Analytical methodologies for mercury in environmental monitoring

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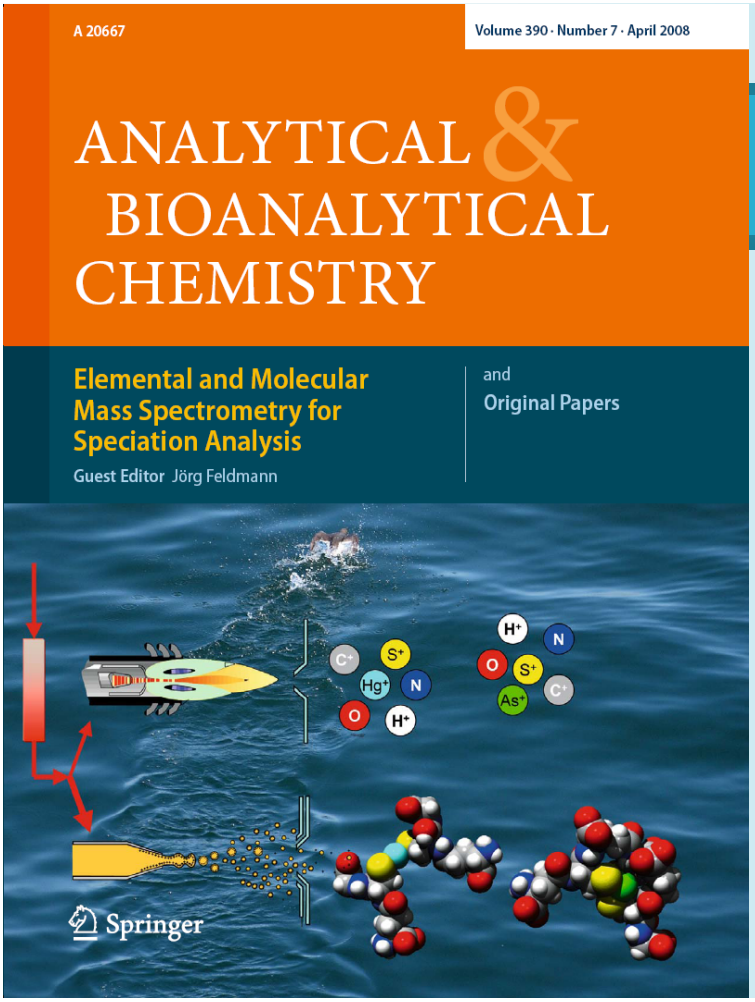
Trace Element Speciation Laboratory Aberdeen
Cromarty, May 2008

Special thanks to:

Instrumentation & lab

- 4 ICPMS (2 ICP-qMS, ICP-TOFMS, HR-ICPMS)
  - GC-ICPMS, HPLC-ICPMS,
  - Laser ablation-ICPMS
- 3 ES-MS (ES-qMS, ES-IT-MS, Orbitrap)
  - Coupled to HPLC and parallel to ICPMS
- AFS
  - GC-pyrolysis-AFS, HPLC-UV(ox)-AFS
- 3 AAS (2 FAAS, 1 GFAAS)
- GC-MS, 4 GC-FID
- Spring 2009 (clean lab, Cat II Microbiology, synthetic lab)
- (access to 400 & 600 MHz NMR)



- We try to understand environmental processes at a molecular level
- Molecular forms of metals in biota and in the environment.

outline

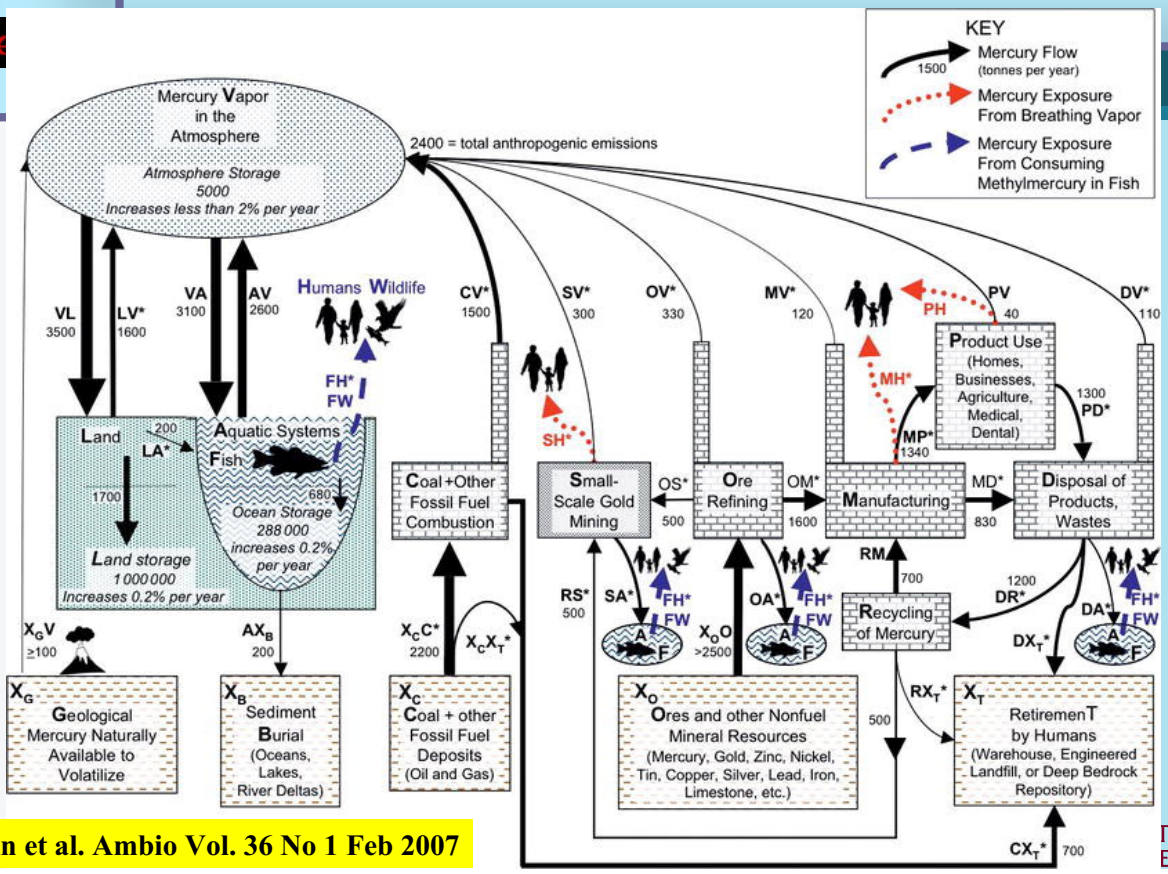
- Sources and sinks of mercury
- Analytical methodologies
- Target samples
  - Sampling, storage
  - Sample preparation
- Discussion points

Mercury

- No known essential biological function
- Industrial use: mercury switches, thermostats, thermometer, medications, preservatives, antiseptics, pesticides…
- Amalgamates with gold and silver: use in mining and as a dental fillings
- Geogenic as ore (Cinnabar) and as trace element in coal: Partition of volatile Hg into air during coal combustion
- High-level Hg exposure produces serious neurological problems in adults and in children born to mothers with high mercury levels

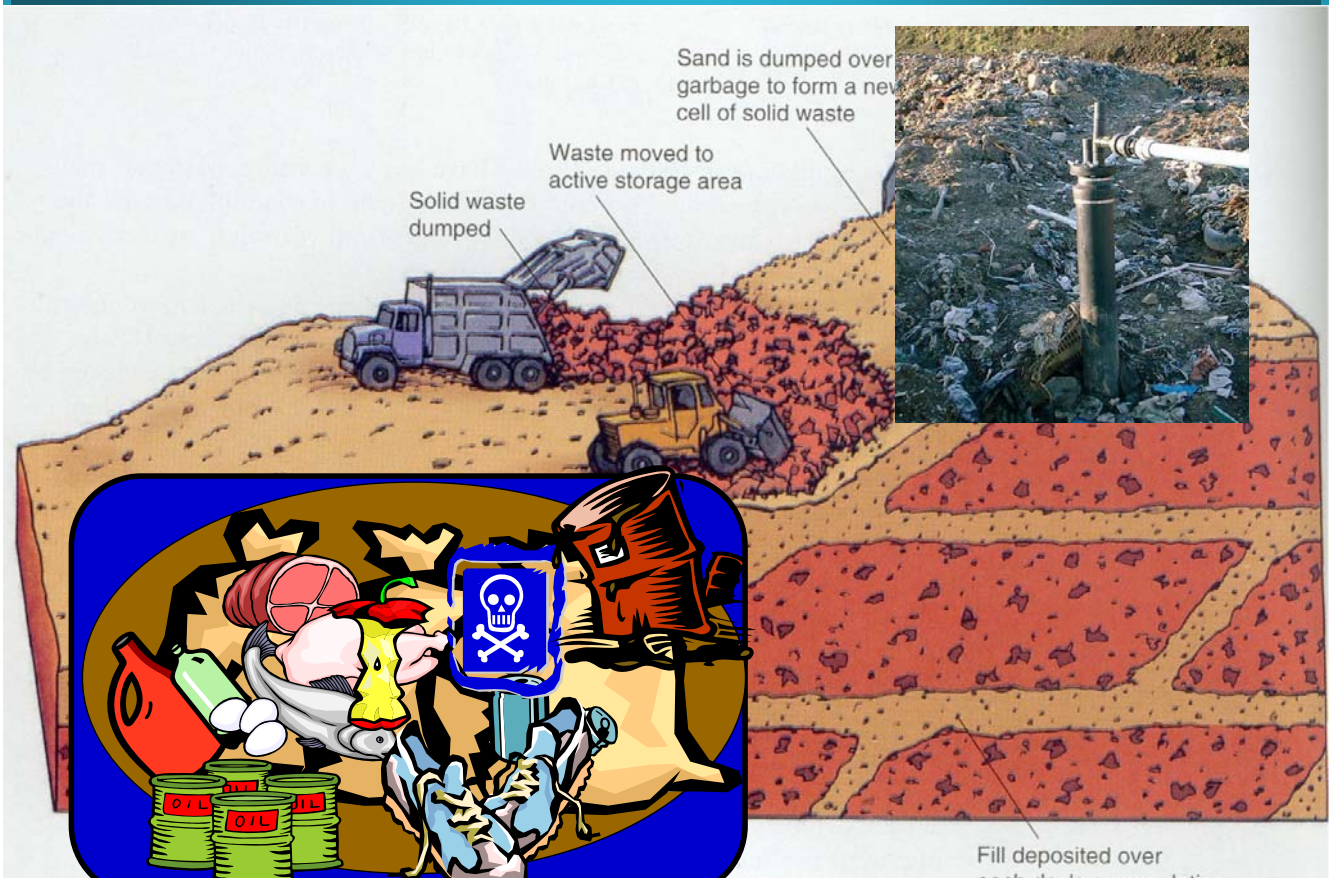
❖ **Mercury is a global pollutant!**

Mercury in the Environment

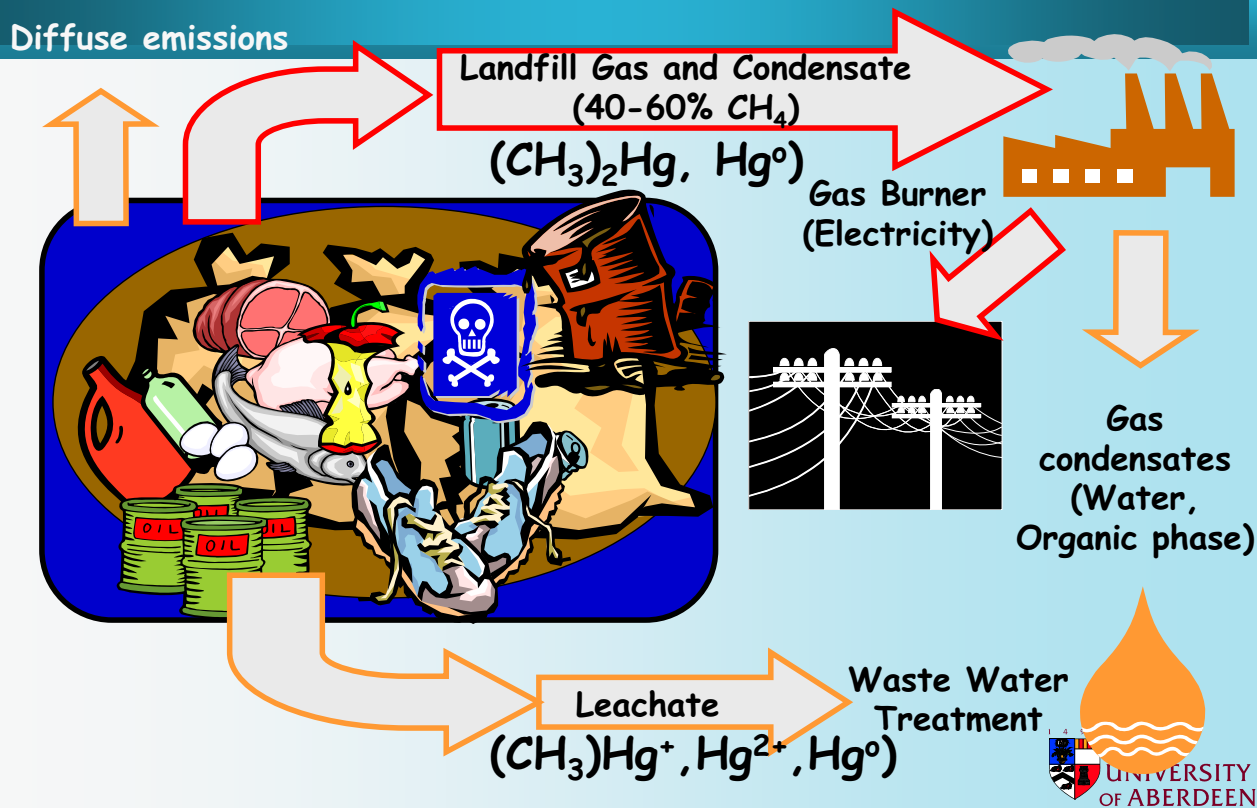


E.B. Swain et al. Ambio Vol. 36 No 1 Feb 2007

Waste Disposal on landfills



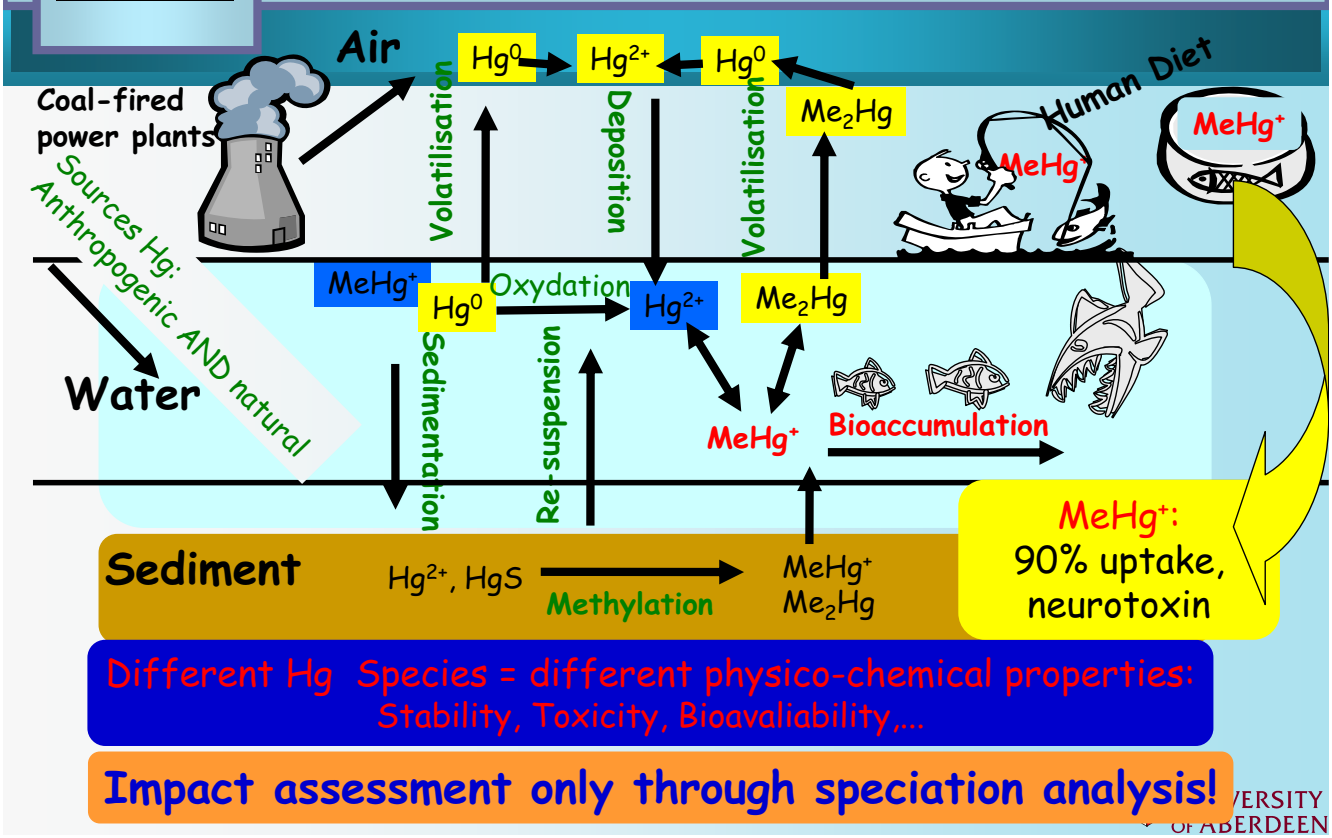
Emissions from Landfills



Waste streams which are potentially large diffuse mercury sources

- natural gas residues
  - Scales in pipelines
  - Dust from filters, charcoal, condensates
- Coal, sewage sludge and waste incineration waste products, furnace slags
- Waste products from mercury catalysts
- Paints, batteries, switches, light sources biocides, pharmaceuticals, cosmetics
- Amalgam fillings
- Thermometer, manometers,...

The Aquatic Mercury Cycle



Speciation of mercury

- Toxicity: $MeHg^+$ >> inorg. Hg.
- Source: Coal burning and waste incineration.
- Bioaccumulation in the food chain.
- Biomethylation: Inorg. Hg -> $MeHg^+$
- ML: Tot Hg: 0.5 mg/kg (*1.0 mg/kg).

→ preditorial fish mainly MeHg

Seawater
0,005 µg/L (2% $MeHg^+$)

Plankton algae
11 µg/kg (25% $MeHg^+$)

Zooplankton
11 µg/kg (25% $MeHg^+$)

Anchovy
40 µg/kg (90% $MeHg^+$)

Ref: Bjerregaard, 1988

Hg speciation in biological and environmental samples

Table 1. Typical total and methyl mercury concentrations in environmental and biological matrices, compiled from US Environmental Protection Agency data (32).

	Hg_{Tot}	$CH_3Hg(II)$
Air	1–170*	0–40*
Precipitation	4–90†	0.04–0.6†
Fresh water	0.2–15†	0.04–0.8†
Sea water	0.3–15†	0.01–0.5†
Soil	8–406‡	0.3–23‡
Ocean sediments	2–2200‡	0.06–70‡
Lake sediments	10–750‡	0.3–30‡
Fresh water fish	30–330§	28–310§
Marine fish	10–1300§	10–1240§

* ng m^{-3}; † ng l^{-1}; ‡ ng g^{-1} dry weight; § ng g^{-1} wet weight

US Environmental Protection Agency. 1997. Mercury Study: Report to Congress, Vol. III, Fate and Transport of Mercury in the Environment. EPA-452/R-97-005. US EPA, Office of Air Quality Planning & Standards and Office of Research and Development.

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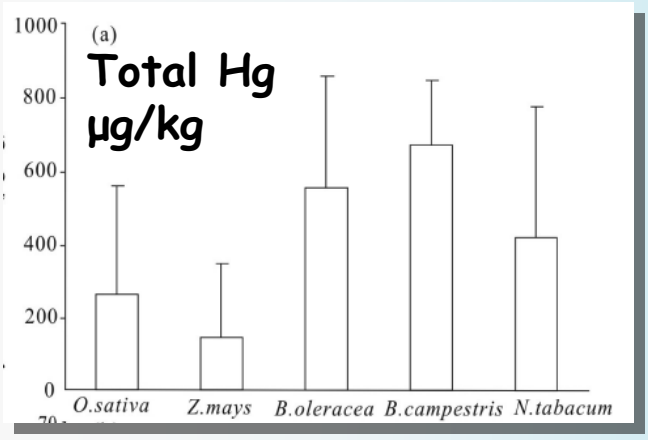
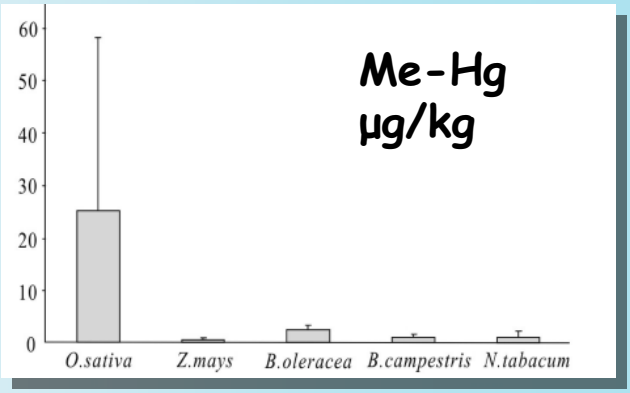
Rice: a methylmercury hyperaccumulator an emerging problem ?

→WHO RfC 1.6 µg/kg bw/week → 0.23 µg/kg bw/d →14-16 µg/d





→US-EPA: 0.1 µg/kg bw/d → 6-7 µg/d
25 µg/kg rice with 300 g rice daily = 7.5 µg/d

J. Agric. Food Chem. **2008**, *56*, 2465-2468

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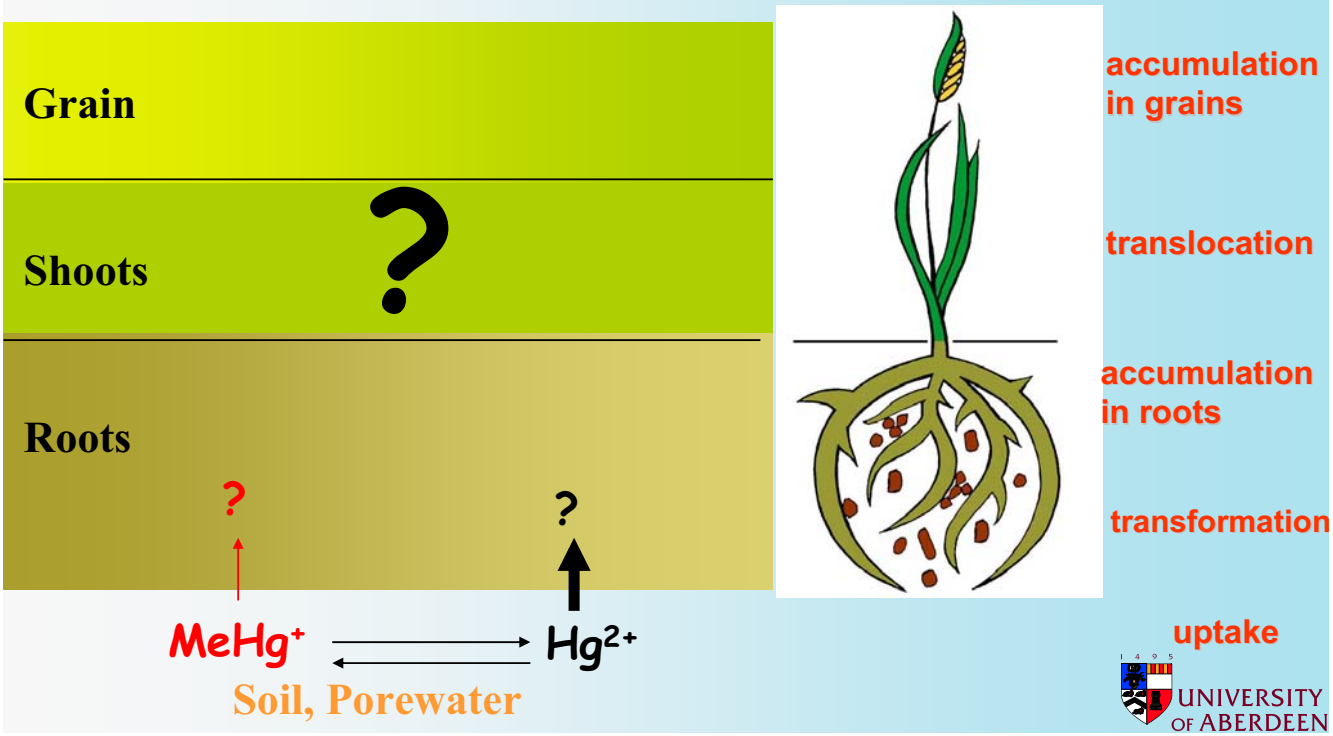
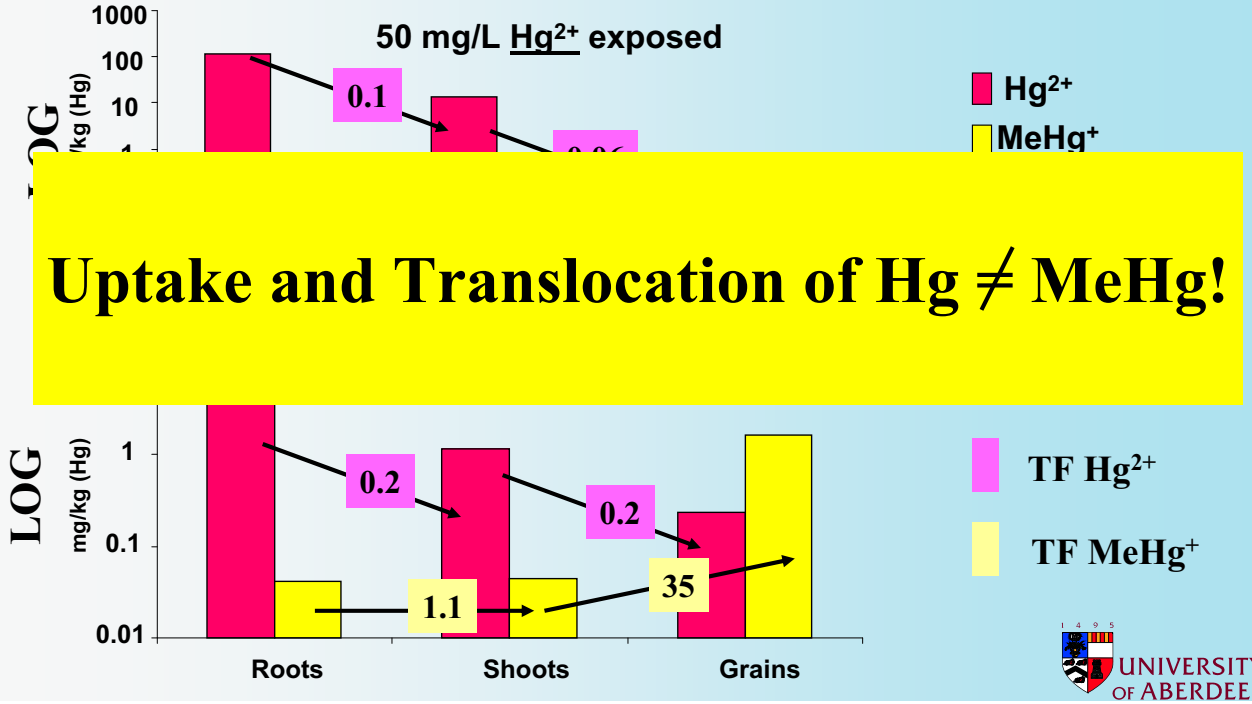
Preferential accumulation of MeHg in rice

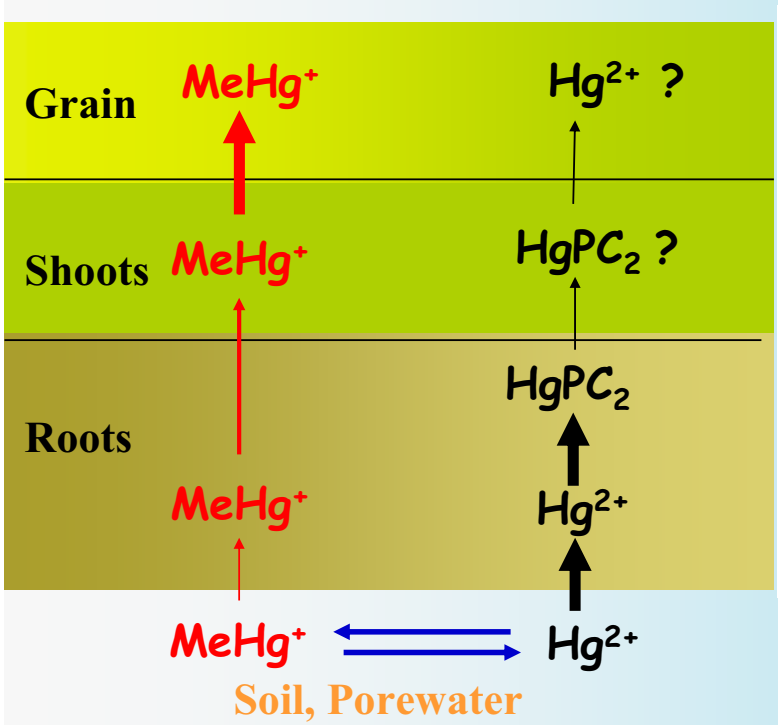


Why do we find MeHg in rice?

Hg^{2+} and $MeHg^{+}$ in roots, shoots and grains by GC-ICP-MS after derivatisation with $NaBPr_4$



Preferential accumulation of MeHg in rice



accumulation in grains

translocation

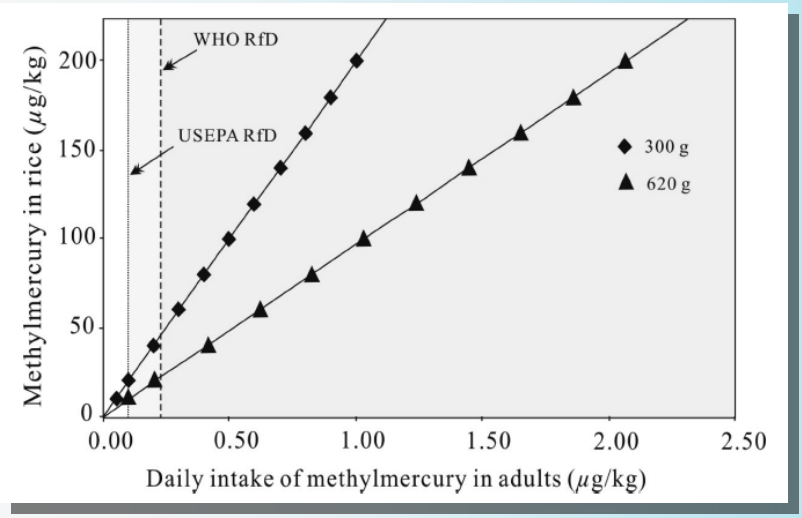
accumulation in roots

transformation

uptake

Importance of rice as the major staple food

- Staple food which accumulates MeHg.



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Biomonitoring of mercury

- Long term exposure:
  - Inorganic mercury: liver and kidney & other organs
  - Methylmercury: brain but also liver and kidney
- Exposure over weeks
  - Hair and nail analysis: mainly methylmercury
- Snapshot exposure
  - Blood (3-4 days)
  - Urine (only for Hg^{2+})

Biomonitoring of exposure – blood analysis

- Gives a direct and relevant value (no external contamination)
- Temporal variability, hence blood levels give only a snapshot
- Invasive sampling
- Storage of blood samples complicated (sample and mercury speciation might change)
- Transport and handling is restricted (infection problem)

Biomonitoring of exposure: hair analysis

- Limitations:
  - lack of precision and accuracy of hair analysis results,
  - external contamination (exogenous vs endogenous) and lack of reliable washing process
  - Inter-individual variations,
  - lack of correlation with health effects,
  - lack of believable reference intervals ?

Biomonitoring of exposure: hair analysis

- Advantages
  - it accumulates high levels of mercury (up to 300 times higher than blood)
  - it is easy to collect by relatively non-invasive methods
  - Comparable data available
  - Hair is stable specimen
  - Hair is easy to store and to transport
  - It gives an integral value rather than a snapshot
  - Can be used as an historical archive (segmental analysis)

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Hg Analysis: Instrumentation

- Atomic Absorption Spectrometry (AAS)
- Atomic Fluorescence Spectrometry (AFS)
- Total Mercury Analyzer (gold trap-pyrolysis-AAS)
- Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

Requirements for **AFS** and **AAS**: Hg must be in the elemental form (Hg^0);

→ (Chemical) Reduction of Hg^{2+} prior analysis;

→ (Pyrolytic) Reduction and breaking Hg-C bond of MeHg prior analysis

→For speciation chromatography is needed but sequential extractions are possible!

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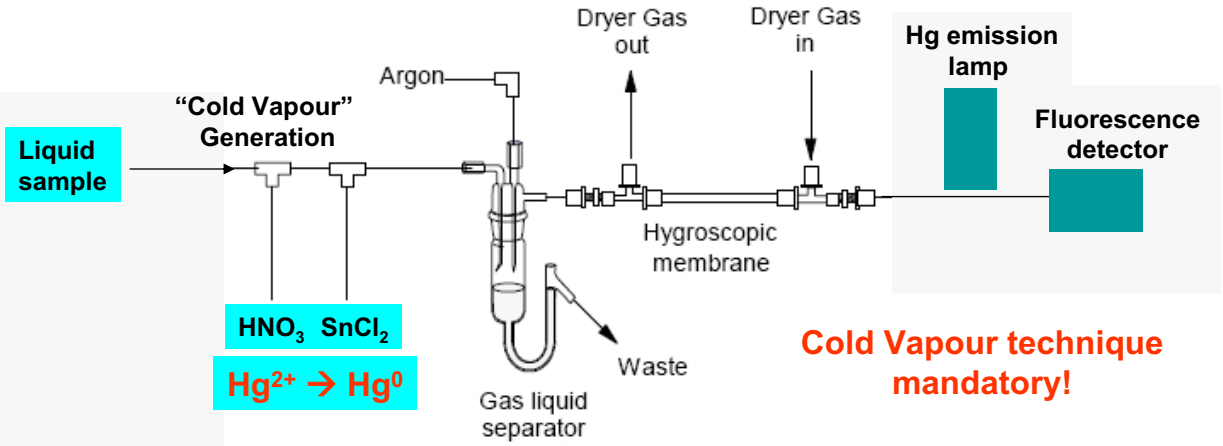
Hg Analysis

Principle of Atomic Fluorescence Spectrometry (AFS)



Sample digestion prior analysis: ~ 0.1 – 0.5 g sample + 3-5 mL HNO_3 (69%, ultrapure)
Digestion in μwave (30 min @ 65°C), dilute to 2-5 % HNO_3 (as appropriate)

LOD (in solution): ~ 10 ng/L (ppt)

LOD (in solid sample): < 1 μg/kg (< 1ppb)
CV-AAS ~ 10 μg/kg

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Gold traps for Hg accumulation

- Mercury amalgamates with gold, and gold traps have often been used for the collection and enrichment of mercury and mercury species.
- All volatile mercury species can be collected on gold traps → gold traps are not species specific!
- Use of gold traps: Improvement of LOD by enrichment; Direct gas sampling;
- Pyrolysis system needed for AFS or AAS detection after gold trapping!
- Speciation after GC also needs a pyrolysis step!

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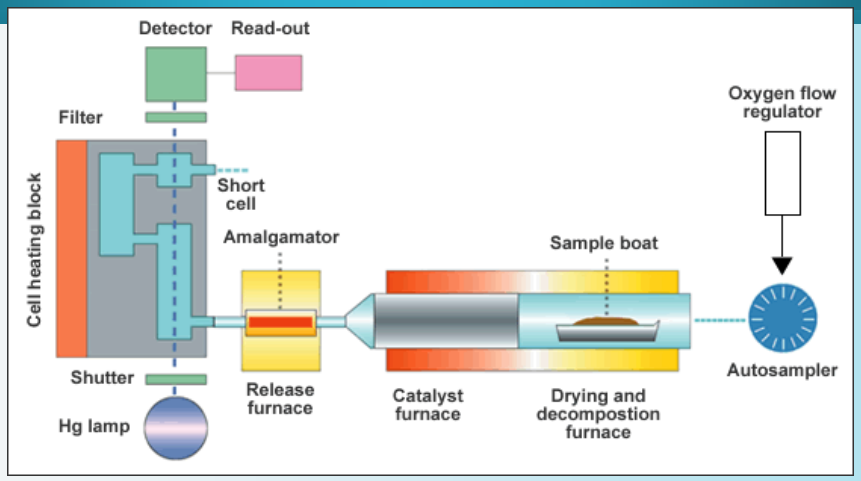
Direct Mercury Analyser



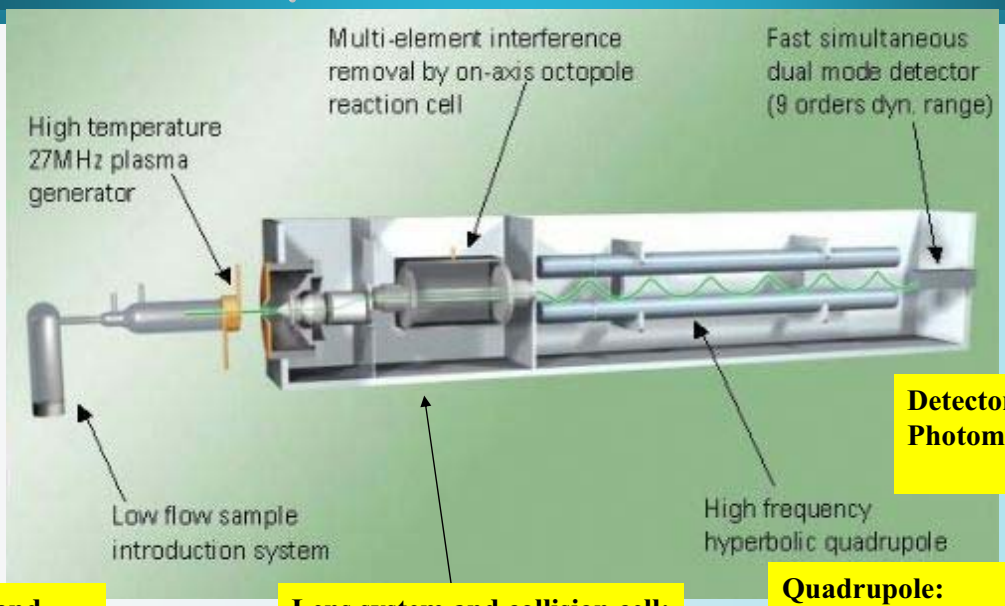
- Liquid or solid samples **(no sample preparation but limited sample mass <100 mg)**
- Sample is dried in an oxygen stream inside a controlled heating coil.
- Combustion gases are decomposed on a catalytic column at 750 °C.
- Mercury vapour (Hg^0) is collected on a gold amalgamation trap, subsequently desorbed
- Mercury is determined using atomic absorption spectrometry at 254 nm
- LOD < 1µg/kg (sub-ppb)

Mercury Analysers for Gas Analysis

Automatic Sampling/monitoring
Use of Gold trap for amalgamation
LODs < 0.1 ng/m^3 (5 pg absolute)

US EPA RfC: 0.0004 mg/m^3

Inductively-Coupled Plasma - Mass Spectrometer (ICP-MS)



Detector: Photomultiplier

Plasma and Sample introduction

Lens system and collision cell: Ion guiding and focusing

Quadrupole: Separation by m/z ratio: Only one selected m/z reaches the detector

LOD for Hg (in solution) < 10 ng/L (ppt)

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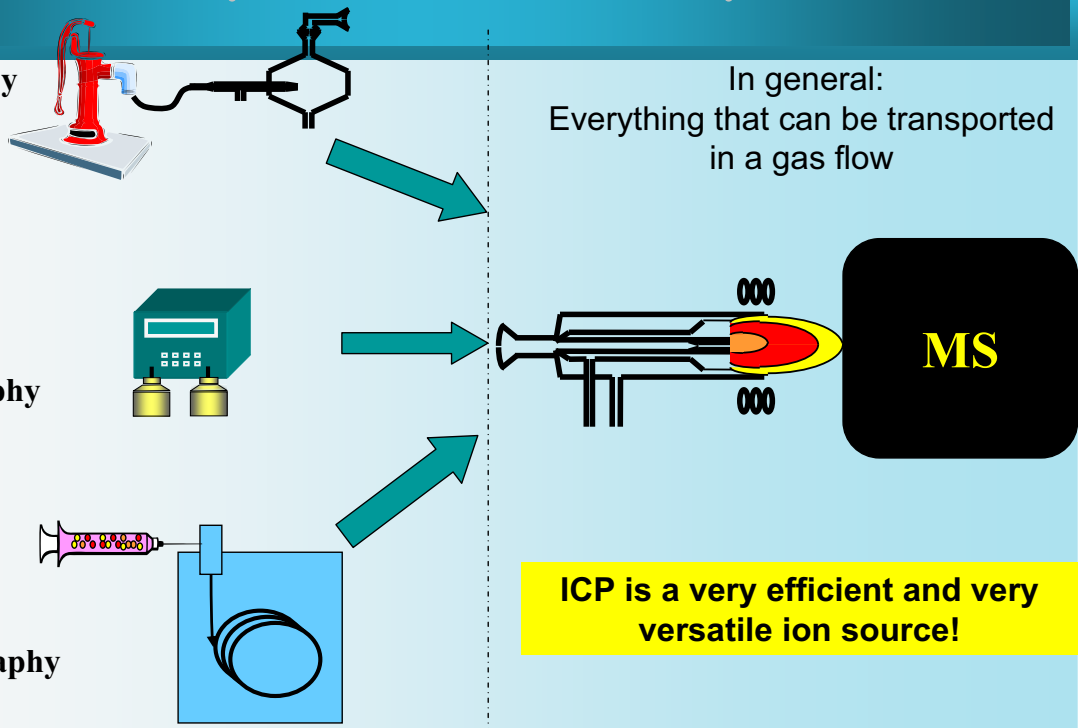
The ICP : Sample introduction possibilities

Liquid, direct by pneumatic nebulisation

Liquid, chromatography

Gas, Chromatography



In general:
Everything that can be transported in a gas flow

ICP is a very efficient and very versatile ion source!

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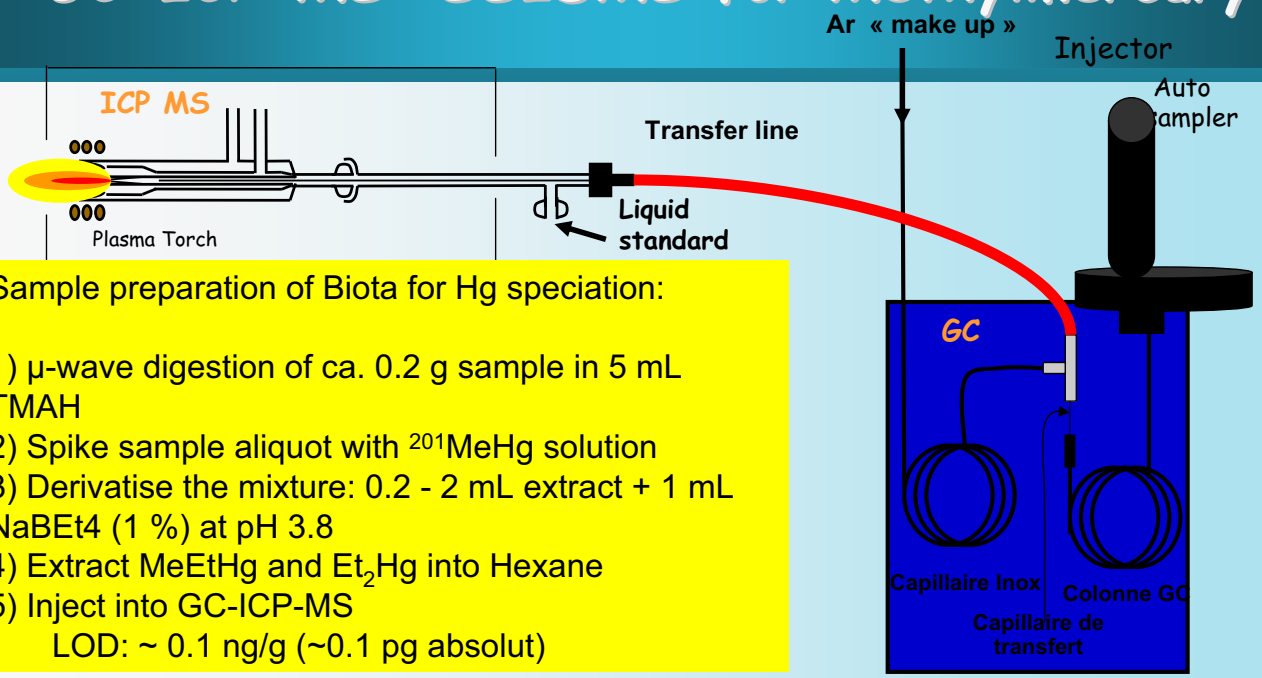
GC-ICP-MS: SSIDMS for Methylmercury



Sample preparation of Biota for Hg speciation:

1) µ-wave digestion of ca. 0.2 g sample in 5 mL TMAH
2) Spike sample aliquot with ^{201}MeHg solution
3) Derivatise the mixture: 0.2 - 2 mL extract + 1 mL NaBEt4 (1 %) at pH 3.8
4) Extract MeEtHg and Et_2Hg into Hexane
5) Inject into GC-ICP-MS
LOD: ~ 0.1 ng/g (~0.1 pg absolut)

Ethylation of mercury species with $NaBEt_4$:

$MeHg^+ + NaBEt_4 \longrightarrow MeEtHg$

$Hg^{2+} + NaBEt_4 \longrightarrow Et_2Hg$

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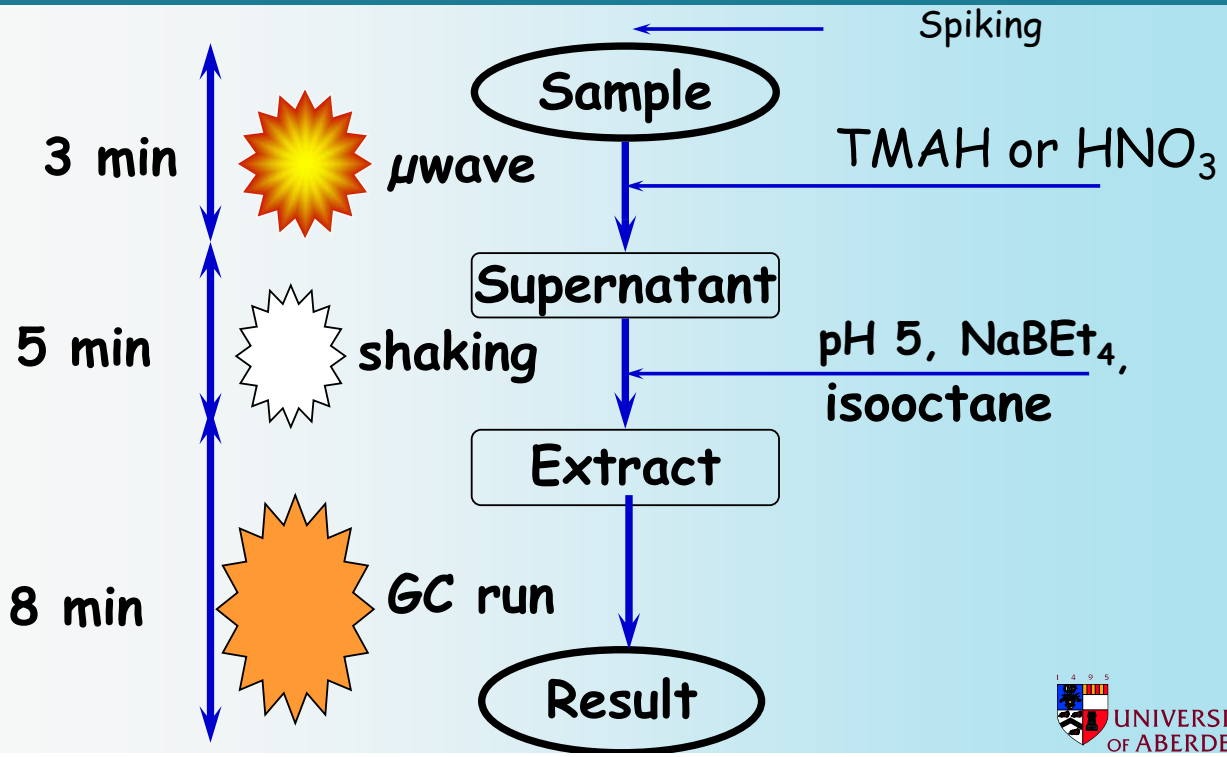
µwave preparation of sediments and biological tissues for Hg speciation



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Gas chromatography coupled to ICP/MS - Speciation analysis and advanced techniques

SAMPLE TREATMENT

- Lyophilisation
- Microwave extraction with HNO_3 6N - 3min 40W
- Derivatization with $NaBEt_4$
- Analysis by GC-ICPMS

Hg speciation in sediments Or sewage sludge

PERFORMANCE OF THE METHOD

	MMHg	Inorg. Hg
Detection limit (ng/g)	0.11	0.24
Reproducibility (%)	2	3

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Species integrity during derivatization?

- Does the species react quantitatively with the reagent ($MeHg^+$ → MeEtHg)?
- Is the derivatization matrix dependant ?
- Is the extraction of MeEtHg into the solvent quantitative?
- Is $MeHg^+$ stable or does it transform: demethylation of MeHg to Hg^{2+} or Hg^0, or formation of Me_2Hg?

→ A need for a reference method

→ **The solution: Species-specific isotope dilution (SS-IDMS): Spike of $Me^{201}Hg$**

→ **Calculation of transformation factors using a double spike: Spike of $Me^{201}Hg$ AND $^{199}Hg^{2+}$**

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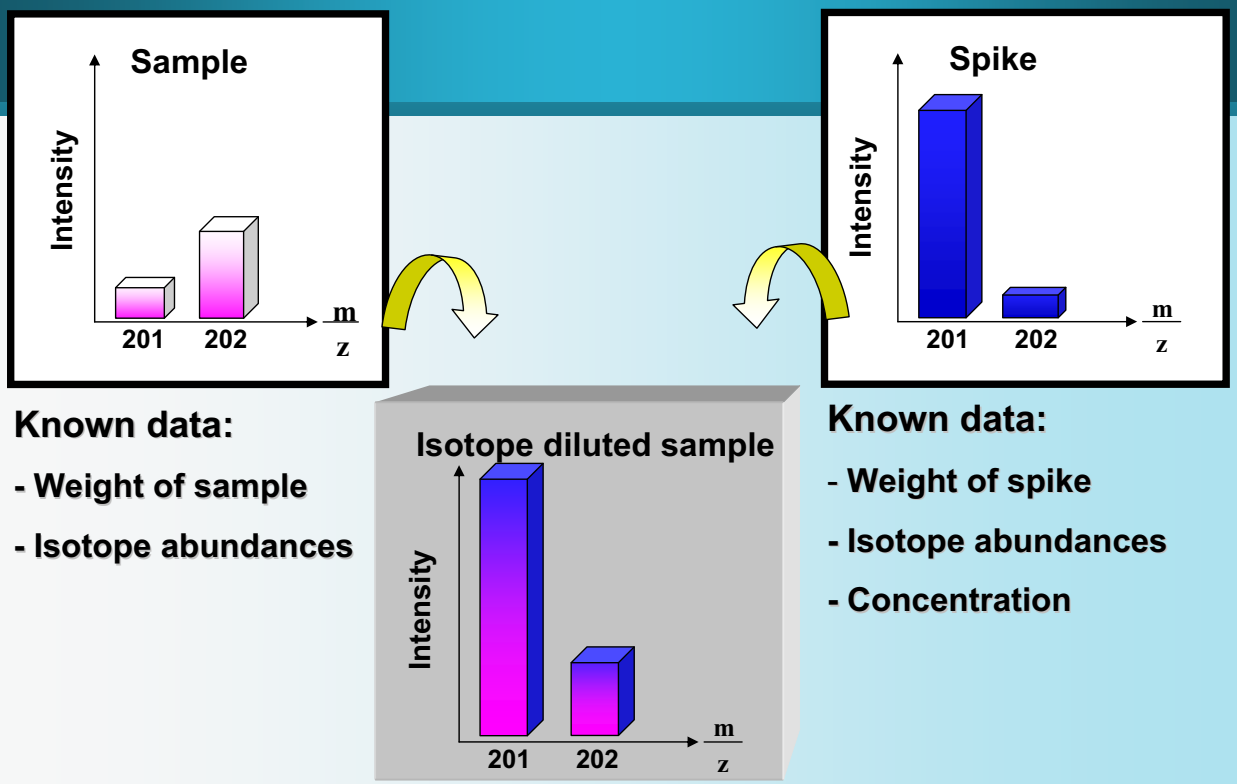
The principle of IDMS for mercury species analysis



Known data:

- Weight of sample
- Isotope abundances

Known data:

- Weight of spike
- Isotope abundances
- Concentration

To be measured: Isotope ratio

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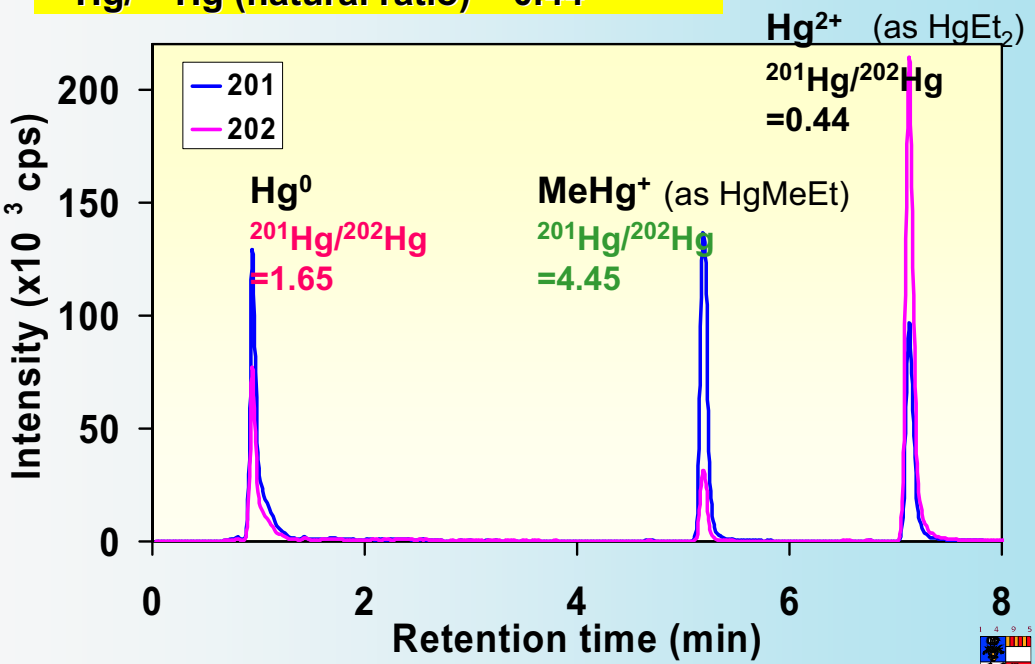
tesla

$MeHg^+$ analysis in seawater by SSIDMS using GC/ICP-MS employing a $^{201}MeHg^+$ spike

$^{201}Hg/^{202}Hg$ (natural ratio) = 0.44



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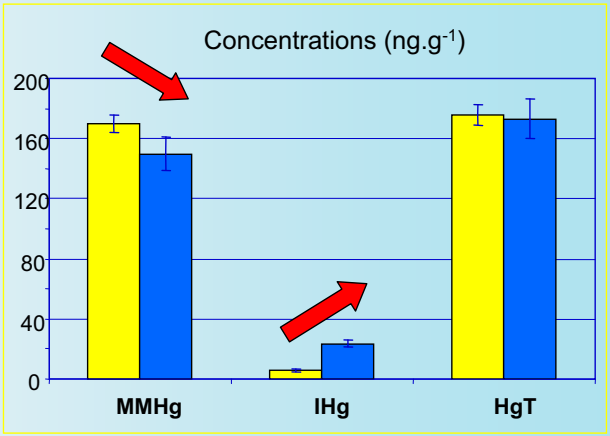
$MeHg^+$ analysis in biota by SSIDMS using GC/ICP-MS

Comparison of a single ($^{201}MeHg^+$) and double spike ($^{201}MeHg^+$ plus $^{199}Hg^{2+}$)

- Using one spike speciated isotope dilution, over estimation of MMHg concentration
- Using double spike speciated isotope dilution, correction of methylation during sample preparation
- Determination of methylation yield (F2) of 10±2% and demethylation yield (F1) of 0.01±0.02%



Monperrus et al, ICMGP proceedings, 2004

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IDMS: what are the advantages?

- Matrix interferences are eliminated as spike and analyte are the same element
- Corrects for analyte loss during sample preparation (non-quantitative recovery)
- Corrects for non-quantitative derivatisation/clean-up steps and dilution errors
- Species-specific IDMS can detect and correct for species transformation during sample preparation steps
- Measurement on Isotope ratios are far more precise (RSD ~ 0.2%)
- The final result is very precise and accurate
- → IDMS is considered an "absolute method"…

Isotope spike is the perfect internal standard

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What did we learn from species-specific GC/ICP-IDMS of methylmercury ?

- ☺ The use of isotopically labelled species identifies species transformations
- ☺ Even if species transformation takes place, accurate quantification is possible by species-specific ICP-IDMS
- ☺ Species-specific ICP-IDMS can best be used for validation of analytical methods for elemental speciation

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outline

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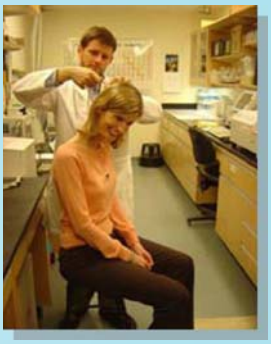
Pre-cleaning for mercury analysis blank management

- Pyrex and/or Teflon dishes should be soaked in acid baths at a temperature of 80°C, rinsed using ultra-high purity water, dried in a drying oven at 100°C and stored in polyethylene bags until use
- gloved hands should be used to manipulate all samples (dirty hands-clean hands principle)
- samples should be stored in polyethylene bags at 4°C and in the dark

Sampling and storage of hair

- Hair cut from occipital area
- 50-100 mg sufficient but ideally 200 mg
- No washing method necessary due to problems associated with indistinguishable exo and endogenous mercury
- Immediately placed in labelled Ziplok bags with usual precautions
- Storage and transport at ambient temperature
- Homogenization or segmental analysis



→ Suggestion is even to use pooled samples

Hair analysis – total Hg

- US-EPA 3052
  - 20 mg of hair in pressure microwave vessel
  - 4 replicates
  - Add 9 mL conc. HNO_3 sealed an irradiated to 180^{0}C for 10 min. and diluted to 20 mL
  - Filtered (0.22 µm) and stored at 4^{o}C
  - L.o.d. 0.05 µg/g if CV-AFS or ICPMS is used (D.L. 0.05 ng/mL)
- Relevant Hg concentration: 0.1 µg/g (10 % of critical value of 1 µg/g).

Hair analysis – speciation

- Sample amount 100 mg ideally more
- 10 mL of 4 *M* HNO_3, 10 min. Microwave 100^{o}C, filtered and diluted to 20 mL
- External Calibration: HPLC-ICPMS or derivatisation GC-ICPMS
- MeHg (99 ± 6 %), total Hg: 106 ± 7 %)
- Hg^{2+} → $MeHg^+$ 4 %, $MeHg^+$→ Hg^{2+} 6 %
- Alternative sample preparation:
  - 5 mL of 100 mg in 2*M* HCl, 4 h at RT, mechanical shaking, centrifuged diluted to 20 mL
- L.o.d. 0.1 µg/g for individual species when ICPMS is used (D.L. 0.8 ng/mL)

J. Anal. At. Spectrom. (2009), **24**, 83

QA/QC for Hg speciation in hair

- Double spiking with $^{199}Hg^{2+}$ and $^{201}HgMe$ (Species-specific isotope dilution analysis) using ICPMS hyphenated to HPLC or GC

- IAEA-085 (methyl-Hg in hair)
  Certified total Hg is 23.2 ± 0.8 µg/g, and CH_3Hg as Hg is 22.9 ± 1.0 µg/g

  and estimated Hg^{2+} is 0.1 – 0.5 µg/g.
  (additional IAEA-086 and NIES-13 available)

Relevant concentration: 1 µg/g

Biota – total Hg

- 0.1 g sample (d.w.) in 3 mL HNO_3
- Overnight at RT
- 15 min at 65°C and 15°C at 75°C (open glass vessels)
- Dilution to 20 mL
- Directly CV-AFS or ICPMS
- Memory wash with CH_3CH_2SH

Fish/Biota - speciation

- 0.1 g sample (d.w.) in 5 mL TMAH
- Aliquot 1 mL 30 min microwave in glass container at 65°C
- Buffer with acetate to pH 3.9 propylation or pH 4.9 ethylation for derivatisation
- Organic solvent (iso-octane 1 mL)
- Add reagent 1 mL 1 % $NaBEt_4$ or $NaBPr_4$
- L.o.d. 0.005 µg/g (0.1 ng/mL GC-ICPMS)

→ Fish in most cases not necessary to do speciation analysis since most of Hg is MeHg. Conservatively assuming all as MeHg for risk assessment

→ Rice ?

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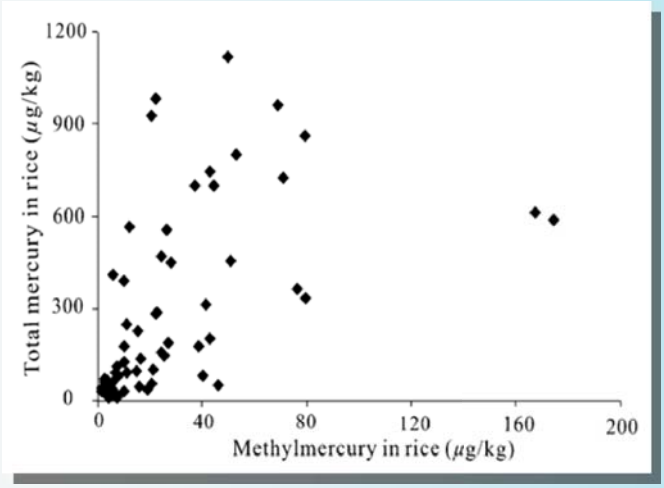
Rice- speciation necessary



→So far no extensive knowledge

→But it seems that there is no direct correlation

→Speciation analysis necessary to estimate risk

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Mercury in soil/sludge/sediment

- General observations
  - Methylmercury high in non-contaminated areas (0.10-30 %)
  - Anthropogenic contamination (cinnabar mining or chlor-alkali plants < 0.001 %)
- → speciation analysis essential !

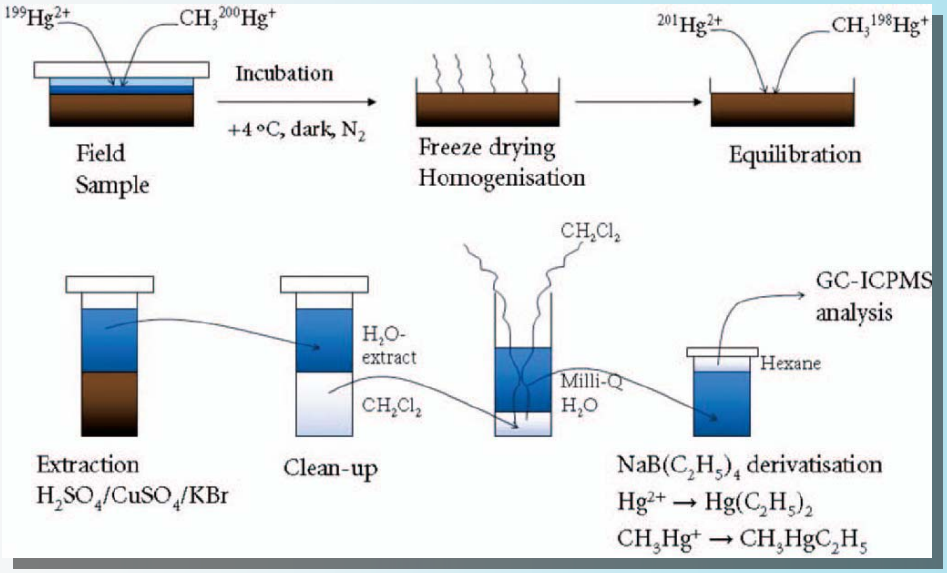
Soil sampling and storage

- Samples transferred to polyethylene bags, frozen at -20°C within 24 h of collection and later freeze-dried (lyophilized).
- Samples should be homogenized and sieved through a 50 µm nylon screen or use the < 2mm fraction
- Concentrations of THg determined on the fine soil fraction only

Total Hg in soil

- Total Hg should be digested under oxidising conditions
  - 1 g is useful for soil due to heterogeneity
  - (e.g., 80°C using a 7:3 (vol/vol) mixture of concentrated nitric and sulfuric acid in a closed PTFE bomb using microwave).
  - Dilution 1:1 and measurement using CV-AFS
- Speciation for inorg. Hg and MeHg
  - Water distillation (problem of species integrity)
  - Extraction in organic solvent as bromide $HgBr_2$ and MeHgBr

Hg-speciation in soil



→Species specific IDMS possible since danger of loosing species integrity

Mercury in soil for totals

- QA/QC
  - NIST SRM 2709 Montana soil 0.625 ± 0.190 mg/kg
  - NIST SRM 2711 San Joaquin soil 0.140 ± 0.080 mg/kg

Vaccination

- Preservative Thimerosal
  - When injected, it disintegrate quickly to ethylmercury
- 0.01 % Hg !!
- 1 mL injection = 100 µg Hg
- Analysis directly with propylation
  - EtHgPr, MeHgPr, $HgPr_2$ using GC-ICPMS

outline

- Sources and sinks of mercury
- Analytical methodologies
- Target samples
  - Sampling, storage
  - Sample preparation
- Discussion points for heterogeneous sampling

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Mercury determination in heterogeneous waste materials

What is the aim of the analysis?
- determine the overall Hg contamination in the waste material?
- determine the Hg contaminated proportion of the waste material?
- determine different Hg species: Hg^0, Hg^{2+}, MeHg…

- What is the sample like?
- Can one identify possible Hg “hotspots”?
- Sort material out prior Hg determination?

How to take a representative Sample?
How much is a representative sample?
How to homogenize a heterogeneous sample?

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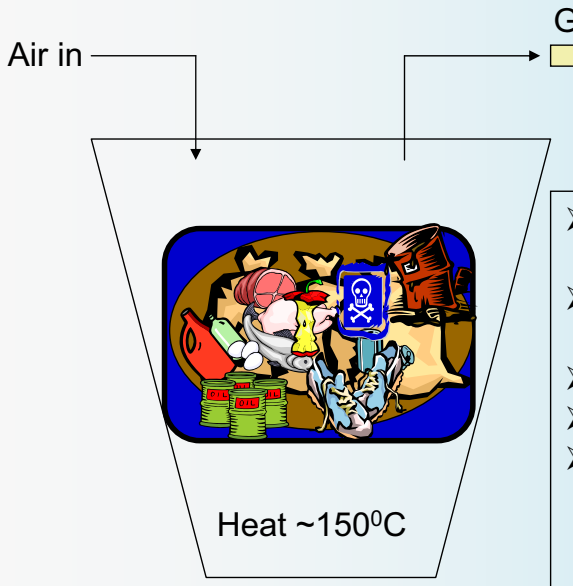
Mercury determination in heterogeneous waste materials: Hg^0 analysis



- Place homogenised and roughly ground waste material (10-100 g?) in a closed vessel
- Connect air inlet to vessel and air outlet to a gold trap
- Establish air stream ~0.1-1 L/min
- Heat vessel ~ 1h to volatilise any Hg^0
- Remove gold trap and measure using a pyrolysis unit and AFS (w/o re-focusing)

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Mercury determination in heterogeneous waste materials: Hg total and speciation analysis

➢Produce a homogeneous sample, mixed and finely ground (Hg^0 might get lost in the process!)

➢Hg total can be determined using a total mercury analyser or digestion followed by AFS, AAS or ICP-MS determination

➢MeHg can be determined using extraction and derivatisation methods followed by GC-ICP-MS or GC-pyro-AFS

OR:

- **Sort Waste Material into Hg-containing and Hg-free**
- **Determine Hg total and Hg speciation in highly Hg containing fraction**
- **Estimate proportion Hg/Hg free waste material**
- **Estimate overall Hg content and risks**

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What should we do?

1. Proficiency tests for THg and MeHg analysis for nationally identified expert labs
2. Collection of pooled human hair samples (controls and exposed subjects in each country)
3. Rice from exposed regions (5-10 samples per country)
4. Collection of a integral waste streams (fly ash, sewage sludge)??
5. Residues from extraction of energy (water geothermal or charcoal from natural gas, etc.)
6. Special samples (vaccines with Hg preservatives)???

Suggestions for the different countries

- Burkina Faso
  - Fish and rice from gold mining area & hair
- Cambodia
  - Fish and rice from gold mining or landfill & hair
- Chile
  - Population around tailings dams, irrigation water ?
- Pakistan
  - Air/water samples from chlor/alkali production & hair from workers
- Philippines
  - Energy sources or gold mining sources & hair

Hair samples

- Each country samples three "hot spots" each 20 hair samples (incl. two controls)
- 200 mg each
  - Subdivide into 2 subsamples (not by cutting!!)
  - Clearly mark the start and the end of the hair growth
  - Take data (questionnaire, worker for how long, many hours, sex, age, fish/rice consumption)
  - Ethic committee??

If possible rice sample

- Rice from three hot spots and two control areas (from mill or farmer), each three samples (20g each).
- Questionaire about the pre-use of irrigation water

Other samples

- Fish
- Sewage sludge from treatment plan
- Sediment from setting ponds

Burkina Faso

- 4.5 t Hg /y
- 40 % water, small scale mining, released to ponds
- No data about human exposure
- No data about Hg in fish

Cambodia

- 3 – 120 kg Hg/y only from fuel/energy sources
- Crude oil 10-300 mg/t release 2-72 kg Hg
- Majority from intentional use of mercury 8485 kg and 1182 kg from small gold mining
- Majority solid waste

Chile

- 361-4xx t of Hg
- Majority is from metal production however not much associated with copper mining and production
- but main source is from mine tailings in region II and IV where also exposure to people exists.

Pakistan

- Main release from chlor-alkali cells (21,000 t)
- Mercury cells should be phased out.
- Listed only in use w/o any release
- No exposure data available

Philippines

- 5500 t released into atmosphere (globally)
- Primary metal production (32 %) 75 t/y mainly gold mining w/o exposure data
- Energy resources (20%) 3-4 g Hg/MWh
- Release air 45 %, water 17%