

# **NIMD Forum 2016**

## **Pathomechanism of Methylmercury Toxicity**

### **~Various Approaches to the Problems~**

December 6-7, 2016

Venue: Minamata Disease Archives, Conference Hall

55-10 Myojin, Minamata City, Kumamoto 867-0055, Japan

National Institute for Minamata Disease

# NIMD Forum 2016



## Pathomechanism of Methylmercury Toxicity

### ~Various Approaches to the Problems~

#### Organizing Committee

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**[December 6]**

9:30 -9:35	Opening address	Yasushi Mochizuki (NIMD, Japan)
9:35 -9:45	Brief introduction	Masatake Fujimura (NIMD, Japan)
9:45 - 10:55	Session 1	<p><b>Chair</b>  Michael Aschner (Albert Einstein College of Medicine, US)  Akira Naganuma (Tohoku University, Japan)</p> <p><b>Speaker</b>  1-1 Schuichi Koizumi (University of Yamanashi, Japan)  Regulation by microglia of methylmercury-evoked neuronal degeneration (メチル水銀によって惹起される神経変性のミクログリアによる制御)  1-2 Matthew D. Rand (University of Rochester School of Medicine and Dentistry, US)  Myogenic targets in methylmercury neuromuscular toxicity (メチル水銀の神経筋毒性における筋原性標的)</p>
10:55 - 11:15	Coffee break	
11:15 - 12:25	Session 2	<p><b>Chair</b>  Sandra Ceccatelli (Karolinska Institutet, Sweden)  Akira Naganuma (Tohoku University, Japan)</p> <p><b>Speaker</b>  2-1 Yasukazu Takanezawa (Kitasato University, Japan)  The role of autophagy against methylmercury toxicity (メチル水銀毒性におけるオートファジーの役割)  2-2 Michael Aschner (Albert Einstein College of Medicine, US)  The Role of skn-1 in methylmercury-induced latent dopaminergic neurodegeneration (メチル水銀によって誘導される潜在的ドーパミン神経変性におけるskn-1の役割)</p>
12:25 - 13:40	Lunch	
13:40 - 15:25	Session 3	<p><b>Chair</b>  Jean-Paul Bourdineaud (Bordeaux University, France)  Toshiyuki Kaji (Tokyo University of Science, Japan)</p>

		<p><b>Speaker</b></p> <p>3-1 Fusako Usuki (NIMD, Japan) Mild endoplasmic reticulum stress preconditioning modifies intracellular mercury content through the upregulation of membrane transporters (軽度の小胞体ストレス前処置は膜輸送体の発現を増加させ細胞内水銀量を変動させる)</p> <p>3-2 Sandra Ceccatelli (Karolinska Institutet, Sweden) Developmental exposure to methylmercury induces depression-like behavior and alters neurogenesis (メチル水銀の発達期曝露はうつ病様行動と神経形成変化を誘発する)</p> <p>3-3 Masaaki Nagano (NIMD, Japan) The effects of wheat bran, fructooligosaccharide and glucomannan on tissue mercury concentration after methylmercury exposure in mice (マウスにおけるメチル水銀曝露後の組織中水銀濃度への小麦ふすま, フラクトオリゴ糖およびグルコマンナンの影響)</p>
15:25 - 15:45	Coffee break	
15:45 - 17:30	Session 4	<p><b>Chair</b></p> <p>Matthew D. Rand (University of Rochester School of Medicine and Dentistry, US) Masako Kiyono (Kitasato University, Japan)</p> <p><b>Speaker</b></p> <p>4-1 Masatake Fujimura (NIMD, Japan) Low <i>in situ</i> expression of antioxidative enzymes in brain regions susceptible to methylmercury in rodent models of Minamata disease (げっ歯類水俣病モデルにおける抗酸化酵素低発現のメチル水銀に対する部位特異的な感受性への関与)</p> <p>4-2 Jean-Paul Bourdineaud (Bordeaux University, France) Chemical forms of mercury in human hair reveal source of exposure (ヒト毛髪における水銀の化学形態は曝露源を示す)</p> <p>4-3 Tomoki Takeda (Kyushu University, Japan) Change in fetal hepatic metabolome by maternal exposure to methylmercury: a search for cellular components linking to toxicity (メチル水銀の妊娠期曝露による胎児肝メタボロームの変化: 毒性に結びつく細胞成分の検索)</p>
18:30 – 20:00	Reception	Fukuda farm (Minamata)

**[December 7]**

9:30 - 10:40	Session 5	<p><b>Chair</b>            Schuichi Koizumi (Yamanashi University, Japan)            Fusako Usuki (NIMD, Japan)</p> <p><b>Speaker</b>            5-1 Yukun Yuan (Michigan State University, US)            Is methylmercury exposure an environmental risk factor for epileptogenesis? (メチル水銀曝露は”てんかん”発作の環境危険因子に成りえるか?)            5-2 Takashi Toyama (Tohoku University, Japan)            Methylmercury enhances sensitivity for pressure-overload stress in cardiomyocytes via mitochondrial fission (メチル水銀はミトコンドリア分裂を介して心筋における圧負荷ストレス感受性を増強する)</p>
10:40 - 11:00	Coffee break	
11:00 - 12:10	Session 6	<p><b>Chair</b>            Yukun Yuan (Michigan State University, US)            Masaaki Nagano (NIMD, Japan)</p> <p><b>Speaker</b>            6-1 Sebastien Cambier (Luxembourg Institute of Science and Technology, Luxembourg)            Impact of <i>in utero</i> exposure following dietary exposure to Hg enriched diet            (食物から供給される水銀の子宮内曝露の影響)            6-2 Toshihiro Imada (Keio University, Japan)            Toxicological effects of methylmercury exposure on ophthalmic tissue (メチル水銀曝露の眼科組織への毒性影響)</p>
12:10-12:20	Brief summary	Fusako Usuki (NIMD, Japan)
12:20-12:25	Announcing NIMD Forum 2017	Koji Marumoto (NIMD, Japan)
12:25-12:30	Closing address	Mineshi Sakamoto (NIMD, Japan)

## 1-1 Regulation by microglia of methylmercury-evoked neuronal degenerations

Schuichi Koizumi, Youichi Shinozaki  
University of Yamanashi, Japan.

Recent accumulating evidence show that glial cells dynamically control a big variety of brain functions both in physiological and pathophysiological conditions. Thus, phenotypical changes in glia are important to understand brain functions, but they have received only limited attention. Here, we show that microglia, an immune cell in the CNS, sense low concentration of methylmercury (MeHg<sup>low</sup>), and become toxic for the brain. We previously showed that astrocytes, another glial cells, respond to MeHg and protect neurons against the acute MeHg-evoked neuronal injuries<sup>1,2</sup>). Microglial cells are more sensitive to MeHg and are transformed into more complex phenotypes. We used hippocampal and cortical slice cultures system, and stimulated them with 100 nM MeHg for different time periods. When exposed for shorter periods (2-3 days), microglia became a beneficial phenotype and protected neurons against MeHg neuronal damages<sup>2</sup>). However, when exposed for longer periods (7-14 days), they became rather neurotoxic phenotypes similar to M1 microglia. They increase expression of pro-inflammatory cytokines etc, thereby leading to neuronal death. We found that microglia increased Rho-kinase (ROCK) activities by MeHg<sup>low</sup>. ROCK inhibitors are reported to inhibit several neurodegenerative diseases including MeHg-evoked ones<sup>3</sup>). Interestingly, in the Parkinson's disease model animals, ROCK inhibitors reduced dopaminergic neuronal loss only when microglial cells were present, suggesting an importance of microglia as a promising target of ROCK inhibitors<sup>4</sup>). Similar to this, ROCK inhibitor acting on microglia, and then might rescue MeHg<sup>low</sup>-evoked neuronal damages. It should be noted that microglia has a key role in regulation of neuronal survival and that microglial ROCK is an essential molecule to control MeHg<sup>low</sup>-evoked neuronal damages.

Key words: microglia, neuronal death, ROCK inhibitor.

References:

- 1) Noguchi Y et al., PLoS One, 8, e57898, 2013.
- 2) Shinozaki Y et al., Sci. Rep., 10, 4, 4329, 2014.
- 3) Fujimura M et al., Tox. App Pharmacol, 250, 1-9, 2011.
- 4) Borrajo A et al., Neuropharmacology, 85, 1-8, 2014.

[内容紹介]

演者の小泉先生は、「山梨大学」にて研究を行っておられます。今回の発表は、「メチル水銀によって惹起される神経変性のミクログリアによる制御」についてです。実験動物であるマウス脳切片を用いた研究において、短期間のメチル水銀曝露で誘導されるミクログリアは神経防御に作用し、長期間曝露で誘導されるミクログリアは神経毒性を示すことが明らかになりました。さらに、メチル水銀曝露はミクログリアの **ROCK** 活性を増加させ、**ROCK** 阻害剤が神経毒性を抑制することが明らかになりました。以上の結果から、メチル水銀によって誘導されるミクログリア由来の **ROCK** 活性が、メチル水銀による神経毒性へ関与している可能性が示唆されました。

**Microglia:** ミクログリア

**Neuronal death:** 神経細胞死

**ROCK inhibitor:** ロック阻害剤

[ノート]

## 1-2 Myogenic targets in methylmercury neuromuscular toxicity

Matthew D. Rand and Lisa Prince

University of Rochester School of Medicine and Dentistry, USA

Methylmercury (MeHg) is a ubiquitous environmental contaminant that has long been acknowledged as a potent neurotoxicant. Historic high-level MeHg poisonings have demonstrated that the fetal nervous system is preferentially targeted, as was seen with persistent cognitive and motor deficits in children exposed prenatally. The risks of chronic low levels of MeHg exposure commonly seen in fish eating populations today are less well understood. Furthermore, MeHg targeting of organs other than the nervous system has not been well characterized. To more fully elucidate MeHg toxicity pathways we have implemented whole genome approaches to identify genes associated with MeHg effects on development using the *Drosophila* model. We recently performed a genome wide association (GWA) analysis to search for genes that associate with MeHg tolerance or susceptibility in developing flies. Of the numerous candidate genes identified, several fall into gene ontology categories related to muscle and neuromuscular development. We also discovered a strong phenotype of disrupted myofiber formation in developing indirect flight muscles in flies in response to MeHg. The latter occurs despite normal morphogenesis of several other tissues including neural structures such as the eyes and bristle organs. Transgenic expression of the glutamate-cysteine ligase catalytic subunit (GCLc), a MeHg protective enzyme, in a muscle lineage-specific manner leads to a robust rescue of development of flies reared on MeHg food. We extended these findings to a mammalian system using cultured mouse C2C12 myoblasts undergoing differentiation. Chronic exposure to low levels of MeHg (0.1-0.5 $\mu$ M, 4 days) results in significant inhibition of myotube formation with a corresponding increase in Pax7 and decrease in Myogenin expression, consistent with an overall inhibition of myoblast differentiation. Importantly, we observed that acute exposure to MeHg (1.5-3.0 $\mu$ M, 24 hours) results in a persistent inhibition of myoblast differentiation and myotube formation upon withdrawal and clearance of the toxicant. In summary, we show that myogenic pathways are targeted by MeHg during development thereby implicating muscle as an additional and previously unrecognized target for MeHg toxicity.

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#### [内容紹介]

演者のランド先生は、「米国・ロチェスター大学」にて研究を行っておられます。今回の発表は、「メチル水銀の神経筋毒性における筋原性標的」についてです。ショウジョウバエを用いた研究において、メチル水銀の発達期曝露が筋線維形成を抑制することが明らかになりました。また、実験動物であるマウスの筋細胞を用いた研究において、メチル水銀が Pax7 という因子を増やし、myogenin の発現を減少させることによって筋分化、筋管形成を抑制することが明らかになりました。以上の結果から、筋肉はメチル水銀の標的組織で、メチル水銀の発達期曝露が筋形成に影響する可能性が示唆されました。

Glutamate-cysteine ligase catalytic subunit (GCLc): グルタチオン合成の律速反応を触媒する酵素

Pax7: 筋形成にかかわる転写因子

Myogenin: 筋分化調節遺伝子ファミリーの1つ

Myotube: 筋管

#### [ノート]

## 2-1 The role of autophagy against methylmercury toxicity

Yasukazu Takanezawa, Ryosuke Nakamura, Yuka Sone, Shimpei Uraguchi, Masako Kiyono  
Kitasato University, Japan.

Methylmercury (MeHg) is a widespread environmental pollutant and causes a serious hazard to health worldwide. However, molecular mechanisms underlying MeHg toxicity remain elusive. Macroautophagy (hereafter referred to as autophagy), a major and highly conserved degradation pathway in eukaryotes, prevents the accumulation of misfolded or damaged protein aggregates and damaged organelles in the cytoplasm. Here we focus on the role of autophagy against MeHg exposure. MeHg treatment increased levels of autophagy marker LC3-II and SQSTM1/p62, possibly by acting on the MAPKs signaling pathway. MeHg exposure elevated the number of LC3 puncta in stable GFP-LC3 MEFs and the number of autophagic vacuoles. The accumulation of LC3-II and p62 was much increased by co-treatment of MeHg with autophagy inhibitors, chloroquine and bafilomycin A<sub>1</sub>. This additive effect of MeHg on LC3-II accumulation in the presence of both inhibitors suggests that MeHg stress activates autophagy and promotes autophagosome/LC3-II accumulation. To establish the impact of autophagy in alleviating MeHg toxicity, we assessed MeHg sensitivity in autophagy-related gene 5 deficient (Atg5 KO) mouse embryonic fibroblasts (MEFs). Atg5 KO significantly decreased viability of the cell following 24 h of MeHg treatment. The enhanced MeHg-induced cytotoxicity in Atg5-deficient cells suggests that autophagy protects cells from MeHg-induced cell death. This finding could suggest that autophagy induced by MeHg serves as a protective mechanism to eliminate intracellular MeHg-induced damaged materials. Our data implicate potential of autophagy as a target to establish therapeutic strategies for the treatment of MeHg intoxication.

Key words: autophagy, LC3, Atg5, p62.

References:

- 1) Takanezawa Y et al., *Toxicol. Lett.*, 16, 135-141, 2016.

#### [内容紹介]

演者の高根沢先生は、「北里大学」において研究を行っておられます。今回の発表は、「メチル水銀毒性におけるオートファジーの役割」についてです。実験動物であるマウス胎児線維芽細胞を用いた研究において、メチル水銀はオートファジーを引き起こしますが、オートファジーに関与するタンパク質の 1 種 (Atg5) を欠損させると細胞傷害が増強することが明らかになりました。以上の結果から、メチル水銀によって生じるオートファジーは、メチル水銀毒性を防御する生体防御機能である可能性が示唆されました。

**Autophagy:** 自食作用

**LC3:** microtubule-associated- protein-light-chain-3

(オートファゴソームのマーカー蛋白質. オートファジーの最初のステップでは、アミノ酸飢餓のようなストレスシグナルを受け、オートファゴソームを形成する)

**Atg5:** オートファジーに関与するタンパク質の 1 種

**p62:** オートファジーの障害によって細胞内に集積するタンパク質の 1 種

#### [ノート]

## **2-2 The Role of skn-1 in methylmercury-induced latent dopaminergic neurodegeneration**

Michael Aschner

Albert Einstein College of Medicine, US

Mercury (Hg) is a persistent environmental bioaccumulative metal, with developmental exposure to methylmercury (MeHg) resulting in long-term health effects. We examined the impact of early-life exposure to MeHg and knockdown of skn-1 on dopaminergic (DAergic) neurodegeneration in the nematode *Caenorhabditis elegans*. SKN-1, a the major stress-activated cytoprotective transcription factors, promotes the transcription of enzymes that scavenge free radicals, synthesizes glutathione and catalyzes reactions that increase xenobiotic excretion. Deletions or mutations in this gene suppress stress resistance. Thus, we hypothesized that the extent of MeHg's toxicity is dependent on intact skn-1 response; therefore skn-1 knockout (KO) worms would show heightened sensitivity to MeHg-induced toxicity compared to wildtype worms. In this study we identified the impact of early-life MeHg exposure on Hg content, stress reactivity and DAergic neurodegeneration in wildtype, and skn-1KO *C. elegans*. Hg content, measured by Inductively Coupled Plasma Mass Spectrometry, showed no strain-dependent differences. Reactive oxygen species generation was dramatically increased in skn-1KO compared to wildtype worms. Structural integrity of DAergic neurons was microscopically assessed by visualization of fluorescently-labeled neurons, and revealed loss of neurons in skn-1KO and MeHg exposed worms compared to wildtype controls. Dopamine levels detected by High-performance liquid chromatography, were decreased in response to MeHg exposure and decreased in skn-1KO worms, and functional behavioral assays showed similar findings. Combined, these studies suggest that knockdown of skn-1 in the nematode increases DAergic sensitivity to MeHg exposure following a period of latency.

Key words: dopamine, neurodegeneration, skn1, worms.

References:

1) Martinez-Finley et al., *Neurochem. Res.*, 38, 2650-60, 2013.

[内容紹介]

演者のアシュナー先生は、「米国・アルバートアインシュタイン大学」にて研究を行っておられます。今回の発表は、「メチル水銀によって誘導される潜在的ドーパミン神経変性における skn-1 の役割」についてです。線虫を用いた研究において、神経変性の防御因子である skn-1 がメチル水銀によるドーパミン神経毒性の防御に重要な役割を果たしていることが明らかになりました。以上の結果から、skn-1 はメチル水銀の重要な標的分子である可能性が示唆されました。

Dopamine: ドーパミン（神経伝達物質の1種）

Neurodegeneration: 神経変性

Skn1: Nrf2 の線虫ホモログ（転写因子の1種）

Worms: 線虫

[ノート]

### **3-1 Mild endoplasmic reticulum stress preconditioning modifies intracellular mercury content through the upregulation of membrane transporters**

Fusako Usuki

National Institute for Minamata Disease, Japan

Mild endoplasmic reticulum (ER) stress preconditioning protects cells against methylmercury (MeHg) cytotoxicity by inducing integrated stress responses.<sup>1)</sup> Here, we showed that mild ER stress preconditioning modifies intracellular mercury content. We examined the expression of genes encoding membrane transporters that affect the cellular influx of MeHg; genes encoding methionine transporters, including L-type amino acid transporter 1 (*LAT1*), *LAT3*, and sodium-coupled neutral amino acid transporter 2 (*SNAT2*); and the gene encoding a membrane transporter that affects the efflux of MeHg, an ATP-binding cassette transporter cassette C subfamily 4 (*ABCC4*), in MeHg-susceptible myogenic cells. The cells were preconditioned with an ER Ca<sup>2+</sup>-ATPase inhibitor thapsigargin for 16 h before MeHg exposure. Results of quantitative PCR (qPCR) showed that mild ER stress upregulated the mRNA expression of the abovementioned genes and that the upregulation of *ABCC4* was much higher than that of other genes. Results of western blotting showed that mild ER stress upregulated *LAT1*, *SNAT2*, and *ABCC4*. Intracellular mercury content corresponded to changes in the expression of these membrane transporters. Analysis of the role of integrated stress responses, phosphorylation of eukaryotic initiation factor 2 alpha, accumulation of activating transcription factor 4 (ATF4), and suppression of nonsense-mediated mRNA decay (NMD) on the expression of the abovementioned genes demonstrated that NMD suppression upregulated the mRNA expression of these genes and that ATF4 accumulation upregulated the mRNA translation of *LAT1*, *SNAT2*, and *ABCC4*. These results suggest that intracellular mercury content can be modulated through the upregulation of genes encoding membrane transporters by promoting ATF4 accumulation and NMD suppression.

Key words: preconditioning, endoplasmic reticulum stress, methionine transporter, *ABCC4*, activating transcription factor 4, nonsense-mediated mRNA decay.

Reference:

1) Usuki F et al., *Sci. Rep.*, 3, 2346, 2013.

## [内容紹介]

演者の白杵は、「国立水俣病総合研究センター」にて研究を行っています。今回の発表は、「軽度の小胞体ストレス前処置は膜輸送体の発現を増加させ細胞内水銀量を変動させる」についてです。メチル水銀に感受性の高い筋細胞を用いた研究において、軽度の小胞体ストレス前処置（プレコンディショニング）が膜輸送体の発現増強を介して細胞内水銀量を低下させることが明らかになりました。さらに、この原因は、活性化転写因子 4 の集積亢進 mRNA 監視機構の抑制によると考えられました。以上の結果から、メチル水銀毒性に影響する細胞内水銀濃度が、軽度の小胞体ストレス前処置によって変動する可能性が示唆されました。

Preconditioning: 前処置

Endoplasmic reticulum stress: 小胞体ストレス

Methionine transporter: メチオニン輸送体

ABCC4: ATP-binding cassette transporter cassette C subfamily 4

(化学物質を細胞から排出する輸送体の 1 種)

ATF4: Activating transcription factor 4, 活性化転写因子 4

NMD: Nonsense-mediated mRNA decay, mRNA 監視機構

## [ノート]

### **3-2 Developmental exposure to methylmercury induces depression-like behavior and alters neurogenesis**

Sandra Ceccatelli

Karolinska Institutet, Stockholm, Sweden

We have studied the effects of developmental exposure to methylmercury (MeHg) using *in vivo* and *in vitro* experimental models. Pregnant mice were exposed to 0.5 mg MeHg/kg/day via drinking water from gestational day 7 until day 7 after delivery. The analysis of behavior showed depression-like behavior in the MeHg-exposed male offspring. Moreover, we detected long-lasting epigenetic changes of the brain-derived neurotrophic factor (BDNF) gene in the hippocampus of exposed mice. BDNF plays a critical role in promoting neurogenesis. Decreased hippocampal neurogenesis has been linked to the pathogenesis of depression in both experimental and clinical studies. We therefore evaluated cell proliferation in the subgranular zone of the hippocampal dentate gyrus (DG) and observed a decreased number of Ki-67–positive cells, as well as a decreased number of neurons in MeHg-exposed males. To further investigate the effects of MeHg on neurogenesis, we used primary cultures of neural stem cells (NSC) and exposed them to MeHg at nanomolar concentrations (2.5 or 5.0 nM). MeHg inhibited neuronal differentiation, decreased proliferation, and altered the expression of cell cycle regulators, such as p16 and p21, and senescence-associated markers. In addition, there was a decrease in global DNA methylation in the MeHg-exposed cells, supporting the idea that epigenetic changes play a role in the alterations of neurogenesis induced by MeHg. Interestingly, all changes observed in NSC directly exposed to MeHg (parent cells) also occurred in their daughter cells cultured under MeHg-free conditions. Our studies provide evidence for programming effects induced by MeHg in NSC and suggest that developmental exposure to low levels of MeHg may predispose to neurodevelopmental disorders.

Key words: depression, neural stem cells, epigenetic, developmental neurotoxicity.

References:

- 1) Bose R et al, *Toxicol. Sci.*, 130, 383-90, 2012.
- 2) Ceccatelli S et al, *J. Intern. Med.*, 273, 490-7, 2013.



#### [内容紹介]

演者のチェカテリ先生は、「スウェーデン・カロリンスカ研究所」において研究を行っておられます。今回の発表は、「メチル水銀の発達期曝露はうつ病様行動と神経形成変化を誘発する」についてです。実験動物であるマウスを用いた研究において、メチル水銀は海馬形成不全を伴ううつ病様症状を引き起こすことが明らかになりました。また、神経幹細胞を用いた研究において、メチル水銀が神経分化および神経増殖を抑制することが明らかになりました。以上の結果から、メチル水銀の胎児期曝露が神経発達疾患に関与する可能性が示唆されました。

Depression: うつ病

Neural stem cells: 神経幹細胞

Epigenetic: エピジェネティック (DNA の塩基配列の変化なしに遺伝子発現に影響する)

Developmental neurotoxicity: 発育神経毒性

#### [ノート]

### **3-3 The effects of wheat bran, fructooligosaccharide and glucomannan on tissue concentration after methylmercury exposure in mice**

Masaaki Nagano<sup>1</sup>, Masatake Fujimura<sup>1</sup> and Kazuho Inaba<sup>2</sup>

<sup>1</sup> National Institute for Minamata Disease, Japan, <sup>2</sup> Azabu University, Japan

Methylmercury (MeHg) is a neurotoxicant that exists widely in the natural environment. Although it has been revealed that the diet containing 30% wheat bran decreased the total mercury (Hg) concentration in the brain and blood of MeHg-treated mice<sup>1)</sup>, the mechanism remains to be unclear. Intestinal flora plays an important role in the decomposition and fecal excretion of MeHg. It has been reported that bacteroides, bifidobacteria, *Escherichia coli* and lactobacilli have the high metabolic activity of MeHg among strains isolated from the human and rat intestinal tracts<sup>2)</sup>. In this study, we investigated the effect of wheat bran on Hg excretion from the tissue of MeHg-treated mice. We also examined the effect of fructooligosaccharide (FOS) and glucomannan (GM) on tissue concentration after MeHg exposure in mice because FOS and GM are prebiotics. Female BALB/c mice were housed in the metabolic cages, and then urine and feces were collected for 4 weeks after a single administration of MeHgCl (5 mg MeHgCl/kg). At the end of the experiment, mice fed wheat bran diet showed lower total Hg levels in blood, brain and kidney than animals fed the basal diet. Interestingly, the cumulative Hg excretion in urine was increased markedly by wheat bran diet-fed mice, whereas fecal Hg excretion increased slightly. These results demonstrate that wheat bran accelerates urinary Hg excretion and subsequently decreases tissue Hg levels in mice. Next, mice fed 5% FOS diet decreased total Hg levels in brain and liver as compare with animals fed the basal diet, whereas 2.5% GM diet showed no effect. Fecal Hg excretion in FOS-fed mice was markedly higher than in basal diet-fed mice, whereas no difference was observed in urinary Hg excretion. These results demonstrate that FOS accelerates fecal Hg excretion and subsequently decreases tissue Hg levels in mice. The findings suggest that wheat bran and FOS may reduce the levels of Hg in the brain after MeHg exposure and might therefore modify the toxicity of MeHg.

Key words: wheat bran, fructooligosaccharide, excretion, gut microflora.

References:

- 1) Rowland IR et al., Arch. Toxicol., 59, 94-98, 1986.
- 2) Rowland IR et al., Xenobiotica, 8, 37-43, 1978.

[内容紹介]

演者の永野は、「国立水俣病総合研究センター」にて研究を行っています。今回の発表は、「マウスにおけるメチル水銀曝露後の組織中水銀濃度への小麦ふすま、フラクトオリゴ糖およびグルコマンナンの影響」についてです。実験動物であるマウスを用いた研究において、小麦ふすまが水銀の尿中排泄、フラクトオリゴ糖が水銀の糞中排泄を促進することが明らかになりました。今回の結果から、小麦ふすまおよびフラクトオリゴ糖の摂取は、体内からの水銀排出に有効であり、メチル水銀毒性の軽減にも有効である可能性が示唆されました。

**Wheat bran:** 小麦ふすま

**Fructooligosaccharide:** フラクトオリゴ糖

**Excretion:** 排出

**Gut microflora:** 腸内細菌叢

[ノート]

## **4-1 Low *in situ* expression of antioxidative enzymes in brain regions susceptible to methylmercury in rodent models of Minamata disease**

Masatake Fujimura, Fusako Usuki  
National Institute for Minamata Disease, Japan.

Methylmercury (MeHg), an environmental toxicant, induces site-specific neurotoxicity in the brain affecting in particular the cerebellar granule cells (CGCs) and the deep layer of cerebrocortical neurons (dl-CCNs) in adult case of Minamata Disease. However, this site-specific neurotoxicity is not completely understood. In this study, we investigated the molecular mechanism of site-specific neurotoxicity using experimental animal models of the disease. MeHg exposure selectively induced neuronal degeneration in the CGCs of rats and the dl-CCNs of mice, in analogy with what observed in adult patients with Minamata Disease. It has been reported that anti-oxidative compounds prevents MeHg-induced neurotoxicity in experimental animal models, suggesting that MeHg causes neuronal toxicity through oxidative stress. Neuronal cells express a number of anti-oxidative enzymes including copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD), glutathione peroxidase 1 (GPx1), thioredoxin reductase 1 (TrxR1) and catalase. We isolated rat brain regions including CGCs, Purkinje cells, and molecular layer neurons, and mice brain regions including dl and shallow layer (sl) of CCNs and hippocampal neurons using a microdissection system, in order to perform real-time PCR analyses of mRNAs of antioxidative enzymes. We observed that the expression levels of Mn-SOD, GPx1, and TrxR1 were significantly higher in Purkinje cells and molecular layer neurons than in CGCs in rats, and that the expression levels of Cu/Zn-SOD, Mn-SOD and GPx1 were significantly higher in sl-CCNs and hippocampal neurons than in dl-CCNs in mice. Furthermore, immunohistochemistry analysis showed increased expression of Mn-SOD, GPx1, and TrxR1 proteins in Purkinje cells and molecular layer neurones in rats, and increased expression of Mn-SOD and GPx1 proteins in sl-CCNs in mice. These findings suggest that the basal expression levels of antioxidative enzymes contribute to the MeHg vulnerability observed in CGCs and dl-CCNs in adult cases of Minamata Disease.

Key words: site-specific neurotoxicity, antioxidative enzymes, rodent models.

References:

- 1) Fujimura M et al., Arch. Toxicol., 88, 109-113, 2014.

[内容紹介]

演者の藤村は、「国立水俣病総合研究センター」にて研究を行っています。今回の発表は、「げっ歯類水俣病モデルにおける抗酸化酵素低発現のメチル水銀に対する部位特異的な感受性への関与」についてです。実験動物であるラットおよびマウスを用いた研究において、ヒト水俣病におけるメチル水銀に対して脆弱な部位に一致して抗酸化酵素の発現が低いことが明らかになりました。今回の結果から、メチル水銀の選択的神経細胞傷害の原因が、抗酸化酵素の分布の違いによるものである可能性が示唆されました。

Site-specific neurotoxicity: 部位特異的神経毒性

Antioxidative enzymes: 抗酸化酵素

Rodent models: げっ歯類モデル

[ノート]

## 4-2 Chemical forms of mercury in human hair reveal source of exposure

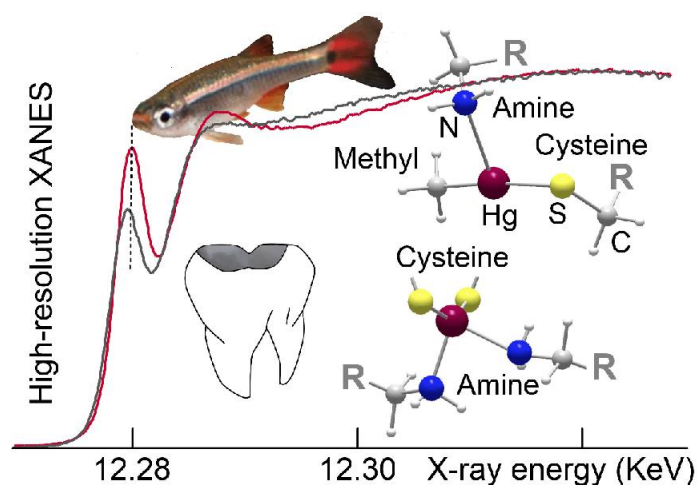
Jean-Paul Bourdineaud  
University of Bordeaux

Humans are contaminated by mercury from different sources through different routes of exposure. Hair is considered, in practice, to contain mainly the methylated organic form linked to fish consumption, whereas blood and urine are the best matrices for quantifying elemental and divalent inorganic mercury acquired through atmospheric contact or from dental amalgams. It will be shown that the chemical structure of mercury in hair depends on the source and can be determined using a high energy-resolution X-ray spectrometer sensitive to C, N, and S mercury ligands. It was even possible to detect contamination from a single amalgam removal by spectroscopic analysis of the mercury spike located by X-ray nanofluorescence on one hair strand and dated to the removal event. The sensitivity of the spectrometer could be harnessed for unravelling chemical structures of metals in various biological and abiotic media pertinent to medical diagnosis, toxicology, and archaeological to modern forensic science.

Keywords : human hair, methylmercury, ethylmercury, dental amalgam, atmospheric mercury, HR-XANES spectrometry

Reference :

1) Manceau A et al., Environ. Sci. Technol., 50, 10721-10729, 2016.



[内容紹介]

演者のボーディナルド先生は、「仏国・ボルドー大学」にて研究を行っておられます。今回の発表は、「ヒト毛髪における水銀の化学形態は曝露源を示す」についてです。HR-XANES 分光分析という方法を用いて、低濃度の水銀濃度（0.5 ppm）を示す毛髪の水銀が魚介類由来であることを明らかにしました。以上の結果から、低濃度の水銀濃度である毛髪であっても、化学形態を詳細に解析することによってその曝露源を確定できる可能性が示唆されました。

Human hair: ヒト毛髪

Methylmercury: メチル水銀

Ethylmercury: エチル水銀

Dental amalgam: 歯科用アマルガム

Atmospheric mercury: 大気水銀

HR-XANES spectrometry: X線吸収端近傍構造（HR-XANES）分光分析

[ノート]

### **4-3 Change in fetal hepatic metabolome by maternal exposure to methylmercury: a search for cellular components linking to toxicity**

Tomoki Takeda<sup>1</sup>, Masaya Hitomi<sup>1</sup>, Yukiko Hattori<sup>1</sup>, Masatake Fujimura<sup>2</sup>, Hideyuki Yamada<sup>1</sup>

<sup>1</sup>Kyushu University, Japan, <sup>2</sup>National Institute for Minamata Disease, Japan

Gestational exposure to methylmercury (MeHg) at lower dose produces a variety of developmental disorders in the offspring. However, the toxic mechanism remains to be fully elucidated. A number of cellular components, such as nutrients and hormones, stimulate the maturation of tissues during the fetal and neonatal period. Therefore, it is possible that MeHg disrupts the above stimuli in fetuses, resulting in the abnormality of postnatal phenotypes. To search the components linking to MeHg-induced fetotoxicity and its sex difference, we initially performed a metabolomic analysis focusing on the fetal liver. Giving MeHg-containing water (0.1-5 ppm) to pregnant rats during gestational day (GD) 0 and GD20 altered a number of hepatic components both in male and female fetuses at GD20, and the number of the components changed was greater in male fetuses than that in females. This analysis also suggested that MeHg increases the level of corticosterone, a stress hormone, to retard fetal growth only in male fetuses. An increase by MeHg in the level of corticosterone was observed in the serum, hypothalamus and cerebrum. Such change seems to be due to the reduced metabolism, because MeHg attenuated the hepatic expression of sulfotransferase 1A1 and UDP-glucuronosyltransferase 1A1 only in male fetuses. In agreement with the evidence that high level of corticosterone downregulates the secretion of growth hormone (GH), MeHg reduced the serum concentration of GH only in male fetuses, and also caused low body weight. In contrast, in female fetuses, melatonin, an anti-oxidative hormone, was increased by MeHg treatment. These results suggest that 1) MeHg causes toxicity in fetuses by sex-specific changes in cellular components associated with tissue damage and protection, and 2) an induction of corticosterone due to a reduced expression of metabolizing-enzymes is one of reasons for undergrowth of male offspring.

Key words: fetus, metabolomics, corticosterone, sex difference.



[内容紹介]

演者の武田先生は、「九州大学」にて研究を行っておられます。今回の発表は、「メチル水銀の妊娠期曝露による胎児肝メタボロームの変化：毒性に結びつく細胞成分の検索」についてです。実験動物であるラットを用いた研究において、メチル水銀の胎児期曝露が、雄のコルチコステロンを増加させることによって成長阻害を示すことが明らかになりました。今回の結果から、メチル水銀の胎児期曝露が雄特異的な成長阻害を引き起こす原因が、コルチコステロン増加に起因する可能性が示唆されました。

Fetus: 胎児

Metabolomics: メタボロミクス, 代謝学

Corticosterone: コルチコステロン (副腎皮質から分泌される糖質コルチコイド)

Sex difference: 性差

Growth hormone: 成長ホルモン

Melatonin: メラトニン

[ノート]

## 5-1 Is Methylmercury exposure an Environmental Risk Factor for Epileptogenesis?

Yukun Yuan

Michigan State University, USA

Epilepsy or seizure disorder is one of the most common neurological diseases in humans. Genetic mutations in ion channels and ligand-gated receptors and some other risk factors such as brain injury are linked to epileptogenesis, however, the underlying causes for the majority of epilepsy cases remain to be identified. Gene-environment interaction is believed to play a critical role in the etiology of epilepsy. Exposure to certain environmental chemicals has been shown to promote or facilitate the development of seizures or epilepsy. Clinical and epidemiological evidence suggests that methylmercury (MeHg), a prominent environmental contaminant, may also be a potential risk factor contributing to epileptogenesis since patients with acute or chronic MeHg poisoning had a significantly higher incidence of epileptic seizures. This is especially true for children with developmental MeHg exposure. Consistently, animals with prenatal MeHg exposure displays increased susceptibility to seizures. Here we examined changes in neuronal excitability of layer II/III neurons in cortical slices of rat following early postnatal MeHg exposure. Our results showed that 40% of the rats exposed to 0.75 or 1.5 mg/kg/day MeHg subcutaneously for 30 days beginning on postnatal day 5 (PND5) display epileptiform activity in layer II/III neurons in cortical slices in a time- and dose-dependent manner. Importantly, this epileptiform activity persisted in 50 - 60% rats even after MeHg exposure had been terminated for 30 days. In contrast, none of the slices prepared from control rats in any group showed epileptiform activity. Thus, these data suggest that early postnatal MeHg exposure *in vivo* altered neuronal excitability and induced a long-lasting hyperexcitability in cortical neurons. Based on our previous observations that MeHg differentially affects glutamatergic and GABAergic systems, we propose that a preferential effect of MeHg on GABAergic inhibitory system, which disrupts the balance between excitation and inhibition in the CNS leading to neuronal hyperexcitability, may be one of the potential mechanisms underlying MeHg-induced changes in seizure susceptibility.

Key words: Methylmercury, environmental risk factors, seizures, epileptogenesis.

[内容紹介]

演者のユアン先生は、「米国・ミシガン州立大学」にて研究を行っておられます。今回の発表は、「メチル水銀曝露は”てんかん”発作の環境危険因子に成りえるか？」についてです。実験動物であるラットの生後5日目から30日間メチル水銀を与えたラットの脳スライス切片を用いた研究において、40%のラットで”てんかん”発作に関する活動電位が示されました。さらに、この原因は、GABA シナプス抑制系への影響によると考えられました。以上の結果から、出生後早期のメチル水銀の曝露が”てんかん”発作の感受性に影響する可能性が示唆されました。

Methylmercury: メチル水銀

Environmental risk factors: 環境危険因子

Seizures: 発作

Epileptogenesis: てんかん原生

[ノート]

## **5-2 Methylmercury enhances sensitivity for pressure-overload stress in cardiomyocytes via mitochondrial fission**

Takashi Toyama

Okazaki Institute for Integrative Bioscience, Japan, Tohoku University, Japan

Electrophiles, which are produced by the disruption of redox homeostasis, modulate protein function by reacting with thiol groups of cysteine, and are associated with pathological events of various tissues including heart. Methylmercury (MeHg) is an environmental electrophile that causes neurologic and developmental diseases. Recent epidemiological studies suggest intake of low level of methylmercury rises cardiovascular risk. These studies suggest that cardiovascular system is vulnerable to toxicity induced by electrophiles. However, the mechanism underlying cardiotoxicity induced by electrophiles is obscure. Using MeHg as a model electrophile, we here examined the mechanism that electrophiles increase risk of cardiovascular dysfunction after pressure overload in mice. We first assessed the effect of MeHg on cardiac structure and functions, however exposure of mice to 1 ppm MeHg for 1 year did not affect cardiac performances. Next we exposed mice to 10 ppm MeHg for 1 week and performed transverse aortic constriction surgery (TAC) to evoke pressure overload stress and following heart failure. Interestingly MeHg significantly exacerbated TAC induced cardiac dysfunctions. Electron microscopic analysis showed abnormal mitochondrial fission in myocardium from MeHg-exposed mice. Biochemical analysis revealed that dynamin related GTP-binding protein 1 (Drp1), a key regulator of mitochondrial fission, is a molecular target of MeHg. Treatment of MeHg (0.5-1  $\mu$ M) induced mitochondrial fission, ROS production and cell death of rat neonatal cardiomyocytes through Drp1 activation. These results suggest that mitochondrial hyper-fission via electrophilic modulation of Drp1 underlies electrophile-induced cardiac vulnerability to hemodynamic load.

Key words: mitochondrial fission, Drp1, covalent modification, pressure overload stress and heart failure

#### [内容紹介]

演者の外山先生は、「岡崎総合バイオサイエンスセンター」において研究を行っておられました（現在は東北大学に所属）。今回の発表は、「メチル水銀はミトコンドリア分裂を介して心筋における圧負荷ストレス感受性を増強する」についてです。実験動物であるラットを用いた研究において、メチル水銀曝露が Drp1 に作用してミトコンドリア分裂を引き起こすことが明らかになりました。今回の結果から、メチル水銀が共有結合修飾を介したミトコンドリア分裂を引き起こし、血行動態に対する脆弱性に影響する可能性が示唆されました。

**Mitochondrial fission:** ミトコンドリア分裂

**Drp1:** ダイナミン関連 GTP 結合蛋白 1

**Covalent modification:** 共有結合修飾

**Pressure overload stress and heart failure:** 圧負荷ストレスと心不全

#### [ノート]

## 6-1 Impact of *in utero* exposure following dietary exposure to Hg enriched diet

Sébastien Cambier

Luxembourg Institute of Science and Technology

Epidemiological studies so far available are contradictory about long-term health consequences of *in utero* and early childhood mercury (Hg) exposure. Those contradictions are linked to the beneficial influence of nutrients from fish, the main cause of Hg exposure, which may counter Hg adverse effects on the developing nervous system. To further investigate *in utero* and early childhood Hg exposure, we exposed rats during fetal life and lactating period *via* maternal transfer followed by early life-stage exposure through food containing naturally Hg contaminated fish such as Aimara (AIM : 76 ng Hg.g<sup>-1</sup>), or a blend of Tuna, Cod and Swordfish (TCS : 35.75 ± 0.15 Hg.g<sup>-1</sup>). The effects of those regimens were first followed on the mothers. Then, from weaning the young rats were either fed with naturally Hg contaminated fish flesh or control diet for 2 weeks before behavioral tests and Hg quantification. Mothers exposed *via* those real exposure scenarios were significantly more accumulating Hg: The highest concentration occurred in kidneys (3 µg Hg/g) for both Hg contaminated diet whereas the ratio of 2 in between TCS and AIM was kept in the other organs. The Hg accumulation pattern between the different organs in the offspring showed an evolution regarding the feeding mode. Indeed, at birth liver showed the highest bioaccumulation (0.08 and 0.17 µg Hg/g, for TCS and AIM) which changed to kidneys at the weaning (0.05 and 0.1 µg Hg/g) to the end of the exposure *via* the enriched diet (1 and 2.9 µg Hg/g). Significant effects were also visible with a higher growth rate of the newborn rats and impacts on their behavior compared with the control. Furthermore those effects of the diet on the growth and behavior are different between male and female. In conclusion, those results suggest that fish diet has a real beneficial contribution to the growth of the newborn rats but is not suppressing the deleterious effects of the Hg on the nervous system development.

Keywords: *in utero*, early childhood, real exposure scenarios.

#### [内容紹介]

演者のキャンバー先生は、2010年に3ヵ月間、国立水俣病総合研究センターに滞在し、共同研究を行ったことがあります。現在は、「ルクセンブルク・科学技術研究所」において研究を行っておられます。今回の発表は、「食物から供給される水銀の子宮内曝露の影響」についてです。実験動物であるラットを用いた研究において、メチル水銀を含む魚肉（Aimara 等）を母ラットに摂取させ、胎児ラットにメチル水銀の子宮内曝露を行った結果、生後の神経行動に影響がでることが明らかになりました。今回の結果から、魚肉摂取は生後の成長には良い影響を与えるものの、含有するメチル水銀が神経系の発達に対して影響を示す可能性が示唆されました。

**In utero:** 子宮内

**Early childhood:** 幼児期早期

**Real exposure scenarios:** 実際の曝露状況

#### [ノート]

## **6-2 Toxicological effects of methylmercury exposure on ophthalmic tissues**

Toshihiro Imada

Keio University School of Medicine, Japan.

Methylmercury (MeHg) is a potent environmental toxicant causing various pathologies. However, there was little information regarding to the toxicological effect of MeHg on ophthalmic tissues. To address this issue, effect of acute exposure of relatively high dose of MeHg on rat ophthalmic tissues were evaluated. MeHg exposed to the rats at the dosage of 20-ppm by administration in drinking water for 3 weeks. In ophthalmic tissues, measurement of tissue mercury concentration and pathological examination were performed. The pathological examination was carried out with gross macroscopy, histopathology, and transmission electron microscopy (TEM). In addition, the lacrimal gland (LG) was subjected to the measurement of mitochondrial content and reactive oxygen species (ROS). Mercury concentration was significantly higher in the LG ( $41.26 \pm 9.01$  ppm) and lens ( $34.92 \pm 11.85$  ppm) than in other ophthalmic tissues (cornea, iris, and retina.  $< 10$  ppm). LG was atrophied and the weight was significantly decreased to approximately 65 % of that of the control group. In other ophthalmic tissues, there was no difference in gross macroscopic findings between MeHg group and control group. Histopathological observation of LG showed the size of acinar cells reduced in MeHg group. In the reduced acinar cells, the appearance of cytoplasmic vacuole and nuclear invagination of cytoplasm were also observed. TEM findings showed that organelles, especially in the mitochondria, were decreased and their cristae structure was extensively disrupted in MeHg group. Mitochondrial content in the LG was significantly lower and ROS generation from LG was significantly higher in MeHg group compared to control group. These results demonstrated that LG is most susceptible tissue to MeHg toxicity among ophthalmic tissues due to its high accumulation rate of mercury. Further investigation focusing on the transport mechanism of mercury into LG is useful to extrapolate these toxicological information to humans.

Key words: lacrimal gland, mitochondria, reactive oxygen species.



[内容紹介]

演者の今田先生は、「慶応義塾大学」において研究を行っておられます。今回の発表は、「メチル水銀曝露の眼科組織への毒性影響」についてです。実験動物であるラットを用いた研究において、メチル水銀の曝露によって水銀が涙腺に蓄積され、腺房細胞の縮小化が引き起こされることが明らかになりました。さらに、メチル水銀の曝露は、涙腺におけるミトコンドリアの減少および活性酸素種の増加を引き起こすことも明らかになりました。以上の結果から、眼科組織の中で涙腺がメチル水銀の標的になる可能性が示唆されました。

Lacrimal gland: 涙腺

Mitochondria: ミトコンドリア

Reactive oxygen species: 活性酸素種

[ノート]

## Brief Summary

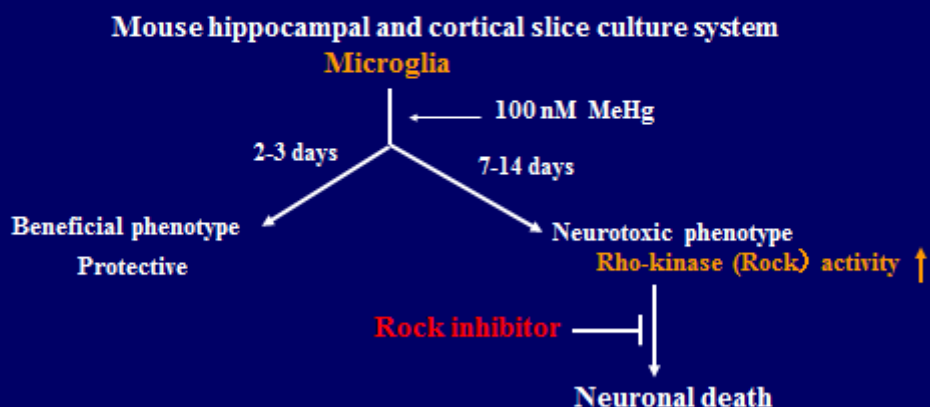
### Theme

#### Pathomechanism of Methylmercury Toxicity ~ Various Approaches to the Problems ~

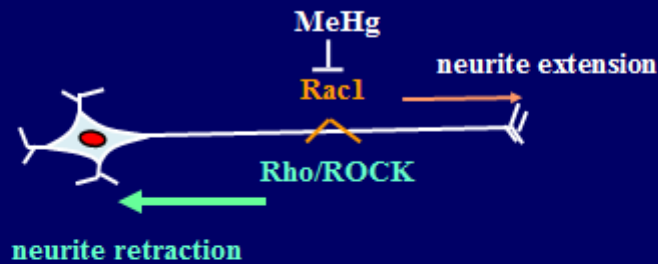
- 1) MeHg toxicity in the central nervous system
- 2) Myogenic targets in MeHg toxicity
- 3) Autophagy in MeHg toxicity
- 4) Effect of factors on tissue Hg content
- 5) Sex-specific changes by maternal MeHg exposure
- 6) Hair mercury analysis for exposure source
- 7) MeHg and pressure-overload stress in cardiomyocytes
- 8) Ophthalmic tissue and MeHg toxicity

#### 1) MeHg-toxicity in the central nervous system

##### 1-1 Regulation by microglia of methylmercury-evoked neuronal degeneration (Dr. Koizumi)



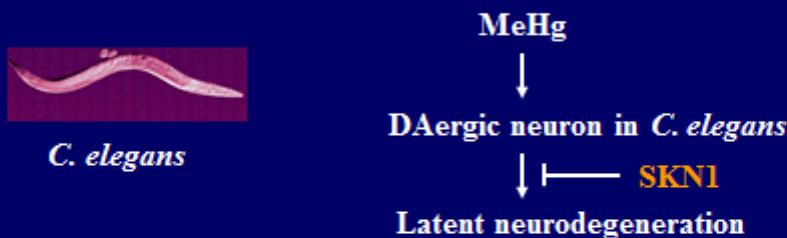
Ref. Differentiated neurons



MeHg downregulates **Rac1** which is known to promote neuritic extension  
 ↓  
 └─ **Rho/Rock inhibitor**  
**Axonal degeneration & apoptotic neuronal cell death**

(Fujimura et al., *Toxicol Appl Pharm*, 250: 1-9, 2011)

2-2 The Role of *skn-1* in methylmercury-induced latent dopaminergic neurodegeneration (Dr. Aschner)



- Early-life exposure to MeHg in *skn-1* knockout *C. elegans*  
 ROS ↑↑, loss of neuron (+), dopamine level ↓  
 Hg content →, behavior ↓

**Skn-1**: *c. elegans* orthologue of mammalian **Nrf2**  
 stress-activated cytoprotective transcription factor

**3-2 Developmental exposure to methylmercury induces depression-like behavior and alters neurogenesis (Dr. Ceccatelli)**

**1) Pregnant mice:**

↓ ← 0.5 mg MeHg/kg/day via drinking water  
 Male-offspring: depression-like behavior  
 epigenetic change of BDNF gene in the hippocampus  
 hippocampus neurogenesis ↓

**2) Neural stem cells (NSC):**

↓ ← 2.5 or 5 nM MeHg  
 Proliferation & neuronal differentiation ↓  
 altered expression of cell cycle regulators  
 and senescence-associated markers  
 global DNA methylation ↓

**Programming effects induced by MeHg in NSC**

**4-1 Low in situ expression of antioxidative enzymes in brain regions susceptible to MeHg in rodent models of Minamata disease (Dr. Fujimura)**

- 1) Microdissection and qPCR (Cu/Z-SOD, Mn-SOD, GPx1, TrxR1)
- 2) Immunohistochemical analysis

Cerebellum in rat model:

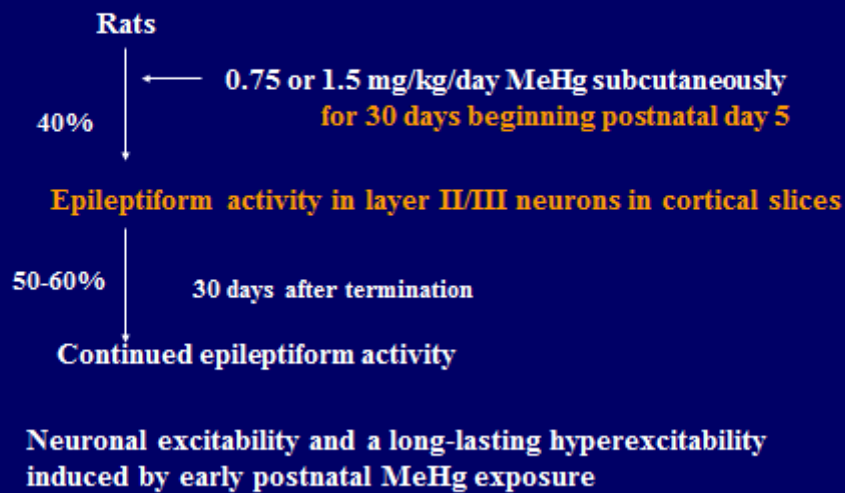
Mn-SOD, GPx1, TrxR1  
 granule cell layer  
 ^  
 Purkinje cells layer  
 molecullar layer

Cerebral cortex & hippocampus in mouse model:

Cu/Zn-SOD, Mn-SOD, GPx1  
 deep layer  
 ^  
 shallow layer  
 hippocampus

**Contribution of the basal expression level of antioxidative enzymes to the site-specific neurotoxicity in brain**

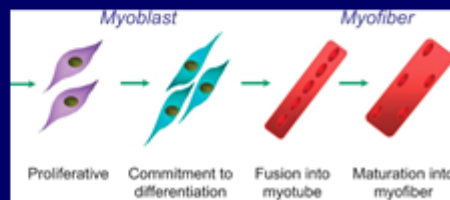
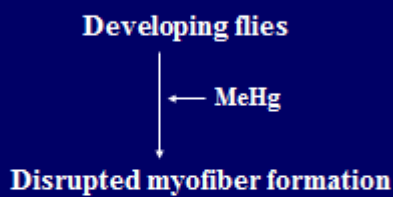
**5-1 Is MeHg exposure an environmental risk factor for epileptogenesis? (Dr. Yuan )**



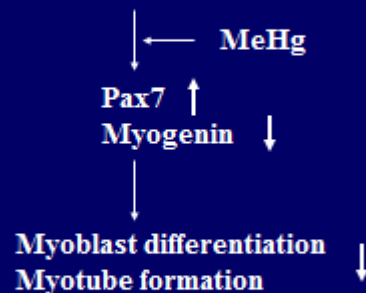
**2) Myogenic targets in MeHg toxicity**

**1-2 Myogenic targets in methylmercury neuromuscular toxicity (Dr. Rand )**

**1. Drosophila model:**

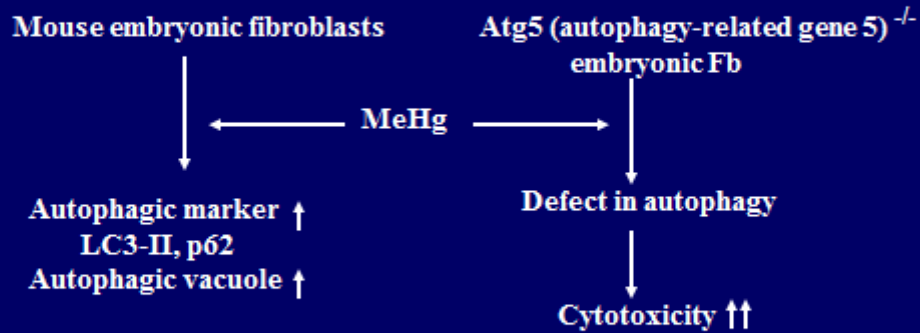


**2. Mouse C2C12 myoblasts:**



### 3) Autophagy in MeHg toxicity

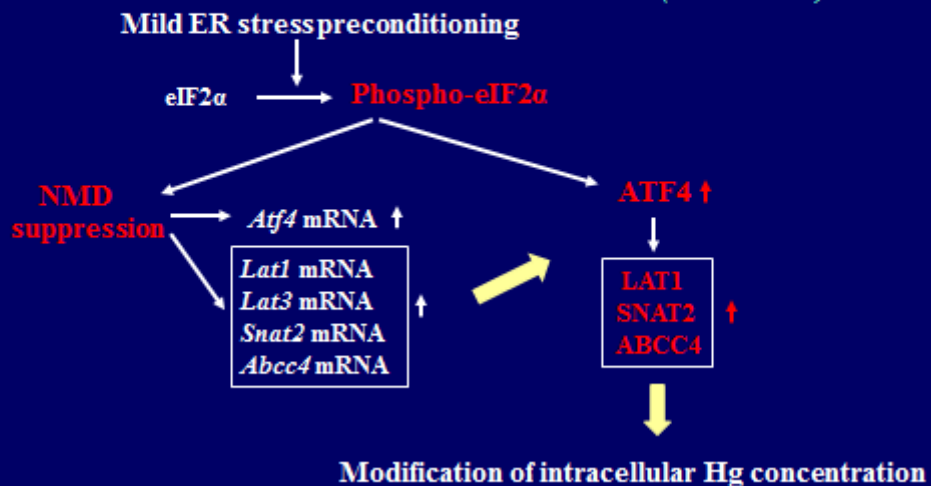
#### 2-1 The role of autophagy against methylmercury toxicity (Dr. Takanezawa)



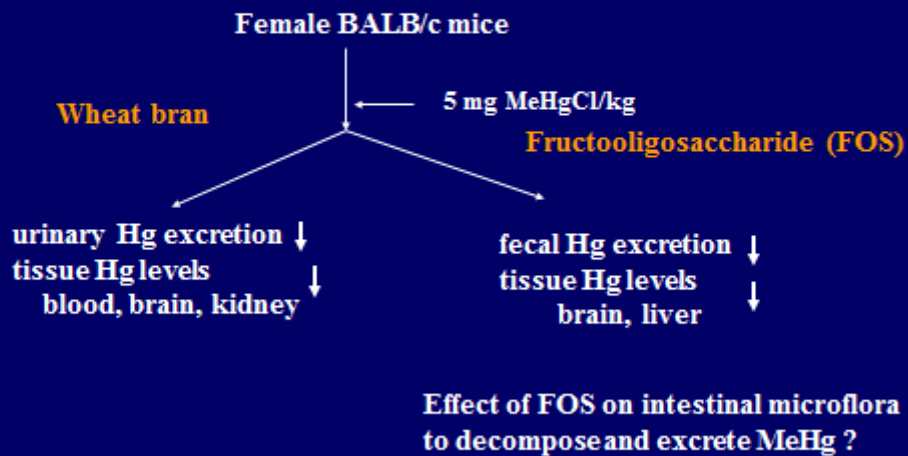
**Protective effect of autophagy** in MeHg cytotoxicity through elimination of intracellular MeHg-induced damaged materials

### 4) Effect of factors on tissue Hg content

#### 3-1 Mild endoplasmic reticulum stress preconditioning modifies intracellular Hg content through the upregulation of membrane transporters (Dr. Usuki)



3-3 The effects of wheat bran, fructooligosaccharide and glucomannan on tissue mercury concentration after MeHg exposure in mice  
(Dr. Nagano )



5) Sex-specific changes by maternal exposure to MeHg

4-3 Change in fetal hepatic metabolome by maternal exposure to MeHg: a search for cellular components linkage to toxicity  
(Dr. Takeda)

Metabolomic analysis on fetal liver (at GD20)

Pregnant rats

← 0.1-5 ppm MeHg via drinking water (GD 0-20)

**Sex-specific changes:**

**Male:**

corticosterone in serum, hypothalamus, cerebrum ↑

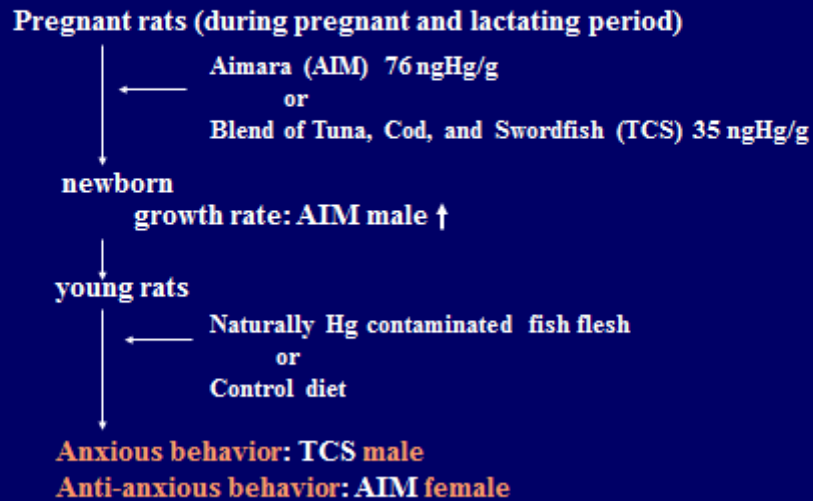
growth hormone ↓

low body weight ↓

**Female:**

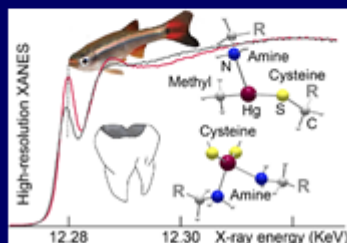
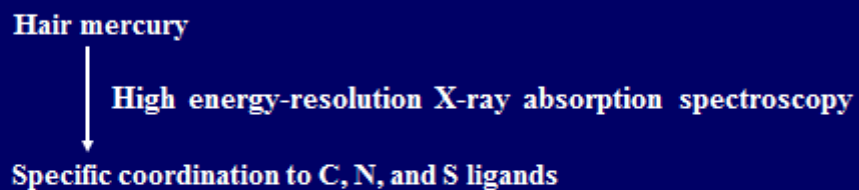
anti-oxidative hormone melatonin ↑

*6-1 Impact of in utero exposure to following dietary exposure to Hg enriched diet (Dr. Cambier)*



**6) Hair mercury analysis for exposure source**

*4-2 Chemical forms of mercury in human hair reveal source of exposure (Dr. Bourdineaud)*



**HR-XANES,**  
High energy-resolution X-ray absorption  
near edge structure



## 7) MeHg and pressure-overload stress in cardiomyocytes

### 5-2 Methylmercury enhances sensitivity for pressure-overload stress in cardiomyocytes via mitochondrial fission (Dr. Toyama)

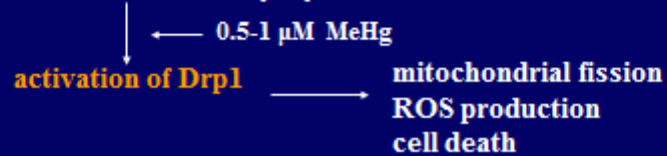
#### 1) *in vivo* study (mouse)

- 1 ppm MeHg for 1 year: no effect on cardiac performances
- 10 ppm MeHg for 1 week + transverse aortic constriction (TAC) surgery  
**pressure overload stress and following heart failure model**

abnormal mitochondrial fission in myocardium  
Dynamamin related GTP-binding protein1 (**Drp1**)  
a key regulator of mitochondrial fission

#### 2) *in vitro* study

rat neonatal cardiomyocyte



## 8) Ophthalmic tissue in MeHg toxicity

### 6-2 Toxicological effects of methylmercury exposure on ophthalmic tissue (Dr. Imada)

Rat

← 20 ppm MeHg via drinking water for 3 weeks

Ophthalmic tissue

Hg content: **Lacrimal gland**, lens > cornea, retina

**Lacrimal gland:**

atrophy

reduced size of acinar cells

cytoplasmic vacuole, nuclear invagination of cytoplasm

mitochondria ↓

ROS ↑

## **Theme**

### **Pathomechanism of Methylmercury Toxicity ~ Various Approaches to the Problems ~**

- 1) MeHg toxicity in the central nervous system
- 2) Myogenic targets in MeHg toxicity
- 3) Autophagy in MeHg toxicity
- 4) Effect of factors on tissue Hg content
- 5) Sex-specific changes by maternal MeHg exposure
- 6) Hair mercury analysis for exposure source
- 7) MeHg and pressure-overload stress in cardiomyocytes
- 8) Ophthalmic tissue and MeHg toxicity

# **5<sup>th</sup> Conference on Prenatal Programming and Toxicity**

**(PPTox V)**

## **NIMD Mercury Session**



### **Exposure Assessment and Health Effects**

Chairs: Irina Zastenskaya (WHO-EURO, Germany) and Mineshi Sakamoto (NIMD, Japan)

Mineshi Sakamoto (NIMD, Japan):

Mercury, selenium, docosahexaenoic acid, and vitamin E profiles in maternal and cord blood

Nozomi Tatsuta (Tohoku University, Japan):

Birth weight of male infants is susceptible to prenatal exposure to methylmercury

– Tohoku Study of Child Development -

Irina Zastenskaya (WHO-Euro, Germany):

Critical points in the planning of a global approach to monitor human exposure to mercury and its compounds

## Mercury, selenium, docosahexaenoic acid, and vitamin E profiles in maternal and cord blood

Mineshi Sakamoto

National Institute for Minamata disease, Japan

Methylmercury (MeHg) is a neurotoxicant, and the developing brain of a fetus is known to be especially susceptible to it. The placenta strongly regulates the biochemical composition of the fetal circulation derived from the mother. Therefore, the study of the characteristics of the profiles of mercury in fetal circulation as well as those of selenium, vitamin E (VE), and docosahexaenoic acid (DHA), potential protective factors against the toxicity of MeHg, are important to evaluate the high susceptibility of the fetus. The objectives of this study were to: 1) Investigate the characteristic of the placental transfer of nutrition by comparing biochemical composition in the fetal and maternal circulations to evaluate placental MeHg transfer. 2) Investigate the difference of the status of Hg, Se, VE, and DHA in fetal blood with those in maternal blood to evaluate the background of the high sensitivity of a fetus to MeHg toxicity. Blood samples were separately collected from the maternal and umbilical vein at parturition from 54 mother-infant pairs of Japanese. The characteristics of fetal circulation were low contents of lipid components and fatty acids and high contents of amino acids, including methionine, compared with those in maternal circulation. Mercury in cord blood (7.26 ng/g) was higher than in maternal blood (185%). Selenium in cord blood (153 ng/g) was similar to maternal blood. On the other hand, VE (0.31 mg/dl) and DHA (57.9 $\mu$ g/ml) in cord blood were lower than maternal blood (45% for VE and 22% for DHA). These results showed that the ratios of selenium/mercury, DHA/mercury, and VE/mercury were lower in fetal circulation than those in maternal blood. Not only the approximately two times higher Hg but also the lower ratios of protective factors such

as selenium, VE and DHA against mercury in fetal circulation may be a cause of the high susceptibility of a fetus to methylmercury toxicity.

# Birth weight of male infants is susceptible to prenatal exposure to methylmercury

– Tohoku Study of Child Development –

Nozomi Tatsuta

Tohoku University, Japan

**Objective:** The effects of prenatal exposure to polychlorinated biphenyls (PCBs), methylmercury, and lead on birth weight remain controversial. The aim of this study was to clarify whether these chemicals affected the birth weight of male and female Japanese infants.

**Method:** We have been conducting a prospective cohort study, the Tohoku Study of Child Development, to examine the prenatal effects on child neurodevelopment of concurrent exposure to hazardous chemicals such as PCBs, methylmercury, and lead. To establish an optimal study population, the eligibility criteria included a singleton pregnancy, Japanese as the mother tongue, and neonates born at term (36-42 weeks of gestation) with a birth weight of more than 2400 g and no congenital anomalies or diseases. We enrolled 687 pregnant women in this study with their written informed consent, and 599 mother-child pairs were registered according to the eligibility criteria. In this study, of the 489 pairs had complete information on PCBs, mercury, and lead concentrations in cord blood, as well as birth weight, and possible confounders such as gestational age and fish/seafood intake. Maternal intake of fish/seafood during pregnancy was assessed using a food frequency questionnaire that was administered by trained interviewers.

**Results:** The mean birth weight of all infants was 3082 (range, 2412-4240) g, and the gestational age was  $39.5 \pm 1.3$  (SD) weeks. The median values of biomarkers in cord-blood were 46.0 (18.6 - 113.8) ng/g-lipid in total PCBs, 10.1 (5th and 95th percentiles,

4.3 - 22.4) ng/g for total mercury, and 1.0 (0.6 - 1.7) µg/dL for lead. Birth weight was significantly heavier in the 252 male babies than in the 237 female babies, so the below analyses were carried out in the male and female babies separately. A negative association between total PCBs and birth weight was observed in both male and female babies ( $p < 0.01$ ), even after adjusting for possible confounders. However, a negative association of total mercury with birth weight was found only in the male babies ( $p < 0.05$ ). There was no significant relationship between lead concentration and birth weight in the two groups.

**Discussion:** Birth weight appears to be affected by prenatal PCBs exposure in Japanese male and female babies, and the effect of methylmercury exposure on male fetal growth may be stronger than in females. Male fetuses are suggested to be more susceptible to methylmercury than their female counterparts. Concerning the effect on fetal growth, further studies are necessary to clarify the toxic mechanism of such chemicals, and also to scrutinize sex difference in the outcomes attributable to other environmental pollutants including methylmercury.

## Critical points in the planning of a global approach to monitor human exposure to mercury and its compounds

Irina Zastenskaya

World Health Organization (WHO)-Euro, Germany

Several critical questions need to be answered when developing a harmonized approach to assessment of human exposure to mercury on a global scale using human biomonitoring (HBM). Among them, what are the critical health-end points from the public health perspective; how to interpret data obtained in a global HBM study at a national and international level and how to communicate health risks at individual level; and, what policy advice to provide in light of precautionary principle? While mercury is known to affect many systems and organs, depending on its form, dose and life-stage, the most critical effect from the public health perspective is neurodevelopmental disorders linked to a low level environmental exposure. Mercury HBM has been used to assess exposure to mercury for the last 40-50 years. In national and regional HBM programs, different population groups have been involved: in the EU-funded projects COPHES and DEMOCOPHES, a target group was mothers and their children aged 6-11 years; the HBM program in Germany focused on 3-14 years old children; different age groups (1-5, 6-11 and 12-19 years old, and adults) are included in the US HBM program. In numerous HBM studies with a wide range of objectives, different ages and population groups (children, workers, pregnant women, disable and poor) were involved. Scientific data obtained over the last 15 years let to conclude that both, acute and chronic mercury exposure can cause adverse effects during any period of life; but, the strongest link has been revealed between the low-dose prenatal exposure to mercury and neurodevelopmental and neuropsychological effects in later life, especially in terms of developmental delay, behavioral disorders, cognitive impairment including memory and learning abilities. Therefore, for the purpose of a global mercury monitoring scheme,



WHO has proposed to focus on assessing prenatal exposure to mercury during the last trimester of pregnancy. Involving mothers who have just delivered a child in maternity hospitals provides additional opportunities to offer a nutritional advice as well as counselling on potential medical follow-up in cases of newborns with high exposure to mercury. Another challenge in developing a global mercury monitoring plan is to cover as many exposure sources as possible. In the currently implemented pilot HBM survey, several locations with different predominant sources of population exposure to mercury have been identified based on the results of previous studies, environmental monitoring data, and analysis of the existing knowledge on mercury sources. These include: populations with high-consumption of marine (Ghana) and fresh water (Karelia, Russian Federation) fish, and other potentially contaminated food (China); artisanal and small-scale gold mining (Mongolia and Costa Rica); power plants and waste management (India); primary mercury mining (Kyrgyzstan); unknown source of exposure (Costa Rica); and, chlor-alkali plant (Croatia).

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