and reference studies may be done as required. Details are explained in the Section5 Health survey.

1-3 Analytical epidemiological study

Analytical epidemiological methods, such as case-control studies, are also helpful in identifying contamination sources.

In case-control studies, interviews are conducted with patients and healthy reference subjects. The various living habits of the 2 groups, including work, living location history, and eating habits, are compared. In this way, factors often seen in patients but not seen in healthy subjects can be elucidated. In addition, other studies are done as necessary in order to investigate the relationship between identified factors and mercury contamination. If multiple factors are identified, an investigation for common items among factors may be helpful in identifying the contamination source.

In case-control studies, the selection of suitable patients and reference subjects has a large impact on the reliability of the study. When there are many patients, all patients do not need to be considered. Rather, the study should center on patients with remarkable symptoms, or those with numerous

symptoms though not necessarily major (based on the opinion of doctors capable of neurological observation; hereafter called typical patients). Mercury measurements for hair, blood, urine, and other samples from patients and reference subjects should be taken to confirm that the problematic mercury exposure only happened to the patients. When studying the results, it is important to divide the subjects into typical patients and others. Moreover, once highly contaminated subjects and mercury poisoning subjects are identified, a cohort should be established and conduct periodic health checks for a long period into the future in order to investigate the long-term health effects.

2. Identification of the source of exposure

2-1 Epidemiological investigation

Epidemiological survey: Epidemiological studies are useful for determining the source of exposure when a patient population has been identified. An epidemiological survey for mercury poisoning consists of the following procedures:

1) medical examinations
2) measurements of total and inorganic mercury levels in blood, urine and hair
3) questionnaire surveys

Mercury poisoning patients are identified on the basis of neurological findings, and mercury exposure levels, distinguishing between organic and inorganic mercury poisoning. Patients are then compared with unaffected groups to identify the differences in demographic characteristics (gender, age, race and ethnicity), residence place of living, occupation, family history, dietary habits and so on.

Neurological findings are the most useful information for identifying methylmercury poisoning, followed by the mercury levels in hair. Inorganic mercury may be methylated in the natural environment. Once methylmercury produced by the methylation of inorganic mercury in the soil reaches the water system, methylmercury is biologically concentrated by the food chain and can reach high levels. Therefore, even inorganic mercury contamination may give rise to patients with methylmercury poisoning. However, the methylation of inorganic mercury and subsequent biological concentration occurs over a long time period (several months to years). Therefore, methylmercury poisoning is unlikely to occur in the early phases of inorganic mercury contamination of the environment.

Analysis of time distribution: Generally speaking, the analyses of chronological and geographical distributions are useful for identifying the cause of epidemics. This is also true in identifying the source of mercury contamination. According to the literature, an adult with body weight of 50 kg consuming 1 mg of methylmercury a day needs at least 30 days to accumulate 25 mg of methylmercury in his/her body, which is the threshold level for developing sensory disturbances. As the rate of accumulation differs from person to person, it is unlikely for an outbreak of methylmercury poisoning to be concentrated within 1 or 2 weeks. Although more than one case of mercury poisoning may occur in a family, the family clustering of methylmercury poisoning is unlikely to be observed in a short period of time, i.e., 1 or 2 weeks. Note here, however, that it is sometimes difficult to determine the onset of mercury poisoning as symptoms and signs gradually develop and last for a

long time period once they develop.

Geographical distribution: For identifying the cause of an outbreak, analysis of geographical distribution is also useful. It is a good idea to draw a map showing the addresses of patients (or the place where the illness occurs) using computer programs. If a computer program is used, it is also easy to calculate area-specific incidence rates and/or prevalence rates and draw maps using those parameters. When computer programs are not available, the addresses of patients can be represented graphically by pins on a map. Note here, however, that the geographical mapping of patients rather than incidence or prevalence is sometimes misleading because patients appear to be clustered in densely populated areas. As mercury poisoning is strongly related to industrial activities and fish consumption, it is necessary to examine whether patients are concentrated in the vicinity of certain factories as well as in the occupations, races and ethnic groups related to large fish consumption.

Gender and age: Symptoms and signs of mercury poisoning in adults are not considered to be dependent on either gender or age. Therefore, the differences in age and sex distributions of patients usually reflect factors related to the environment, lifestyles and occupations. In the case of methylmercury, poisoning may occur due to in-utero exposure during pregnancy. The clinical features of such children are different from methylmercury poisoning in adults.

Section 5 Health survey

Item 1 Health survey in contaminated area (population)

1. Objective

The major objective of a health check survey in a contaminated area (population) is to identify the source of mercury contamination or assess health effects. In certain instances, the survey is expected to play a role as an official measure of health care management. The methods of surveying are dependent on their objectives.

When the outbreak of the illness is already noted, epidemiological investigations are useful for identifying the source of contamination. However, in such circumstances, it is requested that the contamination source be elucidated as quickly as possible so that the outbreak can be contained. Although ideally a census survey examining all the residents is conducted in order to collect complete and accurate information, it is practically impossible to examine a large number of subjects in detail in a short period of time. As attempting a large-scale and in-depth investigation is likely to waste precious time in the early phases of an epidemic, it is a good idea to start an investigation that can be relatively easily implemented.

Once the source of the contamination is identified, the focus of attention will be shifted to the complete ascertainment of patients and health care of the exposed population.

2. Investigation for identifying the source of contamination

2-1 Emergency survey

It is unavoidable for an emergency survey to be incomplete in case assessment and to be limited in its nature because it is completed in a short time. In such cases, it is best to avoid the time wastage that occurs through aiming at perfection. As a study method, it is better to visit hospitals and clinics to interview physicians and review medical records (active method). The survey should be planned such that it can be completed within a few days or 1 week at most. Although interview with patients will not be sought, in principle, some patients may be interviewed to collect detailed information. Needless to say, it is necessary to exercise caution in interpreting the results of this survey due to its preliminary nature.

This survey is conducted in order to collect the following information, which is readily available:

1) the information to be recorded in patient registration form, including the time of the occurrence of the illness, the time of diagnosis, the name and address of the hospital, the name of the physician, the symptoms and signs, and illness history
2) demographic characteristics, including sex, age, family, race, ethnicity
3) residential history, occupational history, family history and so on

2-2 Main study

If the emergency survey described above fails to identify the source of contamination, a full-fledged study should be initiated. If the source of contamination is narrowed down by an emergency survey, a health survey of the subjects with those characteristics should be carried out in order to identify the patients completely. For example, if patients are concentrated in a town or a factory, a census survey of all the residents in the town or all the workers in the factory should be conducted. If a particular group of subjects is suspected to be highly exposed on the basis of the results obtained from the surveys of the environment, lifestyles, residential history, and occupational history, those subjects should be closely examined. In the Minamata and Niigata incidents, fishermen were of such a group.

Although a census survey takes a long time, this survey should be completed within 1-2 weeks, or 1 month maximum, considering the urgent nature of such a survey,

2-3 Case-control investigation

In an epidemiological investigation where the source of mercury contamination is to be identified, it is necessary to compare cases with unaffected subjects. One approach is a census survey, examining the entire population. However, a census survey is a time-consuming, labor-intensive, and costly operation. A relatively efficient approach compared to a census survey is a case-control study. In this approach, a sample of people (control subjects) selected from the base-population, which is the target population of the study, are compared with diagnosed patients. The control subjects thus selected are called the control series. In a case-control study, the accuracy of the data to be obtained is highly dependent on the selection of controls. In principle, the control series should be randomly selected from the base-population of the study (population control). Note here, however, that even if cases are concentrated in a particular area, the controls should be selected from a much wider area. In a case-control study where the control series does not have a sufficiently wide distribution of exposure

level, its case-control comparison is meaningless. Such a case-control study is similar to a study that follows only exposed subjects. Needless to say, such a follow-up study does not allow the health risk in the exposed and control groups to be evaluated. By the same talken, it is not a good idea for a case-control study investigating environmental contamination to use neighborhood controls, i.e. subjects living in the neighborhood of the patients. If it is difficult to randomly select controls from the base population of a study, hospital controls selected from patients other than mercury poisoning, may be used as a control series (hospital control). Hospital controls should be selected from patients or outpatients of hospitals that are not necessarily the same as that at which the mercury-poisoning patients were diagnosed. In the comparison of mercury poisoning and controls, there are no illnesses that are particularly inappropriate. However, it is a good idea to select controls from various illnesses rather than a few selected ones. Here again, it is necessary to select controls from a sufficiently wide area. If a small hospital is used as a source of controls, the controls may be selected from a small area, which may correspond to the area where cases are concentrated. Generally speaking, it is difficult to select hospital controls such that the control series represents the base population from which cases were ascertained. Therefore, a hospital-based case-control study is not suitable for an exploratory investigation for which there is no specific hypothesis. One of the advantages of using hospital controls is the fact that it is easy to collect scalp hair, blood, and nail.

Controls are selected to match mercury-poisoning patients with respect to gender and a 5-year category of age. It is desirable to use five times as many controls as cases. However, it is not a good idea to reduce the number of patients in order to maintain this ratio.

3. Health survey for health risk assessment

3-1 Study subject

In a health survey conducted for health risk assessment, study subjects should be residents in areas and workers in factories that are possible mercury exposure sites. The choice of study area or study population is of profound importance. In the case of the Minamata incident, the area that was contaminated with methylmercury was defined on the basis of observations of cats having abnormal neurological functions as well as abnormalities in fish, including the observation of floating fish caught in Minamata Bay and the Yatsushiro Sea. The abnormalities in animals were observed prior to the development of neurological disorders in human. In the Niigata incident, the areas suspected to be contaminated were identified on the basis of the experiences in Minamata. Exposed subjects in the possibly contaminated areas were identified on the basis of mercury measurements of scalp hair. If the exposed population is defined too strictly, the assessment of patients may be incomplete. If patients are found in the areas not included in the survey, and the survey is expanded as a result, residents may have the impression that the situation is not under control. However, it is acceptable to start from the highest exposure group and then move on to groups that are considered low-exposure. If the study population is defined too broadly, residents may be unjustifiably concerned about the detrimental effects on their health, and the study may become excessively expensive.

3-2 Control group

In health risk assessment, it is necessary to assess not only typical cases but also atypical cases so that case assessment is complete. When the excess risk of typical mercury poisoning is to be evaluated, it is sufficient to examine contaminated populations only because the incidence of patients in uncontaminated groups is negligible. However, it is difficult to distinguish atypical cases from other abnormalities even if the level of mercury exposure is known. Therefore, quantitative risk assessment should be conducted to compare exposed and unexposed groups. Ideally, the control group should be exactly the same as the exposed group in all respects aside from exposure. As it is practically impossible to select such a population, a control group should be selected in such a way that its geographical environment, lifestyles, occupation and so on are similar to the exposed population. Even if some population characteristics are different in the exposed and control groups, it is possible to eliminate biases possibly derived from those differences using statistical methods. Note here, however, that if the selection of exposed and control subjects is related to health status, the biases derived cannot be eliminated by statistical methods.

4. Ascertainment of patients

4-1 Methods

In surveys conducted in order to identify the source of contamination, it is more efficient to focus on typical cases. However, when assessing health effects, it is acceptable to include atypical cases in case ascertainment so that all the cases are identified. The following methods are available to identify patients:

1) Active registration

In the active method, investigators visit hospitals and clinics to interview medical workers, including physicians, and review medical records. This method, which allows information to be collected by telephone, facsimile and Internet, is suitable for an emergency survey. If possible, a patient registration form is sent to medical institutions beforehand, and retrieved at a later date on a periodical basis. In this approach, it is necessary to pay sufficient attention to the accuracy of diagnosis when analyzing the obtained data because more than one physician, including non-neurologists, may make clinical diagnoses, and it is impractical to unify the criteria of diagnosis beforehand.

2) Health survey

It is possible to conduct a health check survey to identify patients. This method is suitable for a survey in which the patients in a certain population are completely ascertained using unified criteria. Its major drawback is its high cost.

3) Passive registration depending on reporting by physicians

In this method, physicians are asked to report patients with the illness of interest to investigators after diagnosis. This method is suitable for long-term monitoring of the occurrence of the illness. It is usually difficult to identify all cases by this approach unless a concerted effort is made.

4-2 Use of uniform registration form and unification of diagnostic criteria

Clinical information and other relevant information should be recorded on a uniform registration

form (refer to Chapter3 Section5 Item1 2-1 and Chapter6 Item4). The diagnosis of mercury poisoning should be based on the unified criteria using the defined clinical features and the results of clinical laboratory examinations. The diagnostic criteria are shown in Section 7 Conditions, Symptoms, and Treatment for Poisoning, Chapter 1 Introduction.

When identifying the patients of mercury poisoning, note that the diagnostic criteria may be different from time to time even if a single physician makes the diagnoses. Needless to say, the consistency of diagnostic criteria is quite doubtful when different physicians make diagnoses, particularly when they are not certified neurologists.

In preliminary surveys conducted in the early phases of a study, case assessment is often based on clinical diagnoses without unified diagnostic criteria. Such situations are the very occasions when it is important to record data on uniform registration cards so that the information can be reviewed easily later.

5. Items and procedures of examination

5-1 Items of examination

The items and procedures for examination are dependent on the situation. The health survey should be conducted under the supervisions of the same organization as environmental surveys so that the two surveys are coordinated.

The health check surveys should include the following procedures:

1) Interview using a questionnaire

An interview is conducted by a trained interviewer using a questionnaire, obtaining information on demographic characteristics including gender, age, race and ethnicity, past history, family history, residential history, occupation, working conditions, and lifestyles including dietary habits, name, contact address and telephone number.

When it is difficult to interview subjects themselves because of poor health conditions and so on, family members are interviewed. Prior to the interview survey, interviewer should undergo a few days training. A computerized questionnaire for the interview, provided by CDC (Center for Disease Control and Prevention: 1600 Clifton Rd. Atlanta, GA 30333, U.S.A), is useful for the interview. The questionnaire is available in English and Spanish. When interviewing is difficult due to shortages in resources, the use of self-administrative questionnaire in the survey is an option, although the reliability of information obtained from this method is questionable. This approach cannot be used in areas where the illiteracy rate is high.

2) Medical examinations by physicians

Physical and neurological examinations should be conducted.

3) Determination of mercury

Other than neurological findings, the most useful data for identifying methylmercury poisoning is the mercury levels in scalp hair samples. A long hair is cut at the root. The hair sample is cut into 1 cm lengths and subject to mercury measurement.

In the case of methylmercury poisoning, mercury levels in urine are also important.

4) Consultation

If the survey is expected to play a role as a measure of health care management, health consultation should also be conducted.

5-2 Location

We recommend that residents be invited to a common location, such as an assembly hall, to undergo their health checks. Everybody, including those without any symptoms, should undergo the health check. Another approach is to have a medical team including physicians visit the residents at home and conduct medical examinations. Those hospitalized should be included in the survey with the consent of attending physicians, particularly when the physicians are not neurologists.

On some occasions, home visiting is the primary means of conducting the survey. An example is a situation where there is a tendency to hide patients for sociological and economical reasons. Indeed, in the early phases of the surveys for the Minamata incident, a home-visit survey was conducted in the Minamata bay area, and proved to be very effective. When a full-fledged census survey is difficult in terms of cost, labor and time, a possible approach is to visit clinics and hospitals where patients belonging to the study population are likely to visit. In this approach, it is highly likely that atypical cases will not be included.

5-3 Data analysis

Using the data obtained from surveys, various items including family history, job, working conditions, residential history, and lifestyles including dietary habits of known cases and unaffected subjects should be evaluated. In epidemiological investigations, it is necessary to collect information not only from patients but also from unaffected subjects so that comparisons between the two groups can be made. In an emergency survey, interviews may be limited to patients as the survey should be completed in a short period of time. Even in such situations, the comparison is made using existing data for the target population (the population to be surveyed). It is often the case that official statistical data on the demographic characteristics of the residents in the area of interest, and occupation-specific population, are already published.

Even if this is not the case, it may be readily apparent that the distribution of a certain factor in patients is strongly skewed.

Item 2 Survey Teams / Organization of Survey Teams

1. Chief officer

As mercury contamination frequently takes place over a wide area, it is essential to obtain cooperation from local authorities when conducting health checks and environmental and ecological surveys. If appropriate institutions are not available, it may be a good idea to designate a chief officer, who is responsible for establishing the system for various surveys.

In order to prevent unnecessary alarm among residents, contact with the media should be conducted under the strict supervision of the chief officer. The chief officer is expected to have the appropriate medicine, biology, and chemistry knowledge to be able to communicate with local and central governments, health-care and medical institutions, and domestic and international research

organizations. However, it should be noted that the chief officer is expected to be an expert organizer rather than have in-depth knowledge of the characteristics of mercury and its health effects.

2. Field managers

In addition to the chief officer, it is necessary to employ field managers that are responsible for health surveys, environmental monitoring, and epidemiological investigations. It is desirable to employ field managers on a different basis from that of the chief officer. The major responsibilities of field managers are listed in table 3-1.

3. Other issues

In addition to the study described above, physicians and other medical professionals who can be temporarily assigned, are necessary for conducting health surveys. In epidemiological investigations conducted to identify the source of contamination, it is desirable to establish collaboration between physicians and the persons responsible for environmental measurements.

Prior to the investigation, it is desirable to ask advice from neurologists with mercury poisoning knowledge, epidemiologists with experience in conducting health surveys for mercury poisoning, ecologists specialized in the environmental and ecological effects of mercury, and analytical chemists specialized in mercury measurements. As it is impossible to obtain the opinions of many experts in emergency situations, it is a good idea to ask for help from the National Institute for Minamata Disease, Japan.

3-1 Table. Survey Team : Personal and Responsibility

Survey system	Work required
Chief officer (full-time position)	Communication with and government agencies, health and medical treatment organizations, research organizations, and the media Establishment of survey systems (people, locations, budgets, etc.)
Field managers (full-time position) Health	Conducting health surveys Securing the services of doctors, nurses, and public-health nurses; scheduling; securing the materials and locations required for health checks; contacting local citizens
Environmental	Conducting environmental survey Collection and management of soil, water, air, biological, and other samples Communication with organizations in charge of measurement
Epidemiological	Execution of epidemiology survey Securing interview survey personnel Securing personnel for collecting samples (hair, blood, etc.) Management of collected samples Communication with organizations in charge of measurement
Doctors capable of neurological medical examinations (part-time position):	Health surveys
Nurses and public-health nurses (non-regular work):	Health surveys, epidemiological surveys
Person in charge of business affairs (full-time position)	Change of business affairs

Section 6 Countermeasures to Prevent Expansion of Pollution Damage

Item 1 Measures for environmental pollution (cleaning, reclamation, etc.)

1. Mercury behavior and countermeasures in the environment

The high mobility of mercury in the environment allows it to disperse throughout the air, water, rock, and soil spheres, from which it accumulates in plants and animals. The accumulated mercury in plants and animals is recirculated back into the natural environment and the various spheres upon their death.

Although mercury has certain characteristic mobility in each sphere, heavy metal contamination of soil and sediment does not spread easily compared to contamination of the atmosphere and water environments. Contamination in soil and sediment therefore tends to accumulate and reach relatively high concentrations. Mercury in contaminated soil can accumulate in plants and animals and therefore enter the global mercury cycle. Especially, sail sphere is important as foundation of plants growth and recirculating materials on the earth.

Although much mercury is discharged into the environment via the atmosphere and waste water, the countermeasures presented on contaminated soil and sediment in this section.

2. Investigation

The state of contamination must first be elucidated in order to execute countermeasures for mercury discharged into the environment. In Japan, standard values have been set for mercury contamination of soil and ground water. The procedures for surveys and countermeasures are defined to comply with water quality standards. An example of the procedures is shown in Chapter6, TableR-3.

3. Countermeasures

Countermeasures for soil and sediment contaminated with mercury can be divided into cleaning processes and solidification processes. Cleaning processes mainly include heat processing and soil washing. A general outline is summarized in Chapter 6, Table R-2.

In Japan, high temperature processing at 600℃ or high is used only rarely. Rather, a low temperature heat processing method for cleaning soil and sediment is being developed wherein mercury is removed through heating contaminated material at 250 ~ 300°C [1)].

4. Heat processing and solidification, and turn-over

The methods of removing mercury from the environment can broadly be divided into heat processing and soil washing. Soil washing and heat processing are the most desirable from the standpoint of restoring the mercury contaminated environment to its original condition. However, the disadvantages of these methods include high cost and difficulty in reusing soil due to significant changes in soil characteristics.

Recently, the low temperature heat processing method (thermal desorption), developed in Japan, utilizes a lower processing temperature (250 ~ 300°C) than all other methods. As a result, soil

deterioration can be suppressed, in comparison to the high temperature processing method (600 ~ 900°C). The processing cost is also low.

In developing countries as well, when soil has been contaminated by mercury, the use of the above cleaning processes is highly recommended. However, processing costs may be prohibitive in many cases. In such cases, solidification, described in the items dealing with countermeasures for waste discharge and treatment are effective alternatives (refer to chapter2, section4, Item2, Item3).

In addition to chemical countermeasures, physical countermeasures are also available, such as turning over the contaminated topsoil or bringing in fresh topsoil. Mercury in soil normally accumulates in the surface layer and does not permeate deeply into the soil profile. Moreover, mercury will generally form stable forms such as mercury sulfide at reduction condition in soil. Therefore, the contaminated surface soil can be turned over and replaced with good soil from lower layers. When the soil is turned over, mercury locked in lower layers is stabilized into forms such as mercury sulfide. The topsoil cover method is also available. With this method the contaminated soil is covered by at least 50 cm of good soil. The thickness of the covering layer varies depending on the application. For example, if the land is to be used for farming, the thickness of the covering layer is determined in terms of possible root depths.

5. Reference

1) A. Matsuyama, H. Iwasaki, K. Higaki, H. Yabata, T. Sano, and H. Akagi, Basic Study on the remediation technology of Mercury compound-Contaminated soil by low temperature thermal treatment, Environmental Science Mercury contaminated sites(ed by R Ebinghaos et al.), pp.421-440, Springer, Berlin (1999)

Chapter 4 Follow-up

Section 1 Environment

Item 1 Environmental monitoring after mercury removal from polluted sites

The remediation of mercury-contaminated sites may change the environment considerably. Due to the possibility of incomplete removal of mercury from contaminated sites, mercury may still represent an environmental hazard. It is therefore necessary to monitor total mercury and methylmercury levels at various locations at the site in order to confirm the effectiveness of remediation.

Item 2 Environmental monitoring

Periodic environment monitoring is required in order to provide confirmation of the above. Although monitoring targets will vary depending on the contamination scenario, normally water (rivers, oceans), sediment (bottom sludge, etc.), and fish are used. Mercury in the atmosphere may also be monitored as required. The total mercury concentration in the ocean is normally 0.5 ~ 3 ng/l, increasing to 2 ~ 15 ng/l near-shore, and 1 ~ 3 ng/l in rivers, lakes, and marshes. Mercury in ocean water mainly exists in mercury chloride complexes. In rivers, lakes, and marshes, although there are differences depending on the environment, 1 ~ 6% of the total mercury is in the form of methylmercury. In Japan, the waste water quality standards for mercury, based on the water pollution control law, set a level of 0.0005 mg/l or less for total mercury. Mercury in sediment exists primarily in the form of mercury sulfide. In the measures taken to remove contamination in Minamata Bay, dredging and burial were executed for sediment containing total mercury concentrations of 25 ppm or more.

Item 3 Fish monitoring

Fish containing methylmercury have been found in all water regions throughout the world. As a result, various countries have established provisional limits for mercury ingested through fish intake. These levels are based on fish intake levels for the country, research into daily mercury doses that do not adversely affect health, and margin of safety. In Japan, total mercury is set at 0.4 mg/kg and methylmercury is set at 0.3 mg/kg (1973). In Canada and America total mercury is set at 0.5 mg/kg and in Sweden and Finland total mercury is set at 1.0 mg/kg. In any case, environmental monitoring, using these standards as reference values, must be continued when attempting to return mercury concentrations to pre-existing levels.

Item 4 Monitoring period

The monitoring period varies depending on the case and should continue for 3 years after levels

have returned to normal or pre-existing values. This monitoring period is required in order to confirm the continuing effectiveness of countermeasures taken when pollution has occurred.

Section 2 Health (local inhabitants)

Item 1 Two contamination accident types

Contamination accidents can broadly be divided into sudden, one-time exposures and long-term, chronic exposures. Examples of the first type are factory explosions, accidents involving leaks in production processes, and ingestion of contaminated food, such as the bread baked with contaminated wheat in Iraq. Examples of the second type are the Minamata disease observed in Minamata and Niigata as well as the long-term ingestion of small amounts of mercury through fish that still occurs today. The Faeroe Islands are another example of long-term exposure that has gained attention. The health effects, development of those effects, and prognosis varies depending on the exposure type and process.

Item 2 Development of main symptoms and body burden caused by accidents

1. Health check and development of symptoms

Persons exposed to mercury contamination (or suspected of exposure) and persons who have developed mercury poisoning symptoms (or suspected of having such symptoms) should be provided with a long-term health management system. In other words, a system should be constructed so that these people can receive health consultations and examinatons when necessary. In addition, periodic (once every 2 years) health checks should be done for these people and help should be provided for health management. Questions, a general physical examination, and laboratory tests should be done during the health check. When the results are abnormal, diagnosis by a specialist should be provided.

This type of long-term health check data should be accumulated over the years to build a database wherein changes in clinical symptoms, accumulated mercury concentrations, and other factors can be observed over time for individuals. The data is also very important in the investigation of symptom changes over time, development of delayed symptoms, and the appearance of later patients.

The major symptoms of long-term exposure to methylmercury nearly all cease to worsen and begin to improve when the exposure is ended. However, the symptoms do tend to continue for a long time. In addition, so-called "delayed Minamata disease" patients who developed symptoms 2 ~ 3 years after exposure have been reported. <Shirakawa et al.> Health effects from methylmercury other than neurological symptoms, which are the critical effect, should not be looked on lightly. Rather, these should also be included in the follow-up investigation. The first reason for this is the possibility that neurological symptoms may be masked by various coexisting symptoms. The second reason comes from the results of animal experiments and the results of autopsies conducted on Minamata disease victims. Although various impairment risks were found, the effects on the liver, kidneys, pancreas,

thyroid gland, and other organs remain controversial. In addition, using current vital statistics, analyses of the cause of death on populations before and after exposure (SMR calculation) and analyses of disease incidences and prevalence should be conducted. These operations are effective in elucidating the overall health picture.

2. Changes in mercury accumulated in the body

Living organisms do not retain absorbed mercury. Rather, the mercury is excreted. The biological half-time is used as an index for the excretion of chemicals from the body. The quantity that is decomposed and excreted decreases over time and the quantity that is metabolized and reduced within the body maintains a certain ratio with the quantity remaining in the body. In two volunteer studies conducted in Sweden, radioactively-labelled methylmercury was ingested orally, and the biological half life was calculated to be 70 days. The values obtained were in good agreement with the estimated values from the methylmercury poisoning that occurred in Iraq.

Since the methylmercury concentration in scalp hair is a good reflection of accumulation within the human body, changes in body burden of methylmercury can be monitored using hair concentrations as an index. According to the criteria outlined in IPCS (WHO),1990, although direct high risks of exposure to methylmercury do not occur in general population, groups ingesting large quantities of fish will experience a related low risk (5%) of neurological impairment in adults when blood mercury levels reach approximately 200 μg/l and hair mercury concentrations reach 50 μg/g. The risk to fetuses is particularly high, with the neurological impairment risk reaching 30% in cases where the hair mercury peak value in the mother is 70 μg/g. This compares to a 5% risk observed with hair mercury peaks of 10～20 μg/g in mothers studied in the analysis of the mercury poisoning that occurred in Iraq.

Hair can be considered to be a supplementary excretion route for mercury. In particular, methylmercury binds strongly to the SH group in the structural protein of hair and is thereby accumulated. As hair grows, the accumulated mercury moves toward the outside and is thereby excreted. As a result, the hair formation process can be used to estimate exposure over time.

Mercury concentrations in blood are also used as an index for estimating levels in acute and subchronic exposures as well as contamination transitions over short time periods. In a WHO study, the total mercury in blood was reported to be 0.5 μg/100ml or less in 74% of a normal population and 5.0 μg/100ml or less in 98% of the normal population.

3. Long-term follow-up

With the IPCS criteria, since fetuses have a particularly high risk, epidemiological studies are required for children of mothers exposed to methylmercury and having peak hair mercury levels of 20 μg/100ml or less. As reported earlier, a relationship was found between hair mercury concentrations in mothers during the gestation period and neurological observations in the children just before school age in a study conducted on the Faeroe Islands. In particular, when methylmercury exposure of a mother during pregnancy is suspected, cohorts must be established for the child after birth and

through childhood. Detailed follow-up regarding mental development must then be done.

For adults, the clinical symptoms discussed earlier include sensory disturbance, weakness, visual constriction and abnormal ocular movement, dysarthria, gait disturbance, and hearing disturbance. Arimura et al.<1996,1999> observed the symptoms of certified Minamata disease patients for 20 years and reported that nearly all of the major symptoms had stopped worsening and began to improve when exposure to the contamination ended. Some worsening neurological symptoms (i.e. weakness of upper extremities, abnormal finger to nose test, abnormal one foot standing) are thought to be due to aging as seen in normal aged subjects. However, improvement of major symptoms, particularly in the aged, is not remarkable and many are reported to remain the same.

Chapter 5 Sampling and Analytical Methods

Section 1 Mercury Analysis and Determination

Item1 Introduction

In order to correctly elucidate and evaluate the degree of mercury contamination, reliable analysis of data based on proper monitoring methods is required as follows: (1) proper choice of sample; (2) appropriate sampling collection; (3) storage and transport; (4) sample preparation techniques; (5) analytical methods/procedures；(6) experienced/trained staff. In addition, when conducting analyses, regular housekeeping must be maintained in order to keep the laboratory clean and glasswares, tools and containers free from contamination. Aside from this, adequate ventilation and personal protective equipment should be provided including facilities to handle chemicals.

Prior to the analyses of actual samples, the accuracy of the method should be checked/validated using the appropriate standard reference materials. Sample collection, storage, and pretreatment are described in items 1 ~ 4 of "Section 2 Monitoring" in "Chapter 2 Pollution Prevention Measures". In essence, this section discusses the important points and parameters to consider when analyzing total mercury and methylmercury in biological samples both from human and animal origin and environmental samples.

Methods for total mercury measurement include absorption spectrophotometry(dithizone colorimetry), cold vapor atomic absorption spectrophotometry(CVAAS), fluorescence spectrometry; and neutron activation analysis.

In absorption spectrophotometry, dithizone forms a chelate with the metal ions and produces a colored organic solution. The intensity of the color depends upon the mercury concentration. Although the method has been used historically due to the simplicity of the procedures, its use has declined greatly with the introduction of the highly sensitive atomic absorption spectrometry in the 1960s. Atomic absorption spectrometry uses the property of metallic mercury to volatilize into its atomic form. This mercury vapor is introduced into an absorption cell and the absorption measured at 253.7 nm.

In neutron activation analysis (NAA), the sample is irradiated with neutrons to form ^{197}Hg. The gamma radiation emitted by ^{197}Hg is then measured by spectrometry. Although NAA is highly sensitive and requires minimal sample preparation, it is not frequently used due to its high cost, the need for a nuclear reactor and an expensive counting apparatus including the safety requirements for handling radioactive materials.

The cold vapor atomic absorption spectrometry (CVAAS) is a much more sensitive method as compared with the conventional flame atomic absorption spectrometry. Aside from this, other advantage includes its ability to measure mercury in the samples using a UV spectrophotometer with a

simple mercury lamp. The first method which is currently the most commonly used, involves sample digestion with strong acids followed by reduction and vaporization of mercury and the other method involves heating and vaporization through direct combustion of the sample[1,2].

For methylmercury and other organomercury compounds, gas chromatography with electron capture detector (GC-ECD) is often used for mercury detection and measurement. This method provides good separation and superior sensitivity for the determination of halogenated organomercury compounds. Conventionally, it has been widely used for the quantification of methylmercury in various types of biological and environmental samples. Briefly the analytical procedures involves the extraction of methylmercury in the samples using an organic solvent and then back-extraction into cysteine or glutathione aqueous solutions, followed by re-extraction into an organic solvent and measurement of methylmercury using GC-ECD[3]. As an alternative, methylmercury can be determined in the final extract, after combustion at elevated temperatures in terms of elemental mercury by cold vapor atomic absorption spectrometry (CVAAS).Recently, a fast and simple method has been reported using hydrochloric acid and toluene extraction for the quantification of methylmercury in hair samples using GC-ECD[4].

In addition, a highly precise and systematic analytical method has been developed for methylmercury determination which is capable of analyzing various samples[5]. In this method, the sample is pretreated by digestion of the sample in an alkaline solution or a similar technique, then methylmercury is separated by dithizone extraction and cleaned-up by back-extraction of methylmercury into an aqueous Na_2S solution. Re-extraction of methylmercuric dithizonate is done with an organic solvent, and subjected to GC-ECD. This method is currently being used in several countries such as Brazil, Tanzania, Philippines and Indonesia for the monitoring of mercury in contaminated areas, and provides the basis to support efforts to solve serious environmental pollution problems. The methods described above are outlined in detail later in this manual.

Whatever methods are used, the quality of the analytical measurements should be checked regularly through the implementation of quality assurance and quality control procedures. One good practice is the regular use of the appropriate certified reference materials. This is highly recommended to ensure the reliability of the results. Currently, a number of standard reference materials are commercially available from the National Institute of Standards and Technology (NIST), the International Atomic Energy Agency (IAEA), and the National Research Council of Canada (NRCC). These reference materials should be used for quality control in the analysis of environmental samples, human biological samples, and other samples.

Item 2 Total mercury

2-1 Quantification by cold vapor atomic absorption spectrometry

Principles

The principle of the reduction and vaporization method involve reducing ionic Hg^{2+} in a sample solution using $SnCl_2$ to generate the elemental form of mercury. The mercury vapor thus generated is introduced into a photo-cell where the absorption is measured at 253.7 nm.

Although measurement sensitivity depends on gas phase quantity within the atomic absorption spectrophotometer, mercury on the 10^{-7}g (0.1 μg) level or lower can be generally measured with good accuracy.

2-1-(1) Water samples[1,2)]

a. Reagents

10% tin (II) chloride solution: Dissolve 10 g of $SnCl_2.2H_2O$ in 0.5N H_2SO_4 to make a final volume of 100 ml.

Mercury standard solution: Dissolve 0.135 g of $HgCl_2$ in 100 ml of 10% HNO_3 and add water to make a final volume of 1000 ml. Dilute this solution 1000 times using 1% HNO_3 and use the resulting solution as the calibration standard. 1 ml of Hg standard solution = 0.1 μg Hg

Prepare a fresh solution for every analysis.

5% potassium peroxidisulfate solution: Dissolve 5 g of $K_2S_2O_8$ in distilled water to make a final volume of 100 ml.

10% hydroxylamine hydrochloride solution: Dissolve 10 g of $NH_2OH \cdot HCl$ in distilled water to make a final volume of 100 ml.

5% potassium permanganate solution: Dissolve 5 g of $KMnO_4$ in distilled water to make a final volume of 100 ml.

Magnesium perchlorate: $Mg(ClO_4)_2$

b. Instruments and equipment

Atomic absorption spectrometer: Instrument with background correction is recommended.

Mercury halide cathode lamp or mercury lamp

Instrument set-up: Figure 5-1-a shows an example of the instrument set-up for a closed circulation system and Figure 5-1-b shows an example of the instrument set-up for an open transfer system.

Reaction Vessel: Use a 300 ml Erlenmeyer flask (a bubbling bottle, BOD bottle, or a separatory funnel can also be used) with a glass ventilation tube equipped with an air diffusion sphere.

Absorption cell: Use a cyrindrical cell 100 ~ 300 mm long × 30 mm outer diameter. Glass or other material can be used but it is recommended that the cell be made of quartz glass.

Diaphragm pump: Capable of introducing air at a flow rate of 1 L or more per minute.

Drying tube: Pack magnesium perchlorate as a drying agent and insert glass wool in both ends to prevent condensation of moisture inside the cell

Connection tube: Use a flexible poly-vinyl chloride tube

Recorder: Any multi-range variable speed recorder that is compatible with the equipment

c. Sample preparation

Transfer an appropriate amount of sample (containing 0.002 mg or less of mercury) into the 300 ml reduction flask and add water to make a final volume of 200 ml. Add 10 ml of sulfuric acid (reagent grade for trace metal analysis) and 5 ml of nitric acid (reagent grade for trace metal analysis), then add 20 ml of 5% potassium permanganate solution. Let stand for about 15 minutes. If the red color of the potassium permanganate disappears, add 1 ml of 5% potassium permanganate solution drop by drop,

until the red color remains for 15 minutes. Then add 10 ml of potassium peroxodisulfate solution and heat in a water bath maintained at 95°C for 2 hours.

Cool it down to room temperature and add 8 ml of 10% hydroxylamine hydrochloride solution to reduce the excess permanganate. Add water to make a final volume of 250 ml.

d. Test procedures and calculations

Test procedures

After preparation of the sample solution, immediately connect the reduction flask into the equipment. Add 10 ml of 10% tin (II) chloride solution, and operate the diaphragm pump to circulate air at a pre-adjusted flow rate of 1 ~ 1.5 L/min. Record the value obtained at a wavelength of 253.7 nm when the absorption index rises and stabilizes. Next, open the bypass valve and continue air circulation until the residual vapor is at a minimum. For the blank test, use 200 ml of water and repeat the above procedures. Record the absorption, and correct the measured reading of the sample. Calculate the mercury concentration in the original sample from the calibration curve for mercury standards.

Preparation of the Calibration curve

Prepare aliquots of 1 ~ 20 ml for mercury standard solution (0.1 μgHg/ml) to obtain 0.2 to 2.0 μg mercury standards. The standards will depend on the range of expected mercury levels.

Place the standards in separate flasks and repeat the procedures for the sample solution preparation and test operations. Record the absorbance values obtained and create a calibration curve showing the relationship between the absorbance and mercury concentration.

2-1-(2) Biological samples [1) 3)]

a.Reagents

Prepare the following reagents in addition to the reagents described in parts 1 and 2 of "a. Reagents" in "2-1-(1) Water samples"

10% urea solution: Dissolve 10 g of NH_2CONH_2 in distilled water to make a final volume of 100 ml.

20% hydroxylamine hydrochloride: Dissolve 20 g of $NH_2OH \cdot HCl$ (analytical reagent grade) in distilled water to make a final volume of 100 ml.

b. Instruments and equipment

Instruments used for this analysis are shown in Figure 5-2.

Light source: Mercury hollow cathode lamp or mercury lamp

Absorption cell: Use a quartz glass tube with an internal diameter of 20 ~ 30 mm × 100 ~ 300 mm long × or a poly-vinyl chloride tube with quartz glass affixed at both ends.

Diaphragm pump: Use a pump that can introduce and circulate air at a rate of 1.5 L/min or more. Use collodion to coat metallic parts that come into contact with the sample gas.

Connection tube: Use a flexible poli-vinyl chloride tube with an internal diameter of 8 mm.

U-shaped tube for drying: Use a U-shaped tube containing magnesium perchlorate in granule form as shown in Figure 5-2

Test solution bottle: Use a 250 ml bottle with a glass filter plate.

Palladium chloride tube: Soak the inside of the glass tube measuring around several centimeters in

length with 1% palladium chloride solution. Dry and pack with a sufficient quantity of glass wool. Replace the tube when mercury removal capability decreases.

c. Sample preparation

Weigh a sufficient quantity of sample (20 g for fish, 0.5 ~ 1 g for hair, 1 ~ 5 ml for blood, and 50 ~ 100 ml for urine). Place in a 250 ml round bottom flask. Add 10 ml of water and then 20 ml of nitric acid. Mix lightly. Carefully add 20 ml of sulfuric acid, then attach a reflux condenser (20 ~ 30 cm long), and heat on a wire gauze over a direct flame until the formation of the brown fumes ceases. If the solution does not turn colorless ~ faint transparent yellow, allow to cool, then add 5 ml of nitric acid and heat again. Repeat this procedure until the solution becomes colorless ~ faint transparent yellow. After cooling, add 50 ml of water and 10 ml of 10% urea solution. Reflux for 10 minutes then allow to cool. Add 1.0 g of potassium permanganate and let stand for 10 minutes with occasional mixing. Repeat this procedure until the purplish red color of the solution persists, then heat for 20 minutes. Cool after the purplish red color of the solution disappears. Add 1.0 g of potassium permanganate and again heat for 20 minutes. If the color still disappears, repeat the addition of potassium permanganate to the mixture and heat the solution for 2 more times. After cooling, add 20% hydroxylamine hydrochloride solution until the color is clear and transparent. Transfer the solution into a different container. Rinse the flask and reflux condenser with water then add the washing to the original sample. Add water to make a final volume of 300 ml. Use this solution for analysis.

Prepare a blank sample using the same procedures.

d. Test procedures and calculations

Test procedures

Collect an appropriate amount of sample solution and perform the procedures described in part “d” of "2-1-(1) Water samples".

Preparation of calibration curve

Prepare specific concentrations of mercury standard solution and perform the procedures described above. Record the absorbance obtained, plot and create a calibration curve showing the relationship between absorbance and mercury concentration.

2-1-(3) Sediment and soil samples [1) 4)]

a. Reagents

Use the reagents described in "a. Reagents" of "2-1-(2) Biological samples".

b. Instruments and equipment

Use the instruments and equipment described in "b. Instruments and equipment" of "2-1-(2) Biological samples".

c. Sample preparation

Accurately weigh 5 ~ 10 g of dehydrated or dried sample. Place the weighed sample in a flask with a reflux condenser attached. Add 50 ml of nitric acid (1+ 1) (reagent grade used for trace metal analysis), and slightly heat to decompose the organic materials. Cool to room temperature. Add 10 ml of 5% potassium permanganate solution. Reflux for 1 hour. If the reddish purple color disappears, cool the

solution then add 5 ml of potassium permanganate and heat again. Repeat these procedures until the reddish purple color persists for about 10 minutes.

Cool the solution to about 40°C. Add a drop of 20% (w/v) hydroxylamine hydrochloride solution to reduce the excess potassium permanganate. Filter the solution by using a glass cotton or glass fiber filter paper. Add water to obtain the desired volume, and use the solution for analysis.

d. Test procedures and calculations

Test procedures

Collect an appropriate amount of sample solution (0.1 ~ 1.0 μgHg) and perform the procedures described in part “d” of "2-1-(1) Water samples".

Preparation of the calibration curve

Do the operations stated in part “d” of "2-1-(1)".

2-2 Quantification by cold vapor atomic absorption spectrometry (CVAAS) with gold amalgamation [1-3)]

Principles

One advantage of this method is its ability to quantify mercury with direct heat processing without any pretreatment procedures. In principle, mercury vapor is generated by direct combustion of the sample. Mercury released from the sample is concentrated by amalgamation. The amalgam is heated to 800°C to free the mercury vapor and measured by CVAAS.

For unique circumstances where mercury is bound in silicates or other matrices that may not thermally decompose, validation of direct analysis of the solid may be confirmed with total decomposition. In place of aqueous mercury standards, solid reference material with a certified value for mercury may be used for calibration. In areas where mercury levels is an existing problem, the background signal may be significantly increased.

2-2-(1) Solid and liquid samples

a. Reagents

Mercury standard: Dissolve 135.4 mg of $HgCl_2$ in 0.001% L-cysteine to obtain a final volume of 1000 ml. This can be stored in a cool, dark place for 1 ~ 2 months. One ml of this solution contains 100 μg of mercury.

Mercury standard solution: Dilute the mercury standard in 0.001% L-cysteine to prepare 0 ~ 200 ng/ml concentration. Prepare a fresh standard solution for every analysis.

Additive: At the time of use, mix (A) alumina and (B) calcium hydroxide ($Ca(OH)_2$) + sodium carbonate (Na_2CO_3) in a 1:1 ratio and heat at 950°C for 30 minutes.

b. Instruments and equipment

Use a mercury analyzer shown in Figure 5-3 which automates the process from mercury-gold amalgamation to the measurement by a CVAAS.

c. Sample preparation

In principle, no sample preparation is required with this method. Simply use a sample to match the

sensitivity of the measuring equipment/analyzer. Alternatively, other mercury vapor introduction systems may be applied. The detection scheme can be used for the analysis of the individual form of mercury that have been separated by an appropriate method or instrument.

d. Test procedures and calculations

Test procedures

Spread approximately 1 g of additive (A) on a ceramic or quartz combustion boat. For solid samples, place 10 ~ 300 mg of finely cut or homogenized sample on the boat. For liquid samples completely soak 0.1 ~ 0.5 ml of sample into additive (A). Add about 0.5 g of additive (A) then about 1 g of additive (B) on top. Place the boat in a combustion furnace, heat at 800 ~ 900°C while introducing air or oxygen at 0.5 ~ 1 L/min to release mercury which can then be collected in a tube. Heat the tube to about 700°C to produce mercury vapor and measured by a CVAA analyzer. Record the absorbance and define the result as "A". Separately, add an additive to a magnetic combustion boat, repeat the procedures and measure the absorption, and define the result as "A_b".

Preparation of the Calibration curve

Use the standard mercury solution as samples in the above procedures, and measure the absorbance. Plot and create a calibration curve. Find the value for A - A_b on the curve and calculate the mercury concentration in the original sample.

Procedural Notes

Advantages of this method include high sensitivity (detection limit near 5 ng), the ability to measure samples without pretreatment and measurement within 10 ~ 15 minutes.

Furthermore, during sample combustion acidic gases including other materials generated that may interfere with measurement are removed. The additives (A, B) are also used to prevent the dispersion of samples during rapid temperature increase.

2-3 Quantitation by CVAAS using semi-automatic mercury analyzer [5) 6)]

Principles

The principle of this method involves reducing ionic Hg^{2+} in sample solutions using tin (II) chloride to generate metallic mercury. The sample is then aerated and the mercury vapor generated is introduced into a cell. The absorption is measured at 253.7 nm. Up to this point, the method is the same as with the circulation method. However, as shown in Figure 5-4, a four-way valve, which separates the pump from the sample solution bottle and the alkali solution, is completely closed. The reducing agent is added to the sample solution, then air is circulated for 30 seconds to evenly distribute the mercury vapor. Allow the concentration of mercury vapor to come to an equilibrium. The valve is then opened and the vapor phase is introduced into the absorption cell as a single unit. In comparison to the circulation method, this method offers a higher sensitivity and greater accuracy.

2-3-(1) Water samples [5)]

a. Reagents

Distilled water: Distilled water from deionized water in a glass

Toluene: $C_6H_5CH_3$ (reagent grade used for residual pesticide analysis)

Nitric acid - perchloric acid (1+1): Mix 100 ml of $HClO_4$ (reagent grade used for trace metal analysis) to 100 ml of HNO_3 (reagent grade used for trace metal analysis). Store in a cool and dark place.

Sulfuric acid: Reagent grade used for trace metal analysis.

20N sulfuric acid: Measure approximately 350 ml of distilled water in a 1 L volumetric flask then gradually add 600 ml of H_2SO_4 (analytical grade for trace metal analysis) while mixing in an ice bath. Allow to cool to room temperature and add distilled water to make a final volume of 1000 ml.

1N sulfuric acid: Gradually add 30 ml of H_2SO_4 (reagent grade used for trace metal analysis) to distilled water to make a final volume of 1000 ml.

1N hydrochloric acid: Add 90 ml of HCl (reagent grade used for trace metal analysis) to distilled water to make a final volume of 1000 ml.

10N sodium hydroxide solution: Dissolve 400 g of NaOH (analytical reagent grade) in distilled water to make a final volume of 1000 ml.

5N sodium hydroxide: Dissolve 20 g of NaOH (analytical reagent grade) in distilled water to make a final volume of 100 ml.

0.1N sodium hydroxide solution: Dilute 10N NaOH 100 times using distilled water.

0.5% potassium permanganate solution: Dissolve 0.5 g of $KMnO_4$ (analytical reagent grade) in distilled water to make a final volume of 100 ml.

10% hydroxylamine hydrochloric acid solution: Dissolve 10 g of $NH_2OH \cdot HCl$ (analytical reagent grade) in distilled water to make a final volume of 100 ml.

10% Ethylenediaminetetraacetic acid tetrasodium salt(EDTA) solution: Dissolve 10 g of $C_{10}H_{12}N_2O_8Na_4 \cdot 4H_2O$ (analytical reagent grade) in distilled water to make a final volume of 100 ml.

0.1% L-cysteine solution: Dissolve 10 mg of $HSCH_2CH(NH_2)COOH \cdot HCl \cdot H_2O$ (analytical reagent grade) in 10 ml of 0.1N NaOH. Use freshly prepared solution for analysis.

Methylmercury standard solution in toluene: Dissolve 12.5 mg of CH_3HgCl in toluene to make a final volume of 100 ml. Dilute this solution 100 times using toluene to prepare a concentration of 1 ppm methylmercury chloride in toluene. One ml of this solution contains 1.0 μg of Hg.

Methylmercury-cysteine solution: Mix 0.5 ml of 1 ppm methylmercury standard solution in toluene and 5 ml of 0.1% L-cysteine in 0.1N NaOH into a 10 ml pyrex glass test tube with glass stopper. Shake for 3 minutes. Centrifuge at 1000 rpm for 3 minutes and discard the organic phase (top layer). Seal the tube, and store in a cool and dark place. One ml of this solution contains 0.1 μg (100 ng) of Hg. (Prepare a fresh solution every month)

0.01% dithizone (diphenylthiocarbazone) solution: Mix 0.011 g of $C_6H_5N{:}NCSNHNHC_6H_5$ with toluene in a 200 ml separatory funnel. Dissolve to make a final volume of 100 ml. Immediately add 50 ml of 0.1N NaOH to extract the dithizone to the aqueous layer (bottom layer). After separation, transfer the aqueous layer to a glass container with glass stopper. Add a drop of 1N HCl to make the solution slightly acidic (blackish green colored crystals will precipitate) and mix with 100 ml of toluene to obtain a purified 0.01% dithizone solution. Allow to settle, discard the bottom layer and seal. Store in a cool, dark place (Prepare a fresh solution for every analysis).

10% tin (II) chloride solution: Dissolve 10 g of $SnCl_2 \cdot 2H_2O$ (analytical reagent grade) in 1N HCl to make a final volume of 100 ml. Aerate with N_2 gas to remove mercury from the solution (50 ml/min for 20 ~ 30 minutes).

b. Instruments and equipment

Instruments and equipment 1 ~ 6 are the same as those used in "2-3-(2) Biological and environmental samples" and "2-3-(3) Hair samples"

Mercury analyzer: Semi-automatic mercury analyzer (Sanso Seisakusho Co., Ltd.)

Hot plate: Capable of attaining surface temperatures up to 200°C.

Sample analysis flask: 50 ml thick walled-measuring flask made of Pyrex (150 mm total height, 13 mm inlet diameter)

10 ml pyrex glass test tube with glass stopper

Volumetric flasks: (10, 100, and 1000 ml)

Serological or volumetric pipettes: (0.1, 0.5, 1, 5, 10, and 20 ml)

1L separatory funnel

Rotary evaporator

Magnetic stirrer

All laboratory glasswares and sample containers to be used in the analysis should be thoroughly washed with 0.5% $KMnO_4$ solution and rinsed with water prior to use.

c. Sample preparation

Transfer 1 L of water sample in a separatory funnel. Add 5 ml of 20N sulfuric acid and 2 ml of 0.5% potassium permanganate solution. Mix and let stand for 5 minutes. Neutralize using 10 ml of 10N sodium hydroxide and mix with 2 ml of 10% hydroxylamine hydrochloride solution. Let it stand for 20 minutes. Neutralize with 2 ml of 10% EDTA and then add 5 ml of 0.01% dithizone solution. Mix and stand to allow complete separation. Discard the aqueous layer (lower phase). Transfer 3 ml of the organic layer to a sample digestion flask. Using a rotary evaporator, immerse the flask in water bath at 60°C and evaporate to dryness under a reduced pressure. Add 1 ml of distilled water, 2 ml of HNO_3-$HClO_4$ (1:1) and 5 ml of H_2SO_4. Place boiling chips and heat on a hot plate at 200±5°C for 30 minutes. Allow to cool then add water to obtain a desired volume and analyze the resulting solution using CVAAS. Prepare a separate 1 L mercury free water as the blank sample and another 1 L mercury-free water spiked with 200 μl (corresponding to 20 ng as Hg) of methylmercury-cysteine solution (0.10 μgHg/ml) as the standard. Follow the above procedures and use the resulting solution for total mercury analysis.

d. Test procedures and calculations

Test procedures

The total volume of the solution needed for total mercury analysis is 10 ml. Before analyzing X ml of blank sample, standard sample and the actual sample solution for total mercury, attach a calibrated dispenser to the semi-automatic mercury analyzer ready to dispense a known volume of distilled water in an amount calculated to obtain a total volume of 10 ml. Gently add X ml (solution +water, maximum of 10 ml) of each solution to the reaction vessel, stopper and then add 1 ml of tin (II)

chloride solution from the accessory syringe. Press the start button of the analyzer. The diaphragm pump will operate and the generated mercury vapor will be circulated through the four-way cock between reaction vessel and acidic gas collection bottle. After 30 seconds, mercury vapor introduced into the absorption cell will be measured automatically by turning the 4-way stop cock by 90°. When the maximum peak height has been recorded, the sample from the reaction vessel can already be discarded and purged with air to remove the residual mercury vapor. Press the reset button and start the next measurement.

Calculation

The peak heights obtained after measuring fixed volumes of X ml (normally 10 ml) from the blank , the standard sample and the actual sample for total mercury analysis are labelled as Pb1, Pstd, and Ps, respectively. The total mercury in the original water sample (ng/l) is calculated using the following formula:

Total mercury concentration in original water sample (ng/L) = 20 ng × (Ps - Pb1)/(Pstd - Pb1) × dilution factor × 1/sample water content (L)

2-3-(2) Biological and environmental samples

a. Reagents: (refer to "a. Reagents" in "2-3-(1) Water samples")

b. Instruments and equipment: (refer to "b. Instruments and Equipment" in "2-3-(1) Water samples")

c. Sample preparation

This method is applicable to biological (fish meat, blood, urine, human biopsy samples, etc.) and environmental samples (sediment, soil, etc). Accurately weigh out the sample (0.5 g or less wet weight, 1 ml for urine samples) into a digestion flask, add 1 ml of distilled water (not required with urine samples) and mix with 2 ml of nitric acid - perchloric acid (1:1), and 5 ml of sulfuric acid. Place boiling chips. Wipe the flask to ensure that residual chemicals were not spilled onto the surface. Heat the solution on a hot plate at 200±5°C for 30 minutes. Allow to cool then add water to obtain a fixed volume and use the resulting solution as the sample. Simultaneously prepare a blank and standard solution by measuring 0, and 1.0 ml (corresponding to 0.10 μg of Hg) of methylmercury-cysteine solution (0.10 μg Hg/ml) . Add 1 ml of distilled water then mix with 2 ml of nitric acid-perchloric acid (1:1), and 5 ml of sulfuric acid. Follow the same procedures as indicated above for the preparation of the sample solution.

d. Procedures and calculations

Procedures

Procedures are the same as those described in "d. Water samples" in 2-3-(1).Measure a fixed volume of X ml (up to 10 ml, normally 5 ml) of the blank, standard for total mercury measurement, and actual sample (or actual sample diluted with blank solution). The peak heights (mm) thus obtained are labeled, respectively, as Pb1, Pstd, and Ps. The total mercury concentration in the sample (μg/g or ml) can then be calculated using the following formula.

Total mercury concentration in the sample (μg/g) = 0.1 μg × (Ps-Pb1)/(Pstd-Pb1) × dilution factor × 1/sample quantity (g).

<For urine samples>

Total mercury concentration in the sample (μg/ml) = 0.1 μg × (Ps-Pb1)/(Pstd-Pb1) × dilution factor × 1/sample quantity (ml).

2-3-(3) Hair samples

a. Reagents (for reagents not shown below, refer to 1 ~ 11 in "a. Reagents" in "2-3-(1) Water samples")

Acetone: CH_3COCH_3 (analytical reagent grade)

Ethanol: C_2H_5OH(analytical reagent grade)

Methylmercury-cysteine solution: Mix 2 ml of 1 ppm methylmercury standard solution in toluene and 2 ml of 0.1% L-cysteine in 0.1N NaOH into a 10 ml pyrex glass test tube with glass stopper. Shake for 3 minutes. Centrifuge at 1000rpm for 3 minutes and discard the organic phase (top layer). Seal the tube, and store in a cool and dark place. One ml of this solution contains 1.0 μg (1000 ng) of Hg. (Prepare a fresh solution every month)

b. Instruments and equipment (the equipment below is used in addition to equipment described in "2-3-(1) Water samples")

Beaker

Vials: 20 ml scintillation vials

c. Sample preparation

Weigh 20 ~ 30 mg of sample in a beaker, wash with neutral soap (1:100) and distilled water then add a small amount of acetone. Under reduced pressure, discard excess water with acetone. Transfer the sample into a 20 ml vial and cut into very fine pieces. Accurately weigh out X mg (normally about 10 mg) of finely cut hair samples into a digestion flask. Add 1 ml of distilled water and mix with 2 ml of nitric acid - perchloric acid (1:1), and 5 ml of sulfuric acid. Place boiling chips. Wipe out the surface of the flask to ensure that residual chemicals were not spilled onto the surface. Heat the solution on a hot plate at 200 ±5°C for 30 minutes. Allow to cool then add water to obtain a fixed volume and use the resulting solution as the sample. Simultaneously prepare a blank and standard solution by measuring 0 and 100 μl (corresponding to 100 ng of Hg) of methylmercury-cysteine solution(1000 ngHg/ml) and follow the procedures in the sample preparation described above. Use the resulting solutions as the blank and the standard sample for total mercury measurement.

d. Test Procedures and calculations

Test procedures

Test procedures are the same as those described in "d. Water samples" in 2-3-(1).

Calculations

Measure a fixed volume of X ml of blank, standard for total mercury measurement and actual sample. The peak heights (mm) thus obtained are labeled respectively, as Pb1, Pstd, and Ps. The total mercury concentration in the sample can then be calculated using the following formula:

Total mercury concentration in sample (ng/g) = 100 ng × (Ps-Pb1)/(Pstd-Pb1) × dilution factor × 1/sample weight (X mg)

Procedural Notes:

1) When preparing water samples for analysis, the addition of hydroxylamine hydrochloride neutralizes the strong oxidizing property of potassium permanganate and the addition of EDTA prevents the interference caused by other metals in the sample. Both are therefore added to protect dithizone from oxidation and unnecessary cross reactions with other metal ions.

2) In this method, perchloric acid is present during the sulfuric acid-nitric acid digestion process.

In normal sulfuric acid - nitric acid digestion, mercury may vaporize during the reaction process. However, in the presence of an oxidizing agent which is incorporated beforehand, mercury vaporization under severe heating conditions can be completely prevented. The use of a long neck (10 cm or more)-thick walled flask as the digestion container will prevent mercury loss even with heating at 200 ~ 250°C.

Item 3 Methylmercury analysis

3-1 Hydrochloric acid - toluene extraction – GC-EDC method [1-3)]

Principles

Hydrochloric acid is added to acidify the sample. Alkylmercury is extracted with toluene, and then cleaned up using the cysteine solution. The cysteine solution is then acidified again with hydrochloric acid and alkyl mercury extracted using toluene and measured using gas chromatography equipped with an electron capture detector.

3-1-(1) Biological samples [1 -3)]

a. Reagents

Toluene: Perform a trial run on toluene $C_6H_5CH_3$ by gas chromatography method to ensure that no other peak co-elutes with the expected retention time for methylmercury.

Internal standard solution: Dissolve an organic chloride compound with a similar retention time as in toluene. As an example, dissolve 0.1 g of p-nitrobenzylchloride ($NO_2C_6H_4CH_2Cl$) in toluene to make a final volume of 1000 ml. Collect 10 ml of this solution and dilute with toluene to make a final volume of 1000 ml.

(1 ml of internal standard solution = 1 μg $NO_2C_6H_4CH_2Cl$)

L-cysteine (1%) - sodium acetate (0.8%) solution: Dissolve 1 g of L-cysteine, 0.8 g of $CH_3COONa \cdot 3H_2O$ and 12.5 g of Na_2SO_4 in distilled water to make a final volume of 100 ml.

Methylmercuric chloride standard solution: Dissolve 0.1 g of CH_3HgCl (standard material) in the internal standard solution to make a final volume of 100 ml. Collect 10 ml of this solution and dilute with the internal standard to make a final volume of 100 ml. Repeat this dilution procedure 3 more times and use the resulting solution as the methylmercury chloride standard.

1 ml of methylmercuric chloride standard solution = 0.1 μg CH_3HgCl = 0.0799 μg Hg.

b. Instruments and equipment

Separatory funnels: 100 and 250 ml

*Volumetric flasks: (*10, 100, and 1000 ml)

Serological or volumetric pipettes: 0.2, 0.5, 1, 2, 5, 10, and 20 ml

Beaker

Homogenizer with 50 ml vials or its equivalent

Gas chromatograph equipped with ECD

Centrifuge

Graduated cylinders: 100 and 200 ml

c. Sample preparation

Place X g of sample (normally 10 g) in a homogenizer vial. Measure 55 ml of water then add an appropriate amount of the water to the vial. Homogenize the sample at medium speed for 3 minutes. Transfer the sample to a 250 ml separatory funnel, wash the vial with the remaining water previously measured. Collect the washing add it to the rest of the sample . Place 14 ml of concentrated hydrochloric acid and 10 g of sodium chloride and add 70 ml of toluene. Extract for 5 minutes using a mechanical shaker. Transfer the solution to a 250 ml centrifuge tube and centrifuge at 2000 rpm for 5 ~ 15 minutes. Collect 50 ml of the toluene layer into a 100 ml separatory funnel and add 20 ml of cysteine - acetate solution, mix vigorously for 2 minutes and stand for 10 minutes. Transfer the aqueous layer (an emulsion may form) to a 50 ml centrifuge tube and centrifuge at 2000 rpm for 5 ~ 15 minutes. Transfer 10 ml of the aqueous layer to a 100 ml separatory funnel then 6 ml of 6N hydrochloric acid and 20 ml toluene are added. Mix vigorously then allow to stand for 10 minutes and discard the aqueous layer. Add a small amount of sodium sulfate (anhydrous) to dehydrate the sample. The final solution is ready for analysis.

With whole blood , serum, brain tissues, and other samples, 2 ml of copper sulfate($CuSO_4 \cdot 5H_2O$) solution (1%), 14 ml of concentrated hydrochloric acid and 10 g of sodium chloride are added. Follow the above procedures as indicated in the preparation of the sample solution for analysis.

d. Gas chromatography (GC) and calculations

GC operations

Inject X μl (normally 5 μl) of sample and blank sample solution into the gas chromatograph. Find the peak heights H and Ho or, when using an internal standard, find the peak height ratios R and Ro.

<Gas chromatography conditions>

Detector: Radioactivity or discharge electron capture type

Column: Glass column 1 ~ 2 m long × 3 ~ 4 mm diameter

Packing: 5 ~ 25% DEGS (Di-ethyleneglycol succinate), 1-4BDS (Butane-1,4-diol succinate)

Column temperature: 150 ~ 160°C

Sample Injection : On-column

Calibration curve preparation and calculation

Prepare a series of methylmercury standard solutions by diluting the stock standard with 10 or 20 ml of internal standard or toluene. Inject X μl of each solution into the gas chromatograph and create a calibration curve using peak height or peak height ratio. Use the curve to determine the methylmercury concentration (μl/g) in the sample.

3-2 Sample combustion - gold trap method [1) 3)]

Principles

By this method, alkyl mercury in the biological sample (particularly fish) is extracted with toluene and re-extracted into a cysteine - acetate solution. To generate mercury vapor which is captured as a metal amalgam, the solution is heated by combustion. The amalgam is heated to free the mercury which is then measured by atomic absorption. In principle, this method can only measure methyl mercury compounds that are soluble in toluene and can be re-extracted into cysteine-acetate solution. These includes ethylmercury, and other alkyl mercury compounds.

3-2-(1) Biological samples

a. Reagents

Cysteine - sodium acetate solution: Dissolve 1 g of L-cysteine, 0.8 g of $CH_3COONa \cdot 3H_2O$ and 12.5 g of Na_2SO_4 in distilled water to make a final volume of 100 ml.

Powdered calcium hydroxide: Heat a suitable amount of commercially available analytical reagent grade $Ca(OH)_2$ in an electric furnace at about 500°C for 3 hours to remove any mercury content.

Mercury standard solution: Use Mercuric chloride ($HgCl_2$) to make a stock solution (100 mg /L) and a standard solution (1, 0.1 mg /L)

b. Equipment

See the extraction apparatus shown in Figure 5-5.

c. Sample preparation

Homogenize an appropriate amount of biological sample and weigh X g (usually 10 g). To the sample, add 30 ml of water and 0.1 g of papain(1:350). Place in a 37°C isothermal water bath for 2 hours. Transfer the solution in the extraction set-up as shown in Figure 5-5. Add 30 ml of 6N hydrochloric acid and 50 ml of toluene. Heat on the sand bath and extract continuously for approximately 4 hours. Once the extraction is completed, carefully add water to the extraction apparatus to wash the toluene off. Transfer the toluene extract to a separatory funnel and then wash the condenser and other parts of the extraction set-up with toluene. Combine the toluene washings with the rest of the extract and wash with 20 ml of 20% sodium chloride solution. Add 10 ml of cysteine–sodium acetate solution and mix vigorously for 2 minutes. Allow the solution to settle, centrifuge if necessary and discard the upper toluene layer. Use the resulting solution as the sample for analysis.

d. Test procedures and calculations

Mix 0.1 g of powdered calcium hydroxide to an appropriate amount of sample on a quartz boat. Place the sample in the furnace as shown in Figure 5-3. Heat for approximately 10 minutes at 850°C using an oxygen flow rate of 1 L/min. Mercury will be captured as an amalgam on the mercury trap. Increase the temperature of the mercury trap to approximately 700°C, then transfer the released mercury vapor to the absorption cell. Measure the absorbance. Determine amount of the mercury from a calibration curve and calculate the mercury concentration in the original sample.

Procedural Notes:

The presence or generation of acidic materials will interfere with the Hg-Au amalgamation. Calcium

hydroxide is therefore added to prevent this interference.

3-3 Dithizone extraction—GC-ECD method [5) 6)]

Principles

This method was established based on the property of methylmercuric dithizonate to immediately convert into its chloride form as soon as it is analyzed by gas chromatography. Proteinaceous substances are decomposed by alkaline digestion and subsequently, under a slightly acidic conditions, the fatty content are removed using *n*-hexane. This initial sample preparation allows methylmercury to be effectively extracted with dithizone-toluene as dithizonate without the formation of an emulsion. After extraction with dithizone-toluene, methylmercury is back-extracted with a slightly alkaline sodium sulfide solution. The excess sulfide ions are then removed as hydrogen sulfide by purging with nitrogen gas after slight acidification with HCl solution. Methylmercury is then re-extracted with a small portion of dithizone-toluene. The extract is washed with NaOH solution to remove the excess dithizone. The extract is then slightly acidified with HCl and analyzed using gas chromatograph equipped with electron capture detector.

Based on the principle of this method, the addition of 2-3 cm sodium chloride on the injection inlet/port is necessary.

3-3-(1) Biological samples

a. Reagents

Toluene: Pesticide analysis grade

Ethanol: Analytical reagent grade

Hexane: analytical reagent grade

Distilled water: Purified water by ion exchange and distillation process

Methylmercury standard solution: Dissolve 12.5 mg of CH_3HgCl in toluene to make a final volume of 100 ml. Dilute this solution 100 times using toluene and use as the methylmercury standard (seal and store in freezer). One ml of this solution contains 1.0 μg of methylmercury.

0.1% L-cysteine solution: Dissolve 10 mg of $HSCH_2CH(NH_2)COOH \cdot HCl \cdot H_2O$ in 10 ml of 0.1N NaOH (Prepare a fresh solution at each time of use).

Methylmercury-cysteine solution: Transfer 5 ml of 0.1% L-cysteine-aqueous hydrochloride solution and 0.5 ml of methylmercury standard solution in a 10 ml conical test tube with glass stopper. Mix well and then Centrifuge at 1000 rpm for 3 minutes. Suction off the toluene (upper layer). Seal, and store in a cool, dark place (Prepare a fresh solution every month). 1 ml of this solution contains 0.1 μgHg.

0.01% dithizone (diphenylthiocarbazone) solution: Dissolve 0.011g of $C_6H_5N{:}NCSNHNHC_6H_5$ in 100 ml of toluene in a 200 ml separatory funnel. Add 50 ml of 0.1N sodium hydroxide and shake to extract the dithizone into the aqueous layer. Allow separation in a dark place. Transfer the aqueous layer (lower layer) in a glass container with glass stoppers and then add 1N hydrochloric acid drop by drop to make the solution slightly acidic (the color turns blackish green). Extract the purified dithizone

with 100 ml of toluene. Allow complete separation and discard the aqueous layer. (lower layer) . Seal, and store in a cool, dark place (Prepare a fresh solution at each time of use).

1N sodium hydroxide solution: Dissolve 40 g of special grade NaOH in distilled water to make a final volume of 1000 ml.

0.1N sodium hydroxide solution: Dilute 1N NaOH solution in distilled water to obtain a 10 fold dilution.

Alkaline sodium sulfide solution: Weigh 0.15 g of special grade $Na_2S \cdot 9H_2O$ in a 10 ml Pyrex test tube with glass stopper and dissolve in 10 ml of distilled water. Use the solution as the sodium sulfide stock solution. Store in a cool, dark place (Prepare a fresh solution every month). Ttransfer 0.1 ml of the stock solution into a container with glass stopper, add 50 ml of 0.1N sodium hydroxide and 50 ml of ethanol, and then mix (prepare fresh at each time of use). One ml of this solution contains 5 μg of Na_2S.

Walpole's buffer: Add 600 ml of distilled water to 200 ml of 1M sodium acetate ($CH_3COONa \cdot 3H_2O$) and about 200 ml of 1N hydrochloride. Mix and adjust the pH to 3.0.

1N hydrochloride solution: Mix 90 ml of HCl (reagent grade used for trace metal analysis) with distilled water to obtain a final volume of 1000 ml.

20% Ethylenediaminetetraacetic acid tetrasodium salt (EDTA) solution: Dissolve 20 g of special grade $C_{10}H_{12}N_2O_8Na_4.4H_2O$ in distilled water to make a final volume of 100 ml.

1N potassium hydroxide - ethanol: Dissolve 56.11 g of KOH in ethanol to obtain a final volume of 1000 ml (Store in a cool, dark place).

N_2 gas

*For reagents 8 ~ 14 above, prepare the needed amount in advance then mix and wash with 1/2 volume of toluene before use.

b. Instruments and equipment

Gas chromatograph equipped with electron capture detector

Multi-flow meter: Model V4 flow meter multi-kit (Sugiyama Gen Iriki Co.Ltd)

Centrifuge

Recipro-shaker

Magnetic stirrer

Volumetric flasks: 10, 100, and 1000 ml

Glass containers with glass stopper: 100, 200, 500, and 1000 ml

Pasteur pipettes

Serological or volumetric pipettes: 0.2, 0.5, 5, 10, and 20 ml

Separatory funnels: 100, 200, and 1000 ml

40 ml conical centrifuge tubes with screw caps

10 ml centrifuge tubes with glass stoppers : 100 mm long × 16.5 diameter

Gas chromatographic conditions

Column: Use a glass column (3 mm × 0.75~1.0 m) packed with Hg-20A-Uniport HP (GL Science, 60 ~ 80 mesh) or 10% KOCL-Hg-Chromosorb W (AW/DMCS, Yanaco, 60 ~ 80 mesh). At the injection

port, pack 2 ~ 3 cm of NaCl previously heated at 500°C for 2 ~ 3 hours

Temperature: Column oven: 140 ~ 160°C, injection port: 180°C, Detector oven: 200°C

Carrier gas: N_2, 30 ~ 40 ml/min

All glass wares should be toluene-washed before use.

c. Sample preparation

This method is applicable to protein-rich samples, such as fish meat, blood, and human tissues.

Methylmercury extraction

Weigh X g of homogenized sample (0.5 g or less as wet weight, in the case of dry sample approximately 0.1 g is moistened with 0.5 ml of water) in a 50 ml screw-capped conical centrifuge tube. Add 10 ml of 1N potassium hydroxide–ethanol. Seal tightly and heat in a 100°C isothermal bath for 1 hour with occasional mixing. Allow to cool. Add 10 ml of 1N hydrochloric acid and 5 ml of hexane and shake for 3 minutes (to remove fats) using a recipro-shaker. Centrifuge at 2000 rpm for 3 minutes then suck off and discard the hexane (upper layer). Add 2 ml of 20% EDTA and shake for 3 minutes and then add 5 ml of purified 0.01% dithizone solution. Shake to extract methylmercury as the dithizonate (complex) in the toluene layer (upper layer). Centrifuge at 2000 rpm for 3 minutes then suck off and discard the lower layer.

Clean-up

Add 3 ml of 1N NaOH to the toluene layer, shake (to remove excess dithizone) and centrifuge at 2000 rpm for 3 minutes. Suck off and discard the lower layer (aqueous layer). Repeat the procedure for the clean-up. Let the solution settle for a while, remove the lower layer, and centrifuge again at 2000 rpm for 3 minutes to obtain a clear toluene layer. Transfer a fixed volume of the toluene layer (normally 3 ml) to a 10 ml conical centrifuge tube with glass stopper (washed beforehand using toluene). Add 2 ml of alkaline sodium sulfide solution, and shake to back-extract the methylmercury into the aqueous layer. Centrifuge at 1000 rpm for 3 minutes then suck off and discard the upper toluene layer.

Wash the aqueous layer with 2 ml of toluene, shake for 2-3 minutes and centrifuge at 1000 rpm for 3 minutes. Suck off and discard the toluene layer (upper layer). Acidify with 1N hydrochloric acid (3 ~ 4 drops, see note-1). Bubble the solution by inserting a pasteur pipette which is attached to a multi-flow meter to pass N_2 gas gently at a flow rate of 50 ml/min for 3 minutes. Re-extract the methylmercury with 2 ml of Walpole's buffer solution and purified 0.01% dithizone solution (0.2 ~ 1.0 ml, normally 0.5 ml). Shake for 2-3 minutes and centrifuge at 1000 rpm for 3 minutes, suck off and discard the lower aqueous layer. Add 3 ml of 1N sodium hydroxide to the toluene layer then shake. Let the solution settle, suck off and remove the aqueous layer (lower layer). Centrifuge at 1000 rpm for 3 minutes. Suck off and discard the lower layer as much as possible. Acidify with 2 drops of 1N hydrochloric acid. Vortex mix and use the resulting solution as the sample for GC-ECD analysis. Perform the sample solution preparation protocol for the reagent blank and standard using 0 and 0.20 ml (corresponding to 0.020 μg of Hg) of methylmercury-cysteine. Use the resulting samples as the methylmercury standard, respectively.

<Procedural Note 1>

Separately mix 2 ml of alkaline sodium sulfide solution with several drops of 0.01% dithizone solution

as a pH indicator. Acidify with 1N HCl dropwise until the color changes from yellow to blue. The same amount of 1N HCl added above will be used in the sample preparation.

d. Procedures and calculations

Inject a fixed volume (normally 5 μl) of the blank, standard sample, and actual sample into the gas chromatograph. Label the peak heights thus obtained as Pb1, Pstd, and Ps, respectively. Calculate the methylmercury concentration (μg/g) in the sample using the following formula:

Methylmercury concentration in sample (μg/g) = 0.020(μg) × Ps-Pb1/Pstd-Pb1 × dilution factor/sample weight (X g).

3-3-(2) Hair samples

a. Reagents

Use the reagents below in addition to reagents 1 ~ 5 in "a. Reagents" of "3-3-(1) Biological samples"

Methylmercury-cysteine solution: Mix 2 ml of 0.1% L-cysteine solution with 2 ml of methylmercury standard solution (1.0 μg Hg/ml) in a 10 ml conical centrifuge tube with glass stopper. Shake the mixture in a recipro-shaker for 3 minutes to extract methylmercury. Centrifuge at 1000 rpm for 3 minutes, suck off and remove the upper organic layer. Seal, and store in a cool, dark place (Prepare a fresh solution every month).

One ml of this solution contains 1.00 μg Hg.

2N HCl: Add 180 ml of concentrated hydrochloric acid (reagent grade for trace metal analysis) to distilled water to make a final volume of 1000 ml.

b. Instruments and equipment

Use the items below in addition to 1, 3, 4, and 12 in "b. Instruments and equipment" in "3-3-(1) Biological samples"

Water bath

10 ml glass test tubes with screw caps: 16.5 mm diameter × 105 mm long

Graduated pipettes: 0.5, 1, 5, 10, and 20 ml

Beaker: 100 ml

Vial: 20 ml scintillation vials

Glass wool or cotton

*All glasswares must be washed with toluene before use.

*Gas chromatographic conditions are the same as those described in "3-3-(1) Biological samples".

c. Sample preparation

Place 20 ~ 40 mg of sample in a beaker. Wash using neutral soap and rinsed with distilled water. Add a small portion of acetone to remove the water. Remove the acetone under a reduced pressure. Next, transfer the sample into a 20 ml vial and cut the sample to fine pieces using a scissors. Weigh out X mg (normally about 10 mg) of finely cut sample in a 10 ml glass test tube with a screw cap. Add 2 drops of ethanol to moisten the sample, then insert and press down a small amount of glass wool or cotton using a glass rod. Slowly add 3 ml of 2N HCl on top of the cotton or glass wool to ensure that the hair sample will not be disturbed. Seal and heat in an isothermal bath at 100°C for 5 minutes to

extract methylmercury in the sample. Allow the solution to cool for a while, then shake and centrifuge at 1000 rpm for 3 minutes. Transfer 1 ml of the supernatant into a 10 ml conical test tube with glass stopper and add 2 ml of pure toluene. Shake the test tube on a recipro-shaker for 3 minutes in order to extract the methylmercury from the HCl layer to the toluene layer. Centrifuge the solution at 1000 rpm for 3 minutes, suck off and discard the lower layer. Use the resulting solution as the sample for analysis. Prepare a reagent blank and standard sample by measuring 0 and 0.10 ml (corresponding to 100 ng as Hg) of methylmercury-cysteine solution in 10 ml glass test tubes with teflon lined screw-caps. Acidify with 3 ml of 2N HCl and perform the sample solution preparation protocol described above.

d. Operations and calculations

Inject a fixed volume (normally 5 μl) of blank, standard sample and actual sample (or some dilution thereof) into the gas chromatograph. Label the peak heights thus obtained as Pb1, Pstd, and Ps (mm) respectively. Calculate the methylmercury concentration in the hair sample (ng Hg/mg) using the following formula:

Methylmercury concentration in hair sample (ng Hg/g) = 100(ng) × Ps-Pb1/Pstd-Pb1 × dilution factor × 1/X(mg)

3-4. References

1) The chemical society of Japan-mercury subcommittee ed.(1977): Suigin, Maruzen, Tokyo, pp.15-69.
2) The Pharmaceutical Society of Japan ed.(1990): Standard Methods of Analysis for Hygienic Chemists – With Commentary, pp. 52-58, 626-628.
3) Ministry of Health and Welfare ed.(1991): Standard Methods of Analysis in Food Safety Regulation – Chemistry, pp.184-192.
4) Bureau of Water Quality Management, Environment Agency of Japan ed.(1975): Teishitsu chousa houhou to sonokaisetsu, p10.
5) H. Akagi and H. Nishimura, Mercury speciation in the environment, Advances in Mercury Toxicology (T. Suzuki, N. Imura and T.W. Clarkson ed.), pp. 53-76, Plenum Press, USA (1991).
6) H. Akagi, O.Malm, F.J.P. Branches,Y. Kinjo, Y.Kashima, J.R.D.Guimarães, R.B. Oliveira, W.C. Pfeiffer, Y. Takizawa and H. Kato(1995): Human exposure to mercury due to gold mining in the Tapajós river basin, Amazon, Brazil: Speciation of mercury in human hair, blood and urine, Water, Air and Soil Pollution, **80**, 85-94, Kluwer Academic Publishers Netherlands(1995).

Item4. Appendix: Cautions for Mercury Analysis

For quality and quantity control in mercury determination, the use of IAEA-085, IAEA-086, and IAEA-142 as standard materials is recommended. However, when these types of standard materials are not available, accurately weigh out a reagent mercury compound and prepare the desired dilution. This type of standard sample should be sufficient in the detection of mercury concentrations of contaminated sites in the field. Longer processing times, handling difficulties, large containers and

other problems are encountered when very large quantities of sample are used. Therefore, 1 g or less of fish meat (wet weight), 200 ~ 500 mg of blood, or 10 ~ 100 mg of hair is recommended for use as the sample. When weighing, measures to prevent sample drying and water absorption are required. Prepare a glass-stoppered container (as light as possible). Weigh the empty container and place the sample inside. Weigh again, and subtract the empty weight. With frozen samples and hair in particular, great care is required since errors in weighing cannot be corrected unlike the sensitivity fluctuations in the analytical equipment at the time of mercury analysis.

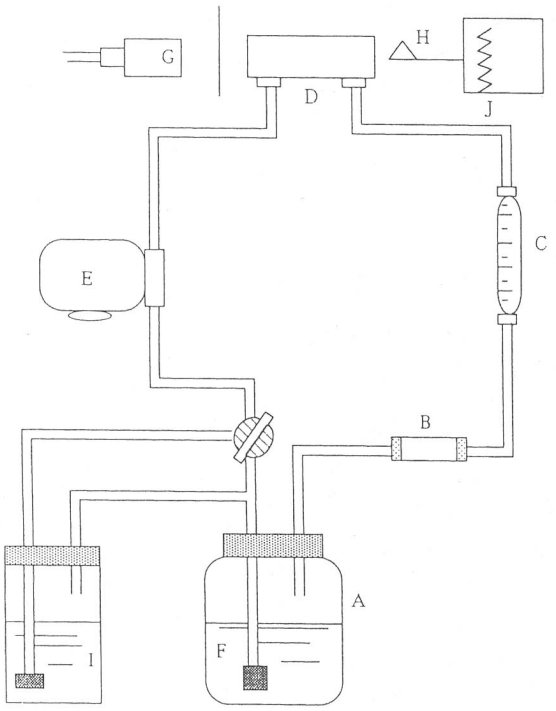

Fig.5-1-a Closed air circulation system

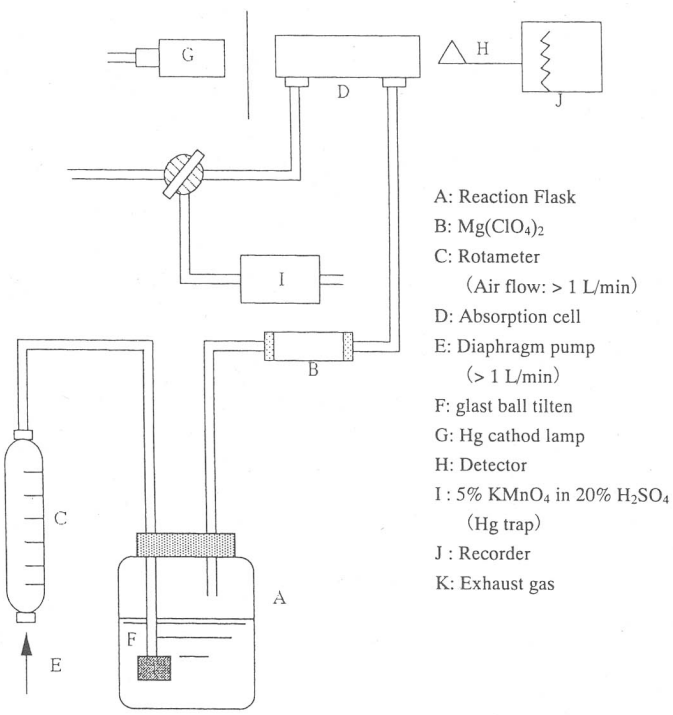

Fig.5-1-b Opened air drain system

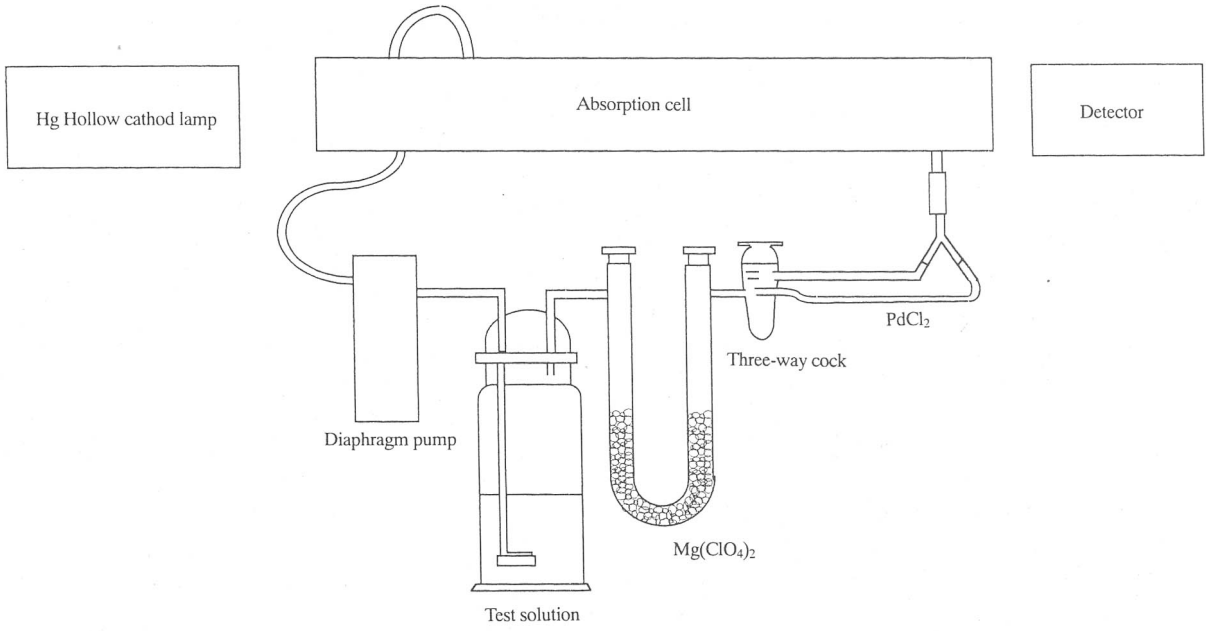

Fig.5-2 Apparatus for mercury analysis by cold-vapor atomic absorption spectrometry

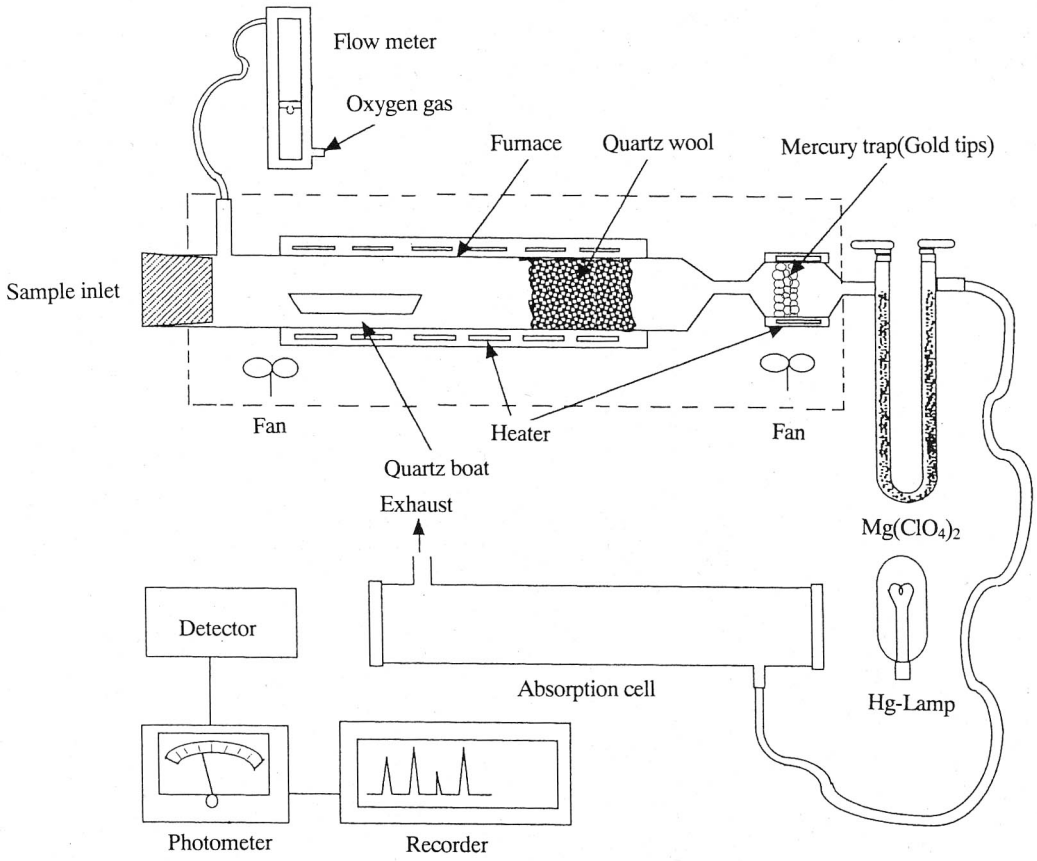

Fig.5-3 Apparatus for mercury analysis by sample combastion Method

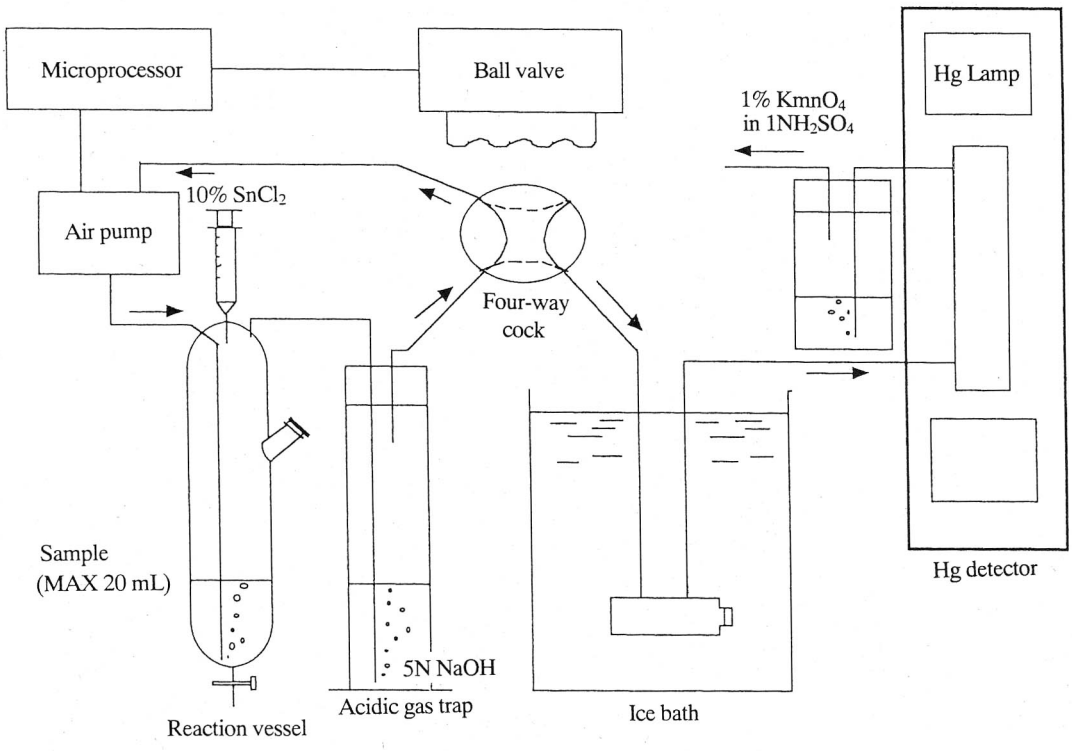

Fig.5-4 Improved apparatus for mercury analysis by cold-vapor atomic absorption spectrometry

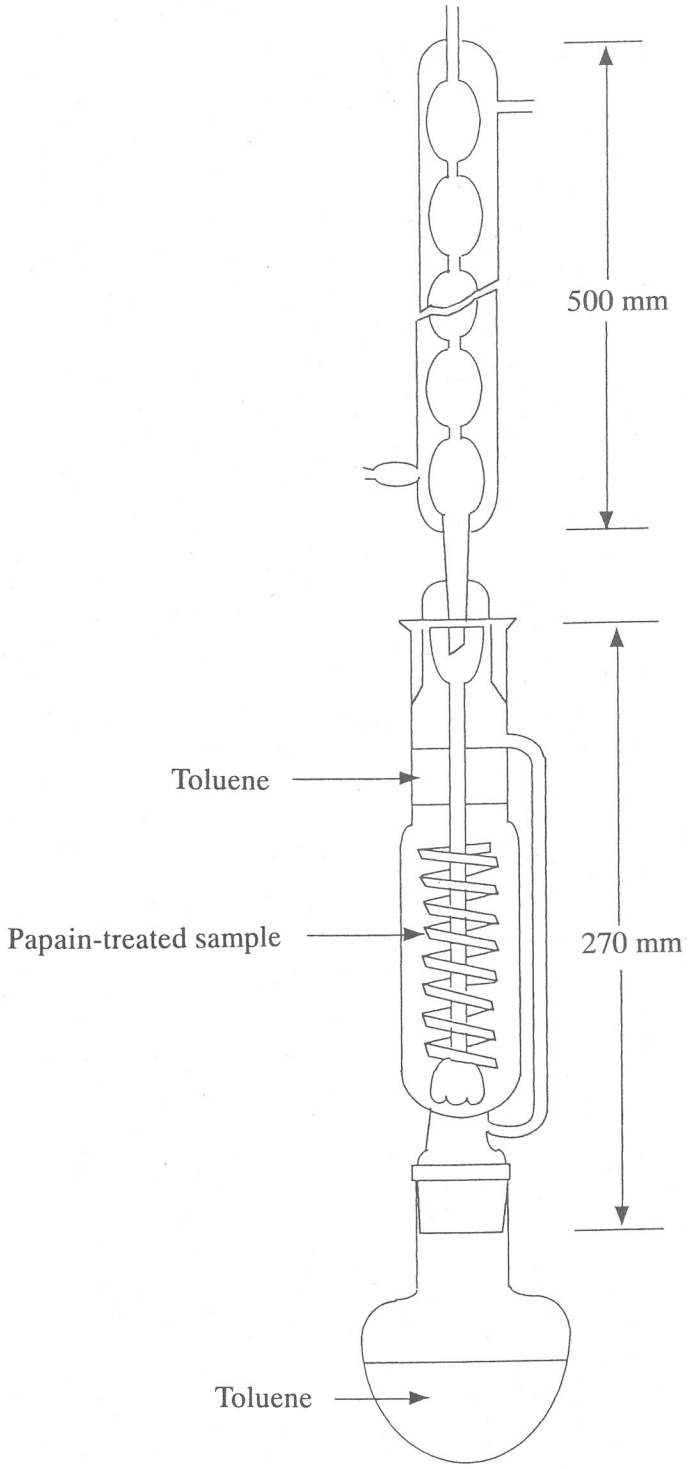

Fig.5-5　Methylmercury extraction apparatus

Chapter 6 Reference Materials

Item 1　Mercury pollution around the world (selected cases)

	Country	Region	Pollution Problems	Effects on Health	References (Connection to National Institute for Minamata Disease and written references)
1	Bangladesh	Chittagong	Mercury particles were found scattered throughout a mercury electrolytic calcined soda factory, which ceased operation in 1996. A high concentration of mercury was detected in the abandoned factory and subsequently mercury pollution spread.	No tests on the effects on residents' health have been conducted.	Aug. 29 ~ Sept. 11, 1997 Local survey (2 people) Jan. 23 ~Feb. 6, 1999 Local survey (2 people)
2	Brazil	The Amazon Basin	Up to now, approximately 3,000 tons of metal mercury has been released into the environment, particularly following the Gold Rush in 1979.	In addition to inorganic mercury poisoning of miners from inhaling large amounts of metal mercury, opposability of the health effects on local residents who eat fishes as the staple food due to the build up of organic mercury deposits in is worsening. It is estimated that over 130 people have inorganic mercury poisoning. The number of miners is said to be between 1~1.2 million people.	Nov. 27 ~ Dec. 3, 1994　International workshop (held in Rio de Janeiro) Dec. 1~26, 1996, Mar. 26~Apr. 11 2 personnel sent to the Amazon basin May 23~28, 1999　Fifth International Mercury Conference (held in Rio de Janeiro)
3	Cambodia	Sihanoukville	Among the personnel involved in the unloading of a ship containing industrial waste including maximum 4,000 ppm of mercury from Taiwan, 1 person died and 10 people were reported to have bad health. The waste, a total of 3,000 tons, is still left on the top of a small hill.	The mercury level in samples from workers　unloading a ship and cleaning up of the dumping site was registered within the normal range. As there have been no symptoms characteristic to The mercury poisoning, it is thought that there is no possibility of such mercury poisoning.	Dec. 24-26, 1998　Local survey (1 person)
4	Canada	Ontario State Quebec State	Organic mercury has been used in pulp sterilizing since the 1940s. In addition, a caustic soda factory has been identified as an origin of pollution. Pathological changes of methylmercury poisoning were found while performing autopsies on two cats.	From 1970, reports have surfaced regarding methylmercury poisoning in residents from settlements in the two states, however Canadian neurologists didn't acknowledge those reports.	Takeuchi, T. et al.: 1979 Takeuchi, T. et al.:1984
5	China	The Province of Ji Lin The Song hua Jiang Basin	Similar to the Chisso and Showa Denko incidents in Japan. Fish and sediments were contaminated by methylmercury from acetaldehyde plants. Hair mercury level was studied among fishermen from the Song hua Jiang basin. Eighteen out of 1,179 people with mercury levels of over 5ppm were found (including levels as much as 113 ppm and 34. 6 ppm).　Two cases of the disease were found in cats.	An official announcement has never been made, but it is said that fish eaters have shown the symptoms of Minamata disease.	Takeuchi, T. et al.: 1984 Chai et al.: 1994
6	China	The Province of Gui zhou The outskirts of Bai hua Lake	Similar to the Chisso and Showa Denko incidents in Japan. The draining of water that contains mercury and methylmercury from acetaldehyde plants has caused pollution. The water containing mercury from the plant is used as irrigation water in paddy fields before entering the Lake Bai hua. There is a concern of contamination in fish species etc.	No report has been shown on the effects on health.	1996 & 97—Joint research conducted with the Gui Zhou Province Environmental Protection Research Institute (2 people sent for technical assistance) Jan. 8~15, 1997, June 20~27, 1997, Oct. 26-Nov. 2, 1997, March 10~17, 1999—Research officers were sent
7	Denmark	Greenland	In 1991, in northern parts and the north pole region, Odense University in Denmark reported methylmercury contamination of fish and seals, the staple food of the indigenous people of Greenland.	No report has been shown on the effects on health.	Hansen, J.C. et al.: 1997
8	India	The Rushikulya Estuary Lake Hussain Sagar	In Ganjam town, mercury was drained into the Rushikulya estuary from a Chlor-Alkaline factory. 1. In effluents from the factory 0.14mg/l 2. 2. Soil 557 ppm 3. 3. Lake Hussain Sagar Plant sediments mercury value 9 µg/l (controlled value 0.2~0.1 µ g/l)	No report has been shown on the effects on health.	Panda, K. K. et al.: 1992 Lenka, M. et al.: 1992 Srikanth et al.: 1993

	Country	Region	Pollution Problems	Effects on Health	References (Connection to National Institute for Minamata Disease and written references)
9	Indonesia	Jakarta Bay	Pollution caused by drainage from factories around the bay. It is a kind of combined pollution including not only mercury but also other compounds such as cadmium, lead, nickel, and soon.	No surveys have been conducted regarding effects of mercury or other heavy metals on health.	Nov. 25~26, 1996 International workshop held Aug. 25~Sept. 11, 1997One person dispatched (technological guidance)
10	Iraq	Central Region	From 1956~60 and again from 1971~72, methyl- and ethyl-mercury poisoning from bread made from organic mercury processed wheat.	In 1971, 6530 people suffered from poisoning after eating bread, and 459 died.	Bakir et al.: 1973 Rustam, H. et al.: 1974 Choi, B. H.: 1978
11	Japan	The outskirts of Minamata Bay, Kumamoto Prefecture	Chisso Minamata Plant drained methylmercury compounds into Minamata Bay. Large concentrations of poisoned marine products were orally consumed by humans which initiated the spread of Minamata disease. Discovered in 1956.	Approximately 2,263 people suffer from Minamata disease (as of June '99—Kumamoto Prefecture 1,775 people & Kagoshima Prefecture 488 people).	Minamata Disease, Kumamoto University: 1968
12	Japan	Agano Basin, Niigata Prefecture	Showa Denko Kanose plant drained methylmercury compounds into Agano River. Large concentrations of poisoned fish and shellfish were orally consumed by humans which initiated the spread of Minamata disease. Discovered in 1965.	Approximately 690 people suffer from Minamata disease (as of June '99).	Minamata Disease, Kodansha Ltd.: 1977
13	Japan	Jintsu River, Toyama Prefecture	Mercury pollution was found in wastewater from a pharmaceutical factory. Total mercury 9,300 ppm and ethyl mercury 13.08 ppm were the levels in sediments in the factory. Levels in water at the drainage entrance to the Kumano river were 2,300 ppm and 31.90 ppm. At the lower reaches of the river, the highest levels of total mercury contamination detected were in the dace fish, maximum 9.40 ppm and average of 5.40 ppm; and Ayu, maximum 5.10 ppm and average 2.40 ppm, all showing an increase.	No report has been shown on the effects on health.	Kawasaki, G. et al.: 1973
14	Kenya		Poisoning from inorganic mercury containing pesticides.	There are two reports of inorganic mercury poisoning in a 2-1/2-year-old boy and a 7-year-old girl.	Brown, J. D. et al.: 1982
15	Kyrgyszian	Khaidarkan	There are concerns regarding inorganic mercury poisoning from mercury mine pollution.	Survey conducted due to reports of an outbreak of mercury poisoning among residents in the region. As a result, no effects on health were verified.	Dec. 4~20, 1996 Two personnel dispatched (Local survey)
16	Italy	Mediterranean Sea	Water drained from 2 Chrol-Alkaline Plants, in Rosignano Solvay (Livorno) and cinnabar mine Southern Tuscany,.	No report has been conducted on the effects on health.	Baldi, F. et al.: 1986
17	New Zealand	Lake Maraetai Waikato River	A pulp factory (Alkaline Chlorine plant) that has been in operation for 19 years releases 10 tons of chlorine in the Lake Maraetai daily and disperses 830 kg of total mercury into the Waikato river every year. The amount of mercury detected in rainbow trout in the area was over 3 ppm.	No report has been conducted on the effects on health.	Weissberg, B. G. et al.: 1973

	Country	Region	Pollution Problems	Effects on Health	References (Connection to National Institute for Minamata Disease and written references)
18	Philippines	Mindanao Island Agusan River	Mercury pollution in the Agusan River with the revitalization of gold mining intensified in the 1980s. Results show drainage downstream of Diwalwal is characterized by extremely high levels of Hg both in solution (maximum 2,906 µg/l) and in bottom sediments (>20mg/kg). People employed in the gold mining industry reached a total of between 80,000~120,000, with small-scale industry engineers in particular using large quantities of mercury (average=52kg/year). Of particular concern was the outbreak of Minamata disease due to the absorption of large amounts of methylmercury, because close to 20 tons of inorganic mercury is drained into the main areas of the rivers and canals.	No report has been conducted on the effects on health.	Nov. 26~27, 1997—An international workshop held in Manila city with 153 people participating from four countries: Japan, The Philippines, Canada and Indonesia Appleton, J. D. et al.: 1999
19	Romania		Pork that was contaminated when fed with sterilized grain containing ethyl mercury was eaten in 1974.	4 people suffered from acute ethyl mercury poisoning, 2 died.	Cinca, I. et al.: 1980
20	Spain	The Motril Region	A paper mill plant caused temporary pollution. The concentration of total mercury in the soil and sediments was 0.117~0.760 ppm. In tested water, the concentration was under 2.088-µ g/l.	No report has been conducted on the effects on health.	Navarro, M. et al.: 1993
21	Sweden	Stockholm	Paper mills continued to flush phenylmercury into the lake from 1940s~1966. Mercury pollution in the working environment was evident between 1940s~1950s. It is likely that methylmercury was created from phenylmercury in the bacteria production stage and then concentrated on fish. The amount reached as much as just under 6 ppm. This amount is enough to infect a cat with methylmercury poisoning within 60~83 days.	15 mercury agricultural plant workers were poisoned.	Ackefors, H.: 1971 Albanus, L. et al.: 1972
22	Tanzania	Lake Victoria (Geita, Mugusu & Victoria Gold Mines)	As the gold rush progressed in the 1980s, it is estimated that 6~10 tons/year of metal mercury used in gold mining were dispersed into the environment. In addition to direct contact of mercury vapor on the body of the gold miners, environmental pollution due to mercury deposits around the lake area, in particular the concentration of organic methylmercury in fish, is concerned.	No report has been conducted on the effects on health.	Ikingura, J. R. et al.: 1996
23	Thailand	Northern part of Thailand, Chao Phya River	Water pollution from many factories around Thai Bay. The Chao Phya River has very low oxygen content. Mercury concentration in the ocean water 1973~74: 0.03-2.38ppb 1975~76: 0.01-0.11ppb 1997: 0.02~2.00ppb (average world wide level: 0.03~0.27 ppb) Mercury concentration in sediments 1973: 49.3 ppb 1974: 23.4 ppb 1975: 0.04~0.15 ppb (average world wide level: 0.27ppb)	No report has been conducted on the effects on health.	Trishnananda, M.: 1979
24	England	The Suburbs of London	1937— methylmercury pesticide plant workers incident	4 workers suffered from methylmercury poisoning.	Hunter, D. et al.: 1940, 1954

	Country	Region	Pollution Problems	Effects on Health	References (Connection to National Institute for Minamata Disease and written references)
25	U.S.A.	New Mexico State Alamogordo	In 1970, people ate contaminated pork that was fed with sterilized grain containing ethyl mercury. The amount of in a family's hair was 1.86~2.40 ppm.	A family was poisoned after eating the pork. A case of congenital Minamata Disease is suspected.	Snyder, R.D.: 1971 Davis, L. E. et al.: 1994
26	U.S.A.	Ohio State	In 1990, a family, which moved into a new apartment, was exposed to mercury vapors due to inappropriate cleaning up of a large amount of spilt metal mercury prior to their arrival.	A 13 year old and 15 year old child contracted neurological symptoms.	Yeates, K. O. et al.: 1994
27	U.S.A.	South Dakota State Lake Ovalle	Drainage of water containing metal mercury from a gold mining company occurred between 1880~1970. (5.5~18kg/day) Fish contaminated. (0.02~1.05ppm)	No report has been conducted on the effects on health.	Walter, C. M.: 1973
28	U.S.A.	California State	Among 97 specimens fish taken from the ocean bed, 19 recorded 0.5 ppm or higher, and 5 recorded 1 ppm or higher of mercury.	No report has been conducted on the effects on health.	Hazeltine, W.: 1971
29	U.S.A.	Lake Erie	Pollution came from a factory water drainage (intensified 1970~). The mercury concentration in the atmosphere was 30μg /m^3. It was 0.5~12.4 ppm at the sediment. Plankton and other sea plants recorded 2.8~3.2 ppm (dry weight), and in fish, the amount was 0.20~0.79 ppm (dry weight).	193 specimens brain tissue taken from people aged over 60 who had lived in the Lake Erie area (people who had no memory of being exposed to mercury vapors in their working environment or by accident) recorded mercury levels of between 0.02~2.27 ppm (average=0.29 ppm).	Pillay, K. K. S. et al.: 1972
30	U.S.A.	South Florida	Sediment total mercury concentration was 1~219 ppm (dry weight) (includes 0.77% methylmercury), fish meat total mercury concentration was 0.03~2.22 (average 0.31) ppm (dry weight) (includes 83% methylmercury). The overall concentration of total mercury in water flowing into Florida Bay (after being filtered) was 3.0~7.4 μg/l (methylmercury 0.03~52%).	The daily intake of fish including 0.31ppm of total mercury is over 70g, meaning there are possibilities of effects on health. No report has been conducted on the effects on health.	Kannan, K. et al.: 1998
a	Denmark	Faroe Islands	The average concentration of mercury in the pilot whale is 3.3ppm, with over 50% being methylmercury. Among 1,023 children, 12.7% were born from mothers whose hair mercury concentrations were over 10 ppm (maximum 39.1 ppm), so that they are suspected suffering mercury poisoning.	After study on 917 children around the age of 7 for mercury poisoning and/or nervous disorders, the possibilities were found of some children being handicapped in terms of memory, physical performance and speech.	Weihe, P. et al.: 1997 Grandjean, P. et al.: 1998
b	Seychelles		Investigations were conducted on developmental disorders in infants suspected of being exposed to the low levels of methylmercury concentration in marine products.	After study on 789 children for the effects of mercury poisoning on physical and mental development, no positive sign has been found. In addition, no clear-cut developmental abnormality was found in 32 infants autopsied.	Lapham, L. W. et al.: 1995 Shamlaye, C. et al.: 1997

a, b: Researchers are examining the effects of a small amount of mercury on children's heath at this stage.

(Surveys conducted up until December 1999: National Institute for Minamata Disease)

Item2 Legal restrictions and standard values of various countries

Item2-1 Regulations and standards on mercury in various countries (Selected).

	subject	Air (occupational)	Air (ambient)	Air (emission)	Water (emission)	Water (drinking)	Food	Waste	Soil
Area									
Argentina		ALKYL MERCURY COMPOUNDS:8 H-TWA:0.01mg/m^3; 15MIN-STEL:0.03mg/m^3			INDUSTRIAL EFFLUENTS-PERMISSIBLE LIMIT:0.005mg/L		0.5mg/kg OF EDIBLE PORTION		
Canada		Alkyl componds,as Hg-TWA:0.01mg/m^3; STEL:0.03mg/m^3; skin absorption.All forms except alkyl,as Hg,Vapor-TWA:0.05mg/m^3	1)The quantity of mercury that the owner or operator of a plant may release into the ambient air from that plant shall not exceed:a)5g/day/1,000kg of rated capacity,where the source of the mercury is the ventilation gases exhausted from cell rooms;b)0.1g/day/1,000kg or rated capacity,where the source of the mercury is the ventilation gases exhausted from the end boxes;or 0.1g/day/1,000kg rated capacity,where the source of the mercury is the ventilation gases exhausted from retorts.2)No mercury shall be released directly into the ambient air from trank.3)The total amount of mercury that the owner or oparator of a plant may release into the ambient air from sources specified above shal not exceed 1.68kg/day (1994)		The owner of a plant may deposit mercury contained in effruent if the actual deposit of mercury in any day does not exceed 0.00250kg/tonne of chlorine times the reference production rate of that plant.(1978)	Maximum Acceptable Concentrations:0.001mg/L(Guidelines .1993)			
Czech repubic		TWA:0.05mg/m^3; CLV:0.15mg/m^3 (calcurated as Hg)(1985)	The substance is classified in the second group of air pollutants(solid inorganic air pollutant)(applies to mercury and its compouds expressed as mercury)(1992)	General Emission Limit:0.2mg/m^3 (1991)	Maximum allowable concentration0.2mg/L (IT applies to industrial wastewater from non ferrous metal industry,and metal surface fishing)(1992)	0.0005mg/L(aplies to mercury and its compouds in drinking surfacer water reserve)(1992)	Maximum Limit:0.1mg/kg (Applies to total feedingstuff),0.02-0.5mg/kg (Applies to specific feedingstuff) (1988)	Maximum Limit:3g/kg dry matter;Waste containing more than MXL of the substance is prohibited to dispose by landfilling.(1992)	MAC:0.6mg/kg dry soil(light soil),0.8mg/kg dry soil(all other soils)(1994)

	subject	Air (occupational)	Air (ambient)	Air (emission)	Water (emission)	Water (drinking)	Food	Waste	Soil
Area									
Germany				As inorganic compounds, must not exceed 0.2mg/m^3 (calcurated as the metal) at a mass flow of>=1g/h.(1986)	Waste water charges are levied by the federal states when waste water discharged into a water body exceeds the threshold values for mercury and mercury compounds of 1ug/L and an annual amount of 100g(1997)	0.001mg/L(calcurated as Hg) corresponding to about 0.005MMole/m^3; permissible tolerance of the measured value+-0.0005mg/L. (1998)	Fish,crustacean,mollusca,and products thereof :0.5-1.0mg/kg wet weight of consumable parts.Applies to the sum of mercury and mercury compounds,calculated as mercury.(1997)	Waste incinertion plants must be constructed and operated in such a way that the emmisison limit values for mercury and its compouds(expressed as mercury)of 0.03mg/m^3(daily average value) and 0.05mg/m^3(half-hour average value) are not exceeded.(1999)	It is prohibited to apply sewage to agricultural soil when the concentration of mercury in the soil exceeds 1mg /kg of soil dry matter
EEC				Waste inicineration plants shall be designed, equipped and operated in such a way that at least the emission limit values in the exhaust gases for mercury and its compounds(expressed as mercury) of 0.05mg/m^3 for new plants and 0.1mg/m^3 for existing plants are not exceeded. (1998)		Limit value for Mercury in surface fresh water used or intended for use in the abstraction of drinking water : 0.001mgHg/L for all categories of water treatment methods. Guideline value:0.0005mgHg/L for all categories of water treatment methods. (1993)	The mean total mercury content of the edible parts of fishery products must not exceed 0.5ppm of fresh product (0.5mg/kg of fresh weight). This limit is increased to 1ppm of fresh product (1mg/kg of fresh product) for the edible parts of the species.(1993)		Limit value for the concentration of mercury in soil to which sludge is applied:1-1.5mg/kg of dry matter in a representative sample of soil with a pH of 6 to 7. (1995)
UK						1ug Hg/L in water supplied from private suppleies for drinking, washing or cooking or for food production purposes.The reguration also specify sampling frequencies.(1992)	The maximum residue level of the pesticide mercury compounds in specified fruits,vegetables and cereals is 0.02mg/kg as Hg.(1994). The sale and use of specified feeding stuffs containing amounts in excess of 0.1-0.5mg/kg referred to a moisture content of 12% is prohibited.(1992)		
India							Fish:0.5mg/kg; Other food products: 1.0mg/kg(1985)		

	subject	Air (occupational)	Air (ambient)	Air (emission)	Water (emission)	Water (drinking)	Food	Waste	Soil
Area									
Japan		Administrative concentration:Mercury and its inorganic compounds(except silver sulfide) 0.05mg/m^3. Alkyl mercury compounds 0.01mg/m^3 (1999)			Maximum limit: 0.005mg/L as mercury (applied to mercury except alkyl mercury). Not detectable as alkyl mercury compounds. (1999)	The consentration of mercury in drinking water surpplied by the water works should not exceed 0.0005mg/L (1999)	marine products (tentative regulation) :total mercury 0.4ppm.Alkyl mercury 0.3ppm. (1999)		Target levels of soil quality through leaching test and content test:0.0005mg/L or less in test liquid.
Kenya							Marine and freshwater animal products: 0.5mg/kg.(1978)		
Mexico		At any workplace where this substance is produced,stored or handled a maximum permissible level of 0.05mg/m^3 must be observed for a period of 8 hours.(1984)				1)0.0005mg/L in receiving waters used for drinking water supply with conventional treatment as well as with disinfection only, and recreation. 2) 0.01mg/L in receivining waters suitable for recreational use,conservation of flora and fauna and for industrial purposes.			

	subject	Air (occupational)	Air (ambient)	Air (emission)	Water (emission)	Water (drinking)	Food	Waste	Soil
Area									
Ruissian		0.4mg/m^3 as mercury (aerosol). Mercury control is obligatory.(applies to copper amalgam). (1990)	0.0003mg/m^3 AV/D (applies to metallic mercury) (1984)			Water surface. 0.0005mg/L(aplies to mercury and its inorganic compouds,calcurated as mercury) hazard class: 1 (1989)	Limits for following food products: Fish products:0.3-0.7mg/kg; Meat products:0.03-0.2mg/kg;Milk products:0.005-0.03mg/kg; Creals:0.01-0.1mg/kg; vegetables,Fruits:0.02-0.1mg/kg; beverages:0.005mg/kg; Child food:0.005-0.15mg/kg(applies to mercury and its compounds,calcurated as mercury). (1982)		2.1mg/kg(translocation criteria of harmfulness) ; lead+mercury - 120.0+1.0mg/kg (1991)
Sweden		Mercury vapour:1D-TWA:0.05mg/m3. Mercury compounds(except alkyl compounds):1D-TWA:0.05mg/m^3 (calcurated as HG),skin absorption.Alkyl mercury compounds:1D-TWA:0.01mg/m^3 (calcurated as HG),skin absorption. (1991)					Fish based foods for infants and young children 0.05mg/kg.(1992)		

Notes : These data were mainly obtained by referensing the UNEP CHEMICALS Data Bank Legal File (http://irptc.unep.ch/irptc/).

: (number) shows Effective year.

: Blanks do not necessarily show nothing about regurations and standards on Mercury.

Item2-2 Standards and regulations on mercury in Japan

Ⅰ Environmental Quality Standards

	name	year	value	note	the current state
	Environmental Quality Standards for air pollution	1973	none		In, 1996,the Central Environment Council designated Mercury and its compounds to be in the priority list of 22 hazardous air pollutants. Setting of air quality standards for mercury and its compounds is now under way.
	Environmental Quality Standards for water pollution	1970, 1975(A standard value is changed)	Total mercury is defined to keep under 0.0005 mg/L Alkyl mercury is not detected.	This is defined by the following considerations; (1) Not exceed permitted quantity as seafood when the fact that mercury in water is accumulated and concentrated into the fish and other marine creatures through food chain is considered. Provisional regulatory standard on fish and other marine creatures (Total mercury is 0.4ppm,Alkyl mercury is 0.3 ppm) as a related factors.(2) A situation of mercury level in natural water (liver, lake, marine, etc.).(3) Accuracy on methods of measurement .	
	Water pollution control law	1975 （enforcement）	Mercury, Alkyl mercury and other mercury compounds: 0.0005 mg/L Alkyl mercury compounds: not detected		
	Environmental Quality Standards for groundwater pollution	1997	Total mercury is defined to keep under 0.0005 mg/L Alkyl mercury is not detected.	Groundwater is used as familiar water resources and important to keep a sound water circulation. This Environmental Quality Standards is applied to all of groundwater, and the same standard values are established as the standard for protecting human health with the 26 substances of the EQS for water pollution.	
	Environmental Quality Standards for soil pollution	1991	Total mercury is defined to keep under 0.0005 mg/L (in test liquid) Alkyl mercury is not detected.	This standard is defined in a point of view to keep an environmental function on soil that clears the quality of water and fosters underground water. So, this standard is the same standard values of the EQS for water pollution. Test liquid is prepared by following methods, mix sample soil with water that is ten times volume of the sample and shake for six hours.	

II Other standards or regulations

	name	year	value	note	the current state
	Provisional regulatory standard for removing bottom sediment	1975		Bottom sediment that contained over 25 ppm of total mercury is object to remove by means of dredging and landfill (blockade, enclosure, etc.).	
	Provisional regulatory standard on fish and other marine creatures.	1973	Total mercury is 0.4 ppm Alkyl mercury is 0.3 ppm (as a reference)	In case marine products exceed 0.4 ppm (total mercury), additional examination for methylmercury is done. If the result exceeds 0.3 ppm, those marine products are judged as objects of exceeding provisional regulatory standard. Restriction and direction relating the catching, the sale and the circulation on market of such marine products are noticed. There is a note that pregnant women, babies and infants need adequate directions on diet relating this provisional regulatory standard applied.	
	Water supply law	1992	Mercury is under 0.0005 mg/L .	JECFA ‘s epidemiological data in 1988 has been a ground that drinking water guideline value on mercury is 0.001 mg/L (total mercury). But, this standard has been set up based on the limit of measurement for keeping former standard “ not detected”.	
	Industrial Safety and Health Law	1972	Density for occupational management on Alkyl mercury compounds is 0.01 mg/m3 and on mercury and inorganic mercury compounds is 0.05 mg/m3.	Alkyl mercury compounds, mercury, and inorganic mercury compounds in the air are objects to be monitored on workspace in industry which deal with them. Density for occupational management on Alkyl mercury compounds is 0.01 mg/m3 and on mercury and inorganic mercury compounds is 0.05 mg/m3. Work space is classified into three divisions by comparing actual measure with density for occupational management. A manager in such an industry has to take an adequate measure to keep laborer’s health in obedience to the class on workspace.	

	name	year	value	note	the current state
	A law for management on waste and cleaning	1971	(1) Technical standards in the final managing facilities.Total mercury is defined to keep under 0.0005 mg/L Alkyl mercury is not detected.	To judge the effect to the quality of underground water, the sample is obtained from underground water around the final managing facilities.	
			(2) Judgement standards relating the management for filling waste Alkyl mercury is not detected.Mercury and its compounds : 0.005 mg/L (in test liquid) as a mercury.	The sample data is obtained from the melted sample after managed. This is defined by the following considerations;a) Harmful substances may be absorbed in soil.b) The management for filling waste is easier than for throwing waste into sea. These standards correspond to the standards of effluent water.	
			(3) Judgement standards relating the management for throwing waste into sea. a) Waste as organic matter Alkyl mercury compounds are not detected. Mercury and its compounds : 0.025 mg/kg as a mercury. b) Waste as inorganic matter Alkyl mercury compounds are not detected. Mercury and its compounds : 0.0005 mg/L as a mercury. c) Waste as acids or alkali Alkyl mercury compounds are not detected. Mercury and its compounds : 0.025 mg/kg as a mercury	Effect of dilution in the sea is considered in these standards.	
	Law for the control of household products containing harmful substances	1973	Organic mercury compounds should not be detected (should not exceed 1 ppm as the background level when measured by atomic absorption spectroscopy) in textile products, in adhesive, paints and waxes for household use, shoe polishes.	Any person should not sell the household products, which do not meet the standards. And such products should be recalled.	

Item 3 Miscellaneous

Table R-1 Comparison of clinical symptoms observed for inorganic and methylmercury poisoning

	Inorganic mercury	Methyl mercury
General condition	Weight loss	-
Face	Erethism	Occasional masked expression
Skin	Pale, sweating, petechial, rash	Sweating
Nails	Atrophy, falling off	-
Eyes	Mercurial crystalline lens, weakness of vision, hyperemia, ptosis	-
Digestive tract distress	Gingivitis, stomatitis, gastritis, appetite loss, vomiting, constipation, diarrhea, metallic taste, hypersalivation	Hypersalivation
Cardiovascular distress	Tunica intima vasorum	-
Arthritis	Arthritis	-
Nose & Throat	Pharyngitis, rhinitis	-
Kidneys	Glomerulonephritis, renal tubule dysfunction	-
Mental symptoms	Erethism (introversion, melancholy, memory loss, insomnia, lack of concentration, irritability)	Mental retardation, personality changes, insomnia, instability, excitability, melancholy
Cranial nerve palsy	Optic neuritis (rare), facial palsy	-
Visual impairment	Constriction of the visual field (mild)	Constriction of the visual field, abnormal pupillary reflex, abnormal stereoscopic vision
Hearing impairment	+ (rare)	+

	Inorganic mercury	Methyl mercury
Impairment of sense of taste	Metallic taste	+
Olfactory impairment	-	+
Dysphagia	Pharyngitis	+
Language impairment	+ (mild, sometimes)	Slow, ataxic
Gait disturbance	Ataxic (mild, sometimes)	Ataxic
Dysdiadochokinesis	+ (mild, sometimes)	+
Limb ataxia	+ (rare)	+
Tremors	+	+
Involuntary movement	+ (rare)	+ (severe)
Convulsions	-	+ (easily stimulated)
Muscle weakness	+	+ (sometimes)
Muscle atrophy	-	+ (rare)
Paresthesia	Muscle pain, joint pain	Numbness
Superficial sensory disturbance	+	+
Deep sensory disturbance	+ (rare)	+
Reflexes	Normal or decreased	Normal or exaggerated (decrease is rare)
Parkinson’s disease	+ (rare)	-

Table R-2 **New heavy metal processing technologies**

	Cleaning principles and basic technology	Applicable soils and contaminants	Technological advantages	Technological disadvantages	Technology type	Remarks
Solidification	Chemicals and cement are added to soil to form chemically stable heavy metal compounds.	<Applicable soils> All contaminated soils	1) Low cost compared to washing processes 2) Simple and easy to manage processing operations.	1) Processing efficiency varies depending on soil conditions at the contaminated site.	1) Phosphate is added and insoluble phosphate minerals (salts) are formed in the soil. 2) Sodium sulfide and other chemicals may be used as the solidification agent.	1) The method was recently proposed in America. *Processing cost is approximately \$50~60/m^3 (varies depending on concentrations and other parameters)
Soil Washing	Contaminated soil is dug up and placed in a wash unit with the goal of separating and reducing harmful materials in the soil and sludge. Various methods are used in the wash unit. One method involves adding liquid washing agents to dissolve or suspend contaminants, which are then separated. Another method involves separating contaminants through contact with high pressure water and other agents.	<Applicable soils> Contaminated soil (with few fine particles), sand, and gravel.	1) When water alone is effective as a washing agent, economical recovery operations are possible. 2) Closed systems can be created and discharge control is possible.	1) Processing efficiency varies depending on soil at the contaminated site. 2) Recovery processes are required since washing agents are used. 3) Washing agent selection varies depending on the soil and contaminants at the site.	1) Methods using washing agents include "Surfactant + acid washing", "Surfactant + hydroxylchloride", "Surfactant + chemical agents", and "Hydrochloric acid and pure water processing". 2) Methods involving contact with high energy include high pressure water injection, steam washing, and combinations thereof.	1) At the EPA (American Environmental Protection Agency), these methods have been extensively evaluated as effective future technologies for processing heavy metal contamination. (Heavy metal processing efficiency) 80 ~ 90% *Processing cost is approximately \$1,000/m^3

	Cleaning principles and basic technology	Applicable soils and contaminants	Technological advantages	Technological disadvantages	Technology type	Remarks
Incineration	Contaminated soil, sludge, and other materials are heated to 800 ~ 1,000°C and the harmful heavy metal in the soil is vaporized and then captured.	<Applicable soils> Applicable to a wide range of contaminated soils, sludge, and other materials regardless of characteristics.	1) Short processing time of 20 ~ 30 minutes. 2) Compatible with high level heavy metal contamination	1) Energy cost is very high 2) Large scale exhaust gas processing facilities are required 3) Heavy metals may be converted to even more toxic materials in the reaction process.	1) Thermal decomposition of harmful materials a. Direct heating and separation method (direct heating on a burner) Direct flame unit Internal flame unit	1) At the EPA (American Environmental Protection Agency), this method has been extensively evaluated as a processing technology for soils contaminated by heavy metals. *Processing cost is approximately \$1,000/m^3
Thermal desorption	Contaminated soil, sediment, and other materials are heated to 250 ~ 300°C to evaporate mercury. The evaporated mercury is then captured.	<Applicable soils> Applicable to a wide range of contaminated soils, sludge, and other materials regardless of characteristics.	1) Short processing times of 20 ~ 30 minutes and low cost. 2) Applicable to high concentration mercury contamination.	1) The method is not applicable to heavy metals, such as hexaralent chromium compounds, with high boiling points	1) Indirect heating and separation method (Externally heated rotary kiln, etc.)	1) At the EPA (American Environmental Protection Agency), these methods have been extensively evaluated as effective future technologies for processing heavy metal contamination. *Processing cost is approximately \$500/m^3
Vitrification	Contaminated soil is heated to 1,200 ~1,500°C to vitrify the soil and contaminations are sealed in the soil.	<Applicable soils> Applicable to a wide range of contaminated soils, sludge, and other materials regardless of characteristics.	1) Applicable to high concentration heavy metal contamination	1) Energy cost is very high. 2) Large scale exhaust gas processing facilities are required.	1) Another method involves inserting an electrode into the soil and passing a current through to vitrify the soil in situ.	1) At the EPA this method is currently only in the research phase due to the extremely high costs involved. *Processing cost is over \$2,000/m^3

	Cleaning principles and basic technology	Applicable soils and contaminants	Technological advantages	Technological disadvantages	Technology type	Remarks
Electrokinetics	Electrodes are placed in the soil and a direct current is generated. Heavy metal ions are then moved through the water in the interval spaces and extracted from wells near each electrode.	<Applicable soils> The method is applicable to soils other than sand, gravel, and heavy clay (sludge).	1) Processing operations are simple. 2) Cost is low compared to washing and incineration.	1) Method is not applicable to areas with low ground water potentials or no water permeability. 2) Electrode deterioration is rapid and heavy maintenance is required.	1) Various materials, including stainless steel and vanadium plating, are used in the electrodes for generating the direct current. The distance between the anode and cathode is normally 8 ~ 13m.	1) The EPA has currently positioned this technology in the research and development phase. 2) Processing efficiency is approximately 1/2 that of other processing methods.
Phytoremediation	Plants that accumulate high amounts of heavy metals are cultivated in the heavy metal contaminated soil. Absorption by the plants is then used to clean the soil.	<Applicable soils> All contaminated soils	1) Processing is simple. 2) Cost is low compared to other processing methods.	1) A long time, on a year scale, is required for cleanup. 2) Cleanup efficiency is low.	1) Processing of plants that have accumulated heavy metals in their systems is important. If left alone, dead plants will decay and return the heavy metals to the environment.	1) Although some effectiveness has been reported, the method is still in the research phase. *Processing cost is approximately \ $30~40/$m^3$
Reactive barrier *for ground water	Heavy metals are made insoluble through a reactive barrier (Principles) A reactive material, such as iron powder, is buried in the down flow region for ground water in a contaminated area. When the water passes through this buried wall, heavy metals are converted to insoluble material and precipitate out.	<Applicable soils> Applicable to all soils	1) Processing operations are easy. 2) Maintenance is easy. 3) Effective use of waste iron, etc.	1) Contaminants remain in the soil.	1) Waste iron, steel slugs, and other materials can be recycled and used as reactive barrier materials. 2) Reactive barrier thickness and depth should be decided after a survey of the site (ground water quality, flow rate, direction, etc.)	1) A model case was used by the EPA and actual data is available.

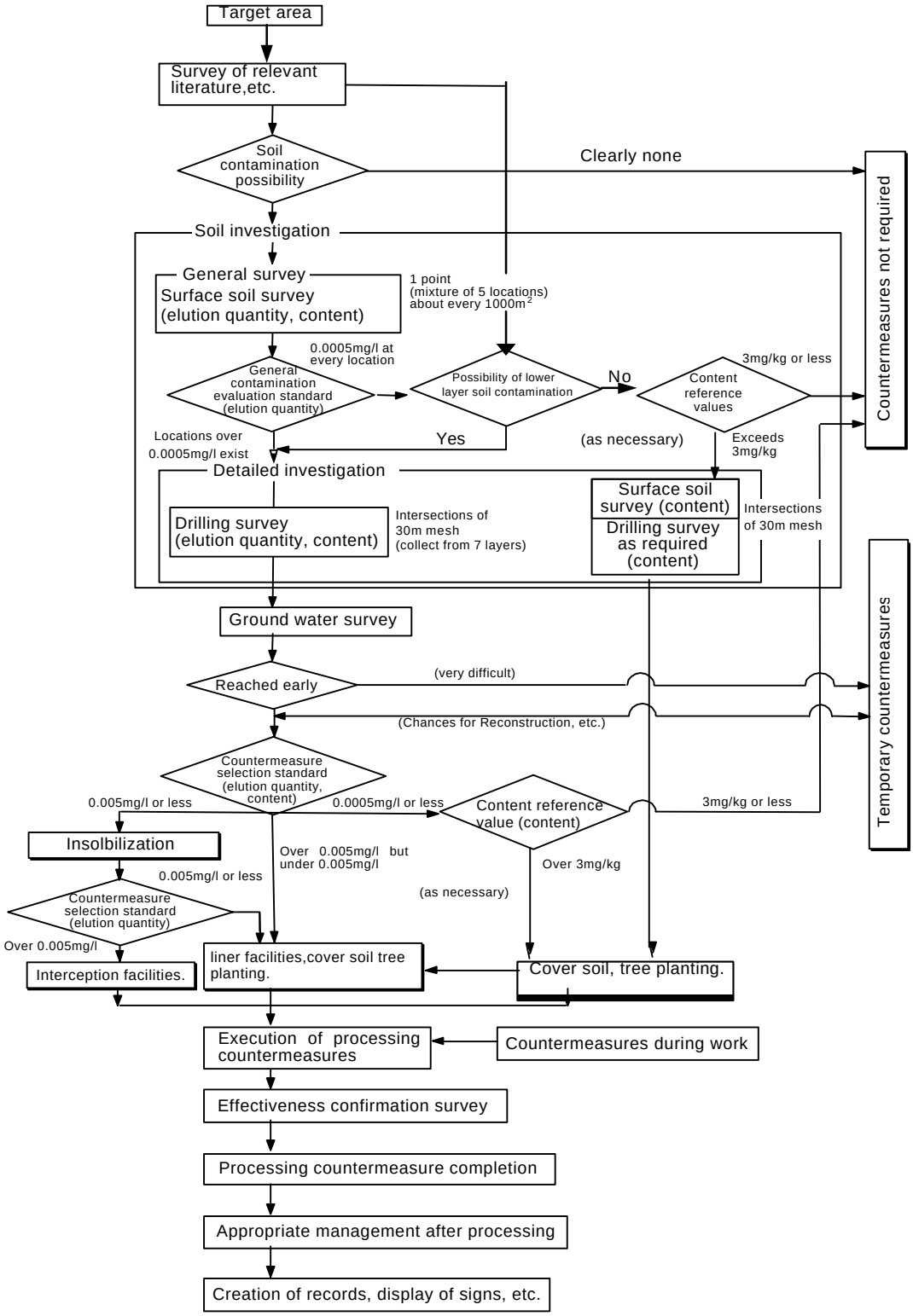

Figure R-1 Flow chart of examination for soil contamination possibility

Item 4 Survey sheet (Example); additionally usable as a registration form

Survey sheet (example)

Name:
Date:
Sex: 1. Male 2. Female
Age: ( ) years

Educational background:
What level of education was completed? Circle the appropriate answer.

1. No education
2. 1 ~ 6 years
3. 7 ~ 9 years
4. 10 ~ 12 years
5. 13 years or more

Work history: Write work description in ( )

1. From _____ to _____ , worked in ( )
2. From _____ to _____ , worked in ( )
3. From _____ to _____ , worked in ( )
4. From _____ to _____ , worked in ( )
5. From _____ to _____ , worked in ( )

Origins:
Where were your mother and father born?

Father ( )
Mother ( )

Where were you born?

( )

Personal history: Write address in ( ) and circle appropriate government of the area.

1. From _____ to _____ , lived in ( ) city town village
2. From _____ to _____ , lived in ( ) city town village
3. From _____ to _____ , lived in ( ) city town village
4. From _____ to _____ , lived in ( ) city town village
5. From _____ to present , lived in ( ) city town village

Medical history:

Family history:

Fish-eating habits:
Write the types of fish eaten 1 or more times per week (including seasonal fish limited to 1 or more times per week during the season) in sequence starting with the most commonly eaten fish type.

1. (　　)
2. (　　)
3. (　　)

About how many times per week do you eat fish?
Circle the appropriate response.

1. Everyday (7 times or more per week)
2. Nearly everyday (5 ~ 6 times per week)
3. Often (3 ~ 4 times per week)
4. Sometimes (1 ~ 2 times per week)
5. Not often (less than 1 time per week)

About how much fish do you eat in a single meal containing fish?
Circle the appropriate response.

1. Very large amount (200g or more/meal; 1 medium mackerel)
2. Large amount (100 ~ 200g/meal; 1/2 medium mackerel or more but less than a full mackerel)
3. Average (50 ~ 100g/meal; 1 cut piece of yellowtail = 50g)
4. Little (20 ~ 50g/meal; 2 small sardines)
5. Very little (10 ~ 20g/meal; 4 anchovies)
6. Almost none (0 ~ 10g/meal)

How much fish did you eat 2 months ago compared to the last week?
Circle the appropriate response.

1. Ate much more 2 months ago (2 months ago >> most recent week)
2. Ate more 2 months ago (2 months ago > most recent week)
3. Ate about the same (2 months ago = most recent week)
4. Ate more recently (most recent week > 2 months ago)
5. Ate much more recently (most recent week>> 2 months ago)

How often do you eat the following per week? Write the number below
In addition, circle the amount eaten each time.

Canned tuna (_____times per week), (0 ~ 1/4 ~ 1/2 ~ 1 ~ 2 cans per time)
Canned salmon (_____times per week), (0 ~ 1/4 ~ 1/2 ~ 1 ~ 2 cans per time)
Canned mackerel (_____times per week), (0 ~ 1/4 ~ 1/2 ~ 1 ~ 2 cans per time)
Canned anchovies (_____times per week), (0 ~ 1/4 ~ 1/2 ~ 1 ~ 2 cans per time)

Do you drink alcoholic beverages?
Circle the appropriate response.

1. Everyday (7 or more times per week)
2. Almost everyday (5 ~ 6 times per week)
3. Often (3 ~ 4 times per week)
4. Somet imes (1 ~ 2 times per week)
5. Not much (less than 1 time per week)
6. Don't drink

Do you smoke cigarettes?

1. Smoke
   1.1 How many cigarettes do you smoke everyday?
   (cigarettes)
2. Don't smoke
3. Quit smoking
   3.1 How long ago did you quit?
   Circle the appropriate response.
   1. Less than 3 months ago.
   2. More than 3 months ago but less than a year.
   3. More than a year.
   3.2 Before quitting, how many cigarettes did you smoke everyday?
   (cigarettes)

Circle any of the following products (makeup and medicines) that you use everyday.

1. Mercury ointment
2. Skin lightening cream
3. Skin lightening soap
4. Mercurochrome
5. Don't use any of the above

Is your hair with a permanent-wave treatment ?

Circle the appropriate response.

1. With a permanent-wave treatment
   1.1 How many times per year do you receive the treatment?
   (times)
2. Without a permanent-wave treatment

Do you dye your hair?

1. Dye
   1.1 How many times per year do you dye your hair?
   (times)
2. Don’t dye.