Determination and Distribution of Methylmercury in Korea using Purge and Trap GC-MS

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Young-Hee KIM

Inorganic Analysis Research Division

MINISTRY OF ENVIRONMENT

National Institute of Environmental Research

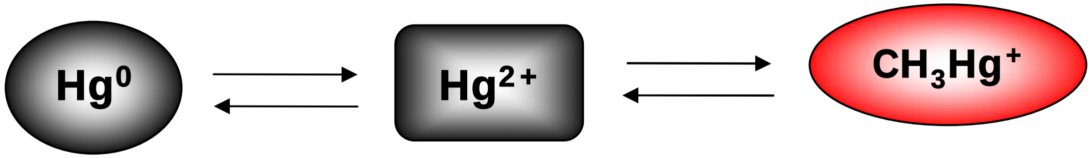
Talk Outline

Content Title

- Introduction of Methylmercury
- Mercury issues in Korea
- Analytical methods for Methylmercury
- Methylmercury distribution in environment
- Current Status and Future Plan

Chemical forms of Hg

- **Elemental :** Hg^0
- **Inorganic:** Hg^{++}, HgX_2, HgX_3^-, HgX_4^{2-}
  ($X = OH^-$, Cl^-, Br^-)
- **Organic:** CH_3Hg^+, CH_3HgCl, CH_3HgOH



Elemental Hg

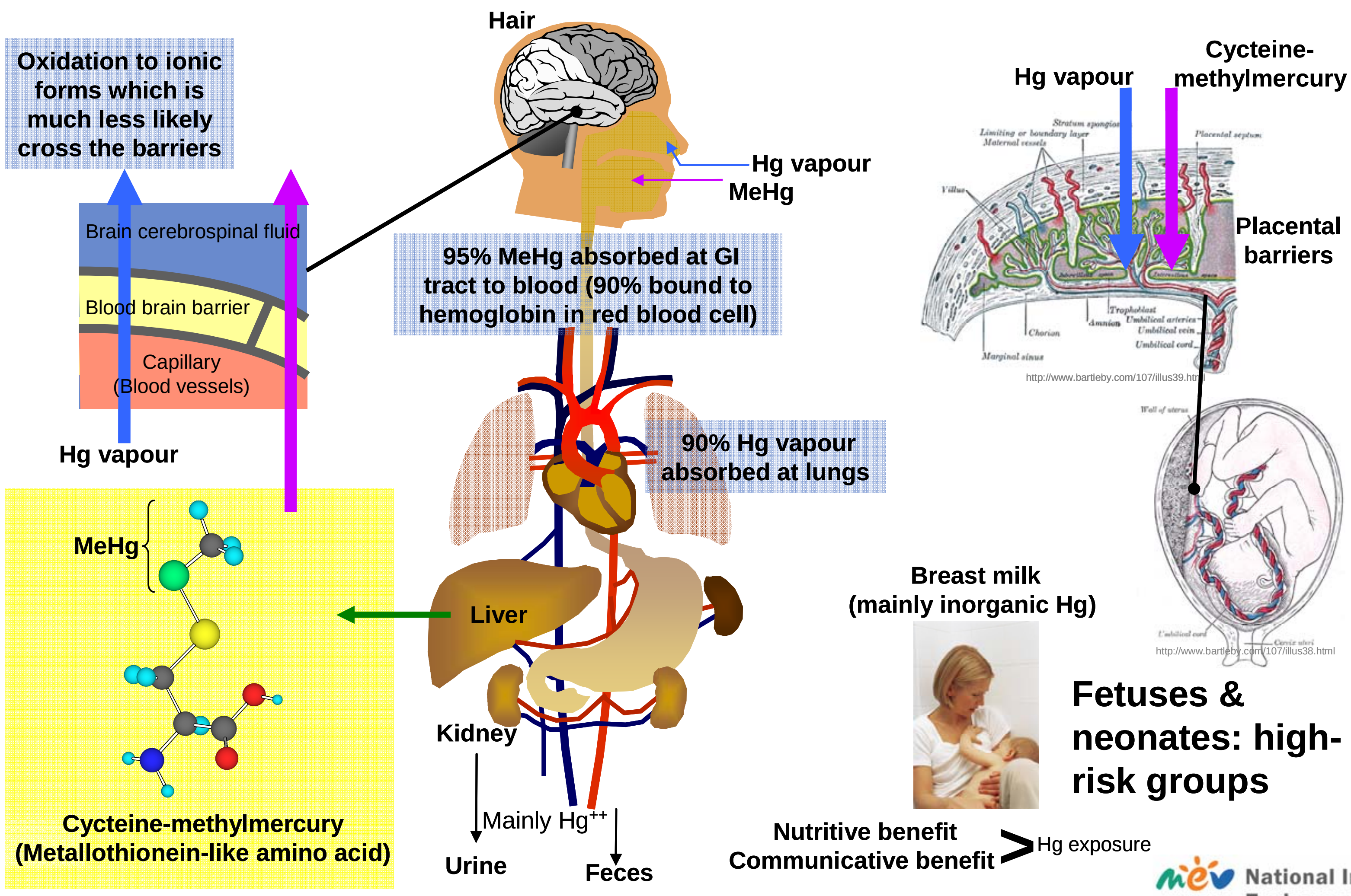
- **Coal combustion, volcano, gold mine**
- **Hg vapor is absorbed by lung & penetrates the brain causing brain damage. It also accumulates in the kidney & cause damages to kidney.**
- **Primary ore is cinnar bar , HgS.**

Methyl mercury (MeHg)

- **MeHg is bioaccumulated through food chains in aquatic systems.**
- **MeHg concentration in fish is the highest in aquatic systems – fish Hg should be measured for aquatic environment assessment.**

Mercury metabolism

MeHg conjugates with cysteine, then easily absorbed as amino acids in the digestive tract & penetrate the brain / fetus - causing disorders in the brain and fetus.



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History of Hg Toxicities

Acute poisoning: Lethal (earlier cases)

- **'Mad Hatters Syndrome':** 19 & 20th century
- **'Acrodynia'** in children: early 20th century
- **Minamata Disease**, Japan: 1950s: 5,000 people affected, Hg-laden fish, Hg used as a catalyst to produce acetaldehyde
- **Iraq:** 1972: 50,000 people affected, Hg-laden fungicide contaminated bread, 5,000 died

Chronic effects

Salonen et al 1995 <u>Circulation</u>

- Hg overload, due to intake of Hg from contaminated fish (Eastern Finnish man) → accelerated progression of cardiovascular disease
- Coronary & cardiovascular death

Hightower 2002 <u>Environ Health Persp</u>

- Patients with hair loss, fainting spells and stomach upset, related to Hg in fish

Minamata disease patient

Fisherman's hand

Congenital Minamata disease patient & her mother

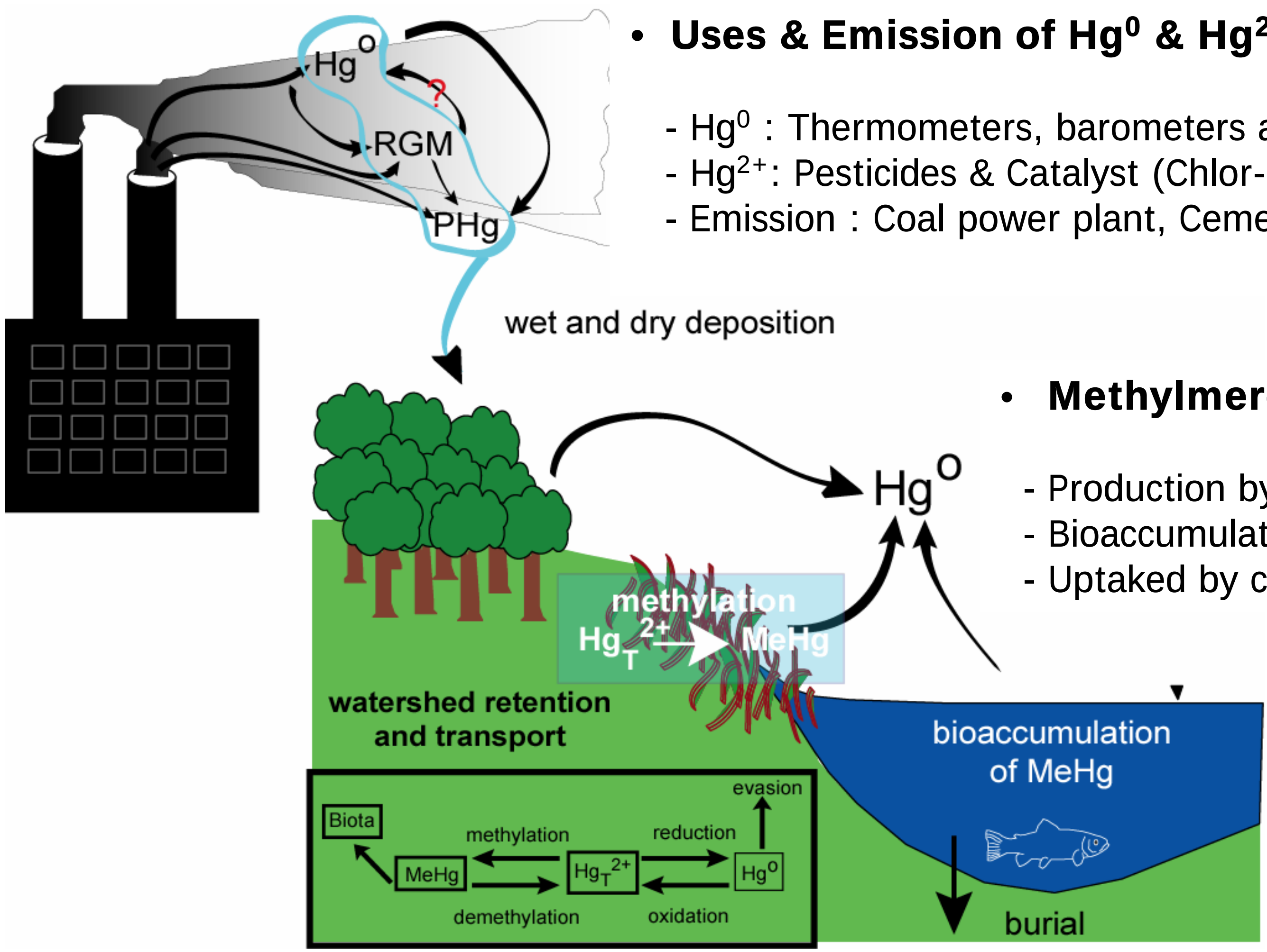
Mercury & Methylmercury in Environment

- **Uses & Emission of Hg^0 & Hg^{2+}**

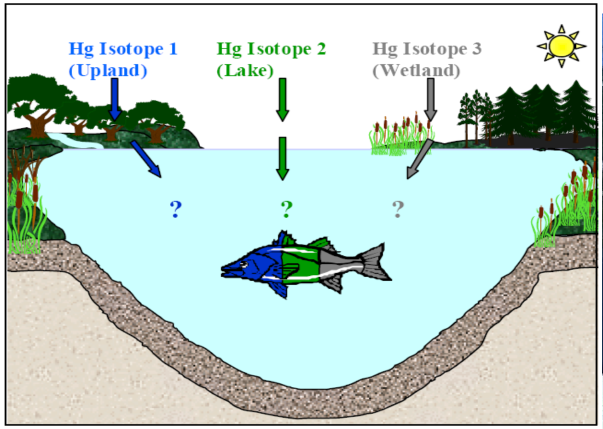
  - Hg^0 : Thermometers, barometers and other electrical switches
  - Hg^{2+}: Pesticides & Catalyst (Chlor-alkali industries etc.)
  - Emission : Coal power plant, Cement production, Incinerators etc.

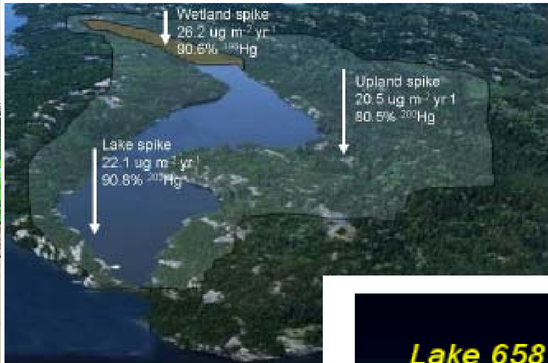
- **Methylmercury Fate & Transport**

  - Production by SRB in sediment
  - Bioaccumulated along with Food Web
  - Uptaked by contaminated fish to human



Environmental Responses to Hg loading

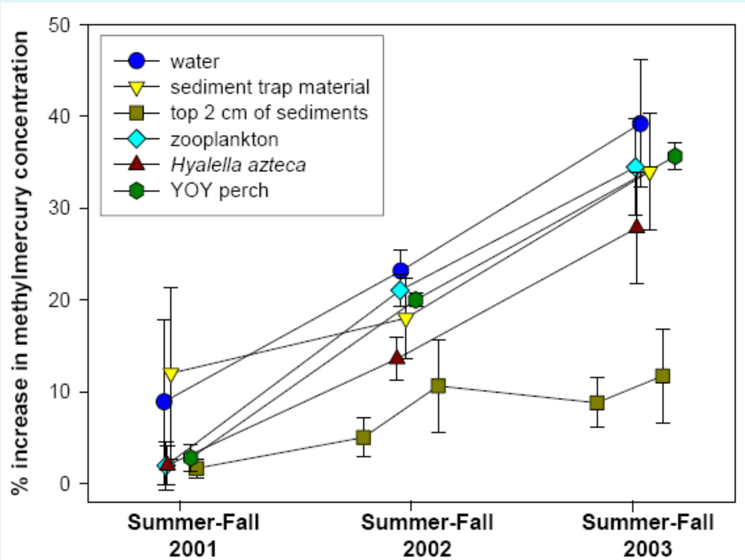




Harris et al. (2008)

METAALICUS PROJECT
Mercury loading to a lake and its watershed

Percent increase in methylmercury concentration due to the 120% extra loading of Hg(II) to the lake



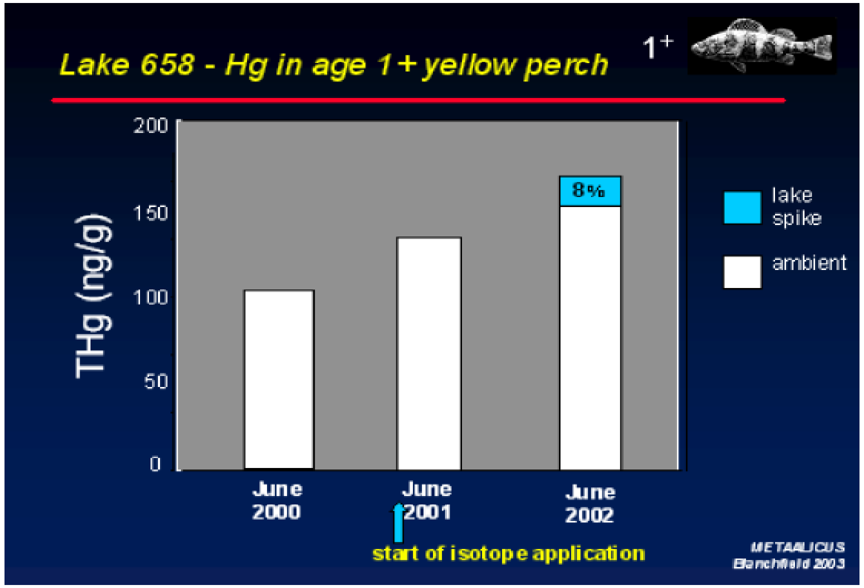


Figure 6. Mercury concentrations in age 1+ yellow perch in Lake 658, for ambient mercury and isotope added to the lake surface (Blanchfield *et. al*)

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International Activities

Mercury issues in UNEP

- **UNEP's Global Mercury Partnership (From the 24th Governing Council)**
  - Artisanal and small scale gold mining, Chlor Alkali process, Coal combustion, Mercury Air Transport and Fate Research, Mercury containing product
  - Expanding Area : Vinyl chloride monomer (VCM) Production, Cement Production, Non-ferrous mining and smelting, Reducing global mercury supply, Waste management
- **Discussion for Adoption of legally binding protocols**

Other international Activities

- Hemispheric Transport of Air Pollutants (HTAP Task Force / UNECE)
- Mercury in the Marine Environment (GESAMP / UNIDO)
- Mercury in Health Care program (WHO)

High Exposures on Mercury

- **Koreans' mercury concentration in blood : 4.34 ppb ('05. NIER)**

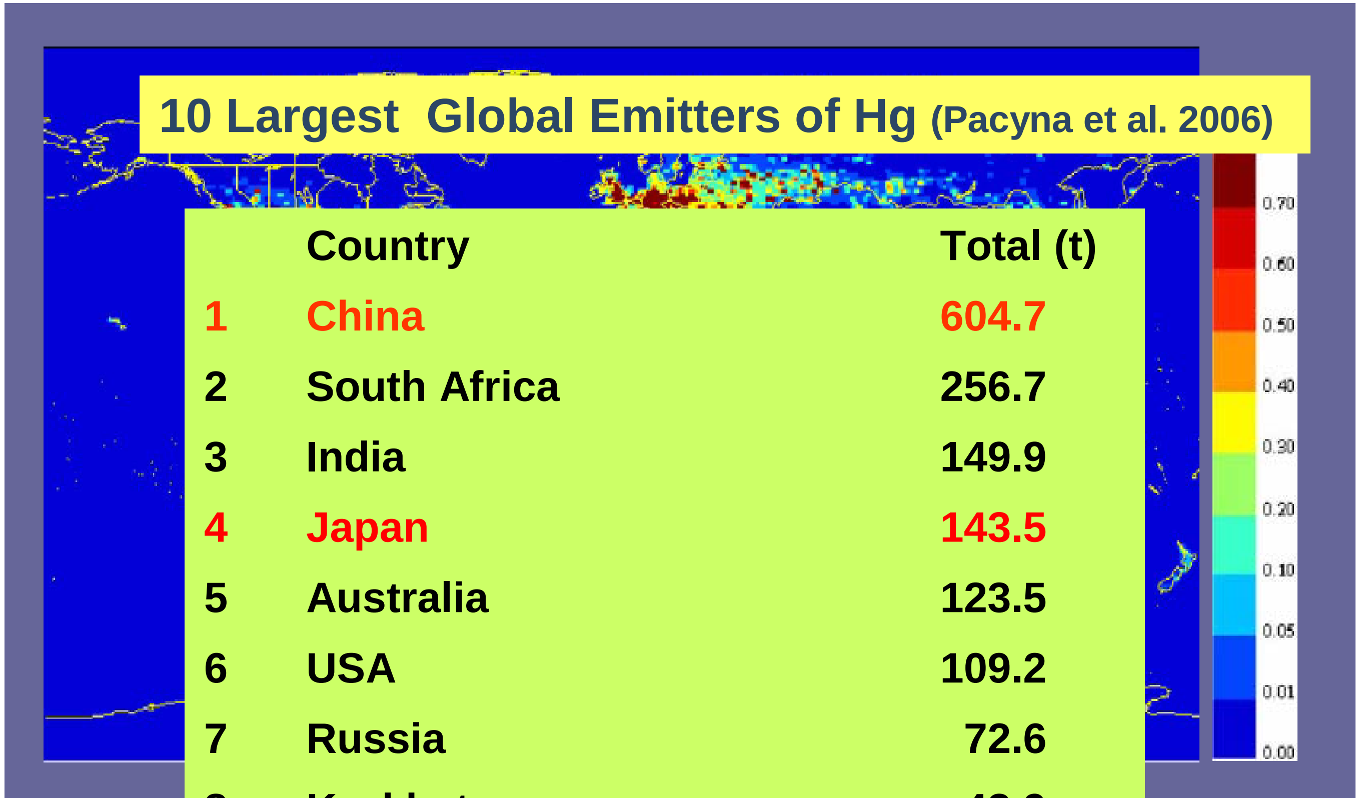
  (USA : 0.83 ppb, Germany : 0.58 ppb)

  - 1/3 of Korean : higher Hg concentrations compared to guideline level of USA EPA (RfD 5.8 ppb)
- **High fish intake rate : 74~94 g/day**

  (USA : 17.5g/day ⇒ 0.3 ppm MeHg of fish: a Tissue Residue Criterion)
- **Total mercury emission : 52 ton/yr ('02, UNEP)**

  (USA :143, China : 533, Japan : 30 ton/yr)
- **Frequent occurrence by long-range transport of Hg**

Global Hg emission

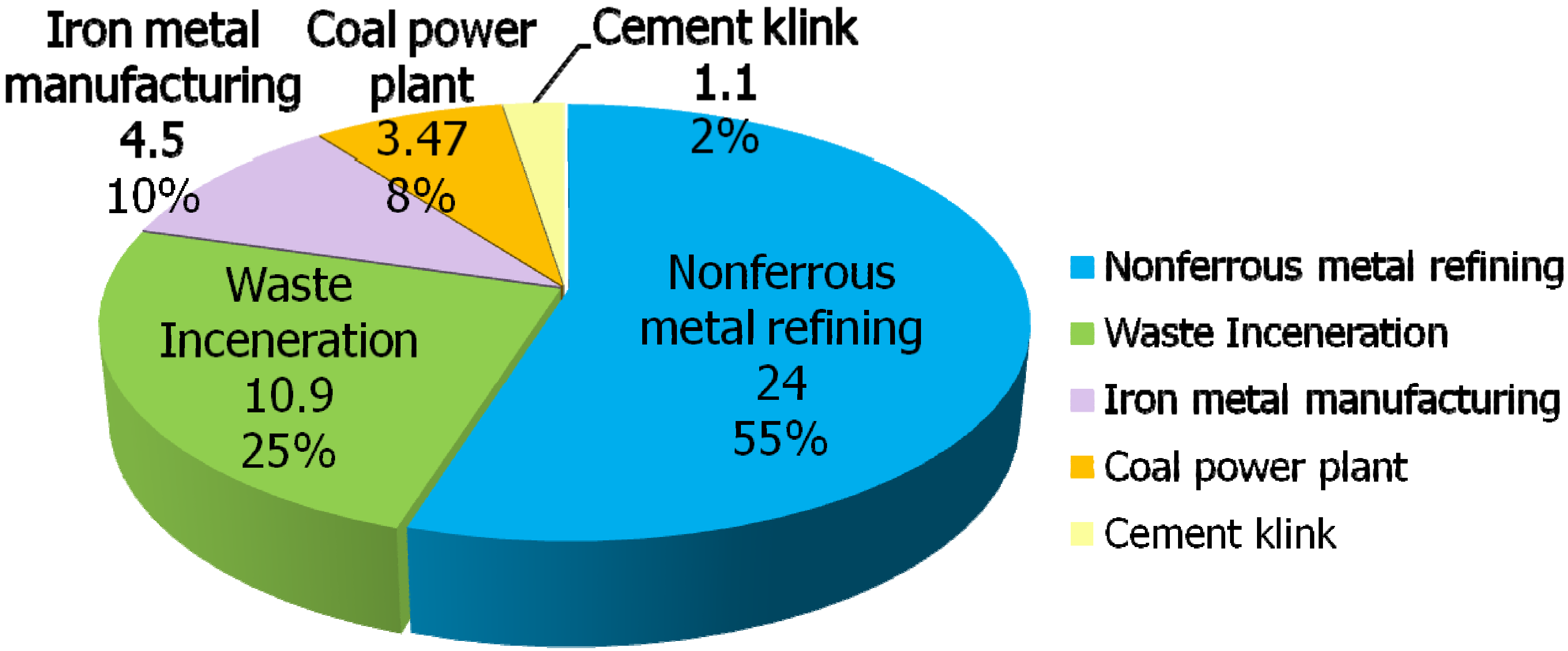


10 Largest Global Emitters of Hg (Pacyna et al. 2006)

	Country	Total (t)
1	China	604.7
2	South Africa	256.7
3	India	149.9
4	Japan	143.5
5	Australia	123.5
6	USA	109.2
7	Russia	72.6
8	Kazkhstan	43.9
9	Korea Democratic Republic	46.0
10	Saudi Arabia	40.7
	Total	1590.7

aroque, *Atmos Environ* (2004)

Mercury Emission Inventory of Korea



The contribution of mercury emissions varies depending on industrial characteristics in each country. (USA: Coal power plant (48%), China: Non-ferrous metal (45%), Japan: Waste incineration (37%))

Mercury Management Policies in Korea

▶ **Battery**

- **Hg-containing battery can not be used more**

▶ **Fluorescent Lamp**

- **Hg content regulation: below 7 mg/per**
- **Recommendation for use of EL mark product**

▶ **Enforcement of Regulations for Hg Emission**

- **including also non-point source emission: 5 mg/Sm^3**
- **Coal-fired power plant, Incineration, boiler : 0.1 mg/Sm^3**
- **Cement/ Steel manufacturing facility : 1 mg/Sm^3**

  : after 2010 -> 0.1 mg/Sm^3

Necessity of Methylmercury Study

Environment

- Emission of Hg^0, Hg^{2+} into Air
- There is no anthropogenic sources for MeHg

Eco-system

Methylmercury Fate & Transport

Where
When
How
How much

Human Health

- >80% of Hg in Fish exist as MeHg (10~1500 ppb)
- >80% of Hg in Human blood exist as MeHg (1~9ppb)

Assessing the effectiveness of Hg control programs is important both in terms of environmental protection and good policy development.

Target of this study

- Establishment of popularized MeHg analytical methods for various matrix

- Investigation of concentration level of T-Hg & MeHg in sediment, freshwater fish and human blood in S. Korea

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Mercury & Methylmercury Background Level

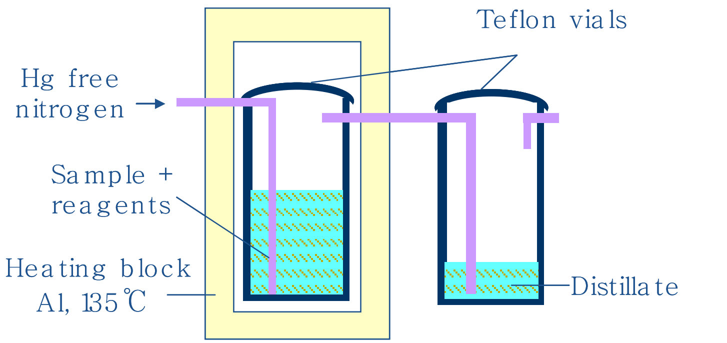
ENVIORNMENTAL MATRIX	T-Hg BACKGROUNG LEVEL	MeHg BACKGROUND LEVEL	% MeHg
AIR (ng/m^3)	1 ~ 170	0 ~ 40	<1%
FRESHWATER (ng/L)	0.2 ~ 15	0.04 ~ 0.8	<10%
SEAWATER (ng/L)	0.3 ~ 15	0.01 ~ 0.5	<5%
SOIL (μg/Kg)	8 ~ 406	0.3 ~ 23	<1%
ESTUARINE SEDIMENT (μg/Kg)	2 ~ 2200	0.06 ~ 70	<5%
RIVER SEDIMENT (μg/Kg)	10 ~ 750	0.3 ~ 30	<5%
FRESHWATER FISH (μg/Kg)	30 ~ 330	28 ~ 310	80%<
SEAWATER FISH (μg/Kg)	10 ~ 1300	10 ~ 1240	80%<
HUMAN BLOOD (μg/Kg)	1 ~ 40	Most of MeHg	80%<
HAIR (μg/Kg)	1000 ~ 5000	Most of MeHg	80%<

Currently used analytical methods for MeHg

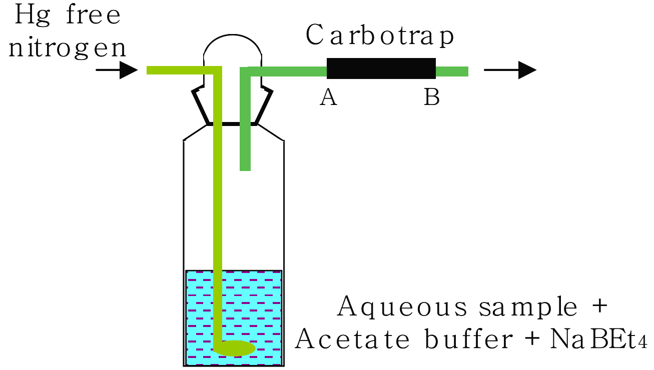
	Westoo (1967)	NIMD (2005)	EPA 1630 (2001)	D.C. Bakter (2007)
Pretreatment	H_2SO_4 (water) KOH (fish)	H_2SO_4 (water) KOH (sediment, fish, blood)	H_2SO_4 (Water,Sediment) KOH (Fish)	KOH (Blood)
Clean-up	Extraction (Toluene-Cystein-Toluene)	Extraction (Dithizone-Na_2S-Dithizone)	Distillation	Extraction (CH_2Cl_2-H_2O)
Separation & Detection	GC-ECD	GC-ECD	GC-CVAFS	GC-ICP-MS
Matrix (Detection Limit)	Water (0.5㎍/kg) Fish (5㎍/kg)	Water, Fish, Sediment, Blood	Water (0.02ng/L) Sediment (0.05㎍/Kg) Fish (1㎍/Kg)	Blood (0.4㎍/L)

Scheme of Distillation & GC-CVAFS method

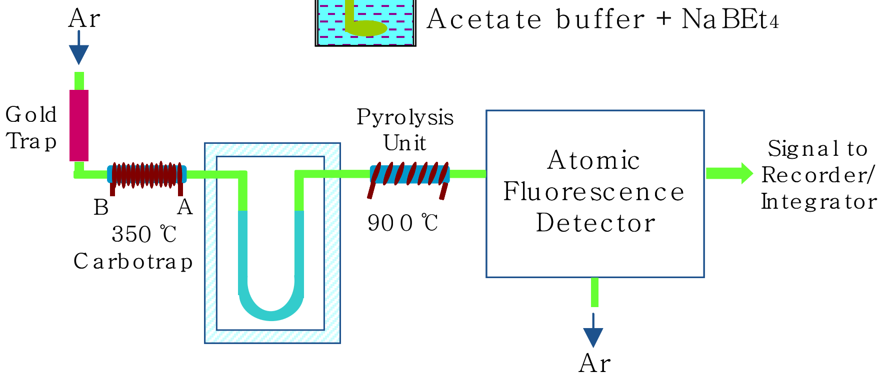
Distillation



Ethylation / Adsorption



Desorption / GC-CVAFS



Methylmercury method using P&T GC-MS

Flow Chart

KOH digestion (Fish, Blood)
1. KOH digestion (Fish, Blood)
2. Dithizone extraction
3. Na_2S back-extraction
4. Ethylation ($NaBEt_4$)
5. P&T GC-MS

H_2SO_4 leaching (soil, Sediment)
1. H_2SO_4 leaching (soil, Sediment)
2. Distillation
3. Ethylation ($NaBEt_4$)
4. P&T GC-MS

* Kim et al, BKCS, 2007, 2293.

Purge & Trap Parameter

Trap	Tenax TA, Supelco
Absorb	40 ℃, 40 mL/min, 15 min
Desorb	200 ℃, 300 mL/min, 3 min
Bake	230 ℃, 300 mL/min, 5 min

GC Parameter

Injector	220 ℃, splitless
Column	DB5-MS (30 m × 250 μm × 0.25 μm)
Carrier gas	He, 1mL/min
GC program	40℃(4 min) to 280℃(5 min) at 15℃ min^{-1}

MS Parameter

SIM mode	MeHg m/z 202,217,246

Method Verification using CRMs (1)

Methylmercury Concentrations (μg/g) in Sediment CRMs					
CRMs	Certified Value	Determined Value		RSD (%)	Recovery (%)
BCR CC 580 (n = 7)	75.0 ± 4.5	GC-MS	74.5±10.6	14.2	85 ~ 108
		GC-CVAFS	73.1±10.1	13.8	83 ~ 111
IAEA 405 (n=7)	5.49 ± 0.53	GC-MS	5.27±0.28	5.5	85 ~ 108
		GC-CVAFS	5.07±0.57	11.3	81 ~ 111

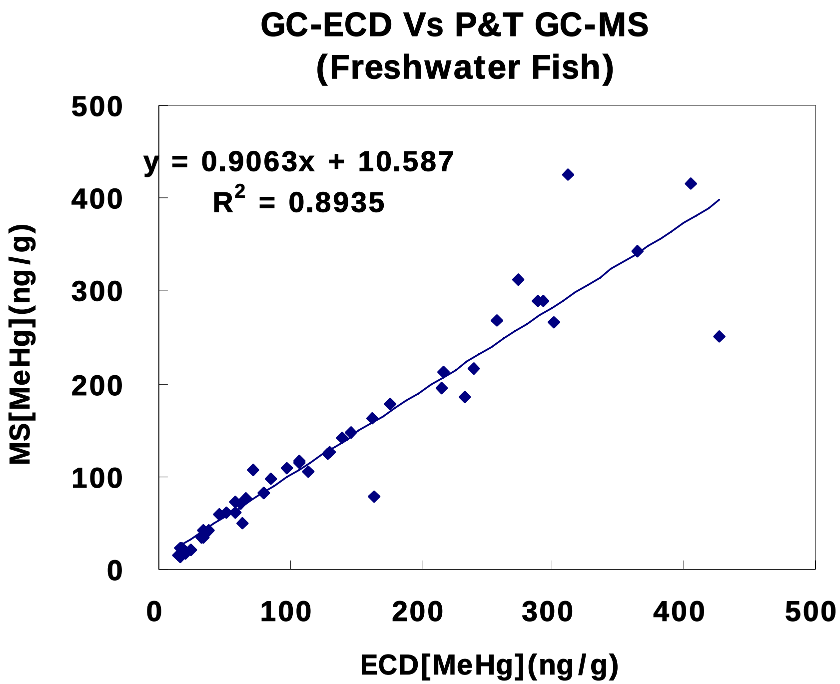
Methylmercury Concentrations (μg/g) in Fish CRMs					
CRMs	Certified Value	Determined Value		RSD (%)	Recovery (%)
IAEA 407 (n = 7)	0.20 ± 0.012	GC-MS	0.19 ± 0.016	3.9	85 ~ 95
		GC-ECD	0.20 ± 0.022	5.7	92 ~ 101
BCR 463 (n=7)	2.83 ± 0.16	GC-MS	2.89 ± 0.26	4.3	98 ~ 108
		GC-ECD	2.76 ± 0.32	5.9	91 ~ 107

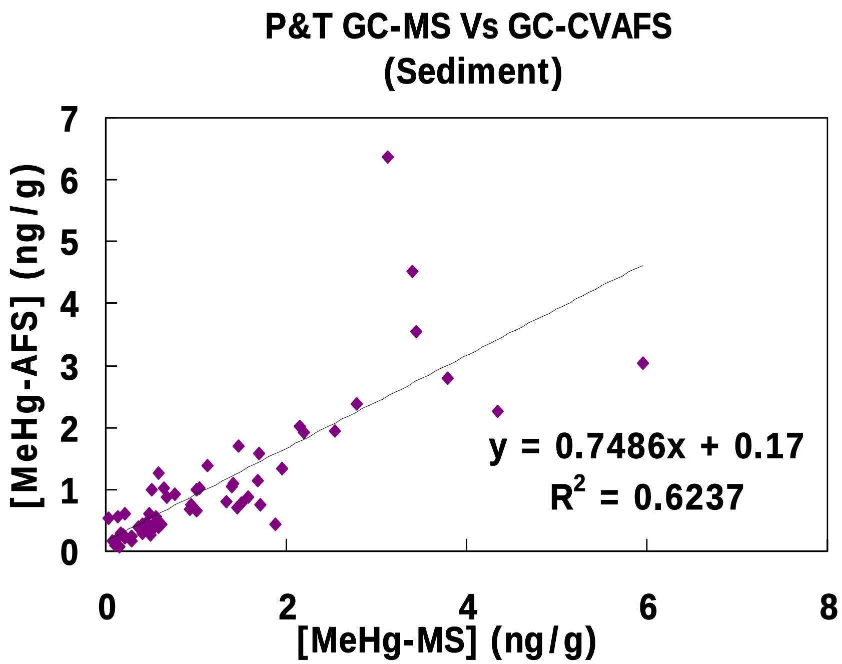
Method Verification using CRMs (2)

Methylmercury Concentrations (ng/g) in Blood CRMs				
Materials	Certified Value	Determined Value	RSD (%)	Recovery(%)
SRM 966 (n = 5)	16.4 ± 1.4	16.6 ± 1.6	4.9	93 ~ 105
M 0605 (n=3)	7.1* (4.6 ~ 9.5)	5.8 ± 0.8	3.3	86 ~ 93
M 0618 (n=3)	26.3* (20.0 ~ 32.3)	23.2 ± 1.6	6.4	79 ~ 91

- The commercially available blood samples were obtained from Centre de Toxicologie du Québec (Québec, Canada). Data from the total mercury analysis and the materials were spiked with methylmercury.

Comparison of P&T GC-MS method





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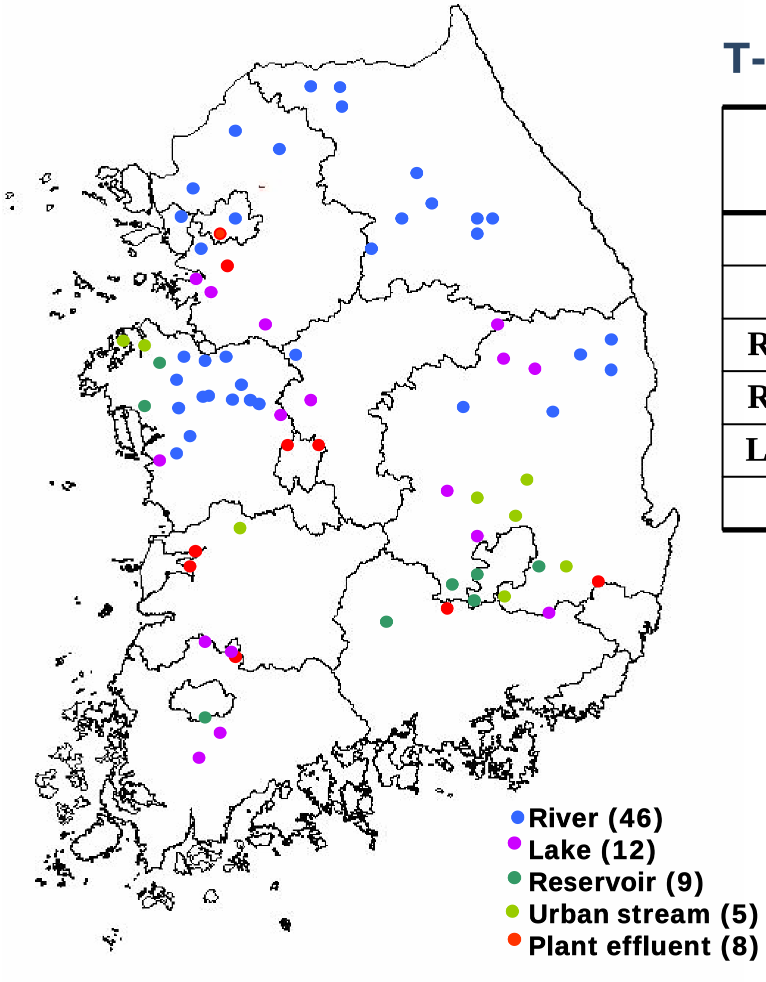
Methylmercury in Sediment



Sediment sampling sites
(Jun. ~ Sep. 2007)

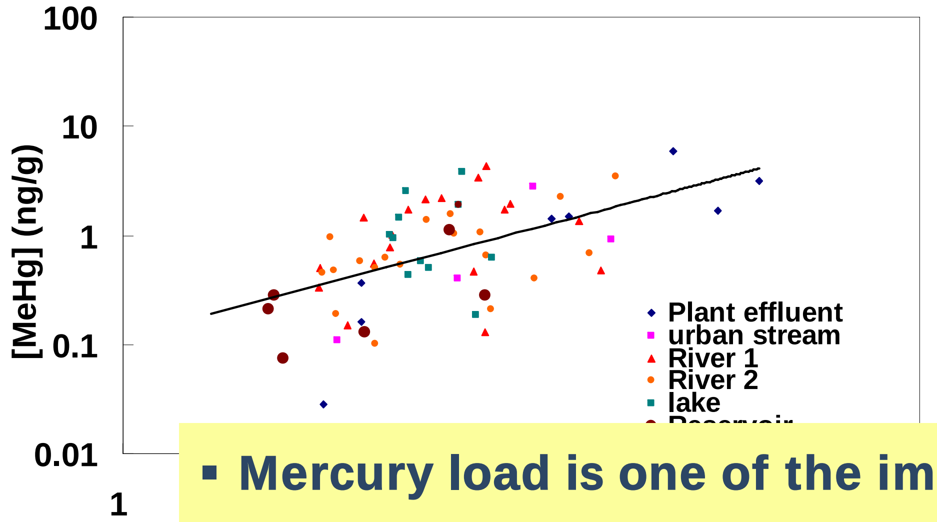
T-Hg & MeHg in Sediment (ng/g, dry weight)

Sampling sites	*T-Hg*		*MeHg [GC-MS]*	
	Average	*Range*	*Average*	*Range*
Plant effluent	**433.4±568.9**	**10.1~1564**	**1.78±1.97**	**N.D.~5.95**
Urban stream	**92.8±114.4**	**7.1~282.6**	**1.06±1.20**	**N.D.~2.78**
River 1 (BOD<3)	**53.5±62.4**	**2.7~251.6**	**1.37±1.15**	**N.D.~4.34**
River 2 (BOD>3)	**57.4±74.8**	**3.6~299.0**	**0.90±0.81**	**N.D.~3.44**
Lake &Reservoir	**27.7±20.9**	**2.4~70.8**	**0.94±0.99**	**N.D.~3.79**
Total	**98.4±232.7**	**2.4~1,564**	**1.16±1.17**	**N.D.~5.95**

* MDL : 0.05 $ng \cdot g^{-1}$

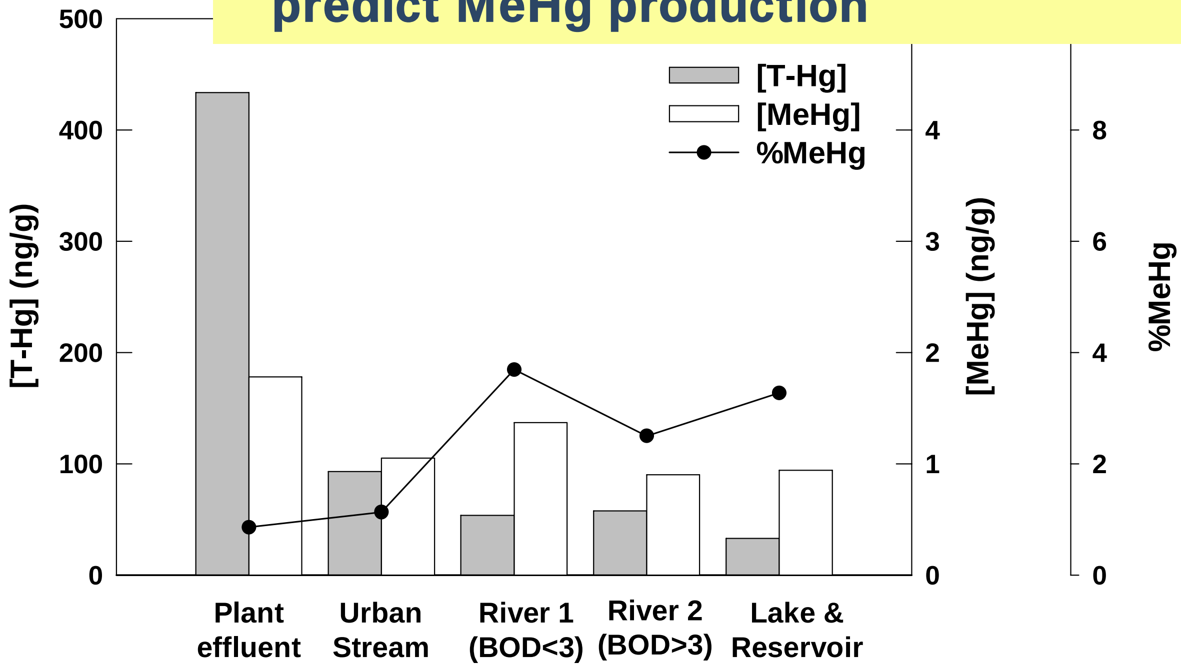
- ***Comparison of MeHg in Sediments***

Sampling sites	*MeHg(ng/g)*	*Ref.*
Florida Everglades, USA	**0.03~10.18**	**Cai et al. (1996)**
Guizhou, China	**1.27~22.5**	**Horvat et al. (2003)**
Minamata, Japan	**0.3~20.0**	**Haraguchi (2000)**
Mobile Alabama river basin, USA	**N.D~3.8**	**Warner et al. (2005)**



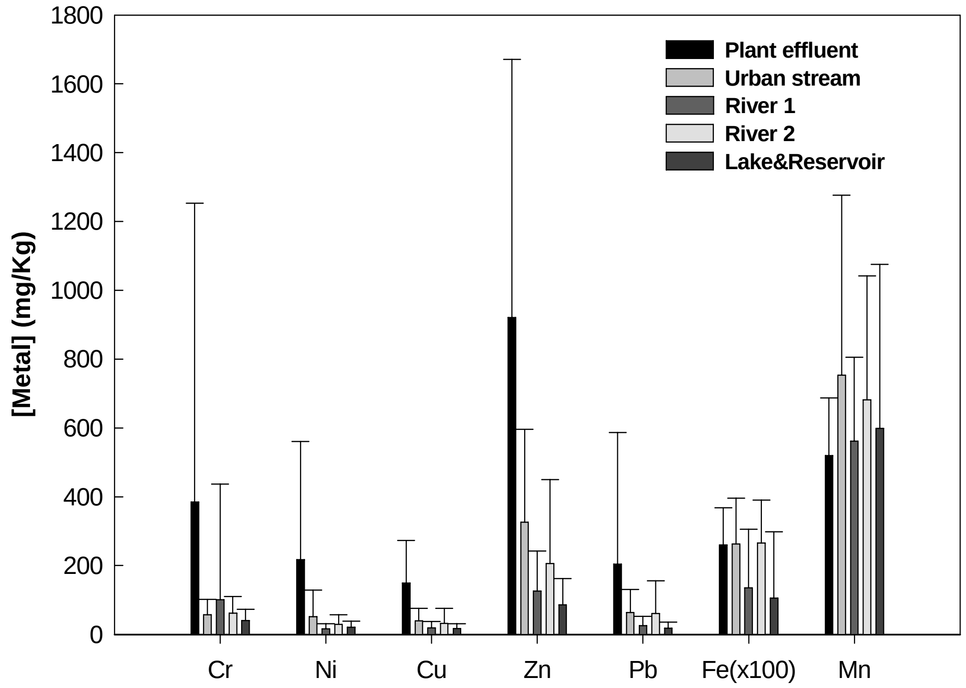
- Statistically significant correlation ($R_{T\text{-}Hg\text{-}MeHg}=0.41$, $p<0.05$),
- A similar value ($R_{T\text{-}Hg\text{-}MeHg}=0.40$) of previous study (Benoit *et al.* 2003).

- **Mercury load is one of the important factor to control MeHg production, but Hg load alone cannot be used to predict MeHg production**



- %MeHg : 2.52±2.39% (n=68)
- The %MeHg in lake and reservoir sites (3.27%) is high compared to plant effluent sites (0.86%) which is due to high total Hg concentration in plant sites.

Sediment metal concentrations & correlations

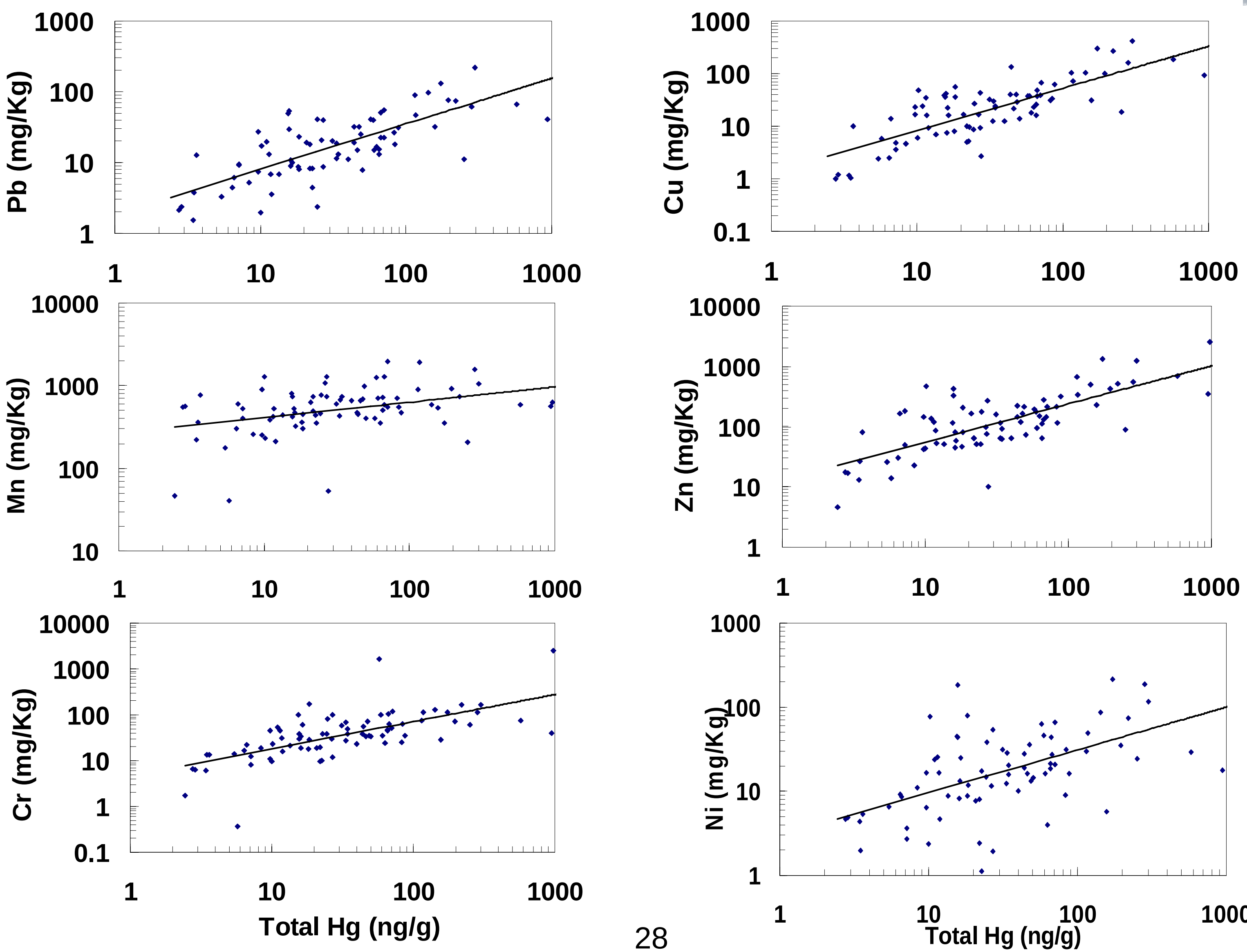


Parameter	*T-Hg*	*MeHg*	*%C*	*%S*
T-Hg	1	0.41	0.41	0.48
MeHg	0.41	1	0.28	NC
%C	0.41	0.28	1	0.67
%S	0.48	NC	0.67	1
Cr	0.39	NC	0.50	0.77
Ni	0.45	NC	0.62	0.89
Zn	0.67	NC	0.59	0.72
Pb	0.51	NC	0.65	0.91
Fe	NC	0.29	NC	NC
Mn	NC	NC	NC	NC

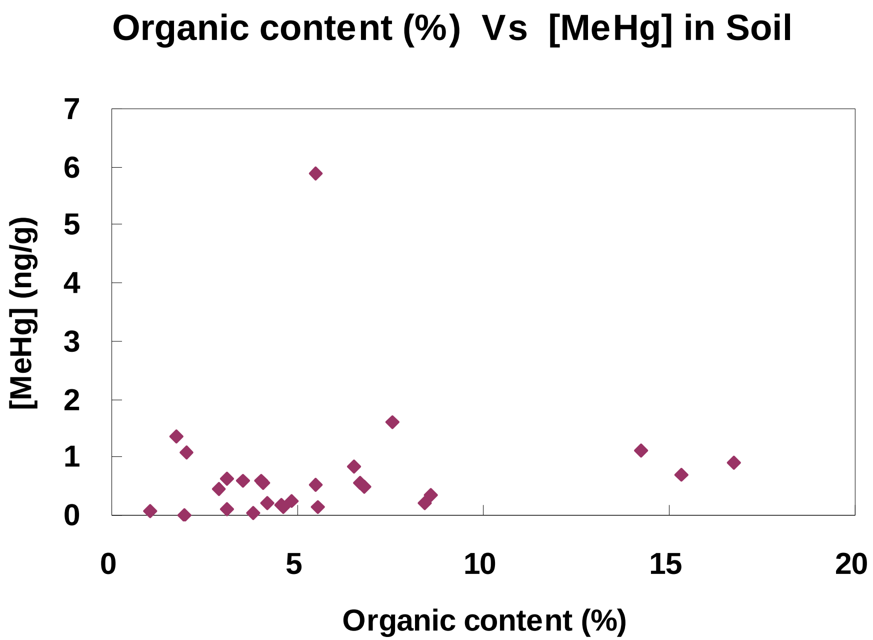
a. NC = no correlation

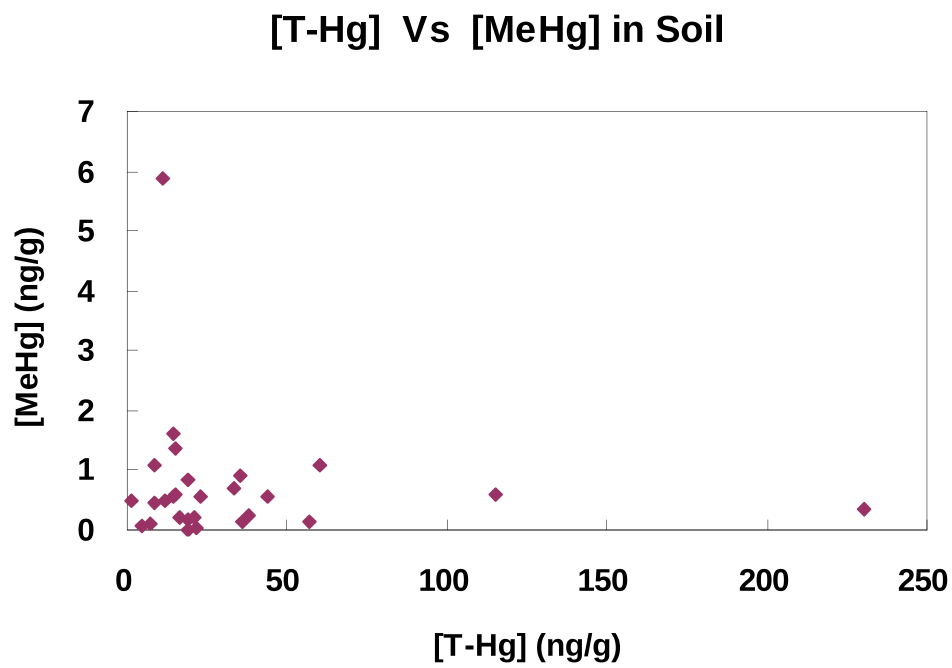
- **Total metal concentrations of plant effluent sites were also relatively high compared to those of other sites**
- **With T-Hg, all parameters showed significant correlation, but with MeHg, no correlation were found.**

Correlation between T-Hg & Metals in Sediment



Analytical Results of MeHg in Soil





- **Among 43 of soil samples, in only 25 samples MeHg were detected.**
- **Total mercury concentrations of soil were in the range of 1.27 ~ 230.40 ng·g^{-1} (mean 34.3 ng·g^{-1}) and methylmercury concentrations were in the range of N.D. ~ 5.88 ng·g^{-1} (<u>mean 0.78 ng·g^{-1}</u>) (n=25).**

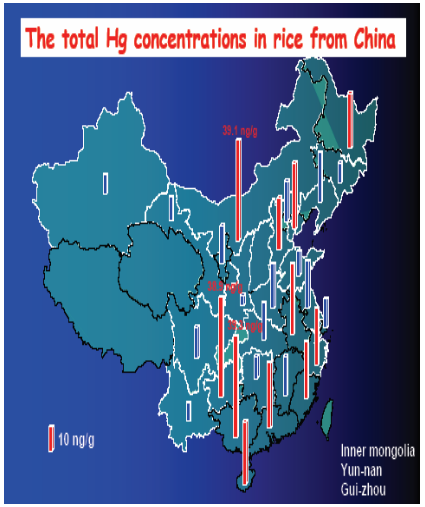
THg and MeHg in crops from Hg mining areas (dw ng/g) in Gui-zhou
(Qiu & Feng, 2005, Appl Geochem)

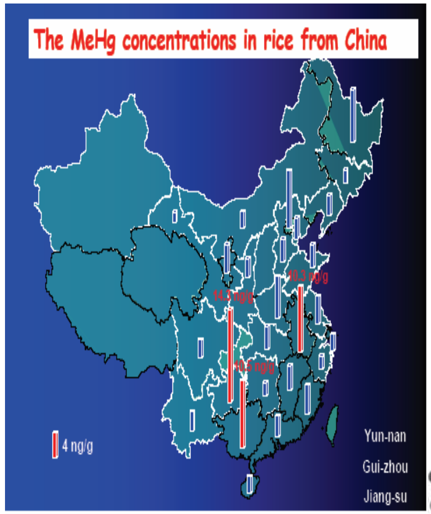
	Species	Min	Max	Mean	n	National Advisory Limit- MeHg
Corn	THg	9.0	572	143	11	20
	MeHg	0.3	1.3	0.7		
Rice	THg	9.0	1408	234	25	
	MeHg	1.1	144	20.1		
Vegetable	THg	103	1156	415	17	10
	MeHg	0.4	4.2	1.9		

MeHg in soil samples from Hg mining areas (dw, ng/g)

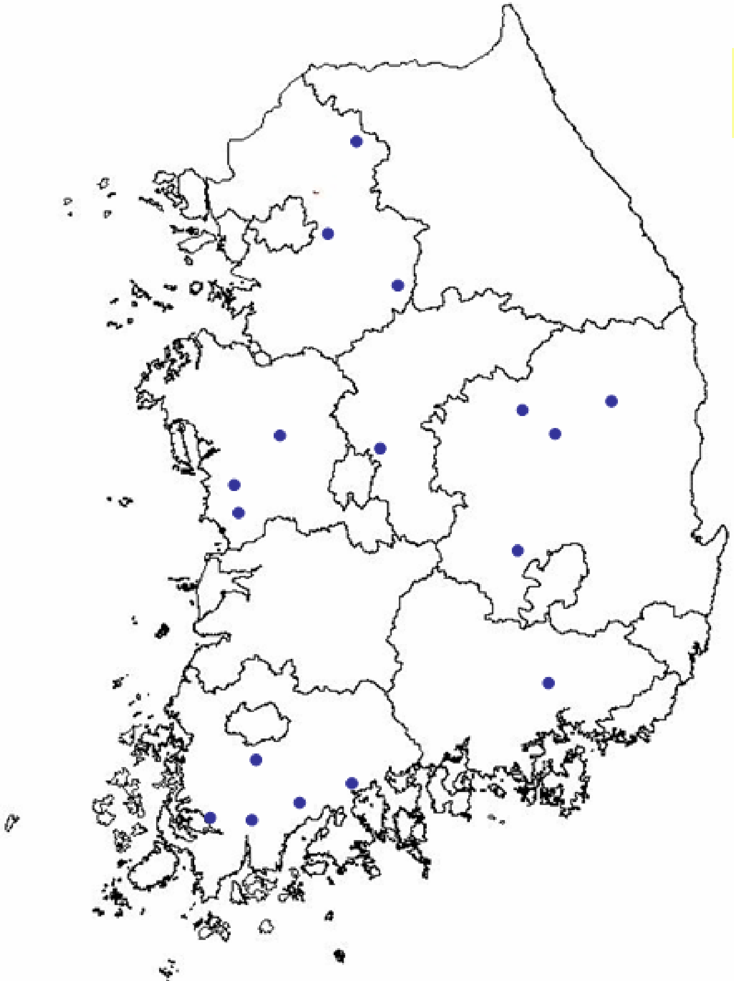
Soil	Range	Mean
Cornfields	0.091-2.3	0.77
Paddies	1.0-20	4.4

Is rice safe for consumption? (GB Jiang, 2006)



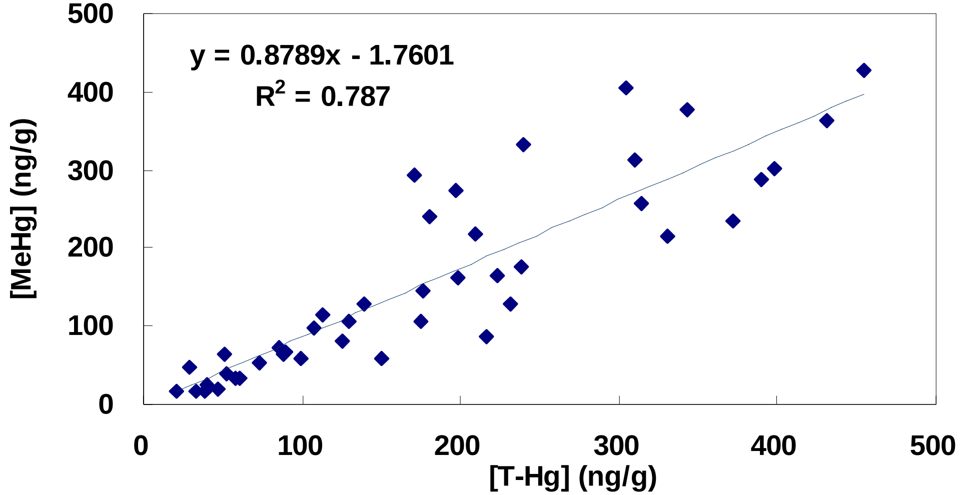


Methylmercury in Freshwater Fish



Freshwater Fish sampling sites
(Jun. ~ Sep. 2006)

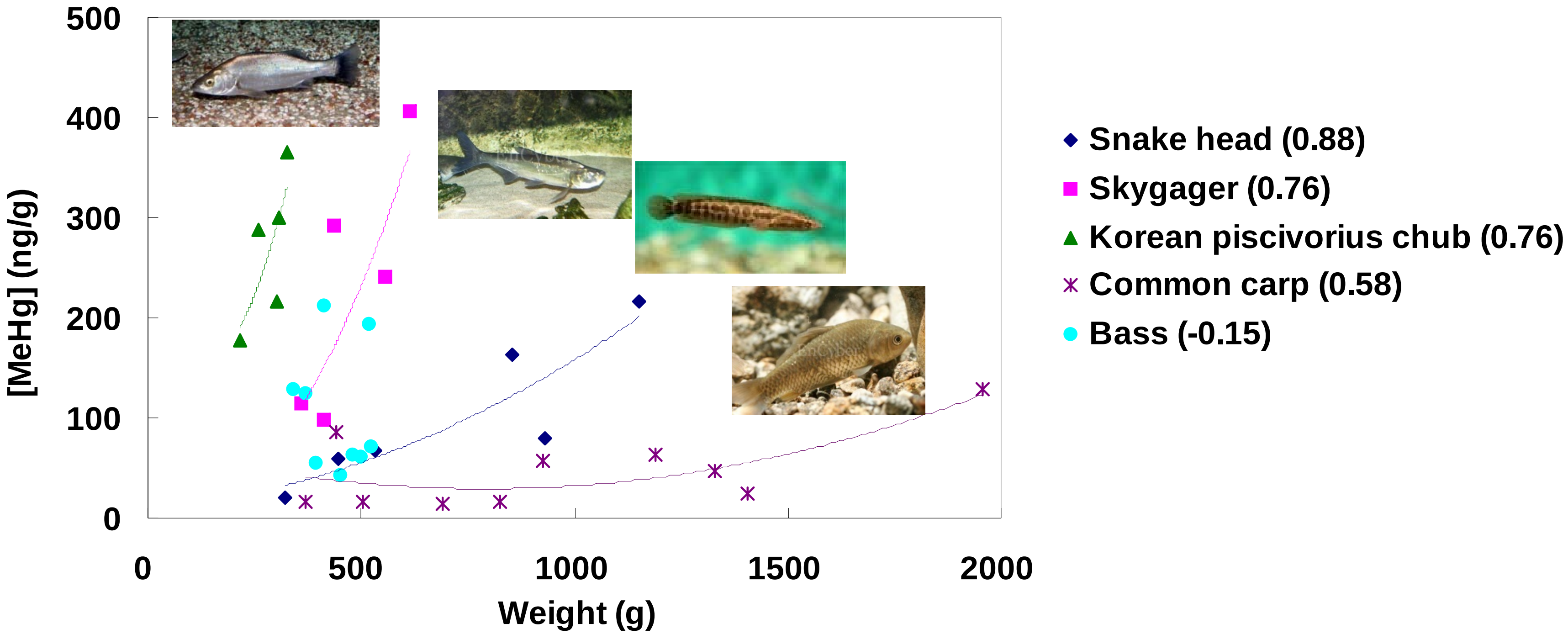
T-Hg & MeHg in Freshwater fish (ng/g, wet weight)



Crusian carp	2	59.9±3.0	42.9±0.7
Common carp	11	49.2±34.4	50.3±41.1
Leather carp	2	35.1±16.7	24.2±10.2
Japanese dace	1	183.16	141.4

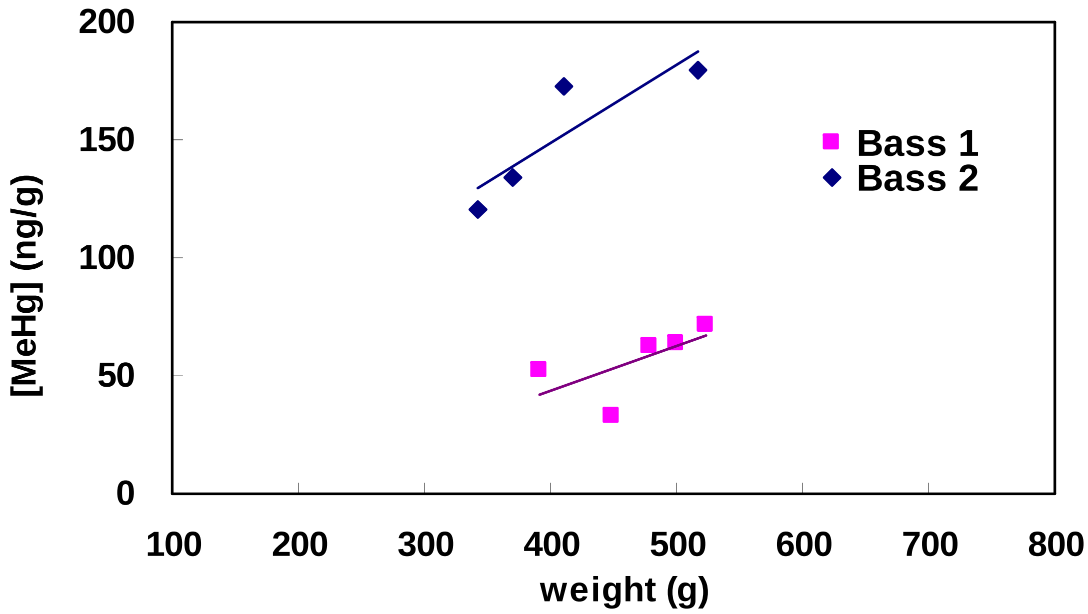
The proportion of methylmercury to total mercury in all fish samples was in the range of 69.1 ~ 103.5% indicated that majority of the total mercury in fish is in the form of methylmercury.

Correlations between MeHg & fish weight (1)



- MeHg concentrations and accumulation rates are increased with trophic level and different species show different patterns.
- MeHg concentrations are significantly correlated with fish body weight (R = 0.58 ~ 0.88, $p<0.05$) except Largemouth bass.

Correlations between MeHg & fish weight (2)

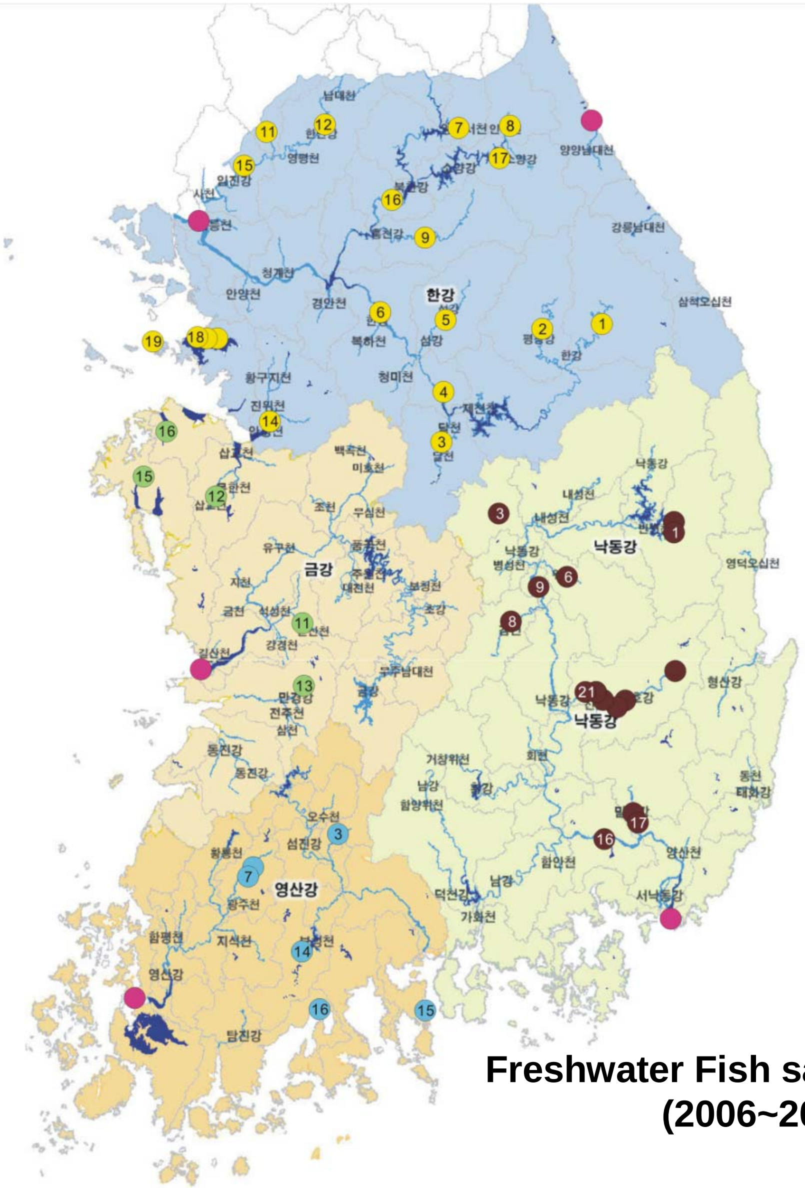


Dam-yang Artificial reservoir

Ju-Nam reservoir

- **Two groups were collected from different locations, i.e. Bass 1 from Ju-Nam reservoir and Bass 2 from Dam-Yang artificial reservoir, which might imply the difference of food availability, methylmercury concentrations in the prey and water chemistry.**

National survey for Mercury in freshwater fish



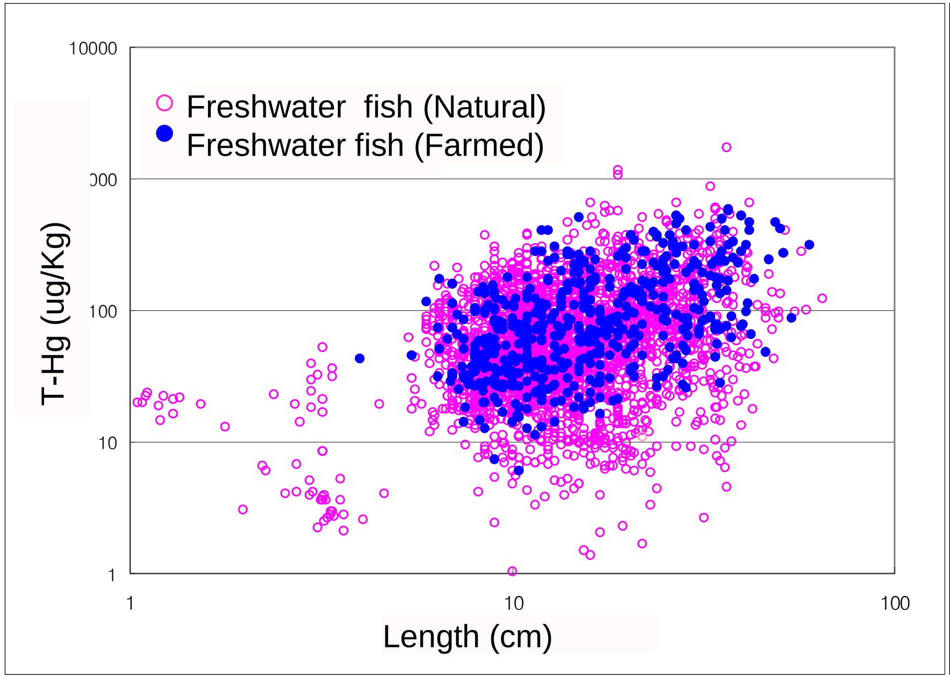
Freshwater Fish sampling sites
(2006~2007)

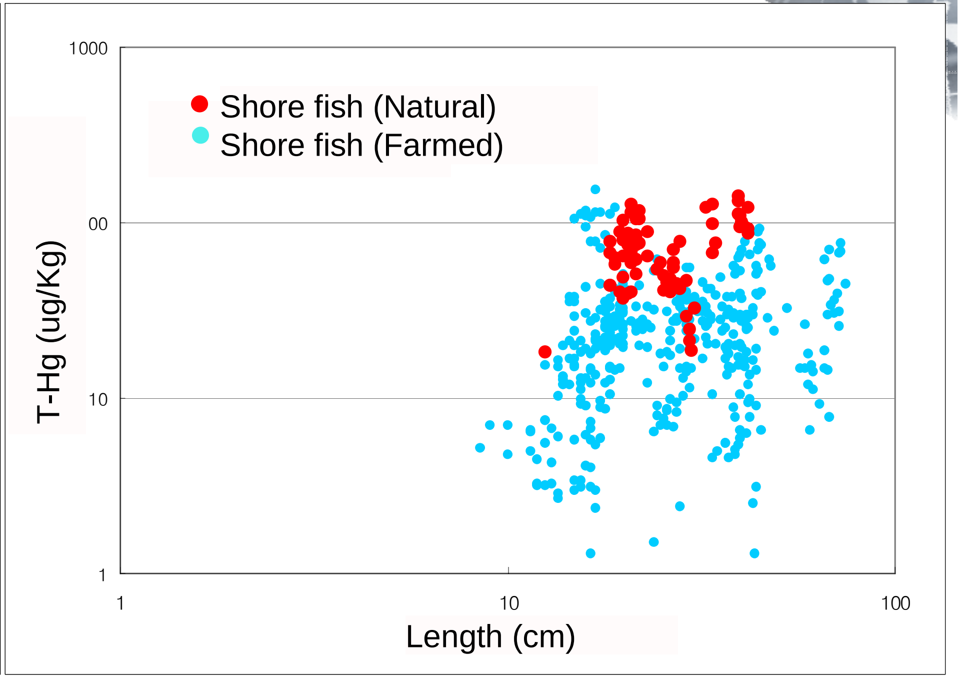
Basin	No of sites	No of species	No of samples
Han River	18	40	905
Geum River	5	17	226
Nak-dong River	15	33	286
Young-san River	4	24	418
Total	45	58	2,004

T-Hg in Freshwater fish (1)

Fishes	n	Length	T-Hg		%MeHg
			Average	Range	
mandarin fish	65	18.3 ± 4.1	173.1 ± 106.9	26.8 ~527	82.0
catfish	79	29.1 ± 7.2	136.2 ± 220.4	18.9 ~1527.6	74.5
snakehead	16	39.3 ± 8.8	109.2 ± 104.7	28.2~414.9	78.9
skin carp	41	20.3 ± 8.1	90.2 ± 77	17.3 ~ 336.7	72.7
Korean dark sleeper	48	11.0 ± 2.9	85.7 ± 99.8	14.5 ~ 480.4	78.8
Korean bullhead	143	15.0 ± 3.5	80.9 ± 63	3.9~ 428.7	75.4
striped shinner	80	10.6 ± 1.8	80.1 ± 49.1	7.5 ~ 279.6	88.1
Coreoperca herzi	87	10.3 ± 2.4	73.3 ± 71	7.4 ~ 424.9	74.4
long-nosed barbel	31	13.4 ± 1.4	66.1 ± 37.9	12.1 ~ 139.5	78.5
korean piscivorous chub	57	11.5 ± 3.5	60.8 ± 39.3	12.5 ~ 153.7	77.1
pale chub	160	10.3 ± 2.1	57.6 ± 35.4	6.9 ~ 189	85.1
crucian carp	226	16.1 ± 6.0	55.4 ± 50.9	4.9~446.2	83.9
largemouth bass	29	15.8 ± 7.6	50.6 ± 32.8	21.5~ 164.4	79.1
black bullhead	57	13.4 ± 4.0	44.6 ± 36.1	9.9~ 274.8	78.9
common carp	68	27.7 ± 11.3	38.8 ± 26.1	4.5 ~133.2	99.8
blue gill	88	9.8 ± 3.1	35.3 ± 15.8	9.3~ 73	82.7
goby minnow	44	13.7 ± 1.8	32.5 ± 19.1	3.8 ~ 89.5	83.0
Goby	104	18.2 ± 3.4	13 ± 11.2	0.4~ 55.8	95.4

Comparison of T-Hg between Natural and Farmed fish



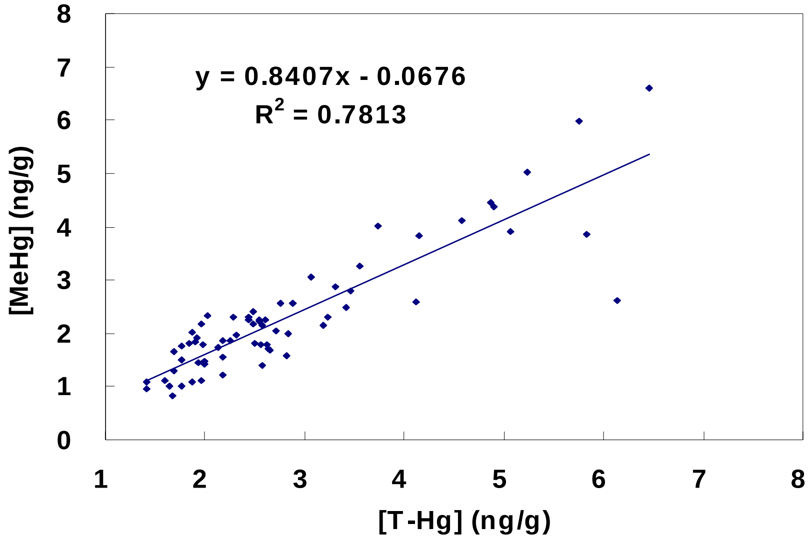


Methylation Triggered by Fish farming practices?

-Methylation triggered by moderate levels of organic enrichment through fish farming (Bay of Fundy, Vancouver) (Sunderland et al, 2006)

-Elevated levels of Hg in demersal rockfishes near salmon farms due to a combination of higher rockfish trophic position and higher Hg levels in prey near farms (coastal British Columbia) (Debruyn et al, 2006)

Methylmercury in Human blood



Mercury exposure study in children is of interest because of continuing neurobehavioral development during this life stage.

The difference between adults and children may be due to differences in toxicokinetics, dose frequency, body size etc.

Mercury levels in children and adults in various regions

Author	*Region*	*Sample*	*Mean of T-Hg* (㎍·L^{-1})
This study	Republic of Korea	85 children aged 10-12 years	2.57 (2.01 for MeHg) (4.34 for adults)
Schober et al. 2003	USA	705 children aged 1-5 years 1709 adults aged 16-49 years	0.34 1.02
Seifert et al. 2000	German	712 children aged 6-14 years 3958 adults aged 25-69 years	0.49 0.77
Batáriová et al. 2006	Czech	333 children aged 8-10 years 1188 adults aged 18-58 years	0.42 0.89

Talk Outline

Content Title

- Introduction of Methylmercury
- Mercury issues in Korea
- Analytical methods for Methylmercury
- Methylmercury distribution in environment
- **Current Status and Future Plan**

Current study

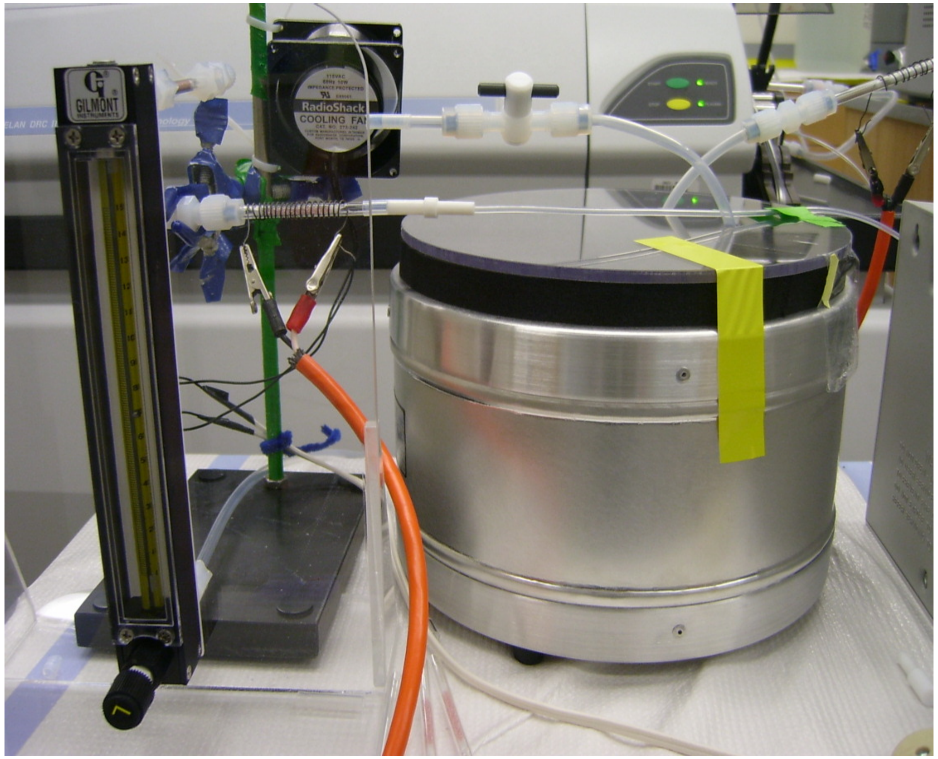
- Tae-An coal power plant (green)
- Studied Reservoir sites (red)
- Control site (Baegreong-do) (cyan)
- Power Plants (yellow)

Sites	TGM (ng/m^3) (n=7)
Tae-An	3.6
Anmyeon-do	4.5
Baegyeong-do	1.2

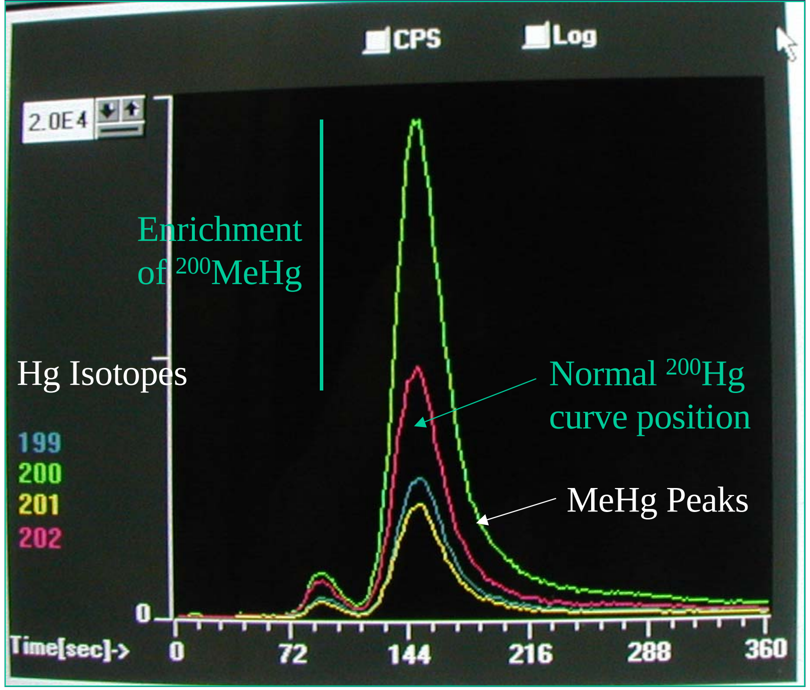
Sampled during 2008. Aug.~Sep.
Global level 1.5~1.8 ng/m^3 (Landis et al. 2002)

Sites	Basin Area (ha)	Capacity (x10^{3}m^3)
Ban-kye	287	446.6
Chang-ki	94	309.1
Shin-doo	138	182.1

Measuring Hg Methylation and MeHg Demethylation Potentials Using Stable Isotopes



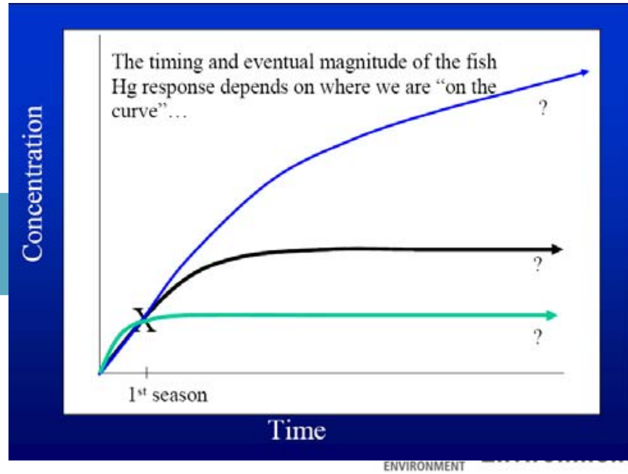
MeHg analysis by aqueous ethylation GC separation and ICP-MS detection



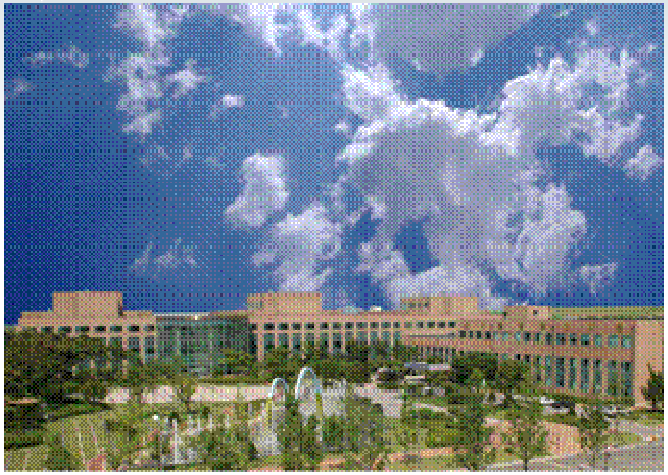
Advantages:
1. Lower concentration can be spiked
2. Both methylation and demethylation can be simultaneously measured
3. Short-term assays are possible (hours) allowing measurement of actual initial rates
4. Steady state concentrations can be related to rate constants, assuming first order processes

Conclusion & Future plan

- **Data gap concerning Hg & MeHg concentrations in different ecological compartments, speciation, bioavailability & toxicity.**
- **Intensive & Long-term Hg & MeHg monitoring are needed to access current status and to evaluate the effectiveness of Hg reduction policies.**
- **Continuing investigation for Hg & MeHg exposure on children and women & Establishment of fish consumption advisories to protect at risk population.**



Thank You !



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