

# The Tardive Health Effects Caused by Methylmercury Poisoning around Minamata

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1. I have been working in Minamata as a medical doctor for about 30 years. I'd like to talk about the tardive effects of methylmercury poisoning around Minamata.

# Onset of Acute Cases

2. Let's begin by looking at cases of acute onset.

# Outbreak of Minamata disease in 1956

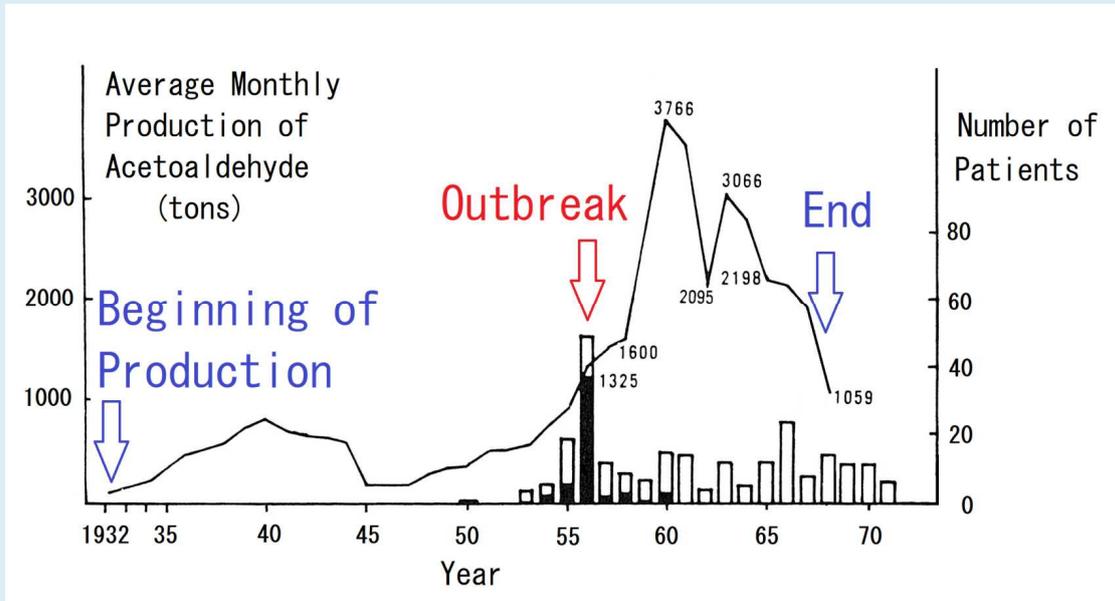
## - Onset of acute cases -



Tokuomi, H. *et al.*: A disease of central nervous system of unknown origin occurred in Minamata district – especially about clinical observations. *Kumamoto Medical Journal*. 31 (supple 1), p.29, 1957. (in Japanese)

3. In 1956, some patients were found to have acute severe and profound neurological disorders, not only somatosensory disturbance, but also walking, hearing, visual disturbances, and so on.

# Outbreak of Minamata disease



4. When we see the acute severe cases in Minamata, we tend to think that the victims might have become abruptly ill after exposure. But we have to remember that even such patients had been exposed for a long time. They had been eating contaminated fish for years.

The Chisso Company began discharging contaminated wastewater into the sea in 1932, 24 years before the outbreak. Do you believe that there were no victims in Minamata before 1956?

My answer is "Yes! there were!" The acute fulminant cases of 1956 were dramatic and very impressive, but they were only the tip of the iceberg. Most cases of the disease develop gradually.

In 1968, when mercury discharge was stopped, only one hundred victims had been recognized as suffering from the disease.

# Onset of MeHg poisoning in severe cases (extreme amount of MeHg)

- Edwards (1865)... one developed severe symptoms one month after exposure for only two weeks, and died one year later in confusion state.

(Edwards, GN.: Saint Bartholomew's Hosp Rep, 1: 141–150, 1865.)

- In an outbreak of the disease in Iraq in 1971, latent periods were from 16 to 38 days.

(Bakir F. *et al.*, Science, 181, 230-241, 1973)

- A professor of chemistry at Dartmouth University, who applied dimethyl mercury solution to a finger on August 14, 1996, developed progressive neurological disorder after 4 months and died on June 8, 1997.

(Nierenberg D.W., *et al.*: N Engl J Med, 338(23): 1672-1676, 1998.)

5. There are other cases that describe this symptom latency after exposure to extreme amounts of methylmercury. One of the first cases in the world, in 1865 in England, became ill one month after exposure. In Iraq, latent periods were from 16 to 38 days. In an accident at Dartmouth University, the onset was 4 months after exposure to dimethyl mercury.

# Chronic Low-Level Exposed Cases

6. My main theme is about chronic low-level exposed cases

# Characteristics of chronic Minamata disease

- Sensory disorders develop slowly, and in many cases in insidious manner. The time of its onset is often unclear.
- When it becomes more serious, it is accompanied by ataxia, visual field narrowing, hearing disorders, dysarthria, taste and olfaction disorders, mental dysfunction, etc.
- Its distribution varies from mild to severe.
- There is a latency period between exposure and onset.
- These symptoms continue chronically, and fluctuation in the degree of symptoms is observed.

7. In the early stages of chronic cases, sensory disorders develop slowly and insidiously. In many cases, the time of onset was unclear. Muscle cramps are often observed. When it becomes more serious, it is accompanied by ataxia, visual field narrowing, dysarthria, taste and olfaction disorders, mental dysfunction, and so on. These symptoms develop gradually and sometimes fluctuate in severity.

# Reports of tardive onset of Minamata disease

- Professor Tsubaki reported cases in which symptoms occurred after a period of several months after exposure to methylmercury (1972).
- Dr. Shirakawa in Niigata reported that subjective symptoms developed after several years (1975).
- Igata reported cases of Minamata disease (1976).

8. In Japan, in the 1970's, some doctors reported the tardive effects of this disease. Tsubaki reported observing effects several months after the end of exposure. Dr. Shirakawa reported several years retardation.

# The Central Environmental Pollution Council of the Environment Agency (Japan, 1991)

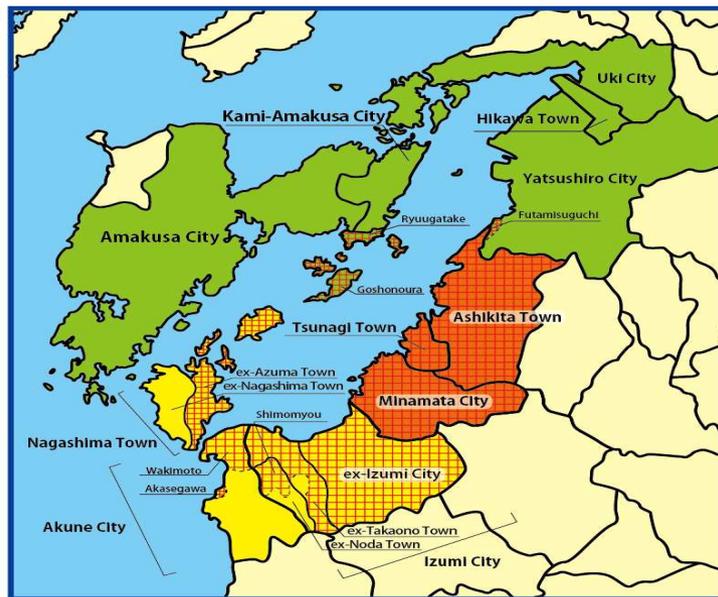
- The period from methylmercury exposure to the onset is ordinarily supposed to be from one month to one year.  
(unsubstantiated claim)

Central Council for Environmental Pollution Control. About Future Measures to Minamata Disease; The Environmental Agency: Tokyo, 1991. (In Japanese)

9. In 1991, without having conducted any clinical or epidemiological studies, the Central Environmental Pollution Council of the Environment Agency of Japan decided that the period from methylmercury exposure to the onset is ordinarily supposed to be from one month to one year. However, I have had a completely different experience. I have examined thousands of residents and patients in and around Minamata.

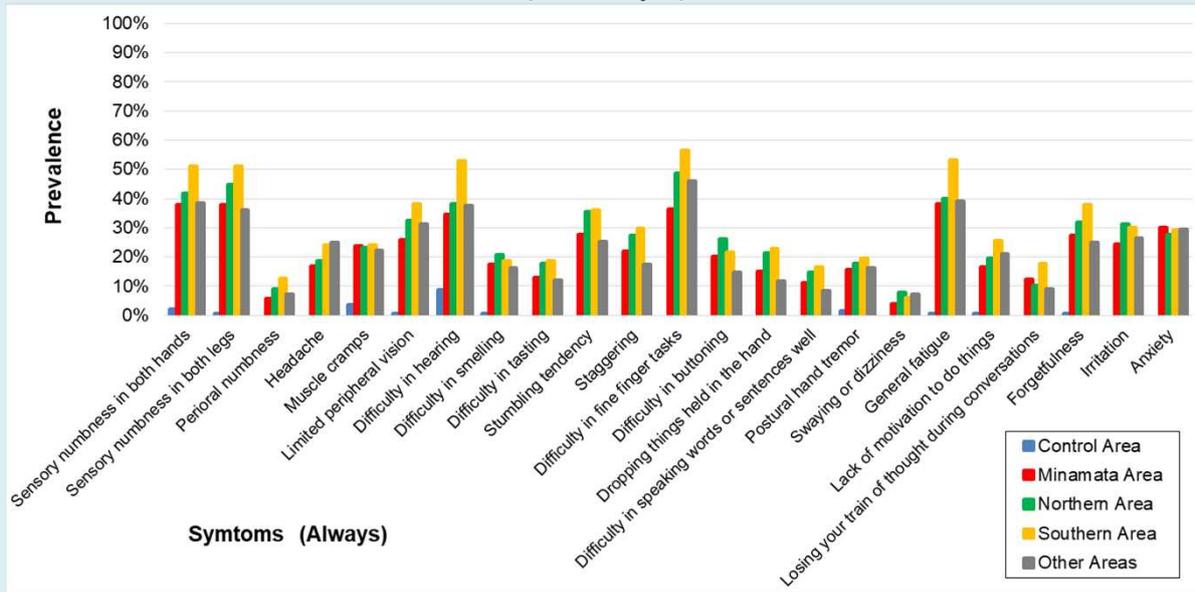
# Survey in 2009

(Takaoka *et al.*: *Toxics*, 6(3), 39, 2018)



10. This map is from an introduction to my study published last year in *Toxics*. In 2009, we studied 973 residents who lived in or had lived in the polluted area. We divided those residents into 4 groups: Central, Northern, Southern, and other districts. Residents from non-polluted areas were used as the control group.

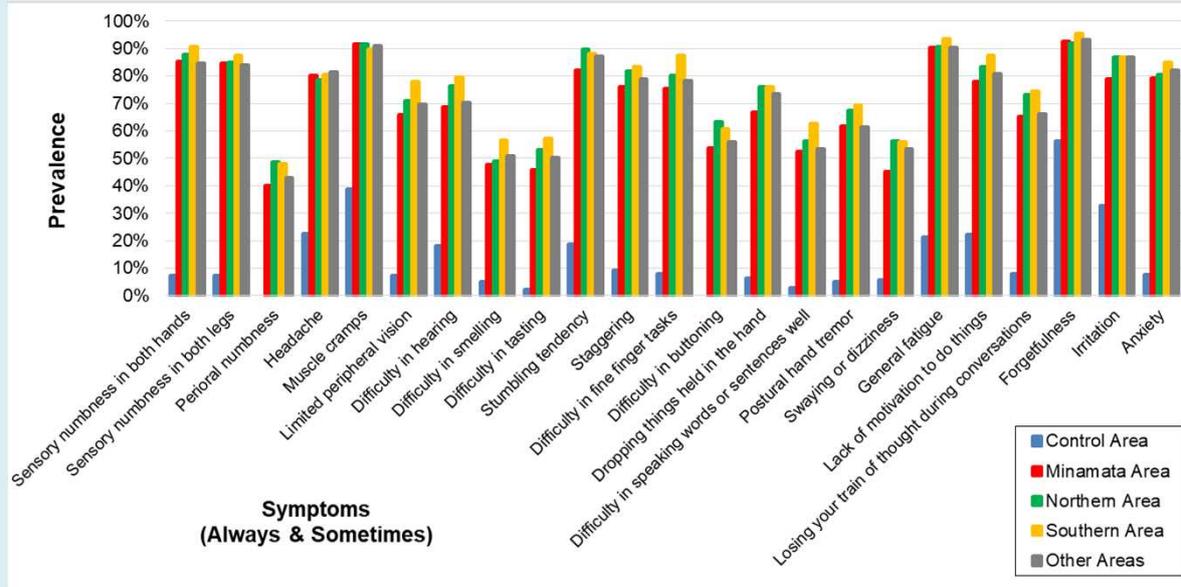
# Prevalence of symptoms (Always)



Takaoka *et al.*: *Toxics*, 6(3), 39, p.11, 2018

11. The complaints "Always" of the four areas were significantly higher in the polluted area groups and the prevalence was very similar among the four polluted area groups.

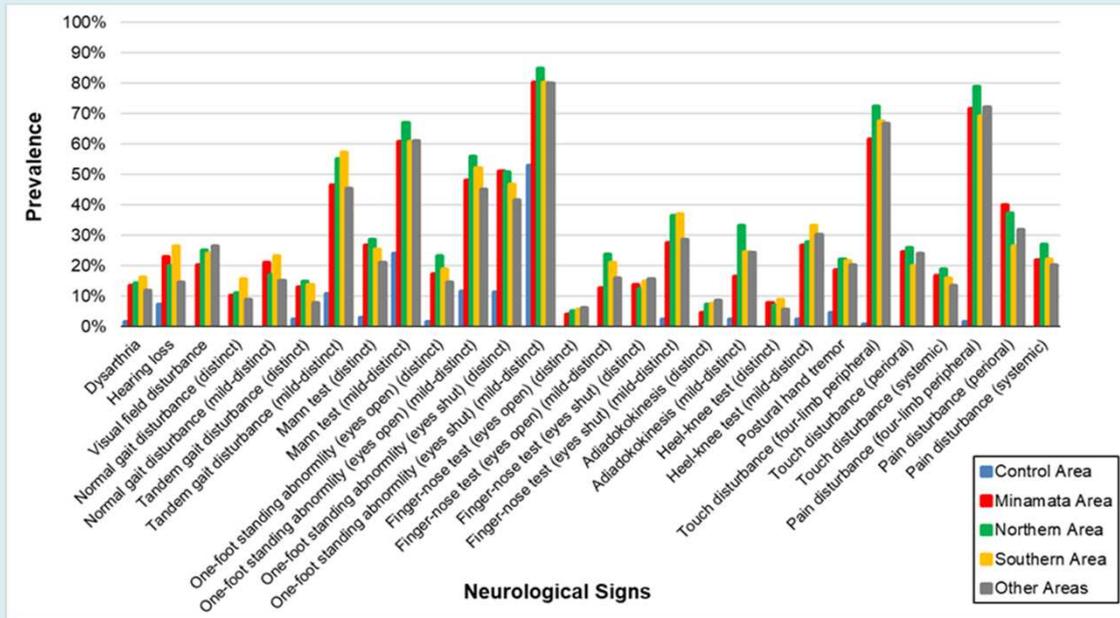
# Prevalence of symptoms (Always and Sometimes)



Takaoka *et al.*: *Toxics*, 6(3), 39, p.13, 2018

12. For the data of complaints of “Always” and “Sometimes”, the prevalence was also very similar.

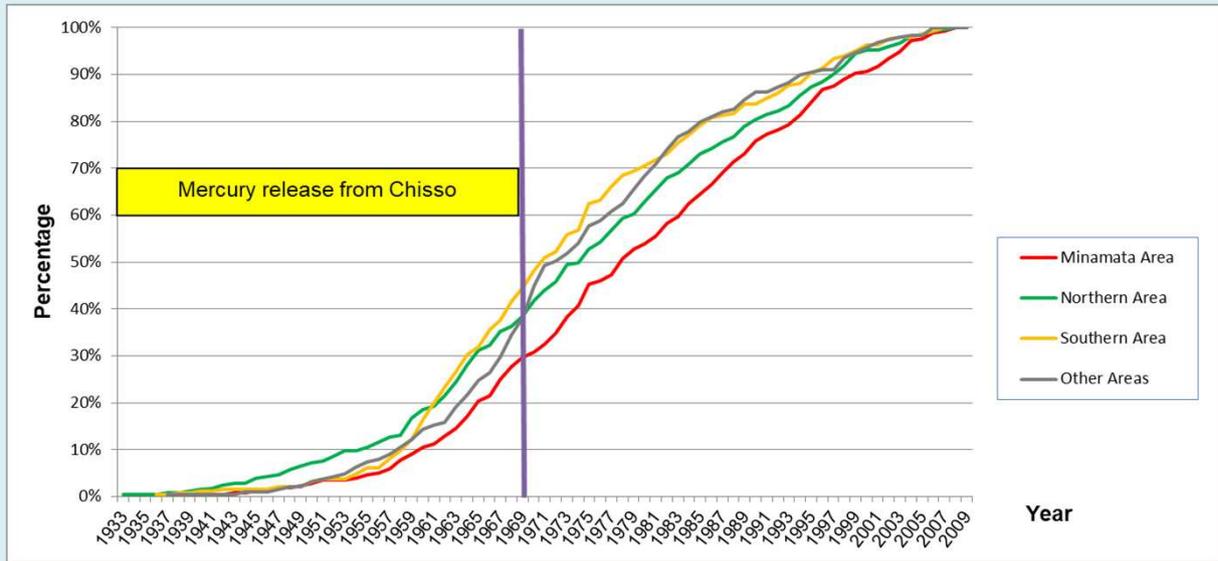
# Prevalence of neurological signs



Takaoka *et al.*: *Toxics*, 6(3), 39, p.16, 2018

13. The same thing can be said for the neurological signs detected by physicians. A government-patronized scholar has explained and emphasized that the increase in new patients was due to the possibility of receiving compensation. This statement is pure supposition and is not based on medical facts. Our research supports the fact that the increase in new patients was mainly due to the appearance of tardive effects from previous exposure to methylmercury as well as victims finally having courage to come forward for recognition despite the risk of discrimination.

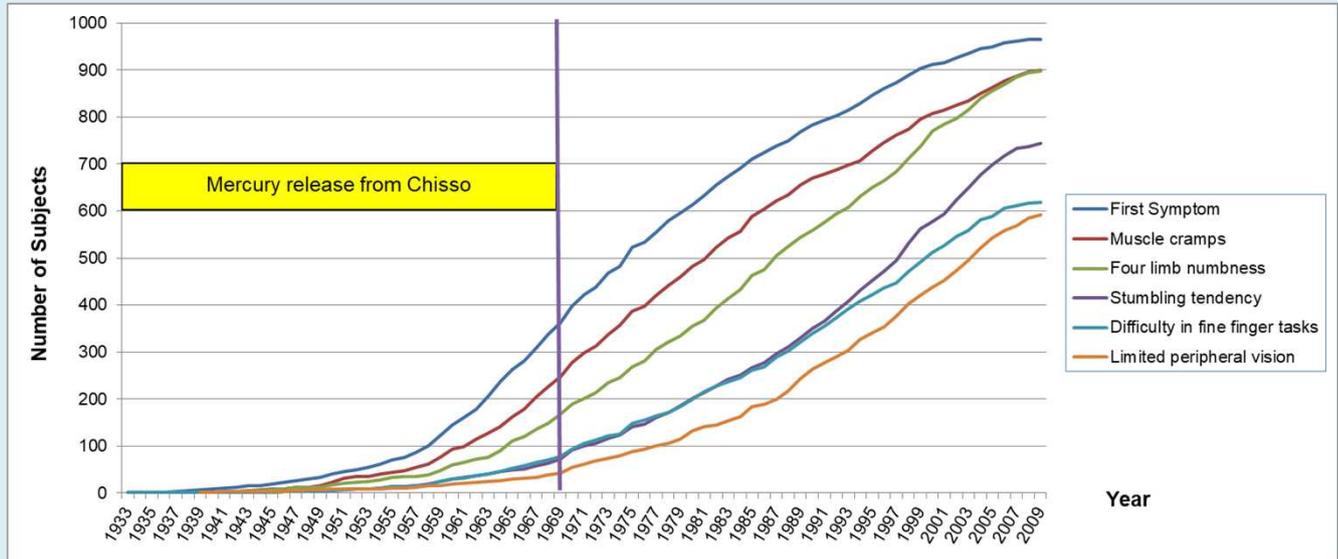
# Onset year of the first symptom in each exposed group (percentage)



Takaoka *et al.*: *Toxics*, 6(3), 39, p.21, 2018

14. This graph shows the onset year of the first symptom in each exposed group. We used a questionnaire to collect this “time of onset” data. Therefore, they might include recall bias. But the cumulative curves of the onset were almost the same among the four exposed groups and therefore, these data are meaningful. Sixty-five percent of the subjects experienced their first symptoms after 1968, when the mercury discharge was stopped. It is difficult to determine at which time, after methylmercury exposure, that a person can be judged to be safe from late developing symptoms.

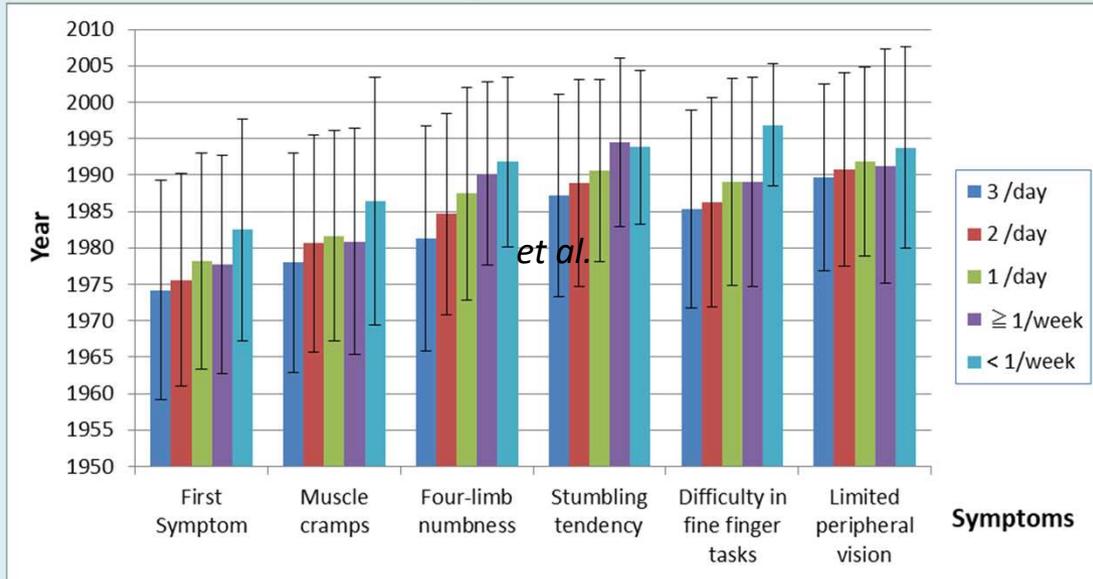
# Onset year of each neurological symptom (actual number)



Takaoka *et al.*: *Toxics*, 6(3), 39, p.21, 2018

15. After the first symptom, muscle cramps, four-limb numbness, stumbling tendency, difficulty in fine finger tasks, and limited peripheral vision occurred.

# Onset of symptoms and frequency of fish ingestion.



Takaoka *et al.*: *Toxics*, 6(3), 39, p.22, 2018

16. This chart shows the relation between onset and frequency of fish ingestion. The frequency of fish ingestion was closely related to the onset year of each symptom. The time of onset increased as the frequency of fish ingestion decreased.

# Latency after Low-Level Chronic Exposure

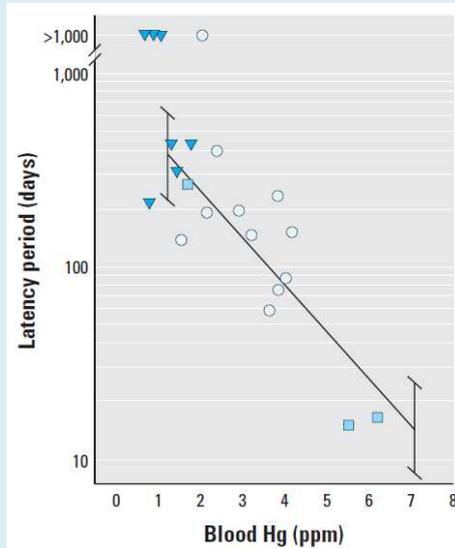
(Rice 1996, Neurotoxicology)

- Rice demonstrated that monkeys receiving a low daily dose of methylmercury for the first 7 years of life developed no signs of poisoning until 13 years of age, that is, after a latency period of 6 years.
- The adverse effects were mild, unlike the severe intoxications discussed above, and consisted mainly of impaired dexterity and clumsiness in handling items of food.

17. Experimenting on monkeys, Rice demonstrated that those having received methylmercury for 7 years developed adverse effects 6 years after the end of exposure. Also, the effects were milder than those from high doses. We feel that this might be similar to what we are seeing in our patients in Minamata.

# The length of the latency period as a function of steady-state blood levels

(Weiss *et al.*: Environmental Health Perspectives, p.853, 2002)



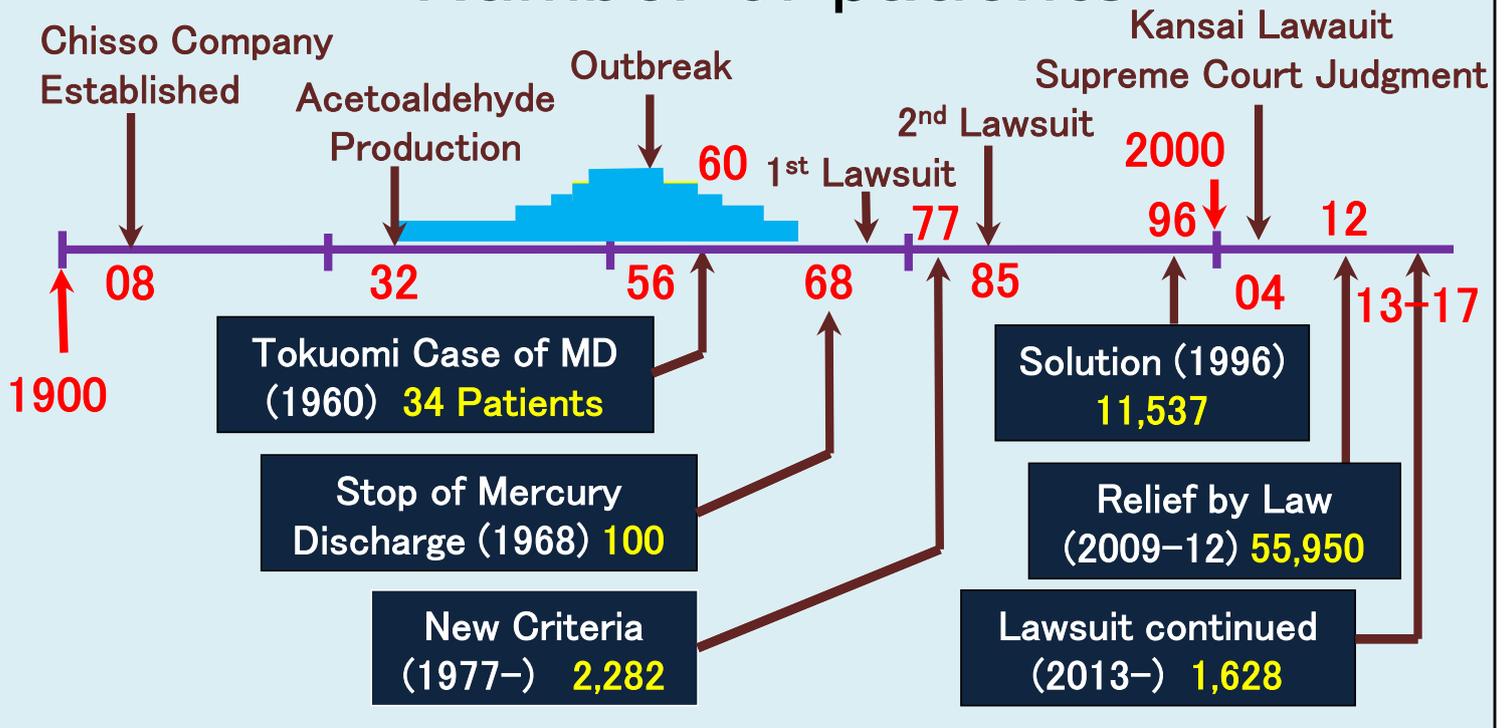
▼ indicates squirrel monkeys from Berlin et al.

○ indicates macaque monkeys from Evans et al.

□ indicates macaques from Shaw et al. as quoted by Evans et al.

18. In experiments on animals subjected to chronic exposure, the latency period became longer as the dose decreased.

# Number of patients



19. Before the Chisso Company stopped discharging contaminated water, only about 100 people had been recognized as having Minamata disease. After 1972, more than 10,000 people had been examined, but only about 2,000 patients were certified. After 2 big lawsuits, 69,000 patients were certified or relieved by 2012.

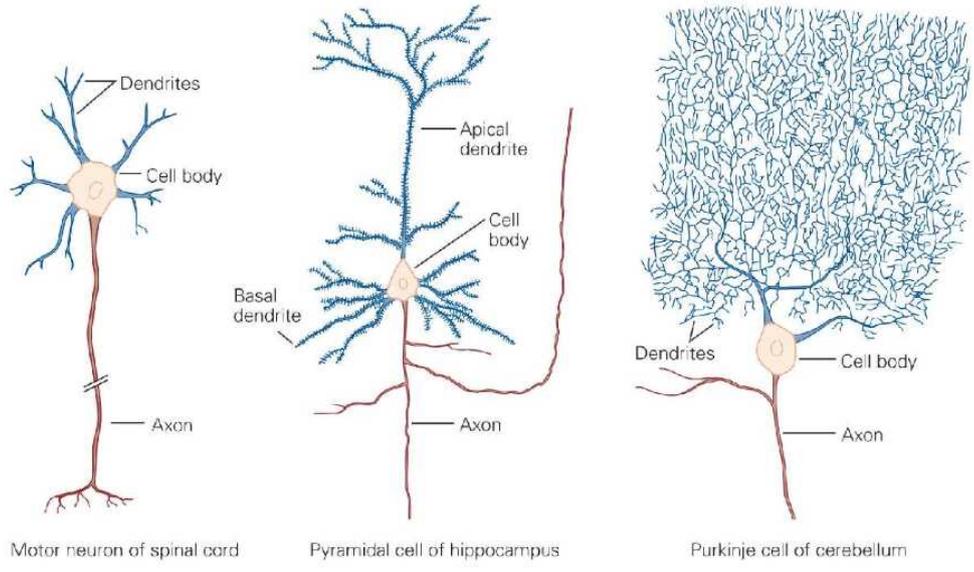
Of course, there are other reasons why new victims continue to be discovered, including the lack of surveys or research by universities and governments as well as discrimination against Minamata disease victims. But the retardation of the onset and the delayed progress of methylmercury poisoning are very important factors in the late discovery of the disease.

# Characteristics of CNS and Methylmercury Intoxication

20. Now, I'd like to talk about the characteristics of methylmercury poisoning on the central nervous system.

# Structure of neurons

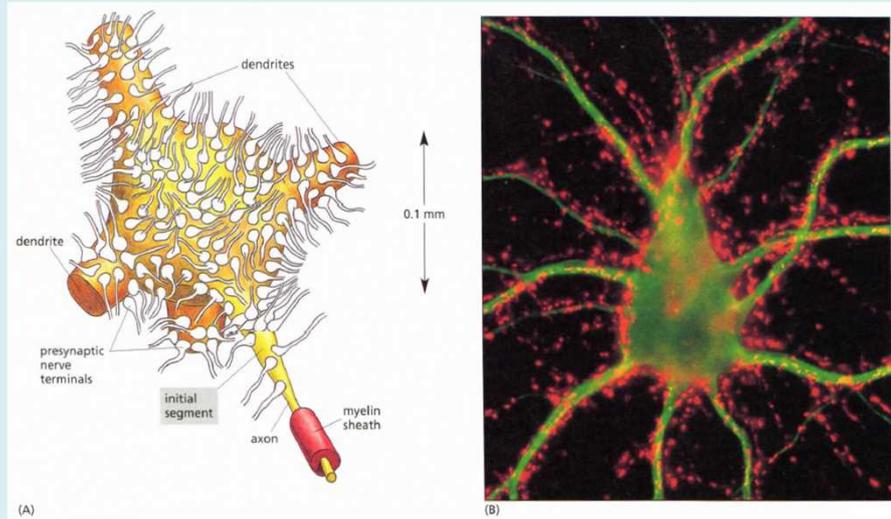
D. Three types of multipolar cells



Kandel's Principles of Neural Science 5<sup>th</sup> Ed. p.25, 2013

21. Neurons have dendrites and axons. One neuron receives input from approximately 10,000 to 1 million connecting synapses.

# Thousands of synapses are formed in spinal motor neuron cell bodies and dendrites



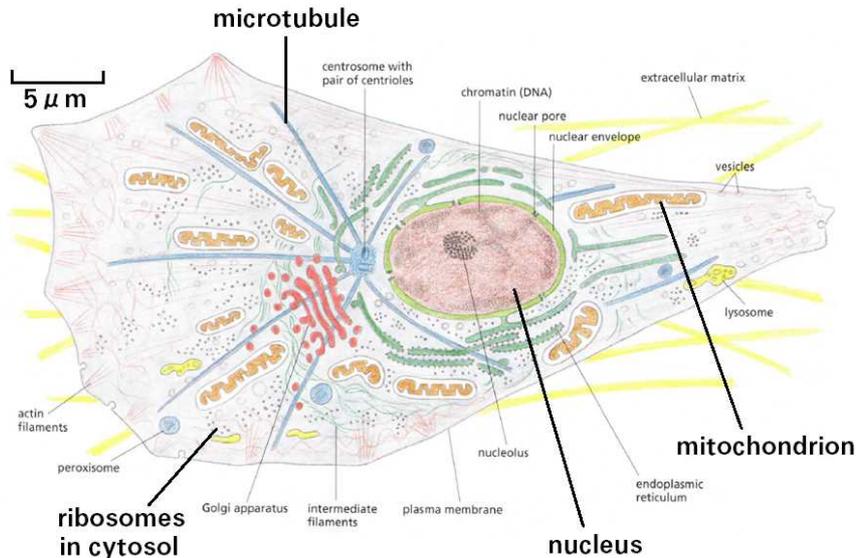
**Green:**  
cell body &  
dendrites

**red:**  
synapses

Molecular Biology of the Cell 6<sup>th</sup> Ed. p.634, 2015

22. The green ones are cell bodies and dendrites, and the red points are synapses.

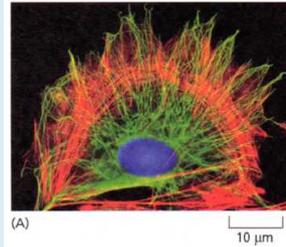
# Structure of a cell



Modified figure from  
Figure 1-25 of  
“Molecular Biology of the  
Cell 6<sup>th</sup> Ed. p.24, 2015”

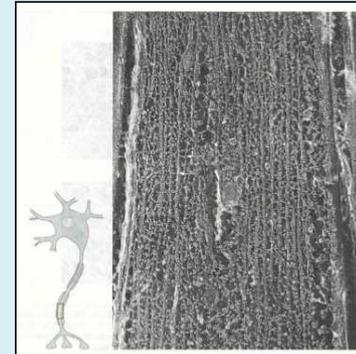
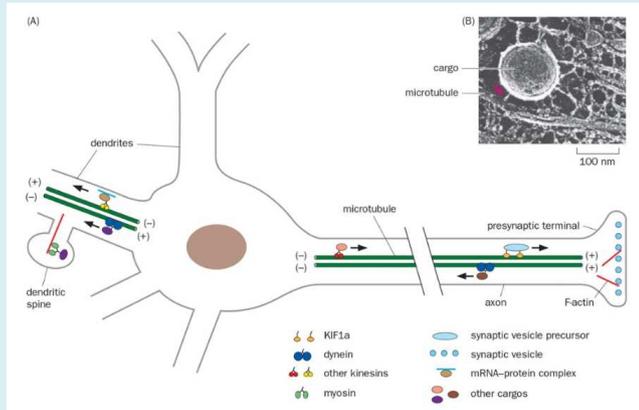
23. This is a cell. We can see the nucleus, mitochondria, and other intracellular organs. Take note of the microtubules in the cells.

# Microtubule



Cytoskeleton  
Blue: Nucleus  
Green: Microtubules  
Red: Actin filaments

Molecular Biology of The Cell,  
6<sup>th</sup> Ed., p.889, 2015



Microtubules in nerve axons

Substances are carried along the microtubules (green) in the nerve axons.

Principles of Neurobiology p.33, 2015

Kandel's Principles of Neural Science  
5<sup>th</sup> Ed. p.78 (in Japanese)

24. Microtubules support the structure of cells. In nerve cells, they play an especially important role in forming the neurites, dendrites and axons.

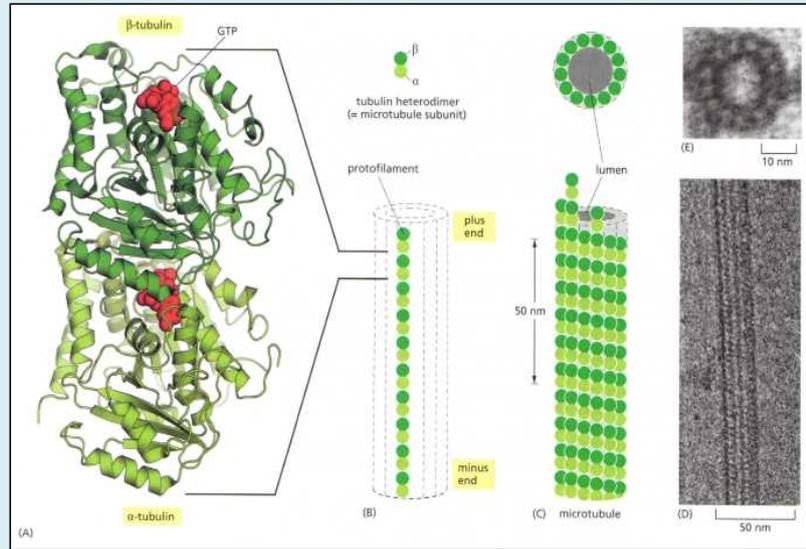
# Molecular structure of microtubules

The tubulin dimer has 15 -SH groups to which methyl mercury can bind.

(Vogel et al.: Toxicology and Applied Pharmacology, 80, 473-486, 1985)



Proteins constituting microtubules  
--- alpha tubulin and beta tubulin

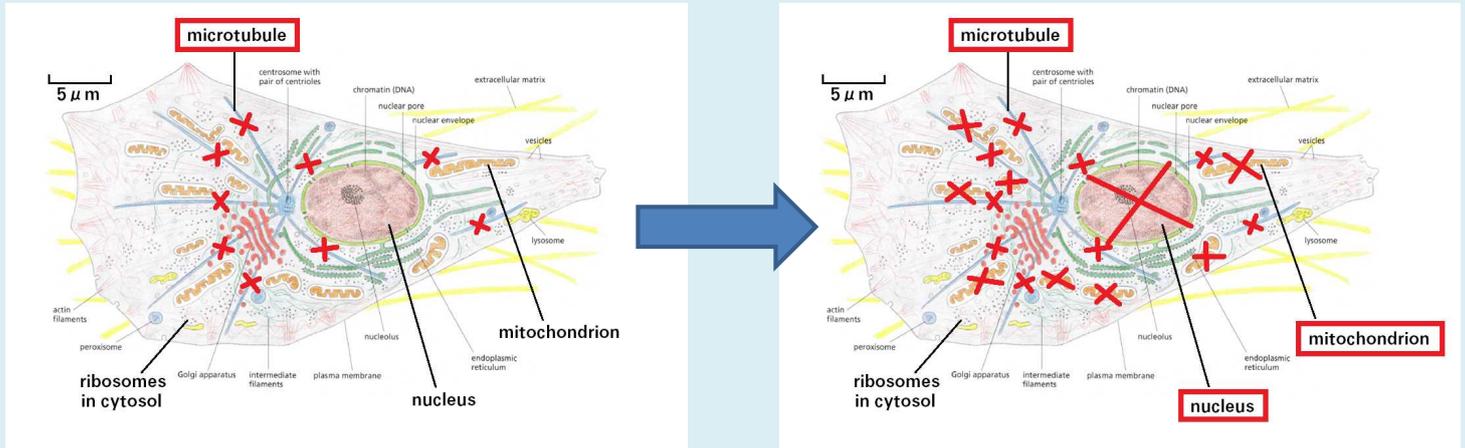


Microtubule

Molecular Biology of The Cell, 6<sup>th</sup> Ed., Chapter 16,p.926, 2015

25. A microtubule is comprised of tubulin protein. Tubulin protein has 15 thiol groups (-SH). Methylmercury is thought to bind with the thiol group and destroy the tubulin and microtubules.

# Microtubule is more susceptible to MeHg than nucleus and mitochondrion



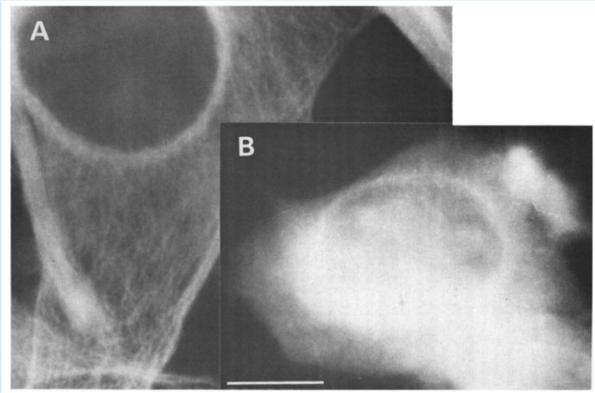
Low Concentration of MeHg

High Concentration of MeHg

Modified figures from Figure 1-25 of "Molecular Biology of the Cell 6<sup>th</sup> Ed. p.24, 2015"

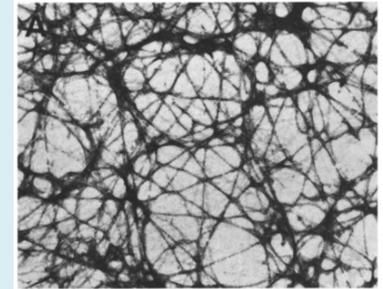
26. Microtubules are more susceptible to damage by methylmercury than the nucleus or mitochondria. It means that nerve cell functions can be decreased even if the cells are still alive.

# Effect of methylmercury on microtubules

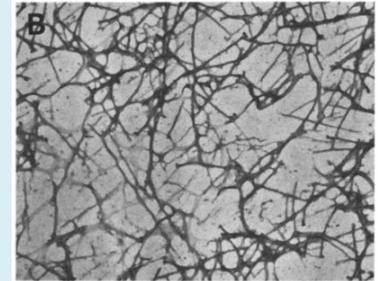


Optical microscope view

A: Control



B: After addition  
of MeHg

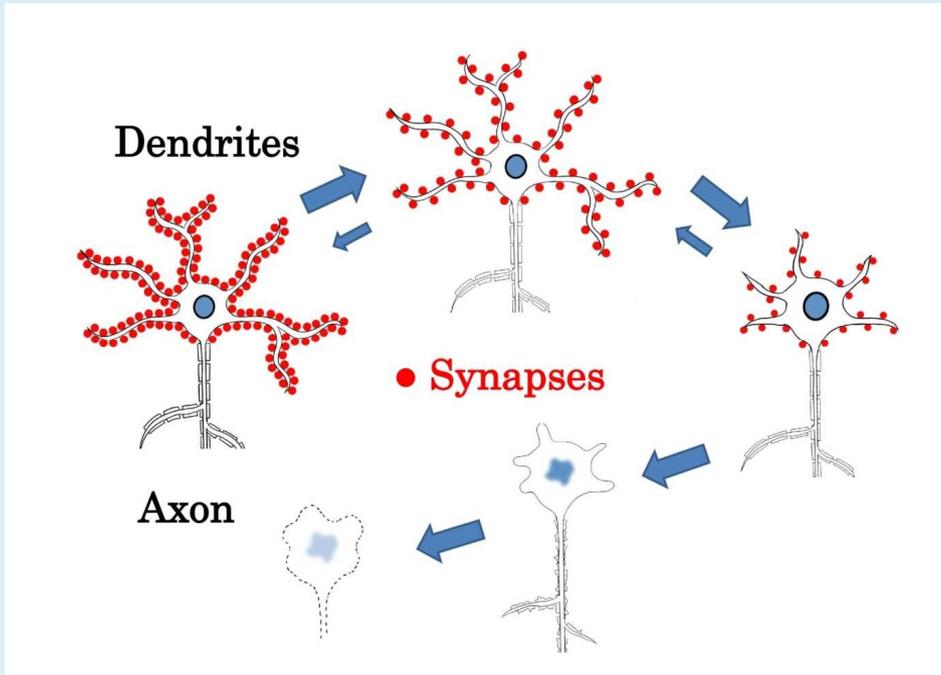


Imura N. *et al.*: Toxicology 17, 241-254, 1980

Electron microscope view

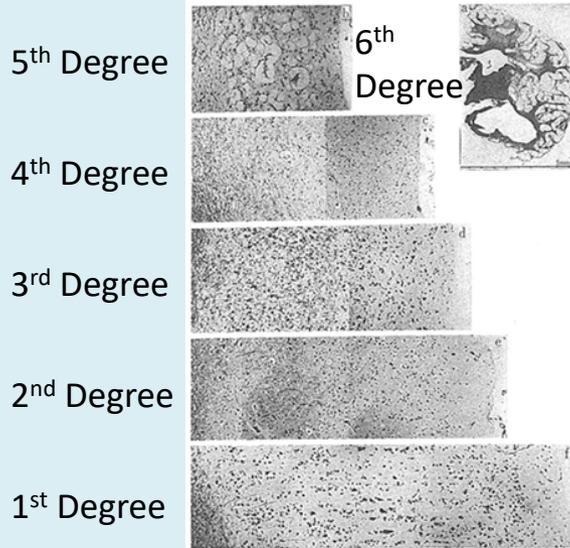
27. These are electron microscope views of microtubules. A is the control. After the introduction of methylmercury, the microtubules decrease as shown in figure B. Interestingly, when the amount of methylmercury is decreased, the microtubules recover.

# Toxic Effect of Methylmercury



28. Therefore, we have to pay attention not only to cell death but also to synaptic disorders and reversible synaptic disorders.

# Pathological classification of brain damage by methylmercury



6th Degree ... macroscopic sponge condition

5th Degree ... microscopic sponge state

4th Degree ... Crotalent

3rd Degree ... >50% dropout of nerve cells

2nd Degree ... 30-50% dropout of nerve cells

1st Degree ... <30% dropout of nerve cells

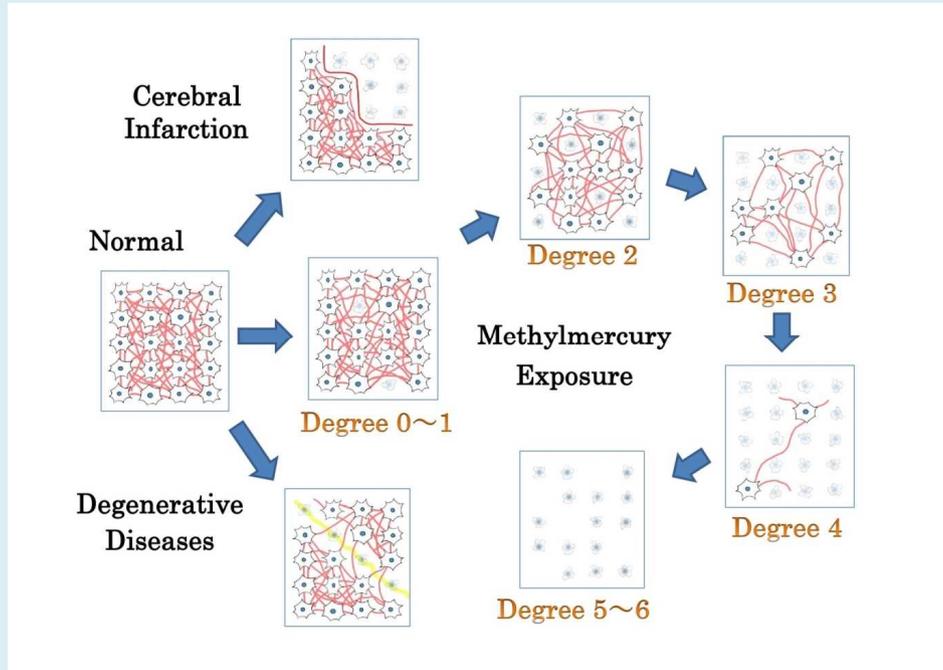
The pathology of Takeuchi and Eto is based on the following items as a basis for disability caused by methylmercury.

- ① Whether neurons are dead or not
- ② Whether or not there is a trace (growth of glia etc.)
- ③ Whether mercury remains

Takeuchi, Eto: Pathology of Minamata Disease. Shinkei Naika 9:111-125, 1978 (in Japanese)

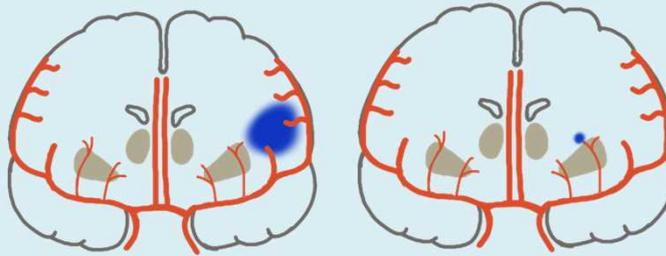
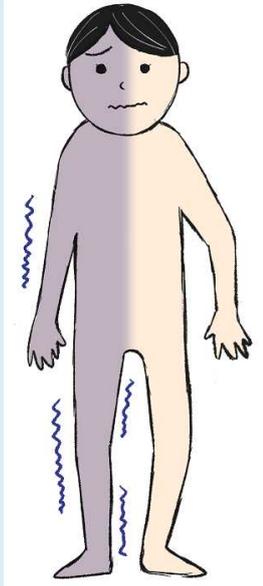
29. Takeuchi classified the microscopic pathological changes of Minamata disease. The classification was based on the degree of nerve cell disappearance. Taking into consideration the role of microtubules, we must accept the possibility of Minamata disease existing without detectable pathological changes in the mildest stages of cell death.

# Single cell necrosis (apoptosis) by MeHg

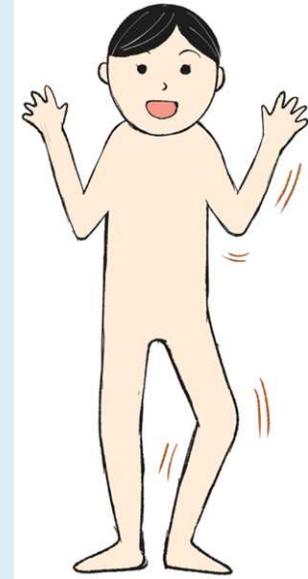


30. This is a schematic view of nerve cell disorder. In cerebral infarction, large numbers of nerve cells disappear immediately. In degenerative disease, specific groups of nerve cells disappear gradually. Differing from cerebral infarction and degenerative disorders, in milder exposure to methylmercury, cells disappear very gradually. Therefore, the signs and symptoms of methylmercury poisoning do not necessarily occur at once. They can be under the threshold for the development of symptoms. Or, symptoms can be reduced by the other surviving or normal cells.

# Brain plasticity: an example of cerebral infarction

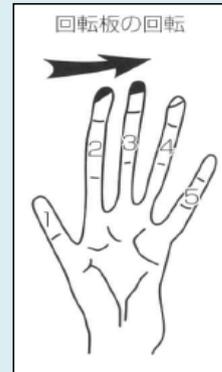
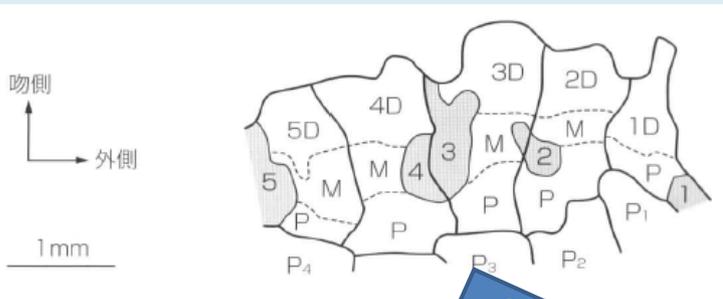


Surviving brain cells compensate  
for diminished or lost functions

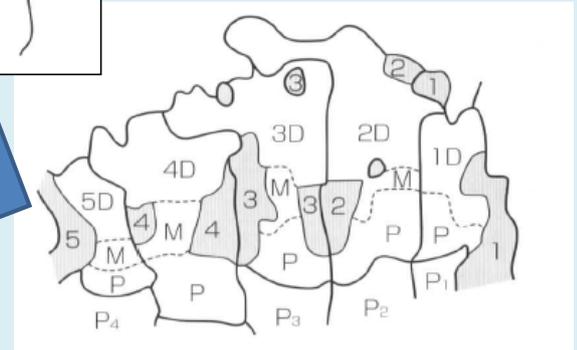


31. The brain, through its plasticity, has the ability to improve its functions. These are samples of cerebral infarction. If the infarction area is large, the hemiplegia or hemiparesis becomes permanent. But if the infarction area is small, dysfunction can recover. Sometimes they can be asymptomatic.

# Brain plasticity: changes in functional receptive area of a monkey's somatosensory cortex by tactile stimulation



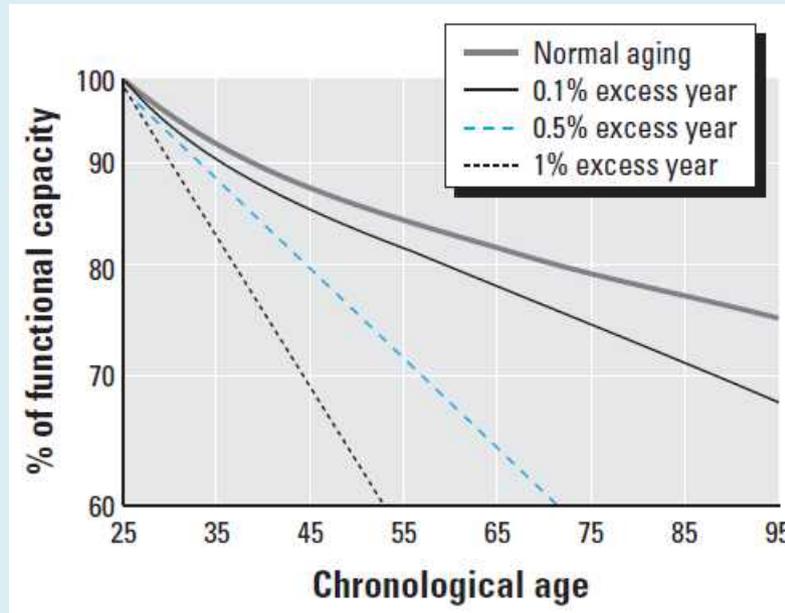
**Stimulus training**



Kaneko, F.: Reorganization of the neural network. Physiotherapy MOOK 16 Brain science and physiotherapy p.59 (in Japanese)

32. In an experiment on a monkey, tactile stimulation was found to change the functional receptive area of the monkey's somatosensory cortex.

# The loss in functional capacity of the brain from 25 years of age onward (Weiss *et al.*, EPH, 2002)



33. The decrease of brain cells or dysfunctions through aging and other factors influences the tardive onset. This is from a paper by Weiss. The uppermost curve depicts “normal” aging. The lower three curves depict the consequences of a slight increase in the rate of loss.

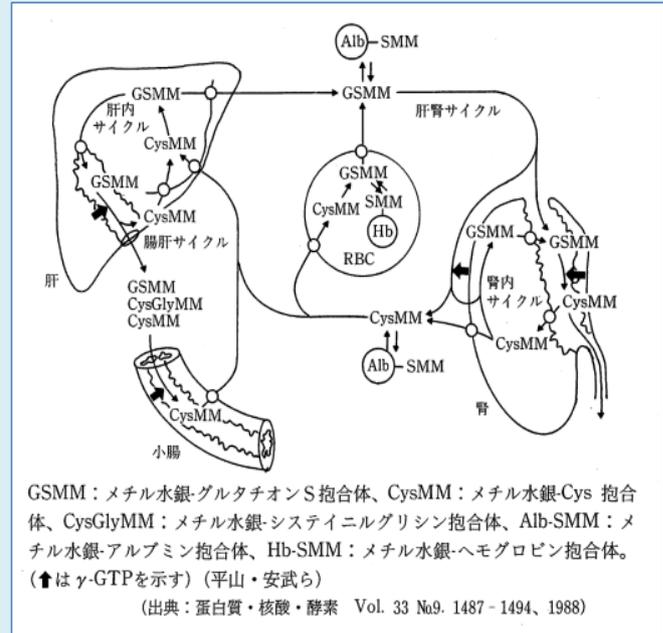
# Other Factors Related to the Tardive Onset

34. There are other factors related to the tardive onset of symptoms.

# Absorption of methylmercury

- Methylmercury contained in food is absorbed at a high rate (95 to 100%) from the gastrointestinal tract.
- Methylmercury after absorption has a high affinity for SH group and binds to protein, cysteine, glutathione and so on.
- The cysteine-methyl mercuric complex is transported to the brain across the blood-brain barrier by a neutral amino acid transport system.
- This is considered to be one of the reasons for showing a strong central nervous system toxicity.

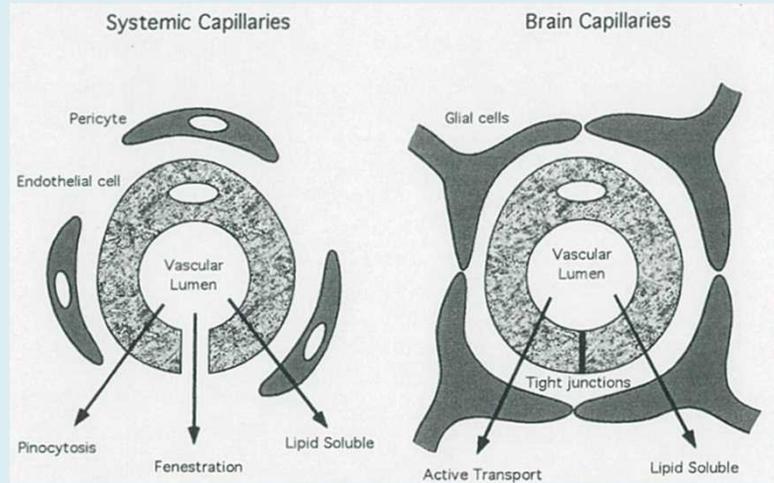
Figure (Right) is from "Sato, H. Ed.: Toxicology Today, p.89, Kinpoudou, Kyoto, 1994 (in Japanese)"



35. Methylmercury is absorbed into body cells not by simple diffusion but by active transport. Methylmercury has a high affinity for the SH thiol group and binds to protein, cysteine, glutathione and so on.

# Migration of methylmercury to the brain and blood brain barrier

- Brain has glial cells in addition to nerve cells.
- Among them, astroglia forms the blood-brain barrier and limits the movement of substances in the blood to the brain.
- However, methyl mercury actively passes through the blood brain barrier.
- Astroglia nourishes nerve cells and regulates neurotransmission.
- It is considered important for synapse development.



Casarett and Doull's TOXICOLOGY, 6<sup>th</sup> Ed. p.536, 2001

36. After monkeys have been exposed to methylmercury, its concentration in their bodies reaches a maximum level after 4 to 8 days. Methylmercury enters not only nerve cells but also glial cells, which can work as buffers.

# Factors related to delayed onset

- Migration of methylmercury to the brain takes time.
- Change of methylmercury to toxic mercury ( $\text{Hg}^{2+}$ ) in the brain tissue takes time
- Brain cell loss goes undetected as the CNS consists of a very large number of cells forming a network
- At low exposure, as microtubules may be damaged without causing cell death it is a possible cause of gradual brain dysfunction
- Functions of central nervous can be maintained by plasticity.
- The decrease in the number of brain cells due to ageing affects the tardive onset.

37. With that, I have reached the end of my presentation on how the brain's characteristics can be a reason for the tardive onset of health effects seen in cases of methylmercury poisoning.

# Tardive Onset is Characteristic of Effects on Health in Methylmercury

-- in both acute and chronic exposure --

38. I think that the tardive onset is a major characteristic of the effects of methylmercury poisoning.