

Recent advances on mercury speciation in aquatic ecosystems, health effects and analytical techniques

Solomon Omwoma,^{*1}; Silah C. Lagat;¹ Joseph O. Lalah,² Philip O. Owuor,³ and Karl-Werner Schramm,⁴

¹*Department of Physical Sciences, Jaramogi Oginga Odinga University of Science and Technology, P.O. Box 210-40601, Bondo, Kenya*

²*Department of Chemical Science and Technology, Technical University of Kenya, P.O. Box 52428-00200, Nairobi, Kenya*

³*Department of Chemistry, Maseno University, P.O. Box 333-40105, Maseno, Kenya*

⁴*Helmholtz Zentrum Muenchen, German Research Centre for Environmental Health, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Munich, Germany*

**Corresponding author email: solomwoma@yahoo.com,*

Abstract

Elemental mercury is not as poisonous as its congeners such as methyl mercury and dimethyl mercury, especially at trace levels. However, understanding the speciation mechanisms of mercury in aquatic ecosystems offers an opportunity to appreciate the dangers emanating from elemental mercury in the environment. Notably, elemental mercury is occasionally used in dental filling therapy which presents it as a non-poisonous element. Recent advances on mechanisms of elemental mercury speciation, health effects of different mercury congeners and analytical techniques of identifying/quantifying them in aquatic ecosystems are outlined. The effects of anthropogenic activities employing the use of elemental mercury such as gold amalgamation are highlighted through a case study. Kenya Gold mines are identified as a potential risk zone amenable to mercury health effects.

1. Introduction

Mercury (Hg), formerly named hydrargyrum and commonly known as quicksilver, is a heavy, silvery-white d-block metallic element that exists as a liquid at ambient temperature and pressure. Mercury has a low melting point of $-38.83\text{ }^{\circ}\text{C}$ and boiling point of $356.73\text{ }^{\circ}\text{C}$. These are exceptional temperatures for a metallic element and they are associated with the fully filled orbitals of $[\text{Xe}]4f_{14}, 5d_{10}, 6s_2$ that make mercury to have weak intramolecular metallic forces.¹

Among the seven naturally occurring isotopes of mercury (^{194}Hg , ^{196}Hg , ^{198}Hg , ^{199}Hg , ^{200}Hg , ^{201}Hg , ^{202}Hg), ^{202}Hg is the most abundant (29.86%) while ^{194}Hg has the longest-lived radioisotope at 444 years half-life.² ^{199}Hg and ^{201}Hg with spins of $\frac{1}{2}$ and $\frac{3}{2}$ are the most studied NMR-active nuclei. Some of the physical properties of Hg include: a) high vapor pressure, Henry's constant = 0.3, b) low solubility in aqueous medium, approximately $6\text{ }\mu\text{g}$ per 100 mL of water at $25\text{ }^{\circ}\text{C}$, c) highly volatile in a liquid:air interface, however, it coalesces into a liquid when placed in an enclosed/diffusion-free system.

Hg has a crustal abundance by mass of 0.8 ppm in Earth's crust, but due to its inability to blend geochemically with other elements, mercury ores are extraordinarily concentrated up to levels of 2.5% mercury by mass.³ Common ores include cinnabar (HgS) and livingstonite (HgSb_4S_8), with cinnabar being the most common ore.⁴

The demand and supply chain for Hg was initiated by the patio process and later pan amalgamation process in treatment of silver and gold ores until the late 19th century and dental filling applications.⁵ Other earlier common uses of Hg that have been discontinued include its use in batteries, fluorescent lights, felt production, thermometers and barometers. Due to depleted sources, the price of Hg in 2006 was \$650 per 34.46 kg flask.⁶ Current demand of Hg is driven by the need for fluorescent bulbs which has encouraged re-opening of deadly cinnabar mines in cities such as Foshan, Guangzhou, and Guizhou province in China.⁷

Sources of mercury to the environment include abandoned mercury mine processing sites. Piles of hazardous wastes comprising of roasted cinnabar can be found in such sites. Water runoff acts as the transport medium of the mercury deposits from such mines to other environmental compartments. However, production of medicines, industrial production of chlorine and caustic soda through the Castner-

61 Kellner process, laboratory uses, mercury vapor lamps and cosmetics may also
62 contribute to anthropogenic levels of Hg to the environment.⁸⁻¹⁰ Burning of coal is also
63 considered as a significant mercury contributor to the environment.¹¹ Approximately
64 75% of environmental mercury is as a result of anthropogenic processes.¹² And there
65 is evidence of annual increase of environmental Hg input over the past two decades.<sup>13-
66 17</sup>

67 In this work, we consider the fate of mercury once it is introduced into
68 environmental aquatic ecosystems. Notably, atmospheric mercury finally finds its way
69 to aquatic systems after a residence time of 27 years; although aerosols can reduce this
70 time significantly.¹⁸
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2.1. From Hg^0 to Hg^{2+} in aquatic ecosystems

The presence of elemental mercury (Hg^0) in an aquatic ecosystem leads to its conversion into inorganic mercury (Hg^{2+}) through precipitation and/or oxidation reactions.²³⁻²⁶ The equilibrium between Hg^0 and Hg^{2+} is maintained by evaporation rates and the different conversion processes.^{24, 27, 28}

Although oxidation of Hg^0 to Hg^{2+} is a negligible process in natural waters, presence of chloride ions facilitates such conversions.²⁹ The oxidation reaction also occur faster in water with high colloid concentration such as the shores of water bodies.³⁰ Aquatic ecosystems with high dissolved oxygen content, and oxidizing agents like carbon dioxide, nitrites, nitrates, iron hydroxides, iron phosphates, sulfur and sulfates, experience high elemental mercury oxidation rates.³⁰

Most mercury salts formed out of precipitation and oxidation reactions are slightly soluble at between 20 °C to 30 °C in aquatic ecosystems.³¹ Some of these salts include $\text{Hg}(\text{CN})_2$, HgCl_2 , $\text{Hg}(\text{ClO}_3)_2$, $\text{Hg}(\text{C}_2\text{H}_3\text{O}_2)_2$, HgS , Hg_2CO_3 , $\text{Hg}_2(\text{CN})_2$, $\text{Hg}_2(\text{ClO}_4)_2$, and Hg_2SO_4 .³¹ Those mercury salts that are not soluble settle down in sediments and/or exist as suspensions and colloids in water systems.³¹

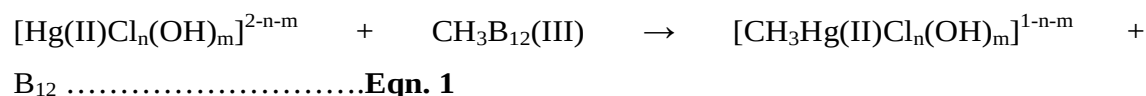
Hg^0 in soils is oxidized aerobically by bacteria like the *Bacillus* and *Streptomyces*.³²⁻³⁴ The resultant aqueous inorganic mercury salts are transported to aquatic ecosystems by either leaching or runoff water.³⁵ Although Hg^0 is volatile and easily evaporates to the atmosphere, if present in surface waters or in wetlands, it is photo-chemically oxidized by UV-B in the presence of O_2 , O_3 , H_2O_2 , Cl^- , SO_4^{2-} , $\text{BrO}^\bullet$, $\text{Br}^\bullet$, $\text{Cl}^\bullet$ and benzoquinones to inorganic mercury salts.^{29, 36-42} Similar reaction mechanisms are also responsible for the deposition of Hg^0 from the atmosphere to land and aquatic ecosystems (see **Fig. 1**).

The divalent Hg^{2+} ion does not exist as a free ion in aquatic systems but as complexed hydroxides and chlorides ($\text{Hg}(\text{OH})^+$, $\text{Hg}(\text{OH})_2$, $\text{Hg}(\text{OH})_3^-$, HgCl^+ , HgClOH , HgCl_2 , HgCl_3^- , HgCl_4^{2-}) depending on the pH of the systems.¹⁸ Some of the Hg^{2+} ion may also exists as HgS or HgS_2^{2-} and HgS_2H_2 or HgS_2H^- in both oxic and anoxic waters.⁴³ Humic substances (dissolved organic matter) are also able to adsorb some of the Hg^{2+} and organic-Hg in aquatic environments.⁴⁴ However, note that the reactions due to Hg^{2+} are first and its various species, particulate phase inclusive, are at equilibrium.

Inorganic mercury is introduced into most food chains through consumption of contaminated water or sediments by aquatic animals and/or benthic organisms.^{31, 45, 46} At higher trophic levels, **in humans**, toxic inorganic mercury may induce excessive perspiration, hyper salivation, renal tubular dysfunction, weight loss, paresthesia of the feet and hands, fatigue, insomnia, erythema, pruritus, neuropsychiatry disorders and progressive weakness in extremities.^{45, 46} However, of great concern is the conversion of inorganic mercury to organic forms by microorganisms, acidic environments and salts like hydrogen sulfide in aquatic environments.⁴⁷⁻⁴⁹

2.2. Methylation of Hg^{2+} in aquatic ecosystems

Methylation of Hg^{2+} takes place in bottom sediments, soils and water.⁴⁹ The methylation process takes place both via the biotic and abiotic pathways.⁵⁰⁻⁵³ The biotic pathway is initiated by sulfate-reducing bacteria.^{52, 54, 55} Biotic methylation takes place in anaerobic conditions which prevail in bottom sediments and wetlands. However, it may also occur in aerobic conditions present in surface waters.^{47, 48} In a pure culture of methanogenic bacterium and methyl **cobalamin** substrate, formations of mono and dimethyl mercury from Hg^{2+} have been reported.⁵⁶ The transfer of methyl from methyl cobalamin (CH_3B_{12}) to inorganic Hg^{2+} has been suggested as the most probable mechanism in the mercury methylation reaction (**see Eqn. 1**).^{56, 57} In this reactions, the electrophile (Hg^{2+}) attacks methyl cobalamine leading to a transfer of the carbanion methyl group to Hg^{2+} .⁵⁷ Methyl cobalamin (MeB_{12}) is produced preferentially by sulfate reducing bacteria (SRB).^{58, 59} CH_3B_{12} can also be consumed by methanogens that use it to methylate Hg^0 .⁶⁰



Optimal biotic methylation conditions include high acidity, high Hg^{2+} concentrations and high temperatures of approximately 35 °C.⁶¹ However, in a lower acidic environment, the electron density of the methyl donor and electrophilicity of the metal ion in the reaction system are altered hence shifting the reaction equilibrium.⁵⁷ In addition, an increase in free sulphide ions (maximum 1.8 mg S^{2-} /g of sediment) shifts the equilibrium towards methyl mercury formation.⁶¹ However, above 1.8 mg S^{2-} /g of sediment, CH_3Hg^+ concentration decreases due to the formation of volatile

dimethyl mercury.⁶² And at a very high concentration of S^{2-} ions, the methylation equilibrium is altered by the formation of the insoluble HgS salt.⁵¹

At the moment, the methylation process in water is speculated to follow a similar mechanism like in sediments.²⁴ The assumption is that in lakes, methyl mercury in surface layers is transported through diffusion and currents from the deeper anaerobic layers (see **Fig. 1**). However, in oceans, some methyl mercury can be formed in aerobic conditions as a result of conversion of dimethyl mercury coming from deeper layers.²⁴ The reactions involving microorganisms lead to the formation of both methyl mercury and dimethyl mercury.^{30, 63}

The selectivity in formation of CH_3Hg^+ or $(CH_3)_2Hg$ depends on the ecosystems pH, $[Hg^{2+}]$, and $[H_2S]$. Whereas rate of formation for CH_3Hg^+ is accelerated at higher pH (pH 4-5) and with a relatively high Hg^{2+} concentrations, $(CH_3)_2Hg$ formation is faster in neutral or alkaline conditions, at a relatively low concentration of Hg^{2+} and in the presence of relatively strong complexing reagents such as H_2S .^{27, 64} As such, in most ecosystems, $[CH_3Hg^+]$ concentration is almost 6000 times higher than that of $[(CH_3)_2Hg]$. Therefore, only 3% of organic mercury occurs as $(CH_3)_2Hg$ species in the unpolluted environment.⁶¹

Abiotic methylation can be achieved with or without irradiation.⁶⁵ Under the irradiation mechanisms, propionic acid, acetic acid, methanol and ethanol donates the methyl groups. However, without irradiation, the methylation process may include methyl cobalamin, trans-methylation (methylated tin compounds) and those with humic substances.⁶⁵ The role of methylated tin compounds and humic substances in methylation of mercury has not yet been studied.

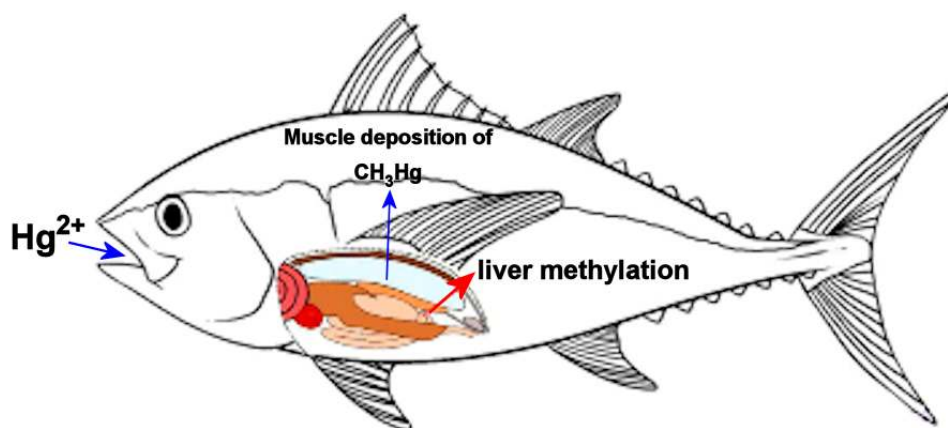


Fig. 2. In vivo Hg^{2+} methylation by fish after a one month dietary exposure followed by two month depuration. The concentration of MeHg continuously increased in the muscles as it decreased in the liver during the depuration period. This was indicative of inter-organ transportation of MeHg from liver to the muscle tissues.⁶⁶

However, for methyl cobalamin, which is a derivative of vitamin B₁₂, the terminal ligand CN is substituted with a CH₃ group. As such, there is a strong correlation between methyl mercury concentrations and methyl cobalamin in bottom sediments.⁴⁸ Notably, methylated tin and lead compounds are potential reagents in abiotic methylation of mercury.^{51, 67} However, note that the abiotic pathway is a contentious topic.⁵¹

Methyl halides (CH₃J), especially CH₃I, occurring at a relatively high concentration in unpolluted waters and produced by algae, has been reported to be involved in direct methylation of mercury.⁶⁷ These compounds are excellent substrates in S_N2 substitution reactions because they are sterically open for attack by nucleophiles with iodide being a very good leaving group. The compounds have been demonstrated as alkylating agents for carbon, oxygen, sulfur, nitrogen, and phosphorus nucleophiles.⁶⁸ However, some experimental data exclude the possibility of mercury methylation through the CH₃J mechanism.⁴⁹ CH₃J can also be involved indirectly in mercury methylation process especially at trace concentrations of Hg^{2+} .^{51, 69}

The carbon-to-metal bond in organic mercury compounds is stable in water due to partly the covalent nature and, although its hydrolysis is thermodynamically favorable, it is kinetically hindered.⁷⁰ As such, CH₃HgCH₃ is unreactive whereas CH₃Hg⁺ predominantly exists as CH₃HgCl, and CH₃HgOH in oxic waters and CH₃HgS⁻ in anoxic waters (**Fig. 1**).

The stability and water-solubility of methyl mercury allows organisms to easily absorb and bio-concentrate it at different trophic levels. In contrast, dimethyl mercury evaporates into the atmosphere where it can be decomposed to methane and elemental mercury by photolysis or oxidized by hydroxyl radicals (**Eqn. 2 and 3**).^{30, 71} And at low pH, (CH₃)₂Hg is easily converted to CH₃Hg.⁷²

There is evidence that in vivo methylation of Hg^0 by organisms such as fish can also occur especially in the liver (**Fig. 2**). Wang et al demonstrates that at least 1.6% of the ingested Hg^{2+} is converted into MeHg and deposited in fish muscles.⁶⁶

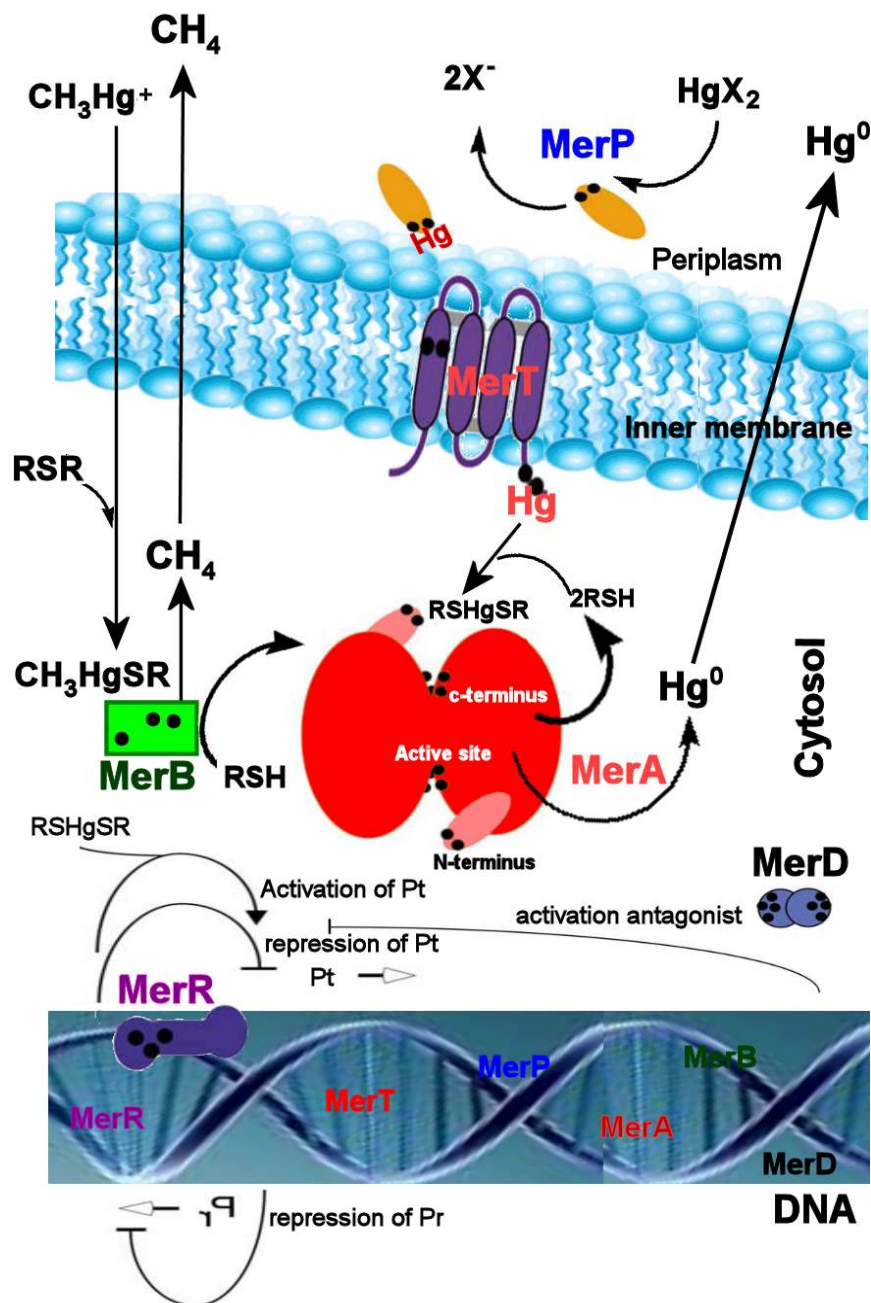
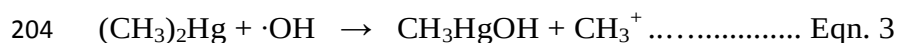
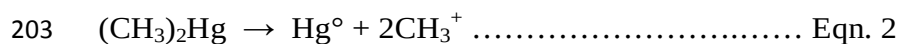


Fig. 3. Degradation/resistance of mercury by bacteria (see text for explanation and/or refer to Barkay et al for finer details).¹¹

The above biotic and abiotic processes, anthropogenic sources of Hg^0 to the environment, various recycling processes of mercury congeners in the environment and bio-concentration of CH_3Hg^+ in various organisms and concentration in plants are illustrated in **Fig. 1**.



205 **2.3. De-methylation of CH₃Hg in aquatic systems**

206 The de-methylation process can proceed through a biological route (microorganisms)
 207 and/or a photochemical route (as a result of irradiation).⁷¹ Biological de-methylation
 208 process is slow as compared to methylation and it is effective in aerobic conditions.³⁰
 209 Since biological de-methylation process involves microorganisms, it is affected by
 210 temperature fluctuations with most surface lake waters having an average of 13.2%
 211 loss daily.⁴⁷

212 The overall ionic equation involved in de-methylation process is shown in **Eqns. 3**
 213 **and 4**. The process is accompanied by hydrolysis of mercury-carbon bond leading to
 214 the formation of Hg^{2+} and methane. The hydrolysis reaction is made possible by
 215 enzymes/photolysis. Whereas CH_3^+ will react with water to give out methane that
 216 eventually escapes to the atmosphere, Hg^{2+} is reduced to the volatile Hg^0 .³⁰



219 De-methylation of CH_3Hg and detoxification of Hg^{2+} by bacteria from its
 220 surrounding environment is a fascinating phenomenon and could be an important de-
 221 methylation in aquatic ecosystem detoxification. Bacteria are able to detox inorganic
 222 and organic mercury compounds in their environment by converting them into volatile
 223 Hg^0 species that escapes to the environment (**Fig 3**).¹¹ The detoxification mechanisms
 224 take place in chromosomes, plasmids, and transposable elements. Key detoxification
 225 enzyme include 1) Mercuric ion reductase (*MerA*) which reduces Hg^{2+} to Hg^0 .¹⁹ 2)
 226 Organic mercurial reductase (*MerB*) that breaks the C-Hg bond.²⁰ Membrane-bound
 227 protein (*MerT*) that helps in the uptake of Hg^{2+} . 3) *MerP* which is a small periplasmic
 228 mercury binding protein that uses its two cysteine residues to displace nucleophiles
 229 like chlorides bonded to Hg^{2+} . 4) *MerR* which binds to Hg^{2+} to provoke allosteric
 230 change protein that is propagated to the DNA of the operator region. This leads to an
 231 under winding of the operator DNA. 5) *MerD* is an antagonist of *MerR* functions.
 232 Interested readers of these section are directed to a much more comprehensive review
 233 article by Barkay et al.¹¹ The most important aspect is that bacteria are able to de-

234 methylate organic mercury compounds. However, this research remains to be
235 laboratory based with no field experiments reported so far.

236 Photochemical de-methylation process takes place under the irradiation of UV-A
237 and UV-B from sunlight with volatile Hg^0 as the main product.⁷³ This irradiation de-
238 methylation reaction rate has been shown to be of first-order with respect to $[\text{CH}_3\text{Hg}^+]$
239 and UV radiation.⁷³ The de-methylation process is inhibited by singlet oxygen-
240 trapping agents.⁷⁴ However, it is evident that in light exposed environments including
241 lakes and wetlands, irradiation de-methylation mechanisms may predominantly
242 prevail.⁷³ Photochemical de-methylation process can be affected by dissolved organic
243 carbon that competes for solar radiation.⁷⁵ In anoxic waters such as wetlands, methyl-
244 mercuric sulfide ion (CH_3HgS^-) has been shown to be readily photo-decomposed to
245 CH_4 and HgS .¹⁸

246 3. Health effects due to mercury and its compounds

247 3.1. Health effects in humans due to Hg^0

248 Mercury in form of Hg^0 easily accumulates in the human body and attacks the central
249 nervous system.⁷⁶ Acute toxicity of Hg^0 is associated with numbness, unsteady limbs
250 (especially hands and legs), tiredness, ringing in the ears, narrowing of the field of
251 vision, loss of hearing, slurred speech, forgetfulness, loss of some senses such as smell
252 and taste.^{25, 77} In most cases, Hg^0 gets into the human body via direct inhalation of
253 gaseous mercury,⁷⁸ use of affected domestic water and/or through consumption of
254 contaminated food (Fig. 1).²⁶

255 3.2. Health effects in humans due to CH_3Hg^+

256 Among the compounds of mercury, organomercury derivatives are highly poisonous
257 and are easily absorbed by the gastric and intestinal organs. They are carried by blood
258 into the brain, liver, kidney and even foetus.⁶³ In most cases, humans accumulate
259 MeHg^+ from contaminated foods such as fish. As such, the recommended level of
260 MeHg^+ in commercial fish is set at 0.3mg kg^{-1} .⁶³ The most stable form of MeHg^+ is
261 CH_3HgCl that is formed favorably in low pH (app. 4-5) regions with high chloride
262 concentrations.⁴⁴ Other forms include CH_3HgOH , and CH_3HgS^- (see section 2.2)

263 Human acute toxicity epidemic of MeHg poisoning occurred in Minamata Bay,
264 (1953) and Agano river basin (1965) both from Japan after bioaccumulation of MeHg

in contaminated fish (Minamata disease).⁶³ Similarly, a sentinel outbreak of MeHg poisoning happened in Iraq (1971-1972) after consumption of contaminated grains resulting in 6500 casualties and 459 deaths.⁶³ Apart from death, other acute deformities include cerebral palsy, mental retardation, deafness, blindness, and dysarthria, especially in children exposed in utero.⁶³

Low level exposure to MeHg has been linked to subtle neurodevelopment.⁷⁹ Therefore, although mercury poisoning epidemics maybe rarely reported, pervasive low level chronic MeHg exposure primarily through fish consumption is a concern.⁸⁰ For instance, in human blood, about 5% MeHg was found in the blood compartment and 10% in brain meaning that MeHg crosses the brain barrier.⁷⁹ Levels in the placenta cord blood were higher than levels in maternal blood levels.⁷⁹ In addition, the foetal brain was found to have 5–7 times higher mercury levels than in maternal blood.⁷⁹

Micro-flora, found in the intestines, metabolizes MeHg to inorganic mercury at the rate of 1% of the body burden per day and the process is insufficient to mitigate MeHg bioaccumulations.⁸¹ However, conversion of MeHg to Hg^{2+} also leads to the slow accumulations of Hg^{2+} that eventually resides in the central nervous system leading to health complications.⁸² Notably, removal of MeHg by the kidneys is negligible leading to extensive enterohepatic cycling of this compound in the body.⁸¹ In addition, MeHg attaches to the sulfur atom of thiol glands making it a water soluble complex that easily enters endothelial cells of the blood-brain barrier with L-cystein.⁸²

MeHg affects the central nervous system in which the initial stage is characterized by a latent period whose duration depends on exposure and accumulated amounts. It is not yet clear what happens during this latent period. However, the first symptoms of MeHg poisoning to the central nervous system is paresthesia which may progress to cerebellar ataxia, dysarthria, constriction of visual fields and loss of hearing.⁸³ Loss of neuronal cells in specific anatomical regions of the brain is linked to the aforementioned signs/symptoms.⁸⁴ The relationship between neurological and developmental defects due to MeHg poisoning is an abnormal muscle tone or tendon reflex that is abundant in boys.⁸⁴⁻⁹¹

3.3. Health effects in humans due to $(\text{CH}_3)_2\text{Hg}$

Dimethyl mercury is equally a very toxic form of organomercury compound. The death of inorganic chemist, Karen Wetterhahn, is directly linked to the spill of

dimethyl mercury on her latex-gloved hands.⁹² Dimethyl mercury easily forms a complex with cysteine which easily crosses the blood-brain barrier. The latent period between exposure and symptoms makes treatment difficult owing to the fact that its elimination from organisms is very slow leading to bioaccumulation.⁹²

3.4. Health effects in humans due to $C_2H_5Hg^+$

The presence of ethyl mercury (EtHg) in human blood is mainly due to the breakdown process of ethylmercurithiosalicylate that is frequently used as an antiseptic and a vaccine preservative.⁹³ Unlike MeHg, health effects of EtHg are not extensively studied probably due to the ability of organisms to clear it from blood metabolically (half-life of 7-10 days).⁹³ EtHg relies on simple diffusion to enter the brain since it does not have the ability to cross the blood-brain barrier.⁹³ Other sources of EtHg in human blood may arise from thimerosal which is a topical antiseptic and a preservative in vaccines routinely given to children.⁹⁴ Thimerosal is made-up of 49.6% Hg by weight which once in the system, it undergoes metabolism to yield EtHg and thiosalicylate.⁹⁴ A pediatric vaccine dose may contain up to 12.5-25 mg of Hg per 0.5 ml of the vaccine and cases of hypersensitivity are observed in some cases after dosage administration.⁹⁴ As such, some countries such as USA have banned the use thimerosal as a preservative in vaccines.⁹⁴ Apart from thimerosal, EtHg may be found in products containing ethyl mercury thiosalicylic acid as a preservative such as cosmetics, including makeup removers, mascara, and eye/ear moisturizers/ointments, antiseptic soap-free cleaners, contact lens solutions, medicated sprays, medications, vaccines, antitoxins, tuberculin tests, desensitizing solutions, adhesives, air conditioner filters, etc.

EtHg may also induce neurodevelopmental disabilities associated with language delay and attention deficit-hyperactivity disorder (more specifically the autism spectrum disorder).⁹⁵ Health effects of EtHg are similar to those of MeHg at higher toxic levels but effects of lower doses remain undetermined.^{94, 96} However, at micromolar concentrations of thimerosal, experiments using cortical human neurons show changes in cell membrane permeability, induced DNA breakages and apoptosis.⁹⁷ Nevertheless, neurologically, health effects due to EtHg are viewed as less toxic than MeHg.⁹⁸

329 There seems to be no studies outlining the possibility of biogeochemical reactions
330 that could lead to the formation of EtHg in the ecosystem from ethyl
331 mercuricthiosalicylate.
332

333 4. Biomonitoring of mercury species in aquatic ecosystems

334 4.1. Biological magnification

335 The process of acquiring food by organisms from their ecosystems is also
336 accompanied by absorption of unnecessary inorganic substances such as Hg and its
337 compounds. Unlike other toxins that might be excreted through metabolic pathways,
338 Hg and especially its organic congeners bioaccumulate in the organisms and their
339 successive trophic levels within their respective food webs (biological
340 magnification).⁹⁹⁻¹⁰¹ It is therefore possible to monitor the contamination process of
341 ecosystems using organisms found in that particular environment.¹⁰² Such information
342 is very important in planning, and conservation efforts by relevant authorities. The
343 process is called biomonitoring and the organisms used are called bioindicators.¹⁰²
344 Invertebrates and periphyton are widely used as bioindicators.¹⁰³⁻¹⁰⁵ However, fish has
345 also been widely used as bioindicators.^{106, 107}
346

347 4.2. The use of invertebrates as bioindicators

348 Earthworms, midges and stoneflies present some of the best invertebrates commonly
349 found in most ecosystems and they can clearly show the contamination level of an
350 ecosystem especially for heavy metals including Hg.⁶ The earthworms might be
351 considered as standard bioindicators for soil due to their ecological importance in most
352 temperate and tropical soils.^{108, 109} As such, they are constantly used to monitor heavy
353 metal concentrations in soils especially mercury and its congeners.^{78, 79}
354

355 4.3. The use of mosses plants as bioindicators

356 Mosses plants do not have real roots and vascular system but obtain nutrients using the
357 entire plant. As such, they mostly absorb nutrients from the atmosphere and
358 precipitation. Hence the use of mosses to monitor atmospheric heavy metal pollution

is a common process¹¹⁰ and has been used intensively to study Hg atmospheric distribution.¹¹¹⁻¹¹³ Nevertheless, most of the cited studies were done in Europe and the North America.¹¹⁴⁻¹¹⁶

4.4. The use of sediments to monitor Hg pollution

Sediments are another important area that should be monitored for accumulation of mercury and its compounds. This is partly due to the partial insoluble nature of most of mercury salts forms on the surface from methylation and other reactions in mercury speciation. In addition, most benthic organisms are exposed to sediment than aquatic water, hence it is an important ecoenvironment that should be continually monitored and evaluated for accumulation.

5. Analytical techniques for mercury speciation studies

5.1. Study design

Study design is an important concept in research and should take place before sampling commence. This insures that the exact point source of the toxicant is determined for the necessary authorities to be informed such that best mitigation measures can be put in place. It is important to differentiate background contaminant concentrations from anthropogenic induced concentrations. Hence a very important component in study design is the control.

Choosing a good control is very critical in mercury speciation studies as mercury is capable of leaching even to underground waters. As such, bore holes and springs may not provide the best controls. Generally, when choosing a control, care must be taken such that the control does not give higher values than the sample. The study should be designed such that the sampling methodology ensures representativeness of the samples collected. In addition, all samples should be collected at least in triplicates from each sampling site to ensure correct representation of the ecosystem being studied.

In the design of aquatic ecosystems experiments, one may prefer to use completely randomized design if only a single aquatic ecosystem at one location is being examined. Under this design, the effect of one primary factor without the need to take into account other extraneous sources of variability is studied. Examples of common primary factors in aquatic ecosystems include season, location (site), aquatic type

(Lentic – slow moving water such as pools, ponds, and lakes; Lotic – rapidly moving water such as streams and rivers; wetlands – areas where the soil is saturated with water), floods, landscape, factories and agricultural fields located near aquatic ecosystems, municipal waste discharge, benthic organisms, aquatic animals and plants.

Nuisance factors are those that may affect the measured result, but are not of primary interest. Examples of nuisance factors that may bring extraneous variability in samples apart from the primary factors in aquatic ecosystem include inexperienced sample collectors who are often employed in sampling, temperature of the aquatic ecosystem, time of day of sampling, flow of the water in the aquatic ecosystem, physicochemical conditions of the aquatic ecosystem, different anthropogenic activities at different locations on the same aquatic ecosystems and others that are study area specific.

Complete block design (CBD) is considered as the basic statistical design for making inferences on treatment means. Treatments/variables are randomly allocated to different experimental units in CBD.^{117, 118} Homogeneous experimental units with respect to the response variable receive equal treatment while heterogeneous experimental units are blocked so as to control extraneous sources of variability. In any experiment, there exist some nuisance factors and the experimenter has the obligation of determining significant nuisance factors to control or keep them in track.

Uncontrollable nuisance factors are blocked so as to eliminate/reduce experimental errors. Basically, it is mandatory when designing an experiment to create homogeneous blocks with constant nuisance factors such that the factor of interest (primary factor) is allowed to vary. Such a design is referred to as a randomized complete block design (RCBD).¹¹⁹ As such, the possibility of assessing/evaluating the effects due to the primary factor without the influence of blocked nuisance factors is achieved. In RCBD, the effect of nuisance factors is catered for in the analysis stage using programs such as the Michigan statistical computing programme (MSTAT-C). In randomization of nuisance factors, the key point is to make sure that in each block, the primary factor occurs at the same number of times as the nuisance factor.¹¹⁹ Therefore, the analysis focuses on variation of the primary factor as the nuisance factor is kept constant (or assumed to vary across the population equally, creating an equal random error).

The purpose of blocking is to avoid undesired effects from other factors that are not of interest. Samples for mercury speciation analysis can easily be affected by nuisance factors such as temperature, pH, land terrain, speed of water, season etc. It is desirable to have the blocks arranged perpendicular to a gradient/extraneous variable for effective randomization.¹¹⁹ Consider each block to be a set of experimental units blocked together such that variations within the block are minimized while variation among blocks is maximized. This kind of blocking yields precise results of the desired primary factor and makes the data more valid for decision making.¹¹⁹ The advantage of using RCBD against CBD lies in the reduction of the variance error while the loss is a decrease in the error of degrees of freedom.¹¹⁹

5.2. Sampling and sample preservation

Collected samples should be well preserved to prevent possible changes of the mercury species during storage and/or during transportation to the laboratory. Solid samples like aquatic animals, plants and sediments should be collected in glass or Teflon large-mouth containers. The containers should be double bagged in opaque brown polyethylene bags and shipped for analysis. Wilder recommends that mercury samples should be collected in an ice box and preserved for not more than 28 days in a freezer at -20 °C.¹²⁰ The solid samples should be homogenized prior to digestion to ensure representative sub-sampling and particle size reduction. Samples such as earth worm tissues, sediment, moss and fish tissues can be homogenized using a mechanical bio-homogenizer. It is highly recommended that air dried solid samples should be used for all the characterization tests.¹²¹

Liquid samples of different types (water, precipitations, urine and blood) for mercury speciation should be collected in amber glass bottles or Teflon bottles. The bottles should be labeled, placed in polyethylene bags and shipped for analysis in an ice box. The samples should be immediately preserved upon receipt in the laboratory by acidifying the samples with 0.20 mL of 12 N HCl per 100 mL of sample.¹²⁰ The samples should then be stored in a sample refrigerator at 4.0 ± 2 °C away from light until analysis. The holding time for preserved liquid samples should not exceed 28 days from the time of sample collection.¹²⁰ Alkinization agents (e.g. NaOH) and oxidizing agents (e.g. NaClO, K₂Cr₂O₇, and KMnO₄) are added to avoid volatilization of mercury from the liquid sample.¹²²⁻¹²⁵

455

456 **5.3. Preparation of samples for analysis**

457 Sample preparation is a complicated operation, depending on type of analyte, sample
458 type and origin, utilized method and technique of final determination. Some of the
459 stages involved include different unit processes such as sample mineralization and/or
460 dissolution, removal or masking interfering substances in the sample, extraction of
461 analytes, purification of the extract, derivatization of the analytes and enrichment of
462 the analytes. Some of these processes can be performed separately or combined in
463 order to save time required for sample preparation and/or reduce the risk of loss by
464 elimination of multiple transfer of solutions, as well as the risk of contamination of the
465 sample; the latter factor being of particular importance when trace analysis is required.
466 The scheme in **Fig. 4** gives a brief step by step procedure for sample preparation
467 before trace speciation analysis and determination of individual organometallic
468 compounds in samples of different types. More information on sample preparation can
469 be found in the following research papers for various samples.^{120, 126-146}

470 **5.3.1. Preliminary sample preparation**

471 Solid samples should first be homogenized. This can be achieved by application of
472 special mills or grinders, after drying or deep freezing in liquid nitrogen. Consequently,
473 some procedures require dissolution of a sample matrix with the purpose of liberating
474 these portions of the analytes, which are adsorbed, complexed or otherwise, bound to
475 the main components of the sample. This operation is most frequently met in handling
476 biological samples, in which the analytes are chemically bonded to the protein chains.
477 Similar situations may take place when sediments samples are treated, taken from
478 sediments rich in humic substances, capable to form complexes with many metals.¹²⁶

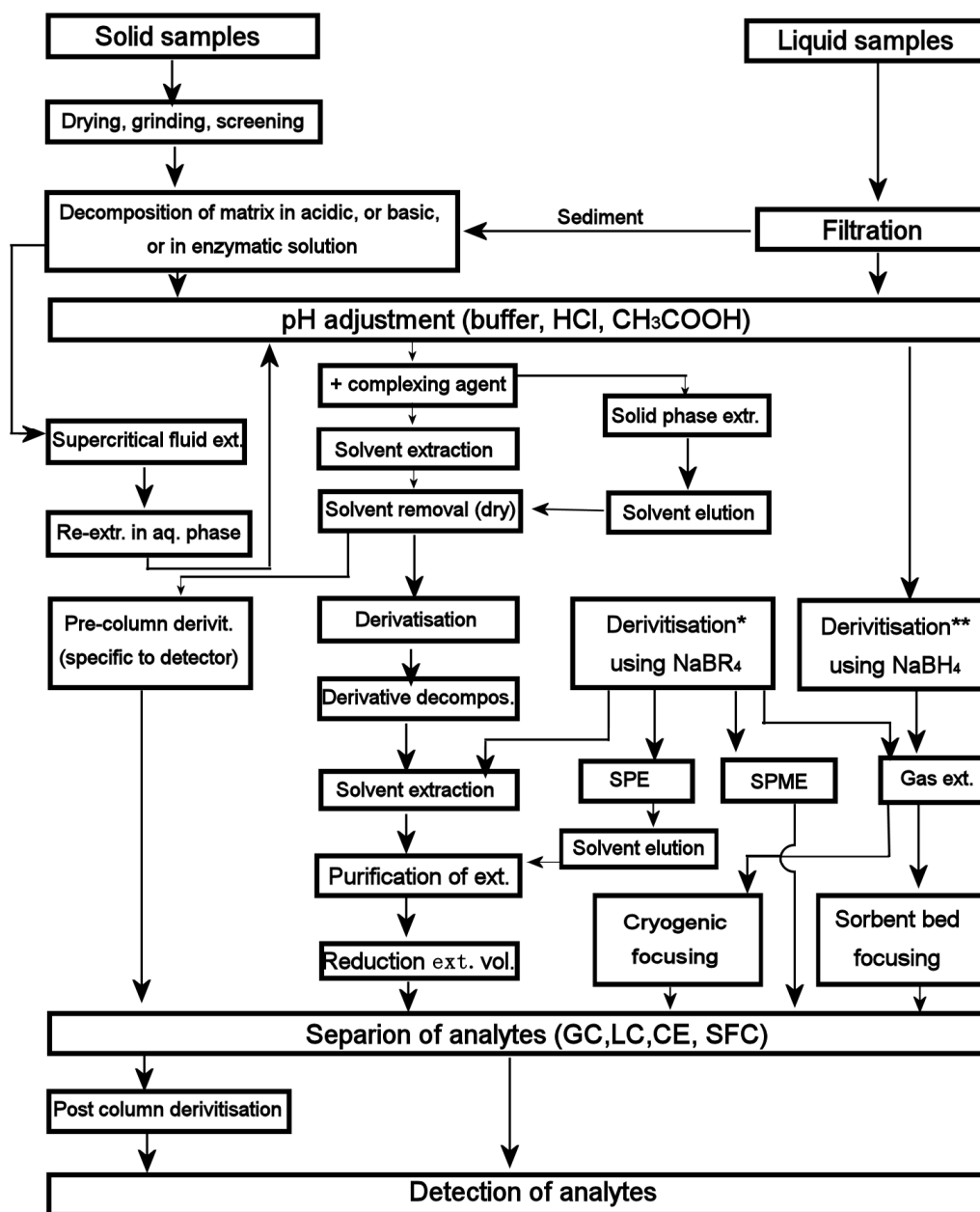
479 In sample dissolution, the most frequently utilized solvents are 1) diluted acids (HCl,
480 CH₃COOH), and their mixtures with alcohols, 2) aqueous or alcoholic solutions of
481 hydroxides (NaOH, KOH, tetramethylammonium hydroxide - TMAH), 3) enzymes
482 containing solutions permitting partial decomposition of a protein matrix for instance,
483 in determination of metal proteins.¹²⁶ Addition of a suitable buffer or certain amount
484 of acid or base solution adjusts the pH of the resulting solution to the desired value.

485 Operation of matrix dissolution used in sample preparation for speciation analysis,
486 despite its formal similarity to mineralization of a sample in cases of determination of

total contents of a component, is usually carried out under much more gentle conditions.¹²⁶ In the latter case, total conversion of all the forms present in the sample to free metal ions is usually required. This is most frequently achieved using concentrated mineral acids (HCl, H₂SO₄, and HClO₄) or their mixtures, which reduce matrix to simple inorganic compounds. The method cannot be employed in case of speciation analysis, as destruction of matrix would mean losing information belonging to the very goal of analysis. Gentle conditions of sample dissolution, used in speciation methods, do not result in matrix destruction, but rather in its partial decomposition and liberating the analytes from macromolecules.¹²⁶

5.3.2. Extraction of analytes

This important step of sample preparation procedure is present in most (if not all) procedures used in trace analysis. It is therefore inseparable from speciation analytics. Extraction of analytes may be included in different phases of the whole process of sample preparation to achieve different objectives. Organometallic compounds such as MeHg and Me₂Hg are present in environmental samples at very low concentrations and as such the principal objective of sample preparation before analysis is to enrich the compounds for ease of extraction and accurate results. To this end, liquid solvent extraction, gas extraction (stripping), solid sorbent extraction, and supercritical fluid extraction are used.¹²⁶ Another objective of this operation is matrix exchange prior to any subsequent step of sample preparation or just before final determination. Other substances that might interfere in speciation analytics are avoided through the extraction process. Analyte enrichment, change of the matrix, and removing interfering substances are the three main targeted objectives in sample preparation.



* R = C₂H₄, C₃H₇,  ** not applicable for lead compounds

Fig. 4. General procedures in mercury speciation analysis. Purification of extract can be done using Al₂O₃ or silica gel and extraction solvents such as dichloromethane etc. SPE = solid phase extraction, SPME = stationary phase microextraction.¹²⁶

Extraction of analytes from aqueous solutions by organic solvents is usually enhanced by addition of complexing agents, like tropolone (2-hydroxy-2,4,6-cycloheptatrienone) or sodium diethyldithiocarbamate (NaDDTC, DDTC). These enhancers form weak

non-polar complexes with ionic forms of analytes originating from sample or as a result of matrix dissolution which are easily transferred to the organic phase.

These additives offer advantageous recoveries when methods including subsequent derivatization of analytes using Grignard reagents, or their extraction to a non-polar solid phase, are employed.¹²⁶ In a case of derivatization using sodium tetraalkyl or tetraarylborates (ethyl borate, propyl borate, phenyl borate), the reaction is usually carried out in a solution obtained directly in the matrix dissolution step, after a suitable pH adjustment. Some authors suggest a possibility of carrying out the extraction of solid samples using supercritical fluids (SFE) without any previous treatment.¹²⁶ Frequently, extraction of analytes is aided by ultrasounds or microwave field energy.

USEPA has developed a method used for specific species extraction in solid samples called Microwave Mercury Extraction (EPA 3200) which follows immediately before analysis of the specific species by HPLC-UV-CVAFS.¹⁴⁷ The main process in this method is extraction of mercury species under the influence of microwaves using a 4.0 M HNO₃ solution. The microwave extraction vessels are loaded with 1.0 ± 0.2 g of the wet or dried sample before addition of 10 mL (4.0 M) HNO₃ solution. The vessels are sealed and irradiated at 100 °C for 10 min, cooled down and filtered through a whatman no. 40 filter and stored at 4 °C until analysis.

It seems worth mentioning that in cases of the determination of total metal contents, as opposed to speciation analyses, extraction of analytes is rarely necessary. It is included, as a rule if only determination of trace amounts of metals is involved (e.g., extraction of gold using ion exchanging resins before its determination from sea water).

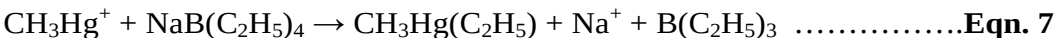
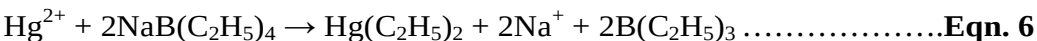
5.3.3. Derivatization of analytes

This operation is included in majority of procedures for determination of mercury compounds.¹⁴⁸ Its objective is to change the chemical form of an analyte which simplifies (or even enables) the subsequent analysis. As mentioned previously, the analytes present in solution after sample dissolution are usually in ionic form. Separation of polar substances, which is the case when ions are involved, may lead to many problems when typical chromatographic systems usually designed for separation of non-polar substances are employed. Ionic analytes may result in peak tailing, which makes proper (or automatic) interpretation of peaks difficult, as well as gradual worsening of separation power of the column, caused by irreversible adsorption of analytes on active sites of the column.¹²⁶ Even if some procedures are available,

permitting separation of analytes without their derivatization prior to separation on specially prepared columns,^{139, 149} they have never enjoyed wide popularity due to their increased labor consumption (column deactivation after several analyses is required). Notably, the derivatization step might increase the risk of error bearing results of analysis due to either loss of analytes or unintentional contamination of samples with analytes.

The aim of derivatization is: 1) facilitating the separation of a mixture of analytes; 2) making detection possible; 3) enhancing sensitivity of the final determination of analytes. While speciation analytics involving gas chromatography employs practically exclusively different types of selective detectors (chiefly spectrometric), when it comes to liquid chromatography, derivatization may make it possible to employ common, universal detectors, e.g., a UV-VIS spectrophotometric detector or fluorescence detector, thus increasing applicability of the latter separation technique; 4) to facilitate or enable analyte enrichment.

Derivatization provides extractable forms of analytes for processes such as stripping, solvent extraction, solid phase extraction or stationary phase micro extraction. Among the methods of derivatization, the following are popular: derivatization utilizing Grignard reagents, sodium alkyloborates and sodium hydroborate. A serious disadvantage of derivatization using Grignard reagents is high labor consumption, due to the necessity of using non-aqueous media.¹²⁶ In most derivatization techniques, adsorption processes of methyl mercury take place at the stationary phase in chromatographic analysis. As such, peak broadening and ghost peaks appear due to this adsorption.¹⁵⁰ Therefore, polar methyl mercury compounds should be converted to nonpolar methyl mercury compounds before chromatographic separation. Sodium tetraethylborate (NaBEt₄) is occasionally used in derivatization of polar methyl mercury compounds to nonpolar ethylated methyl mercury (see **eqns. 6 and 7**).



While the methods using sodium alkyloborates or sodium hydroborates are much simpler (respective reactions occur in aqueous medium), their application is not always possible. Ethylation of analytes using NaBEt₄ does not always provide an unambiguous answer to the question, ‘What kind of analyte was present in the original

sample?' (e.g., Pb^{2+} , PbEt^{3+} , PbEt_2^{2+} , PbEt^{3+} , all will produce PbEt_4 after ethylation), while the reaction with sodium hydroborate is limited to analytes yielding volatile products (this fact excludes it from use in lead compounds).¹²⁶

Nevertheless, derivatization in aqueous medium is such an easy method with new derivatization reagents being tested, like sodium tetrapropyl and tetraphenylborate.¹³⁸ Application of these new reagents brings opportunity to eliminate the aforementioned ambiguities. The derivatization process may be carried out before, after or even during the extraction step. It is usually performed before the separation of analytes; sometimes it takes place prior to the detection step. While derivatization is presented as a step of many methods for determination of mercury compounds, it is relatively/rarely included in procedures for the determination of total metal contents, unless derivatization is counted like such operations as precipitation or reduction of metals to atomic form.

5.3.4. Enrichment of analytes

As previously mentioned (see section 5.3.2), enrichment of analytes, while in different styles, is embraced by all procedures of speciation analytics. Most frequently, this process occurs during solvent extraction of analytes. Other popular methods include: solid phase extraction, supercritical fluid extraction and gas extraction. Each of these methods has its own advantages and disadvantages. Undoubtedly, solvent extraction may be among the most versatile methods. However, it also has its share of disadvantages. First of all, when high enrichment is desired, relatively large samples must be used. In turn, adequately large volumes of liquid solvents are consequently consumed. This solvent (or solvents) must subsequently be separated from the analytes to achieve the desired degree of enrichment.

To this end, solvent evaporation in a stream of inert gas or distillation is usually utilized. Both of these operations are time consuming and cause air pollution either with substances which are neutral to human health or to the environment. Economic considerations also provide arguments against this method. Solvents used for extraction must be of high purity, which means they are of relatively high price. Although they may be recovered (e.g., by distillation), laborious process of their purification must be done. Despite the handicaps, enrichment by solvent extraction remains quite popular due to simplicity and the use of unsophisticated equipment.

Although, there is a trend in analytical chemistry to eliminate this method replacing it by other methods such as analyte enrichment, limited use of solvents is still in practice.

Among such methods, there are: 1) solid phase extraction, 2) supercritical fluid extraction, and 3) gas extraction. Solid phase extraction (SPE) is a method which might become a complete substitute for solvent extraction. Unsophisticated equipment, simplicity, wide selection of sorbents and high values of the enrichment coefficient contribute a wider application of this technique. Additionally, there is always a possibility of analyte enrichment *in situ* and analysis of samples in an on-line mode. Among the limitations, there is a necessity of previous transfer of analytes to a liquid or gaseous phase when solid samples are involved, and also some difficulties in extraction of highly contaminated samples.

Supercritical fluid extraction (SFE) is a method used for analyte enrichment from solid samples. A great and unique advantage of this technique is the opportunity of almost complete control of extraction properties of the supercritical fluid. Unknown to other methods of extraction, this feature creates the opportunity to influence the process, even when in progress. The most common extracting agent is carbon dioxide, pure or with the addition of the so-called modifiers (methanol, acetone, ethanol and dichloromethane). Additionally, other rarely used media are freons, ethane, sulfur hexafluoride, nitrous oxide, methanol, ammonia, and water. The limited number of these substances does not limit versatility of SFE. When using an extracting agent like carbon dioxide, some parameters of the process can be determined such as temperature, pressure, type and amount of a modifier used which best suit our actual needs. Despite numerous and undisputable advantages of supercritical fluid extraction, it is not yet common in analytical laboratories (quite the opposite situation is found in industry). That can be attributed to the high price of instrumentation required.¹³⁴

Gas extraction is a method of analyte enrichment, usually coupled with gas chromatography. The method is employed when analytes are sufficiently volatile or may be suitably transformed by means of derivatization. Gas extraction is one almost perfect method of enrichment, when applicable.¹⁵¹ The technique is used for analysis of both solid and liquid samples. It provides an opportunity of practically complete transfer of analytes from the sample to the separation system, at the same time permitting significant lowering of the detection limit of the method. It eliminates any need for using solvents.

Additional gains are: simple equipment and almost "natural" compatibility with on-line analytical systems. Its most serious disadvantage is limited applicability. As mentioned before, the analytes (or their derivatives) enriched by this technique must indicate suitable volatility, which eliminates any chances for analysis of high molecular weight substances (e.g., biomolecules). A limited repertoire of derivatization reagents also narrows the application of gas extraction for enrichment of analytes. Additional problems may arise not from the type of analytes present in the sample, but rather from the sample matrix. For example, when the sample matrix contains surfactants, the formation of foam during gas extraction may be observed.

In summary, an ideal and perfect method of analyte enrichment does not exist. The repertoire of available ways and means is sufficiently broad to permit an optimal choice of most suitable method in a given case.

5.3.5. Integrated sample preparation procedures

The problem associated with shortening analysis time is closely related to the chosen procedure of sample preparation method. Sample preparation is the bottleneck step of all analytical methods (if it is included), limiting further reduction of time required per single analysis. Among the methods of sample preparation used in speciation analytics, special attention should be paid to integrated (accelerated) sample preparation procedures which combine several sample preparation steps into one operation. Microwave assisted sample preparation procedure has been successfully applied for organotin, organomercury and organolead compounds for samples of different origin.^{126, 128}

Besides shortening analysis time integrated sample preparation procedures are easier for automation and less susceptible to human errors than multistep protocols. Analytes separation is an imminent feature distinguishing procedures belonging to the field of speciation analytics from procedures for determination of total metal contents. The necessity of this step, or stage, is indicated by the very definition of speciation analysis. Liquid and gas chromatographic methods are commonly employed in integrated sample preparation as well as supercritical fluid chromatography.¹⁵¹⁻¹⁵⁴

Attempts have been made to apply capillary electrophoresis for speciation analytics of organometallic compounds.¹⁵⁵ However, its utilization is rather marginal despite the high separation potential. There are several reasons for this situation: For instance, a requirement to use a highly sensitive detector (e.g., ICP-MS) to ensure an adequate

detection limit, due to a small volume of the sample that may be introduced to the detector.^{128, 156} Additionally, in many cases there are no sufficiently sensitive and selective detectors for organometallic compounds.

A similar situation may be observed for the use of chromatography with supercritical fluid as a mobile phase. This technique combines the features of gas and liquid chromatography. Despite its advantages (e.g., a possibility of determination of substances of significantly different boiling points, even non-volatile ones, in a single run), it has not yet found wide application in speciation analytics. The most probable reason is the high cost of required instrument compared to instruments used in competitive methods, like both gas and liquid chromatography which for many reasons and in many cases are better methods.

Liquid chromatography, capillary electrophoresis and supercritical fluid chromatography are irreplaceable methods when separation of high molecular mass, non-volatile, or thermally labile substances is required. It does not mean that applications of liquid chromatography are restricted to these groups of compounds. Many cases are described which utilizes liquid chromatography for the determination of derivatives usually analyzed by gas chromatography.¹⁵⁷ Sometimes liquid chromatography enables analysis of samples without previous derivatization of analytes. Unfortunately, the "Achilles heel" of liquid chromatography is the high value of the detection limit.

This result from the fact that most frequently, detectors used in speciation analytics require introduction of the sample in a gas phase (as a vapor or aerosol).^{128, 157} This requires turning the elute from the column into a form of spray, which leads to losses in analyte contents up to 98% of their original amount, according to some authors.¹²⁸ The leading place among the separation methods employed in speciation analytics is, undoubtedly, occupied by gas chromatography.¹⁵¹ It is used for determination of compounds which are characterized by suitable volatility and thermal stability under separation conditions, or may be transformed to a suitable form. This group includes chiefly alkyl and aryl derivatives of such metals as mercury, lead, tin,¹⁵⁸ arsenic,^{159, 160} and selenium.¹⁶¹ The situation here is paradoxical, as the analytes present in real samples are basically compounds of ionic character, usually polar and of not particularly high thermal stability. Generally, these are not good candidates for determination by gas chromatography. In the field of speciation analytics, using

different reactions of derivatization of the analytes solved the problem. These reactions yield non-polar compounds, sufficiently stable at elevated temperatures, quite suitable for separation by gas chromatographic techniques.

Utilization of gas chromatography in speciation analytics brings several advantages. For instance, there is flexibility in combining this technique with different systems of final determination and/or analyte identification. As opposed to liquid chromatography, it is relatively easy to introduce a whole sample to the detector. Additional advantages favoring this technique are: moderate instrument prices, wide variety of available detectors, and one of the best separation powers.

5.3.6. Final Determination

The last stage in analysis (if one skips the data handling) is identification and final determination of analytes. Different spectroscopic methods enjoy wider application in analysis of metals. Also detectors such as electron capture detector (ECD) or a flame-photometric detector (FPD) are occasionally employed. The type of detector employed is related to the technique of separation of the analytes, although some detectors are of broad or universal use. For instance, the aforementioned ECD and FPD are not used when the analytes are separated by means of liquid chromatography or capillary electrophoresis, while a fluorescence detector is not suitable for gas chromatography. Spectroscopic methods such as mass spectrometry or atomic emission spectrometry with inductively coupled plasma (ICP-MS, ICP-AES) belong to the most universal, sensitive and selective methods of detection utilized both in speciation analytics of organometallic compounds and in determinations of total contents of metals. Due to the low detection limits offered by inductively coupled plasma, techniques of combining this detector with all major methods of analyte separation (GC-ICP-MS, LCICP-MS, SFC-ICP-MS, CE-ICP-MS) have been developed.⁹⁹

In a similar case and gaining wider popularity is the atomic emission detector with microwave induced plasma, MIP-AED. Its coupling with gas chromatography has been solely described to-date. Due to its lower sensitivity (by several orders of magnitude), usage of the atomic absorption detector (AAS) is limited to GC-AAS and LC-AAS combinations.¹⁶² For some metals, specific and highly sensitive detectors exist such as atomic fluorescence detector (AFS) which has been successfully used in determinations of mercury, arsenic, and selenium compounds,¹⁶³ or a flame

photometric detector (FPD), offering an opportunity of selective determination of tin, selenium¹⁶¹ and germanium.¹⁶⁴

Another feature differentiating speciation analytics from determination of total contents of metals is a trend toward utilization of detectors enabling identification of structure of the analyte detected. Among such detectors are mass spectrometers of several types (MS, MS-MS) as well as detectors utilizing the infrared spectrometry principles (IR, FTIR, FFTIR).

5.4. Loss of mercury during sampling and prevention

Volatization of mercury to the environment is a fast and viable process in which mercury can be lost from a sample before analysis.^{124, 165} A significant loss of mercury to the atmosphere during sampling has been reported.^{124, 165} However, this loss significantly reduce on addition of KMnO_4 , NaClO , $\text{K}_2\text{Cr}_2\text{O}_7$, KMnO_4 , and Au(III) to the sample before storage.¹²²⁻¹²⁵

Mercury adsorption to container walls also significantly reduces mercury concentrations in solution. Specifically, polyethylene bottles can accumulate significant amounts unless the sample is acidified with nitric acid, sulfuric acid, or a combination of acid and oxidizing agents such as KMnO_4 , NaClO , $\text{K}_2\text{Cr}_2\text{O}_7$, KMnO_4 , and Au(III) .^{165, 166} It is also possible for mercury to adsorb onto colloidal particles in the sample.¹⁶⁷ Furthermore, if the surrounding environment is contaminated with mercury, then it is also possible for atmospheric mercury to affect sample concentrations. It has been demonstrated that a mercury concentration of 1 mg/m^3 in the air produces 2 ng/m^3 in the associated aqueous phase.¹⁶⁵ And that the addition of acid/oxidants can increase mercury partitioning in favor of the water phase, hence acting as a mercury sink.¹⁶⁷

Hg^0 can easily be oxidized in hydrocarbon solvents especially for samples exposed to oxygen,¹⁶⁸ hence the need to take great care during analytics procedures to avoid errors in speciation data.

5.5. Sampling of mercury from the atmosphere (air)

The need to determine, with precision, the exact point source of mercury into an aquatic ecosystem requires elimination of possible atmospheric contaminations.¹⁶⁹ As such, it is important to collect atmospheric samples near the sampling point for complete data interpretation. In order to collect an air sample, one may use chemically

selective adsorption traps: 1) Chromosorb-W treated with hydrogen chloride (HCl) - retains only Hg^{2+} compounds, 2) Chromosorb-W treated with sodium hydroxide (NaOH) - retains MeHg compounds only and even excludes $\text{CH}_3\text{CH}_2\text{Hg}$ compounds, 3) Glass beads coated with silver – collects Hg^0 by forming an amalgam, and 4) Glass beads coated with gold - retains dimethyl mercury only.¹⁷⁰

The specificity of atmospheric mercury collection by the above chemo-traps was evaluated.¹⁷¹ Chromosorb-W (HCl) and Chromosorb-W (NaOH) did not collect elemental mercury and dimethyl mercury vapors. However, both silver-coated and gold-coated glass beads retained both species completely. Other chemo-trap tubes include: 5) Tenax-GC which is a porous polymer based on 2,6-diphenyl-p-phenylene oxide at room temperature – it retains CH_3Hg compounds only, 6) Carbosieve-B which is a granular form of activated charcoal at 180 °C – it collects $\text{CH}_3\text{CH}_2\text{Hg}$ compounds only, and 7) Gold wire at room temperature – it collects elemental mercury.^{170, 171}

6. Specific methods of determining different mercury species

6.1. Vapor generation atomic absorption spectroscopy (VGAAS)

Different mercury species in aquatic ecosystem can be analyzed using VGAAS. Initially, this method was used solemnly in determination of total mercury, even at trace levels in foodstuffs.¹⁷² The analytical method follows conversion of all the mercurial species to atomic mercury vapor. Using this approach, different mercury species can also be determined in a sample if the species are separated before analysis. We describe briefly how these separations can be achieved and later analyzed differently using VGAAS.¹⁷³⁻¹⁷⁵ The Separation procedures have been used successfully in extracting different mercury species in biological samples.¹⁷⁶

In order to separate inorganic mercury species from an aquatic sample, 1 g of the dry solid sample is homogenized in distilled water using homogenizers like Polytron homogenizer. Then, 0.25 ml of the aliquot (or of water sample) of each homogenate is acidified with 6 M HCl (0.1 ml) and mixed with toluene (1 ml) in 2 ml screw-capped tube with 4 zirconium particles (2-mm diameter) using the micro homogenizing system and centrifuging at 12000 rpm for 1 min. The upper phase is removed before the MeHg in the homogenate is completely removed by addition of 4 ml toluene extractions. The resultant aqueous phase contains exclusively inorganic mercury and is

washed once with petroleum ether (1 ml) to remove residual toluene and neutralized with 4 M NaOH (0.15 ml) and thereafter total mercury is determined using VGAAS.

In order to separate MeHg from a sample, 0.5 g of dry sample (10% w/v) is homogenized in distilled water using a polytron homogenizer. Addition of MeHg-L-cysteine (2.5 μ M which is equivalent to 0.5 ppm as Hg) to each homogenate is done and then 0.25 ml of the homogenate or filtered liquid sample is acidified with 6 M HCl (0.1 ml) in a 2 ml screw capped polypropylene tube. Toluene (1 ml) and 4 zirconium particles (2 mm diameter) are subsequently added to the sample.

Table 1. Experimental conditions for the mercury speciation analysis.

Injection Volume (μ L)	100
Sample flow (mL/min)	1.5
Reductor Flow (mL/min)	4 $\pm$ 1
Blank Flow (mL/min)	8 $\pm$ 1
Detector Gain	1.0

The mixture is then vigorously mixed at 3000 rpm for 2 min using a micro homogenizing system. Thereafter, the samples are centrifuged at 12000 rpm for 1 min and exactly 0.5 ml of toluene solution at the upper phase is transferred to a 1.5 ml polypropylene tube. In this tube, exactly 0.25 ml of 5 mM glutathione in 100 mM sodium phosphate buffer (1 mM EDTA, pH 7.5) is added. The resultant mixture is shaken (5 min) in a microtube mixer; one can use the MT-360 type, to extract MeHg by the aqueous phase as its glutathione conjugate.

Centrifuge the resultant sample (12000 rpm for 1 min) and remove toluene by aspiration; an insoluble material phase is mixed with petroleum ether (0.5 ml) using a vortex mixer for 1 min, then the organic phase is removed by aspiration. The residual petroleum ether is removed completely under an air stream of aspiration. The sample solution thus prepared is diluted by 2 times from the initial homogenate and contains exclusively MeHg. The mercury level in the final sample is determined together with the initial homogenate by VGAAS.

Total mercury in different aquatic environmental samples can also be determined using this method. Solid samples like fish, earth worms, sediments and plants can first be homogenized then placed in an oven set at 80 $^{\circ}$ C until a constant weight is attained.

Thereafter, 0.5 g of the homogenized sample is placed into digestion vessels then 10 ml of HNO₃ is added and allowed to stand in a fume chamber for 30 minutes. The vessels are then sealed and digested for 2 hours then cooled in the fume chamber. Lastly, the extract is transferred into a 100 ml volumetric flask, then topped up to the mark with double distilled water.

All the above extracts are subjected to VGAAS analysis by adding 60 ml of potassium permanganate solution (6%). The samples are left loosely capped for at least 2 hours, followed by slow addition of 15 ml hydroxylamine chloride solution (20 %m/v). Deionized water is added to the sample to make 100 ml by volume. The sample is then analyzed in a VGAAS using the following spectrometer parameters: Wavelength = 253.7 nm, Lamp current = 75 %, Bandpass = 0.5 nm, Background correction = D2 Quadline, Resamples = 5, Measurement time = 4.0 s. While Vapor parameters are: Pump speed = 40 rpm, Gas flow = 200 ml/min, Acid reagent = 50 % HCl, Reductant = 7.5 % stannous chloride in 10 % HCl, Measurement delay = 70 seconds. During these processes: 1) take care as the reaction is exothermic; 2) add hydroxylamine chloride slowly as the solution may foam and eject some sample from the flask.

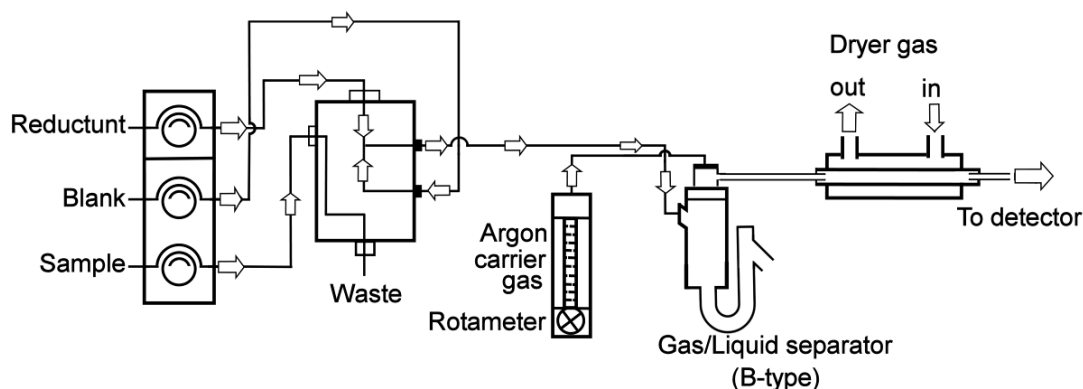


Fig. 5. Diagram of the mercury flow system.¹⁷⁷

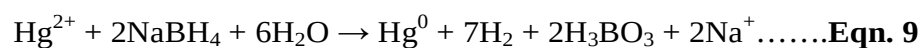
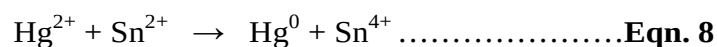
6.2. Use of cold vapor generation atomic fluorescence spectrometry coupled to an online UV post-column oxidation system

Currently, the method used for mercury speciation is the HPLC-UV-CVAFS (cold vapor generation atomic spectrometry coupled to an online UV post-column oxidation

system).¹⁷⁸ The samples for this analysis are prepared/extracted by microwave mercury extraction method described in EPA 3200.¹⁴⁷ The dried or wet homogenized solid samples are weighed (1.0 ± 0.2 g) into microwave extraction vessels (or 1 mL of water sample) and 10 mL of 4.0 M HNO₃ added to each sample. The microwave vessels are then sealed and irradiated at 100 °C for 10 min. Thereafter, the vessels are allowed to cool and the extracts filtered through a whatman no. 40 filter. The extracts are then stored at 4 °C until analysis.

Separation of mercury compounds is achieved by injection of 100 µL of the sample/blank/standard into a reverse phase column (for example ODS HypersilThermo. 250 x 4.6 mm, 5 µm).¹²⁰ The separation mobile phase is composed of methanol: ammonium pyrrolidinedithiocarbamate (APDC) at pH 5.5, (1.5 mL APDC + 10 mL NH₄Ac) in a ratio 80:20. Simultaneous analysis of Hg²⁺, MeHg⁺ and EtHg⁺ is optimized through adjusting the ratio between organic and aqueous phase, however, the mobile phase remains to be of the same composition. Some of the experimental conditions as used by Wilder (2010) are shown in **Table 1**.¹²⁰ APDC is highly stable and it forms stable non-ionic complexes with Hg species suitable for reverse phase separation.

The separated species are converted to Hg²⁺ for determination by CVAFS system. The Hg-C bond in HPLC separated organomercuric compounds is broken by an online oxidation photo-reactor. The reactor consist of a poly(tetrafluoroethylene) (PTFE: 0.5 mm diameter and 12 m long) tube coiled on a UV lamp that generates 150 W of radiation at 250 nm. Emission of non-absorbed radiations from the reactor is prevented by covering it with an aluminum sheet that reflects the emissions back to the system. After passing Hg-C compounds through the reactor, the generated Hg²⁺ is converted to Hg⁰ by acidified stannous chloride (**Eqn. 8**) or sodium borohydride (**Eqn. 9**).^{120, 163}



Introduction of argon gas into the transference line helps to pick up Hg⁰. The gas-liquid mixture is separated in another separator device in which the argon gas mixed with Hg⁰ is taken to AFS detector.

During the reduction step, a multi-channel peristaltic pump running at a constant speed of 100 rpm delivers tin chloride/ sodium borohydride to the sample solutions.

An electronically controlled switching valve helps to alternate between blank and samples for accurate measurements. Thereafter, the gas/liquid separator isolates and delivers the gaseous products to an atom reservoir where subsequent spectroscopic determination takes place (see **Fig. 5** for an illustration). In the detector, mercury vapor absorbs radiation emitted at 254 nm from Hg lamp and fluoresces at its characteristic wavelength of 254 nm enabling its detection. Microwave extracted samples should be oxidized before analysis by adding 50 μ L $\text{BrO}_3^-/\text{Br}^0$ 55% solution.

Total mercury can also be determined using this method by feeding the sample, blank and/or standard directly into the CV generator. However, the volume intake should be increased (<20 mL) due to continuous pumping of the samples until the detection signal is well stabilized. Method detection limits of up to 0.05 $\mu\text{g/L}$ have been recorded.^{120, 163}

6.3. Methyl mercury and dimethyl mercury determination in aquatic water samples using distillation, aqueous ethylation, purge and trap, and CVAFS

Cold vapor atomic fluorescence spectroscopy (CVAFS) has been successfully employed in analysis of CH_3Hg concentrations from various environmental samples including filtered and unfiltered water.¹⁷⁹ Before analysis, the samples undergo distillation, aqueous ethylation, purge and trap and deposition techniques.¹⁷⁹ Although detection limits of up to 0.02 - 5 ng/L have been recorded, much lower limits can be achieved with a smaller sample size. This is particularly so as detection limits (MDL) and minimum level (ML) of quantization depend on the level of background elements rather than instrumental limitations. As such, MDL; 40 CFR 136 for CH_3Hg has been determined to be 0.02 ng/L excluding background elements and matrix interferences. Similarly, the minimum level (ML) is set at 0.06 ng/L. It is possible to achieve lower MDLs of up to 0.009 ng/L if samples and reagents are handled cautiously and only 'for ultra-low level only' distillation equipment are employed.¹⁷⁹

Among the speciation techniques in this method, the following steps are highlighted:

- 1) Collect water samples using specially cleaned and pretested fluoropolymer or borosilicate bottles (100 – 2000 mL).
- 2) If dissolved CH_3Hg is the target species, filter the samples through a 0.45- μm capsule filter.
- 3) Preserve the water samples by adding 4 ml/L of pretested 11.6 M HCl. For saline samples ($[\text{Cl}^-] > 500$ ppm), preserve them by adding 2 ml/L of 9 M H_2SO_4 solution which prevents distillation interferences

caused by excess chloride. 4) Put 45 ml of the sample into a fluoropolymer distillation vessel and add 35 ml of ultra-pure water and distill the sample into a receiving vessel at 125 °C under N₂ environment. 5) Adjust the distillate pH to 4.9 using acetate buffer. 6) Ethylate the resultant sample by addition of sodium tetraethyl borate (NaBEt₄) (**Fig. 6a**).

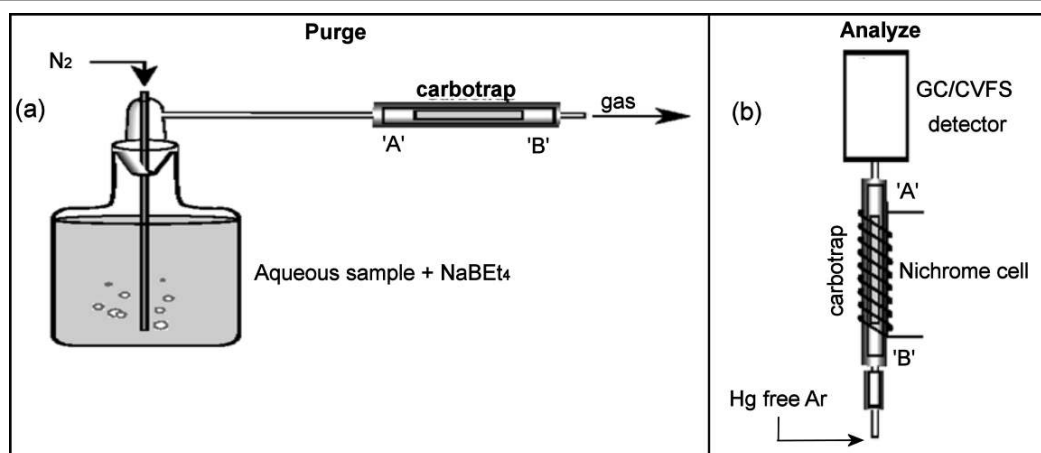


Fig. 6. Schematic diagram of bubbler setup and carbotrap® trap orientation¹⁷⁹.

7) Separate the ethyl analog of CH₃Hg, methyl-ethyl mercury (CH₃CH₃CH₂Hg), by purging with N₂ onto a graphitic carbon (Carbotrap®) trap (**Fig. 6a**). 8) The trapped CH₃CH₃CH₂Hg is then desorbed from the Carbotrap® thermally (using a GC) into an inert gas stream (**Fig. 6b**). 9) The gas stream carries the released CH₃CH₃CH₂Hg to a pyrolytic decomposition column, to convert organo-mercury to elemental mercury, and then into the cell of a CVAFS for detection. 10) Carry out quality tests to validate the results through the use of standards and blank samples (calibration range = 1 -100 pg Hg).

Dimethyl mercury, (CH₃)₂Hg, can also be determined by the same method. However, the following additional steps are necessary. 1) The analysis should be performed on fresh unfiltered and unpreserved water samples. 2) To minimize analyte losses, all efforts must be made to speed the time between sample collection and sample analysis. 3) To minimize contact of the sample with the atmosphere prior to purging, the samples should be purged and analyzed in the field. 4) If it is impossible to do onsite analysis, samples should be collected directly into headspace-free 1 L glass bottles with fluoropolymer-lined caps, and sent via express to the laboratory for

analysis. 5) The sample can have a stable $(\text{CH}_3)_2\text{Hg}$ concentration for up to 48 hours under the dark and cool environments without preservation. 6) Do not collect samples in plastic bottles of any kind (including fluoropolymer) for determination of $(\text{CH}_3)_2\text{Hg}$ (half-life of dissolved $(\text{CH}_3)_2\text{Hg}$ in fluoro-polymer bottles is 6 h). 7) The distillation step is avoided and 1 L of the sample is purged onto a Carbotrap® trap under N_2 environment at a flow rate of 300 mL/min for 30 min. (In most cases, a specially designed 1 L bottle with 24/40 ground glass fitting and fritted bubbler cap is used for purging see **Fig 6**). 8) Analyze the samples within 6 hours after purging. 9) Set the GC oven at 80 °C for $(\text{CH}_3)_2\text{Hg}$ and 110 °C for $\text{CH}_3\text{CH}_3\text{CH}_2\text{Hg}$. This facilitates the separation of Hg^0 from $(\text{CH}_3)_2\text{Hg}$. 10) During analysis, the first peak that appears at 1.0 min belongs to Hg^0 while the second peak at 1.5 min belongs to $(\text{CH}_3)_2\text{Hg}$. The peaks are close to one another hence the need to set an event marker which will signal the start of GC run as positive identification of the analyte is dependent on retention time. 11) While preparing standard samples, it is advisable to use a stock solution prepared by dissolving 1 ppm of $(\text{CH}_3)_2\text{Hg}$ in methanol, or purchase one from Strem Chemical (Newburyport, MA).

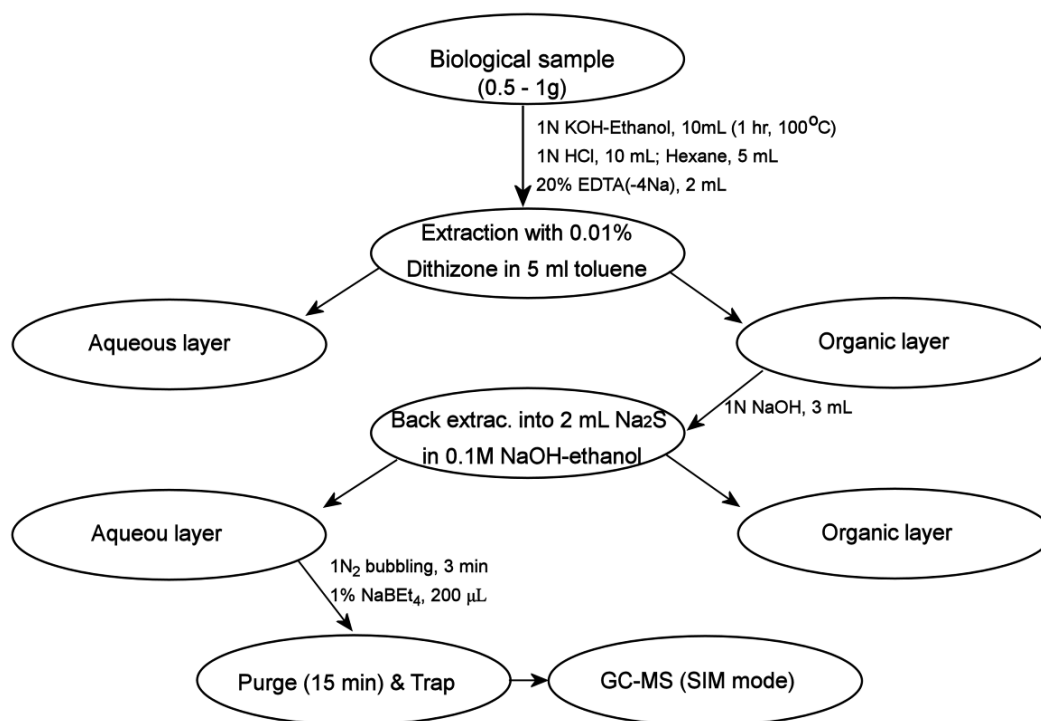


Fig. 7. Schematic flowchart of the purge & trap GC-MS method for the analysis of methyl mercury in biological samples.¹⁴⁵

A good working stock solution of 1 ng/mL maybe prepared by 1:1000 dilution of the purchased stock solution in methanol. Such solutions can be stable for up to 4 years under refrigeration in dark glass bottles. It is important to note that the purchased stock solution will have an approximate concentration hence the need to determine its exact concentration by spiking 1 ng into a bubbler of pre-purged reagent water, and then purged onto a gold coated sand trap.

Analyze total Hg in the sand trap (use EPA Method 1631 for this analysis). Alternatively, one can purchase pre-calibrated working standards from Frontier Geosciences Inc., or equivalent. In most cases, it is advisable to analyze three method blanks after a batch of 20 samples. Note also that since $(\text{CH}_3)_2\text{Hg}$ is rarely detected in the environment, matrix spikes should be low (i.e., 1-5 pg), to assure the ability to measure near the MDL. Furthermore, there are no certified reference materials (CRMs) or second source LCSWs available for $(\text{CH}_3)_2\text{Hg}$. There are no interferences registered so far in $(\text{CH}_3)_2\text{Hg}$ determination, however, volatile organic compounds mostly present in wastewaters/effluents could elute together with $(\text{CH}_3)_2\text{Hg}$ in the GC hence quenching the signals from atomic fluorescence detector. Do not forget to analyze/collect samples in replicate and if a sample is opened, it must be analyzed immediately as $(\text{CH}_3)_2\text{Hg}$ will evaporate. In addition, do not filter the samples prior to analysis and do not store the samples in acidified environment as this will convert $(\text{CH}_3)_2\text{Hg}$ to CH_3Hg . Nevertheless, sample acidification can be done immediately before analysis if both Hg^0 and $(\text{CH}_3)_2\text{Hg}$ are to be analyzed together. Furthermore, note that if samples are stored in light, $(\text{CH}_3)_2\text{Hg}$ decomposes to CH_3Hg^+ , and if stored in plastic bottles, $(\text{CH}_3)_2\text{Hg}$ diffuses into the plastic matrix.

6.4. Determination of methyl mercury in biological samples using dithizone extraction method followed by purge & trap GC-MS

Speciation analytics of mercury mainly involves extraction, separation and detection. GC and LC are well advanced technologies in separation whereas ECD, AAS, AFS and ICP-MS are used for detection.^{27, 180} Extraction of CH_3Hg^+ from water samples has been achieved through solvent extraction and distillation techniques (see section 6.3). However, CH_3Hg^+ extraction from solid and biological samples cannot be

achieved through the above techniques due to matrix interference. For instance, toluene or dichloromethane solvent extraction of solid/biological samples yield very low extraction efficiency.¹⁸¹ The distillation technique leads to transfer of large amounts of volatile organic compounds into the distillate leading to interference at the ethylation stage and GC detection inaccuracies.¹⁸² As such, new techniques have been developed to extract and analyze CH_3Hg^+ from solid biological samples such as the dithizone extraction method combined with GC-MS detection.¹⁴⁵ This method successfully alleviates matrix interference from blood and fish samples with very high extraction efficiencies resulting from dithizone complexation with CH_3Hg^+ .¹⁸¹

Among other analytical procedures, the following steps are highlighted. 1) Dissect/grind all solid samples to a homogenized pastry state (if blood sampled, proceed to step 2). 2) Mix the resultant paste with 1 N KOH-ethanol solution at 100 °C for 1 hour. 3) Cool to room temperature and add 1 N HCl and then wash with n-hexane. 3) Add 20% EDTA-4Na solution into the aqueous phase (this step helps to mask other metal ions in the samples). 5) Add purified 0.01% dithizone-toluene and discard the aqueous layer. 6) Remove the excess dithizone in toluene by washing the product with 1 N NaOH. 7) Transfer a fixed amount of the resultant toluene into centrifuge tubes, add Na_2S solution and centrifuge at 1,200 rpm for 3 minutes. 8) Repeat step 7 three times and in each time, discard the toluene phase, only keep the aqueous phase (Na_2S solution acts as a back extraction agent for CH_3Hg^+). 9) Acidify the resultant solution with 3-4 drops of 1 N HCl and aerate the resultant sample with N_2 at 50 mL/min for 3 minutes (this expels excess sulfide ions). 10) Add ultra-pure distilled water and sodium acetate buffer to the resultant sample followed by sodium tetraethylborate solution. 11) Treat blanks and standards in a similar manner. 12) Inject the samples into the sparser connected on the purge & trap sampler. 14) Monitor the following ions in SIM mode: m/z 202, 217, 246 for $\text{CH}_3\text{HgC}_2\text{H}_5$; m/z 202, 231, 260 for $\text{Hg}(\text{C}_2\text{H}_5)_2$ (during MS detection). 15) Do not forget to calibrate the method using blanks every after a batch of 20 samples and use standards for calibration. Find the summary of the steps in **Fig. 7**.

In a similar way, Sali and coworkers¹⁸³ used dithizone-anchored micro-beads, for adsorption/desorption of different mercury species such as Hg^{2+} , CH_3HgCl , and $\text{C}_2\text{H}_5\text{HgCl}$ from aqueous media. The adsorption of different mercury species was complete in about 60 min with adsorption capacities of 138 mg/g for Hg^{2+} , 221 mg/g

for CH_3HgCl , and 207 mg/g for $\text{C}_2\text{H}_5\text{HgCl}$. The desorption process was achieved through the use of eluent solutions such as a mixture of CuSO_4 and KBr for organomercury species, and HNO_3 for inorganic mercury. And the adsorption/desorption process was recyclable. Ultra-trace analyses of organomercury species using a GC-AAS coupled system were performed.

6.5. Determination of methyl mercury by the GC-ECD method

A relatively cheap and effective method of determining methyl mercury in biological samples such as fish has been developed by the use of GC-ECD.¹⁸⁴ The biological samples are digested in hydrochloric acid followed by extraction in toluene. Methyl mercury can then be determined using GC-ECD with a DB-608 (30 m, 0.53 mm) capillary column. ECD detectors can be very useful instruments in mercury speciation analytics because they are readily available and can directly determine methyl mercury without the need for derivatization. The only disadvantage they face is the adsorption of polar MeHg on active centres of the stationary phase. As such, repeatability, retention times, peak tailing and broadening are frequently reported.¹⁸⁴ However, this disadvantage can easily be overcome by conditioning columns with Hg^{2+} salts, or the use of columns that are inert in nature instead of the packed columns.^{150, 185-188}

Specific experimental procedures include weighing approximately 0.2 g of the biological homogenized sample into 10 mL centrifugation tubes. Acetone (5 mL) is added with vigorous stirring. The resultant mixture is centrifuged at 3500 rpm for 5 min and the supernatant carefully discarded (remove only 4 mL from the tubes). The procedure is repeated twice with acetone and once with toluene. Thereafter, 0.7 mL of hydrochloric acid (1:1 dilution (v/v) and washed with toluene) and 4 mL of toluene are added to the tubes (agitate the aqueous phase with a glass rod to ensure complete digestion before the addition). Transfer the organic phase carefully after centrifugation into a stoppered test tube with 2 g of anhydrous sodium sulphate. Add another 4 mL to the same aqueous phase and repeat the above procedure for at least three times to ensure complete extraction. The resultant extracts are frozen at 4 °C before analysis. Shelf life for the extracts has been determined to be 14 days at 23 °C. Instrument parameters used by the authors were: 2 min 140 °C; gradient 4 °C/min to 160 °C; 2 min at 160 °C; injector temperature 240 °C, detector temperature 300 °C; sample volume of 2 µL; use of external standards in detection.¹⁸⁴

6.6. The use of chronopotentiometric stripping analysis at gold film electrodes after microwave digestion for total mercury determination

EPA recommends stripping analysis in mercury analytics and, whenever possible, *in situ* analysis using some of the well-developed portable Hg analytical instruments.¹⁸⁹⁻¹⁹¹ The mechanisms involved in stripping analysis include two steps. Firstly, the analyte of interest is electroplated onto an inert electrode at a constant potential (deposition step). The pre-concentration step involves either anodic or cathodic processes. For instance, halide deposition on mercury drop electrode hanging in the form of an insoluble film of mercurous chloride is considered to be anodic process. While reduction of a metal ionic species from solution onto a mercury electrode results in amalgam formation and may be considered as an example of cathodic process. Secondly, the oxidation of the analyte from the inert electrode is done (stripping step).

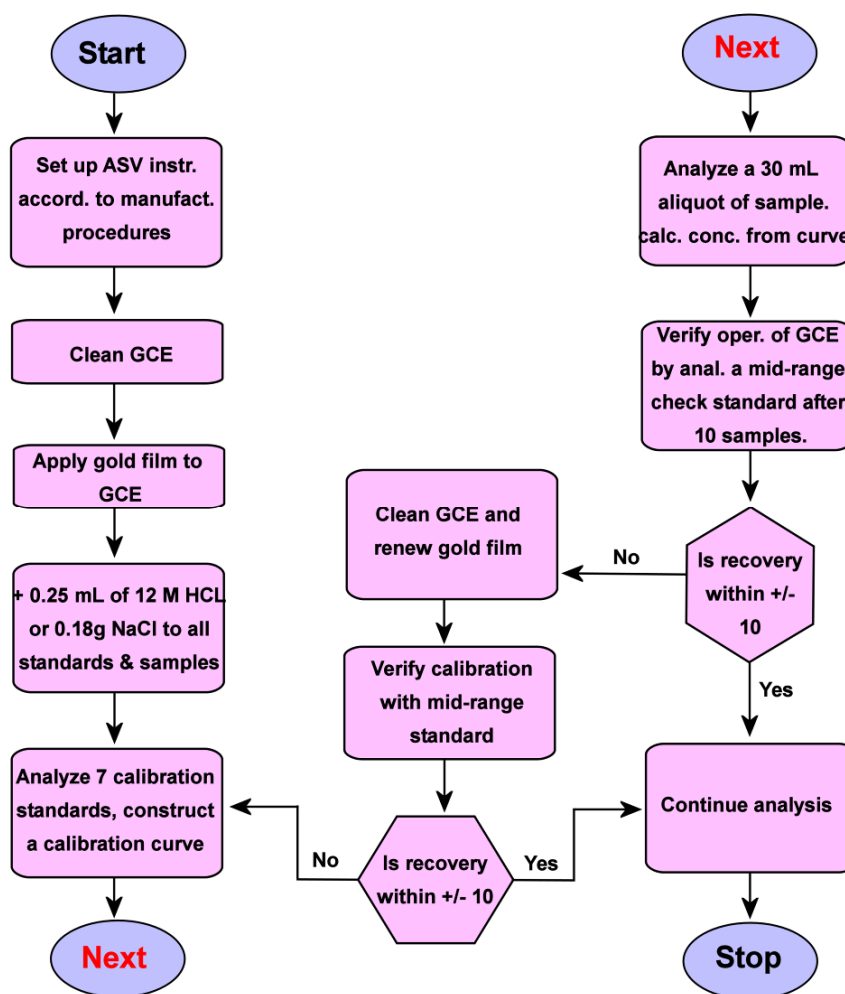


Fig. 8. Schematic analytical procedure for the anodic stripping voltammetry (ASV).
GCE = glassy carbon electrode.¹⁸⁹

The stripping stage uses a voltage scan on the electrode. This process causes stripping of various species in the amalgam (also called electrolytic dissolution) at characteristic potentials.

In summary, this electrochemical voltammetry technique involves the study of a current-potential behavior at an electrode surface, whereby the potential is varied in some systematic manner to cause electro-active chemical species to be reduced or oxidized at the electrode. The current that results from this process is proportional to the concentration of the analyte. However, note that stripping voltammetry may not be appropriate for samples with a concentration exceeding 1 ppm. Such samples should be analyzed polarographically.¹⁹⁰

There exist some sensitive and reliable analytical techniques such as optical and atomic flame spectrometry. However, neutron activation analysis and spark spectroscopy are more reliably used in trace analysis. High instrumentation cost, rigorous sample preparation and selectivity may reduce efficiency of the aforementioned techniques, especially when multicomponent trace analysis in complex sample matrices is required.¹⁹⁰ As such, stripping analysis provides the most viable pathway for trace analysis. Trace components of up to 10^{-5} to 10^{-9} M concentration have been quantified using the electrochemical technique with excellent sensitivity and selectivity.¹⁹²

For reusable electrodes, it is important to clean the electrode (using double distilled water) from previous analyte before use. This step, also called conditioning, involves the application of a specified potential to the electrode for a controlled time. The step removes contaminants not removed at the stripping stage. In order to ensure oxidation of the metals back into solution, it is important to have the conditioning potential as positive with respect to half-wave potentials of the analyte.

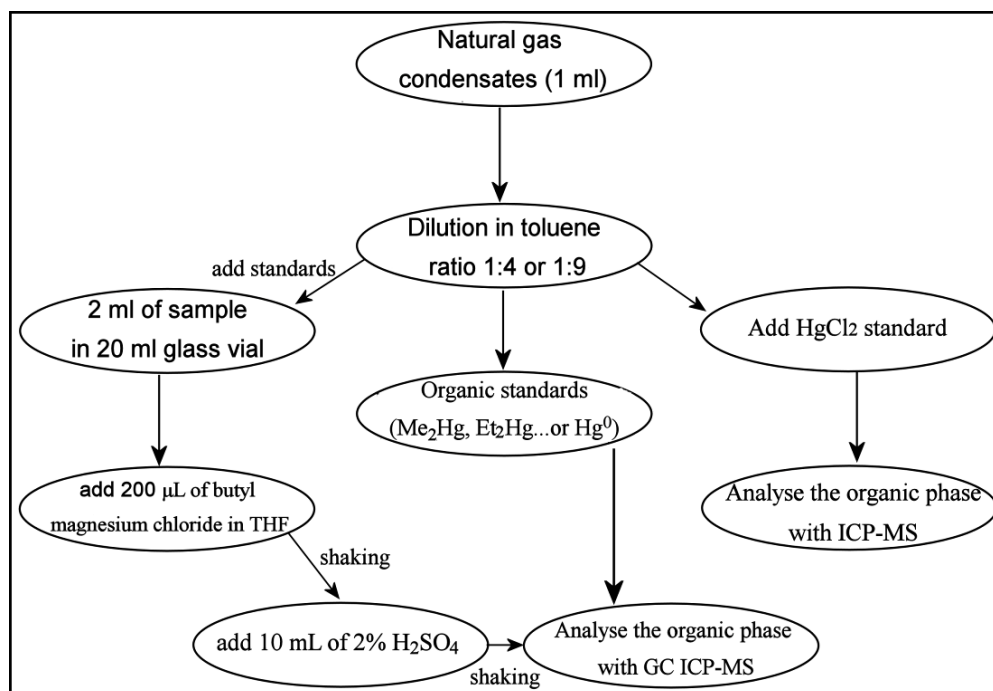


Fig. 9. Schematic representation of mercury speciation studies of natural gas condensates.¹⁹⁴

Furthermore, in order to make sure that a clean electrode surface for deposition step is achieved, the TFME should be formed *in situ*, with the conditioning potential being set positive to the oxidation potential of mercury.

Anodic stripping voltammetry (ASV) has been used to determine the concentration of trace metals such as mercury in aquatic samples.^{189, 193} Solubilization of solid matrices is achieved through acid digestion prior to **quantification** by ASV or by microwave digestion.¹⁹³ However, since this method may not be useful in direct determination of water-insoluble mercury compounds, liberation of such ions is first achieved through chemical processing before the ASV analysis. The MDL for Hg^{2+} is $0.1 \mu\text{g L}^{-1}$ using a 10 - minute plating time and $3 \mu\text{g L}^{-1}$ using a one minute plating time. See **Fig. 8** for the summary outline of the method.

6.7. Detection and speciation studies of mercury in natural gas condensates

Natural gas condensates from petroleum refinery industry are contaminated with various mercury species in the range of 10 – 3000 ng/mL of total mercury, especially mercuric sulfide.^{168, 194, 195} Apart from catalyst poisoning and corrosion of aluminum alloys in steam cracker cold boxes by mercury, products from natural gas condensates

serve as a great source of mercury to aquatic ecosystems.^{194, 196} Besides, the damage caused to industrial plants due to the presence of mercury, **it** has diverse financial implications such as unscheduled shut-downs especially in petrochemical industry. As such, the need to determine the exact species of mercury present in natural gas condensates, **it** is very vital as this will help in understanding the mechanisms of interaction of mercury with equipment, catalysts, mercury removal systems and its final deposits in aquatic ecosystems.¹⁹⁷

The complexity of natural gas condensate matrix coupled with pico-levels of different mercury species makes speciation studies a difficult venture. However, Bouyssiere and co-workers¹⁹⁴ employed capillary gas chromatography with inductively coupled plasma mass spectrometric detection methods to effectively isolate and quantify different mercury species present in natural gas condensates. Firstly, 1 ml of gas condensate is diluted with toluene in the ratio of 1:4 or 1:9. Secondly, 2 ml of the diluted sample in a 20-ml glass vial is mixed with 200 μ l of 2 M butylmagnesium chloride in tetrahydrofuran, followed by shaking for complete butylation before addition of 10 mL 0.5 M sulfuric acid to destroy the excess Grignard reagent. After vigorous shaking, the phases are separated by centrifugation and the organic supernatant placed in an auto-sampler vial and analyzed by GC–ICP-MS. Total mercury is determined by diluting the sample with 2-propanol and analyzed by high-resolution ICP-MS. This method is summarized in **Fig. 9**.

Shafawi and coworkers¹⁹⁸ have developed methods that can be used to remove mercury from natural gas condensates by converting all the species to elemental mercury. In the first method, they adsorb the converted mercury species with alumina-adsorbent impregnated with a metal sulphide and in the second method they adsorb the converted mercury using sulphide impregnated with carbon or molecular sieves.

6.8. The use of atomic fluorescence/absorption spectroscopy (AFS/AAS) in mercury speciation studies

Atomic fluorescence spectrometry has been used to determine the concentrations of mercury at trace levels.¹⁶⁵ In this method, tin(II) chloride is used to reduce different mercury species in solution to Hg^0 .^{199, 200} This reduction method experiences less interference since mercury is separated from solution before measurements. Another

reducing agent is sodium tetrahydroborate (NaBH_4) mostly employed in cold vapor technique (CV-AFS/AAS).¹⁶⁵

The success of this method in mercury determination lies in the ability of mercury to vaporize completely ensuring effective separation from other constituents in the sample. However, noble metals such as Au, Pt, Pd and Ag can interfere with such measurement techniques.²⁰¹ In addition, substances that produce stable complexes with mercury ions and/or those that reduce mercury ions to elemental form may interfere with CV-AAS/AFS measurements unless the complexes are decomposed before the reduction process. Such substances include bromide, iodide, cysteine, sulfide, thiosulfate, and selenium (IV).²⁰²⁻²⁰⁴ Iodide and its related compounds are particularly singled out as the worst interference substances especially for trace mercury analytics.²⁰⁵ In addition, it is noted that the reducing power of tin(II) is more powerful in alkaline medium than acidic medium (Standard redox potentials for tin(II) = -0.93 V at pH = 14 and 0.15 V at pH = 1, using normal hydrogen electrodes).¹⁶⁵

The detection limits in CV-AAS/AFS determinations are very high; therefore, for trace analytics, pre-concentration of samples is highly required. Methods such as amalgamation with metals like copper, silver, gold and the use of liquid traps such as brominating solutions, acidic solution of KMnO_4 , liquid nitrogen, chelating resins and chelating exchangers have been reported.^{165, 206-212} Gold amalgamation offers the best pre-concentration results in addition to the ease of separation at 800 °C.²¹³

The success of using tin (II) reduction method arises from its ability to selectively reduce inorganic mercury ions to elemental mercury in acidic solution, whereas organo-mercury compounds cannot be reduced by this reducing agent. In order to detect organo-mercury species, the use of other oxidants such as $\text{K}_2\text{S}_2\text{O}_8$ or strong UV-light irradiation have been employed to convert organo-mercury to inorganic mercury species.^{203, 214, 215}

6.9. Some of the mercury speciation results obtained using various analytical techniques

Table 2 lists some of the results reported by different researchers on the concentrations of different mercury species in different aquatic ecosystems using some of the analytical methods highlighted above. The analytical methods include: vapor

generation atomic absorption spectroscopy, cold vapor generation atomic fluorescence spectrometry coupled to an online UV post-column oxidation system, aqueous ethylation, purge and trap, dithizone extraction method followed by purge & trap GC-MS and the use of GC-ECD.

7. A Case study of mercury distribution in Kenyan Aquatic environment

The only deposit of mercury in the Kenyan soils was discovered in cinnabar form in a thin veinlet cutting mudstones in 1937, Malindi district.²¹⁶ They discovered it during prospecting operations in a little north of Cottar claims, at Vitengeni. The deposit on subsequent examination by the Mines and Geology Department of Kenya proved to be of very limited extent, and to have shallow depth. The maximum tenor of samples was about ½ percent mercury.²¹⁶

Mercury minerals are therefore rare in Kenya and volcanic eruptions are also not common²¹⁷ hence natural sources of mercury deposition into the Kenyan environment and consequently aquatic ecosystems is not common. Mercury that is present in the Kenyan aquatic ecosystems is of anthropogenic origin such as from thermometers, sphygmomanometer, barometers, cathartics, incandescent lights, batteries, dental amalgam, topical medicines, medical waste incineration, municipal waste disposals, artisanal gold mining among others.^{26, 218-221} And among these artificial sources of mercury, artisanal gold mining is the major contributor. The processes of purifying gold through amalgamation, releases Hg^0 directly into aquatic systems and as mercury vapor during the amalgamation process.^{26, 218-221}

Hg^0 has been used in the amalgamation of gold for centuries.²⁶ During the amalgamation process, approximately 1.32 kg of Hg^0 is lost to different environmental compartments for every 1 kg of gold produced.²²² Nearly 0.5 Kg of the 1.32 Kg loss takes place at the initial amalgamation process and the receiving environmental compartments include soil, streams canals, wells, dams and rivers.²⁶ As discussed in various sections of this document, the introduced Hg^0 will be converted to Hg^{2+} and eventually to organic-Hg in different environmental systems.²⁴ The remaining 0.8 kg of Hg^0 is directly released into the air during the heating stage of Hg–Au amalgam. The Hg^0 released to air is often inhaled by the miners and by their immediate family members including their children.^{26, 78} Due to high volatility of Hg^0 , dangerous levels are readily obtained in air exceeding the set limit of 0.1 mgm^{-3} in air.⁶³

Kenyan artisanal gold miners use the amalgamation process both at the mines and in homesteads.²²³ This activity takes place mainly in the western part of the country, especially Migori, Siaya and Kakamega districts.²²³ The population of artisanal gold miners depends on the gold seasons. About 30000 miners can be encountered at peak periods relative to 10000 miners at low peak periods.²²³ Previous research in Migori and Kakamega gold mines revealed elevated levels of total mercury in aquatic ecosystems.^{220, 221, 223, 224} However, the exact chemistry of mercury in these aquatic ecosystems remains to be a big challenge since mercury toxicity is species dependent.

In the aquatic system, the dangers due to elemental mercury are incomparable to inorganic mercury and to a larger extent, organic mercury. However, there are no specific speciation studies done to determine possible environmental effects from the mercury present in Kenyan aquatic systems. Neither is there any mercury health related research done to this population, hence it is difficult to explain the effects of the identified total mercury to the ecosystem.

Conclusions

This work presents the environmental fate of elemental mercury with a detailed discussion on its methylation processes. Conditions favoring the methylation processes include high acidity, sulphide ions, CH_3J produced by algae, microorganisms and irradiations. Health effects due to the presence of methyl mercury in the environment to humans include a possible attack of the central nervous system. This in turn results to numbness, unsteadiness, awkward movements, tiredness, ringing in the ears, narrowing of the field of vision, loss of hearing, slurred speech, loss of sense in smell and taste, forgetfulness and at higher concentrations, death. Organomercury compounds such as CH_3Hg and $\text{CH}_3\text{CH}_2\text{Hg}$ are highly poisonous and are easily absorbed by the gastric and intestinal organs, and they are carried by blood into the brain, liver, kidney and even foetus.

Various analytical techniques have been analyzed in the speciation analytics of elemental mercury in aquatic ecosystems. Among others, the use of vapor generation atomic absorption spectroscopy, cold vapor generation atomic fluorescence spectrometry coupled to an online UV post-column oxidation system, aqueous ethylation, purge and trap, dithizone extraction method followed by purge & trap GC-MS and the use of GC-ECD method have been explored intensively.

1251 **Recommendation**

1252

1253 The role of methylated tin compounds and humic substances in methylation of mercury
1254 remains a challenge to scientists working in speciation studies of mercury. There is also need
1255 for field experiments to demonstrate the action of bacteria in the demethylation of organic
1256 mercury compounds. Furthermore, there is a need to develop an analytical method that is
1257 robust enough to carry out trace analysis of mercury in environmental samples.

1258

1259 **Table 2:** Some of the results reported by different researchers on the concentrations of different mercury species in different aquatic
1260 ecosystems
1261

Aquatic ecosystem	Sample type	Method of analysis	Method detection limit	Recovery studies	Total Hg	MeHg	(Me) ₂ Hg	Hg ²⁺	Ref
R. Vltava - Zelčín	Fish	CVAAS (T-Hg), GC (MeHg)	1mg/kg (t-Hg) 21mg/kg (MeHg)	ND	0.30 mg/kg	0.27 mg/kg	ND	ND	225
R. Labe - Občíství	Fish	CVAAS (T-Hg), GC (MeHg)	1mg/kg (t-Hg) 21mg/kg (MeHg)	ND	0.36 mg/kg	0.34 mg/kg	ND	ND	225
R. Ohøe - Louny	Fish	CVAAS (T-Hg), GC (MeHg)	1mg/kg (t-Hg) 21mg/kg (MeHg)	ND	0.15 mg/kg	0.12 mg/kg	ND	ND	225
Mississippi wet land	Fresh marsh soil	CVAAS (T-Hg) GCAFS (MeHg)	-	ND	140 µg/kg	4.19 µg/kg	ND	ND	226
Mississippi wet land	Salt marsh soil	CVAAS (T-Hg) GCAFS (MeHg)	-	ND	80 µg/kg	1.34 µg/kg	ND	ND	226
Reservoirs and streams in Korea	Mandarin fish	Dithizone extraction method followed by purge & trap GC-MS	0.9 ng/g MeHg)	53% MeHg	413.1 ± 57.8	219.0 ± 45.7	ND	ND	145
Reservoirs and streams in Korea	Korean piscivorous chub		0.9 ng/g MeHg)	86% MeHg	357.9 ± 75.7	254.2 ± 68.2	ND	ND	145
Reservoirs and streams in Korea	Skin carp	GC-ECD	0.9 ng/g MeHg	88% MeHg	220.4 ± 90.3	194.5 ± 95.4	ND	ND	145
River Water	River Water	Cold Vapor Atomic Absorption Spectrometry after Pre-Concentration	NG	98.0% Hg ²⁺ , 97.5 % MeHg	ND	ND	ND	0.68	227

Effluent 1	Effluent 1	with Bis (2-Mercaptobenzothiazole) Immobilized on Microcrystalline Naphthalene	NG	98.0% Hg^{2+} , 97.5 % MeHg	ND	1P.85	ND	3.72	227
Effluent 2	Effluent 2		NG	97.5 Hg^{2+} 97.1 MeHg	ND	3.67	ND	6.42	227
Venice canal	Sediments station 7	HPLC-UV-CVAFS	12 $\mu\text{g/L}$ for Hg^{2+} , MeHg ⁺	100% MeHg, 150% for Hg^{2+}	7.5mg/kg	BDL	ND	7.5mg/kg	120

1262 NG – Not given in literature, ND – Not Determined

1263 Notes and references

12641. L. J. Norrby, *J. Chem. Educ.*, 1991, **68**, 110.
12652. C. R. Hammond, *The elements in lide*, *CRC Handbook of Chemistry and Physics* (86th ed.), CRC Press, ISBN 0-8493-0486-5, 2005.
12673. K. Hans Wedepohl, *Geochim. Cosmochim. Acta*, 1995, **59**, 1217-1232.
12684. J. J. Rytuba, *Environ. Geol.*, 2003, **43**, 326-338.
12695. R. W. Jamieson, *Domestic architecture and power*, Springer, ISBN 0-306-46176-5, 2000.
12706. W. E. Brooks, *Mercury*, U.S. Geological Survey, USA, 2007.
12717. M. Sheridan, *Green lightbulbs poison workers: Hundreds of factory staff are being made ill by mercury used in bulbs destined for the West*, The Sunday Times, London, UK, 2009.
12738. S. K. Parker, B. Schwartz, J. Todd and L. K. Pickering, *Pediatrics*, 2004, **114**, 793-804.
12749. J. Liu, J. Z. Shi, L. M. Yu, R. A. Goyer and M. P. Waalkes, *Experimental biology and medicine*, 2008, **233**, 810-817.
127610. B. R. Leopold, *Manufacturing processes involving mercury. Use and release of mercury in the United States*, U.S. Environmental Protection Agency, Cincinnati, Ohio, 2002.
127811. T. Barkay, S. M. Miller and A. O. Summers, *FEMS Microbiol. Rev.*, 2003, **27**, 355-384.
127912. W. F. Fitzgerald and R. P. Mason, *The global mercury cycle: Oceanic and anthropogenic aspects*. In: *Global and regional mercury cycles: Sources, fluxes and mass balances* (Baeyens, W., Ed.), Springer Netherlands, 1996.
128213. F. Slemr and E. Langer, *Nature*, 1992, **355**, 434-437.
128314. W. F. Fitzgerald, D. R. Engstrom, R. P. Mason and E. A. Nater, *Environ. Sci. Technol.*, 1998, **32**, 1-7.
128515. P. Lorey and C. T. Driscoll, *Environ. Sci. Technol.*, 1999, **33**, 718-722.
128616. E. B. Swain, D. R. Engstrom, M. E. Brigham, T. A. Henning and P. L. Brezonik, *Science* 1992, **257**, 784-787.
128817. P. F. Schuster, D. P. Krabbenhoft, D. L. Naftz, L. D. Cecil, M. L. Olson, J. F. Dewild, D. D. Susong, J. R. Green and M. L. Abbott, *Environ. Sci. Technol.*, 2002, **36**, 2303-2310.
129018. F. M. M. Morel, A. M. L. Kraepiel and M. Amyot, *Annu. Rev. Ecol. Syst.*, 1998, **29**, 543-566.
129119. O. A. Summers and I. L. Sugarman, *J. Bacteriol.*, 1974, **119**, 242-249.
129220. L. J. Schottel, *J. Biol. Chem.*, 1978, **253**, 4341-4349.
129321. J. K. Schaefer, J. Letowski and T. Barkay, *Geomicrobiol. J.*, 2010, **19**, 87-102.
129422. O. Krystofova, V. Shestivska, M. Galiova, K. Novotny, J. Kaiser, J. Zehnalek, P. Babula, R. Opatrilova, V. Adam and R. Kizek, *Sensors*, 2009, **9**, 5040-5058.
129623. S. De Flora, C. Bennicelli and M. Bagnasco, *Mutat Res.*, 1994, **317**, 57-79.
129724. M. M. M. François, M. L. K. Anne and A. Marc, *Annu. Rev. Ecol. Syst.*, 1998, **29**, 543-566.
129825. C. R. Hammond, *The elements*, In: *Handbook of chemistry and physics* CRC press, Boca Raton, ISBN 0849304814, 2000.
130026. K. J. Timmins, *Artisanal gold mining without mercury pollution*, United Nations Industrial Development Organization, http://www.naturalresources.org/minerals/cd/docs/unido/asm_mercury.pdf 2003.
130327. S. M. Ullrich, T. W. Tanton and S. A. Abdrashitova, *Crit. Rev. Env. Sci. Technol.*, 2001, **31**, 241-293.
130528. N. T. Loux, *Environ. Toxicol. Chem.*, 2000, **19**, 1191-1198.
130629. M. Amyot, G. A. Gill and F. M. M. Morel, *Environ. Sci. Technol.*, 1997, **31**, 3606-3611.
130730. E. D. Stein, Y. Cohen and A. M. Winer, *Crit. Rev. Env. Sci. Technol.*, 2009, **26**, 1-43.
130831. H. L. Clever, S. A. Johnson and M. E. Derrick, *J. Phys. Chem. Ref. Data*, 1985, **14**, 631.
130932. T. Smith, K. Pitts, J. A. McGarvey and A. O. Summers, *Appl. Environ. Microbiol*, 1998, **64**, 1328-1332.
131133. O. S. Anne and S. Simon, *J. Bacteriol.*, 1972, **112**, 1228-1236.
131234. N. V. Hamlett, E. C. Landale, B. H. Davis and A. O. Summers, *J. Bacteriol.*, 1992, **174**, 6377-6385.
131435. S. Omwoma, J. O. Lalah, D. M. K. Onger and K.-W. Schramm, *Environ. Earth Sci.*, 2013, **71**, 4297-4308.
131636. S. E. Lindberg, S. Brooks, C. J. Lin, K. J. Scott, M. S. Landis, R. K. Stevens, M. Goodsite and A. Richter, *Environ. Sci. Technol.*, 2002, **36**, 1245-1256.

131837. J. Thoming, B. K. Kliem and L. M. Ottosen, *Sci. Total Environ.*, 2000, **261** 137-147.

131938. J. D. Lalonde, M. Amyot, A. M. Kraepiel and F. M. Morel, *Environ. Sci. Technol.*, 2001, **35**, 1320 1367-1372.

132139. M. E. de Magalhaes and M. Tubino, *Sci. Total Environ.*, 1995, **170**, 229-239.

132240. J. Munthe, *Atmos. Environ.*, 1992, **26A**, 1461-1468.

132341. C. Seigneur, J. Wrobel and E. Constantinou, *Environ. Sci. Technol.*, 1994, **28**, 1589-1597.

132442. M. Yamamoto, *Chemosphere*, 1995, **31**, 2791-2798.

132543. G. W. Luther III and E. Tsamakis, *Mar. Chem.*, 1989, **127**, 165-177.

132644. R. P. Mason, J. R. Reinfelder and F. M. M. Morel, *Environ. Sci. Technol.*, 1996, **30**, 1835-1845.

132845. D. J. Dyll-Smith and J. P. Scurry, *Med. J. Aust.*, 1990, **153**, 409-415.

132946. M. M. Weldon, M. S. Smolinski, A. Maroufi, B. W. Hasty, D. L. Gilliss, L. L. Boulanger, L. S. Balluz and R. J. Dutton, *West J Med.*, 2000, **173**, 15-18.

133147. T. Matilainen and M. Verta, *Can. J. Fish. Aquat. Sci.*, 1995, **52**, 1597-1608.

133248. O. Regnell, T. Hammar, A. Helg  e and B. Troedsson, *Can. J. Fish. Aqua. Sci.*, 2001, **58**, 506-517.

133449. L. Boszke, G. G  osi  ska and J. Siepak, *Polish J. of Env. Studies* 2002, **11**, 285-298

133550. H. Nagase, Y. Ose, T. Sato and T. Ishikawa, *Sci. Total Environ.*, 1982, **25**, 133-142.

133651. J. H. Weber, *Chemosphere*, 1993, **26**, 2063-2077.

133752. S. Jensen and A. Jernelov, *Nature*, 1969, **223**, 753-754.

133853. J. M. Wood, *Science*, 1974, **183**, 1049-1052.

133954. C. C. Gilmour, E. A. Henry and R. Mitchell, *Environ. Sci. Technol.*, 1992, **26**, 2281-2287.

134055. J. K. King, J. E. Kostka, M. E. Frischer and F. M. Saunders, *Applied and environmental microbiology*, 2000, **66**, 2430-2437.

134256. J. M. Wood, F. S. Kennedy and C. G. Rosen, *Nature*, 1968, **220**, 173-174.

134357. B. Chen, T. Wang, Y. Yin, B. He and G. Jiang, *Appl. Organomet. Chem.*, 2007, **21**, 462-467.

134458. M. Leermakers, M. Elskens, S. Panutrakul, F. Monteny and W. Baeyens, *Netherlands J. Aqua. Ecol.*, 1993, **27**, 267-277.

134659. J. M. Benoit, C. C. Gilmour and R. P. Mason, *Applied and environmental microbiology*, 2001, **67**, 51-58.

134860. S. Hamelin, M. Amyot, T. Barkay, Y. Wang and D. Planas, *Environ. Sci. Technol.*, 2011, **45**, 1349 7693-7700.

135061. G. W. Bryan and W. J. Langston, *Environ. Pollut.*, 1992, **76**, 89-131.

135162. P. J. Craig and P. A. Moreton, *Water Res.*, 1986, **20**, 1111-1118.

135263. G. Guzzi and C. A. La Porta, *Toxicology*, 2008, **244**, 1-12.

135364. M. Galvin, *Water S. A.*, 1996, **22**, 7-18.

135465. T. Hamasaki, H. Nagase, Y. Yoshioka and T. Sato, *Critical Review in Environ. Sci. Technol.*, 1995, **25**, 45-91.

135666. R. Wang, X. B. Feng and W. X. Wang, *Environ. Sci. Technol.*, 2013, **47**, 7949-7957.

135767. R. Ebinghaus, H. Hintelmann and R. D. Wilken, *Fresen. J. Anal. Chem.*, 1994, **350**, 21-29.

135868. G. A. Sulikowski, M. M. Sulikowski, M. H. Haukaas and B. Moon, *Iodomethane*, e-EROS Encyclopedia of Reagents for Organic Synthesis, DOI: 10.1002/047084289X.ri029m.pub2, 2005.

136169. G. Cerrati, M. Bernhard and J. H. Weber, *Appl. Organomet. Chem.*, 1992, **6**, 587-595.

136270. S. N. Davies and R. C. M. DeWiest, *Hydrogeology* Wiley Interscience, Chichester UK, 1966

136371. J. L. Hobman, J. R. Wilson and N. L. Brown, *Microbial mercury reduction. In: Environmental microbe-metal interaction*. Lovley D. R. (ed.) ASM Press, Washington, 2000.

136572. F. J. Black, C. H. Conaway and A. R. Flegal, *Environ. Sci. Technol.*, 2009, **43**, 4056-4062.

136673. P. Seller, C. A. Kelly, J. W. M. Rudd and A. R. MacHutchon, *Nature*, 1996, **380**, 694-697.

136774. I. Suda, M. Suda and K. Hirayama, *Arch. Toxicol.*, 1993, **67**, 365-368.

136875. M. Amyot, D. R. S. Lean and G. Mierle, *Environ. Toxicol. Chem.*, 1997, **16**, 2054-2063.

136976. J. Mitra, E. N. Guerrero, P. M. Hegde, H. Wang, I. Boldogh, K. S. Rao, S. Mitra and M. L. Hegde, *Biomolecules*, 2014, **4**, 678-703.

137177. UNEP, *Global mercury assessment*, UNEP chemicals Geneva, Switzerland, 2002.

137278. UNIDO, *Assistance in assessing and reducing mercury pollution emanating from artisanal gold mining in Ghana*, United Nations Industrial Development Organization Project Document US/GHA/99/128, USA, 2000.

137579. E. Cernichiari, R. Brewer, G. J. Myers, D. O. Marsh, W. Lowell and W. Lapham,
1376 *Neurotoxicology*, 1995, **16**, 4.
137780. M. Rezayi, A. S. Esmaeli and T. Valinasab, *Bulletin of environmental contamination and*
1378 *toxicology*, 2011, **86**, 511-514.
137981. WHO, *Environmental health criteria 101: Methylmercury* International Program on Chemical
1380 Safety. World Health Organization, Geneva, 1990.
138182. WHO, *Environmental health criteria 118. Inorganic mercury*, International Program on
1382 Chemical Safety. World Health Organization, Geneva, 1991.
138383. F. Bakir, S. F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N. Y. Al-Rawi, S. Tikriti, H.
1384 I. Dhahir, T. W. Clarkson, J. C. Smith and R. A. Doherty, *Science*, 1973, **181**, 230-241.
138584. G. E. McKeown-Eyssen and J. Ruedy, *Clin. Invest. Med.*, 1983, **6**, 161-169.
138685. D. O. Marsh, M. D. Turner, J. C. Smith, P. Allen and N. Richdale, *Neurotoxicology*, 1995, **16**,
1387 717-726.
138886. G. J. Myers, P. W. Davidson, C. Cox, C. Shamlaye, E. Cernichiari and T. W. Clarkson,
1389 *Environmental research*, 2000, **83**, 275-285.
139087. P. W. Davidson, G. J. Myers, C. Cox, C. Axtell, C. Shamlaye, J. Sloane-Reeves, E.
1391 Cernichiari, L. Needham, A. Choi, Y. Wang, M. Berlin and T. W. Clarkson, *Jama*, 1998, **280**,
1392 701.
139388. P. W. Davidson, G. J. Myers, C. Cox, C. F. Shamlaye, D. O. Marsh, M. A. Tanner, M. Berlin,
1394 J. Sloane-Reeves, E. Cernichiari and O. Choisy, *Neurotoxicology* 1995, **16**, 677-688.
139589. P. Grandjean, B.-J. Esben, F. W. Roberta, J. J. Poul, W. Pal, D. Frodi and K. Niels, *Am. J.*
1396 *Epidemiol.* , 1999, **150**, 301-305.
139790. P. Grandjean, P. Weihe, R. F. White and F. Debes, *Environmental research*, 1998, **77**, 165-
1398 172.
139991. P. Grandjean, P. Weihe, R. F. White, F. Debes, S. Araki, K. Yokoyama, K. Murata, N.
1400 SØRensen, R. Dahl and P. J. JØRgensen, *Neurotoxicol. Teratol.* , 1997, **19**, 417-428.
140192. M. Boulland and A. Boudeault, *New almaden*, Arcadia Publishing, Chicago, USA, ISBN
1402 0738531316, 2006.
140393. USEPA, *Clean air mercury rule*, United States Environmental Protection Agency, Washington
1404 DC, USA, 2005.
140594. L. K. Ball, R. Ball and R. D. Pratt, *Pediatrics*, 2001, **107**, 1147-1154.
140695. M. R. Geier and D. A. Geier, *Medical hypotheses*, 2005, **64**, 946-954.
140796. N. A. Halsey, *Jama*, 1999, **282**, 1763.
140897. D. S. Baskin, H. Ngo and V. V. Didenko, *Toxicol. Sci.* , 2003, **74**, 361-368.
140998. L. Magos, *Journal of applied toxicology : JAT*, 2001, **21**, 1-5.
141099. W. J. Adams, R. A. Kimerle and J. W. Barnett, *Environ. Sci. Tech.*, 1992, **26**, 1864-1875.
1411100. R. Manly, *Biological indicators*, In: *Environmental analytical chemistry*, Fifield, F.W.,
1412 Haines, P.J. (Eds.) , Black Academic and Professional, Oxford, London, 1996.
1413101. N. A. Campbell, *Biology*, 4th. Ed., The Benjamin/cummings publishing company Inc.,
1414 USA. University of California 1996.
1415102. D. Roth, L. Fay, K. Moyer and R. Warne, *Environmental lands acquisition and*
1416 *maintenance strategy discussion paper* South West Florida Water Management District, USA. ,
1417 1997.
1418103. J. L. Stauber and T. M. Florence, *Marine Biol.*, 1987, **94**, 511-519.
1419104. S. Kaitala, *Water Sci. Tech.*, 1998, **20**, 23-32.
1420105. R. D. Kale, *Earthworms: Natures gift for utilization of organic wastes. Earthworms*
1421 *ecology*, CRC Press, Boca Raton, FinLand 1998.
1422106. Y. Kara, *Int. J. Environ. Sci. Tech.*, 2013, **2**, 63-67.
1423107. C. C. Kaonga, S. S. Chiotha, M. Monjerezi, E. Fabiano and E. M. Henry, *Int. J. Environ.*
1424 *Sci. Tech.*, 2008, **5**, 471-478.
1425108. D. J. Spurgeon, J. M. Weeks and C. A. M. Van Gestel, *Pedobiologia*, 2003, **47**, 588-606.
1426109. R. Lanno, J. Wells, J. Conder, K. Bradham and N. Basta, *Ecotox. Environ. Safe.* , 2004, **57**,
1427 39-47.
1428110. A. Ruhling and T. Germund, *Botaniska Notiser* 1968, **121**, 21-42.
1429111. K. Grodzińska, G. Szarek and B. Godzik, *Water, Air, Soil Pollut.*, 1990, **49**, 409-419.
1430112. L. Thoni, N. Schnyder and F. Krieg, *Anal. Bioanal. Chem.*, 1996, **354**, 703-708.
1431113. E. Otvos, T. Pazmandi and Z. Tuba, *Sci. Total Environ.*, 2003, **309**, 151-160.

1432114. H. Ross, *Water, Air, Soil Pollut.*, 1990, **50**.
1433115. R. Bargagli, D. H. Brown and L. Nelli, *Environl. Polln.*, 1995, **89**, 169-175.
1434116. J. Poikolainen, E. Kubin, J. Piispanen and J. Karhu, *Sci. Total Environ.*, 2004, **318**, 171-185.
1435117. G. F. Giesbrecht and L. M. Gumpertz, *Completely Randomized Design In: Planning, Construction, and Statistical Analysis of Comparative Experiments*, John Wiley & Sons, Inc., 1437 Hoboken, NJ, USA, 2005.
1438118. T. P. Ryan, *Completely randomized design, In: Modern experimental design*, John Wiley & 1439 Sons, Inc., Hoboken, NJ, USA, 2007.
1440119. D. C. Montgomery, *Design and analysis of experiments*, John Wiley & Sons, Inc., ISBN: 1441 978-0-470-45687-3, 2009.
1442120. L. G. R. Wilder, *Speciation of mercury in environmental samples. Master thesis*, University 1443 of Barcelona Catalonia, 2010.
1444121. P. Quevauviller, M. B. de la Calle-Guntiñas, E. A. Maier and C. Cámara, *Microchimica* 1445 *Acta*, 1995, **118**, 131-141.
1446122. S. Shimomura, Y. Nishihara and Y. Tanase, *Bunseki Kagaku*, 1968, **17** 1148-1149.
1447123. S. Shimomura, V. Nishihara, Y. Fukumoto and Y. Tanase, *Eisei Kagaku*, 1969, **15**, 84-89.
1448124. T. Toribara, *Talanta*, 1970, **17**, 1025-1028.
1449125. J. M. Lo and C. M. Wai, *Anal. Chem.*, 1975, **47**, 1869-1870.
1450126. A. Wasik and J. Namieśnik, *Pol. J. Environ. Stud.*, 2001, **10**, 405-413.
1451127. J. A. Stäb, W. P. Cofino, B. van Hattum and U. A. T. Brinkman, *Anal. Chim. Acta*, 1994, 1452 **286**, 335-341.
1453128. R. Łobiński, I. R. Pereiro, H. Chassaigne, A. Wasik and J. Szpunar, *J. Anal. At. Spectrom.*, 1454 1998, **13**, 859-867.
1455129. L. Moens, T. De Smaele, R. Dams, P. Van Den Broeck and P. Sandra, *Anal. Chem.*, 1997, 1456 **69**, 1604-1611.
1457130. R. Fischer, S. Rapsomanikis and M. O. Andreae, *Anal. Chem.*, 1993, **65**, 763-766.
1458131. C. Gerbersmann, M. Heisterkamp, F. C. Adams and J. C. Broekaert, *Anal. Chim. Acta*, 1459 1997, **350**, 273-285.
1460132. L. Liang, M. Horvat and N. S. Bloom, *Talanta*, 1994, **41**, 371-379.
1461133. X. Yin, W. Frech, E. Hoffmann, C. Lüdke and J. Skole, *Fresen. J. Anal. Chem.*, 1998, **361**, 1462 761-766.
1463134. J. Dachs, R. Alzaga, J. M. Bayona and P. Quevauviller, *Anal. Chim. Acta*, 1994, **286**, 319- 1464 327.
1465135. M. Ceulemans and C. A. Freddy, *Talanta*, 1998, **46**, 395-405.
1466136. S. Shawky, H. Emons and H. W. Derbeck, *Anal. Commun.*, 1996, **33**, 107.
1467137. T. Gremm and F. Frimmel, *Water Res.*, 1992, **26**, 1163-1169.
1468138. J. Vercauteren, A. De Meester, T. De Smaele, F. Vanhaecke, L. Moens, R. Dams and P. 1469 Sandra, *J. Anal. At. Spectrom.*, 2000, **15**, 651-656.
1470139. A.-i. Sadiki and D. T. Williams, *Chemosphere*, 1996, **32**, 1983-1992.
1471140. M. Heisterkamp and F. C. Adams, *J. Anal. At. Spectrom.*, 1999, **14**, 1307-1311.
1472141. D. C. Baxter and W. Frech, *Pure Appl. Chem.*, 1995, **67**.
1473142. W. M. R. Dirkx, R. Łobiński and F. C. Adams, *Anal. Chim. Acta*, 1994, **286**, 309-318.
1474143. M. Takeuchi, K. Mizuishi and T. Hobo, *Anal. Sci.*, 2000, **16**, 349-359.
1475144. M. Leermakers, H. L. Nguyen, S. Kurunczi, B. Vanneste, S. Galletti and W. Baeyens, *Anal.* 1476 *Bioanal. Chem.*, 2003, **377**, 327-333.
1477145. J. Lee, Y. Ryu, J. Park, S. Jeon, S. Kim and Y. Kim, *Bull. Korean Chem. Soc.*, 2007, **28**, 1478 2293-2298.
1479146. T. Kerry, *Analysis of ultra-trace level methyl mercury in water by aqueous phase ethylation*, 1480 HG-005-2.7., 2008.
1481147. USEPA, *Method 3200: Mercury species fractionation and quantification by microwave* 1482 *assisted extraction, selective solvent extraction and/or solid phase extraction*, United States 1483 Environmental Protection Agency, Washington DC, USA, 2005.
1484148. M. Burguera, *Talanta*, 1997, **44**, 1581-1604.
1485149. M. J. Vazquez, A. M. Carro, R. A. Lorenzo and R. Cela, *Anal. Chem.*, 1997, **69**, 221-225.
1486150. A. M. Caricchia, G. Minervini, P. Soldati, S. Chiacvarini, C. Ubaldi and R. Morabito, 1487 *Microchem. J.*, 1997, **55**, 44-55.

1488151. J. C. A. Wuilloud, R. G. Wuilloud, A. P. Vonderheide and J. A. Caruso, *Spectrochim. Acta*, 1489 *Part B*, 2004, **59**, 755-792.
1490152. T. L. Chester, J. D. Pinkston and D. E. Raynie, *Anal. Chem.*, 1998, **70**, 301R-319R.
1491153. U. T. Kumar, N. P. Vela, J. G. Dorsey and J. A. Caruso, *J. Chromatogr. A*, 1993, **655**, 340-345.
1493154. U. T. Kumar, N. P. Vela and J. A. Caruso, *J. Chromatogr. Sci.*, 1995, **33**, 606-610.
1494155. H. Greschonig, M. G. Schmid, x000Fc and G. bitz, *Fresen. J. Anal. Chem.*, 1998, **362**, 218-223.
1496156. R. Łobiński and F. C. Adams, *Spectrochim. Acta, Part B*, 1997, **52**, 1865-1903.
1497157. R. Ritsema and O. F. X. Donard, *In sample handling and trace analysis of pollutants: Techniques, applications and quality assurance*, (ed. D. Barcelo), Elsevier Science B.V, 1999.
1499158. B. K. Afghan and A. S. Y. Chan, *Analysis of trace organics in aquatic environment*, CRS 1500 Press Inc., Florida, 1989.
1501159. P. Thomas, L. Christopher and S. Gerhard, *J. Anal. At. Spectrom.*, 1999, **14**, 1501-1504.
1502160. P. Thomas, H. Stephan, L. Christopher and S. Gerhard, *J. Anal. At. Spectrom.*, 1999, **14**, 1-1503 8.
1504161. C.-G. de la, x000F, M. B. as, F. Laturnus and F. C. Adams, *Fresen. J. Anal. Chem.*, 1999, 1505 **364**, 147-153.
1506162. B. Salih, *Spectrochim. Acta, Part B*, 2000, **55**, 1117-1127.
1507163. Y. Cai, *TrAC, Trends Anal. Chem.*, 2000, **19**, 62-66.
1508164. S. H. Jiang, J. H. Liang, J. T. Chou, U. T. Lin and W. W. Yeh, *Nucl. Instr. and Meth. in* 1509 *Phys. Res. A*, 1998, **413**, 281-292.
1510165. H. Morita, H. Tanaka and S. Shimomura, *Spectrochim. Acta*, 1995, **50B**, 69-84.
1511166. C. Feldman, *Anal. Chem.*, 1974, **46**, 99-102.
1512167. S. N. Hamlin, *J. Am. Water Resour. Assoc.*, 1989, **25**, 255-262.
1513168. S. M. Wilhelm and N. Bloom, *Fuel Process. Technol.*, 2000, **63**, 1-27.
1514169. M. E. Brigham, D. A. Wentz, G. R. Aiken and D. P. Krabbenhoft, *Environ. Sci. Technol.*, 1515 2009, **43**, 2720-2725.
1516170. R. S. Braman and D. L. Johnson, *Environ. Sci. Technol.*, 1974, **8**, 996-1003.
1517171. W. H. Schroeder and R. Jackson, *Chemosphere*, 1984, **13**, 1041-1051.
1518172. F. Yolanda, M. Klaus and D. Hazel, *Accurate analysis of foodstuffs using vapor generation* 1519 *atomic absorption spectroscopy and ICP-OES thermo fisher scientific*, San Jose, CA, U SA, 1520 http://www.thermo.com/eThermo/CMA/PDFs/Various/File_53371.pdf 2011.
1521173. L. E. Kaercher, F. Goldschmidt, J. N. G. Paniz, É. M. de Moraes Flores and V. L. Dressler, 1522 *Spectrochim. Acta, Part B*, 2005, **60**, 705-710.
1523174. F. A. Duarte, C. A. Bizzi, F. G. Antes, V. L. Dressler and É. M. d. M. Flores, *Spectrochim.* 1524 *Acta, Part B*, 2009, **64**, 513-519.
1525175. A. S. Luna, E. S. Ralph and C. d. C. Reinaldo, *Anal. Chem.*, 2000, **72**, 3523-3531.
1526176. A. Yasutake, M. Nagano and A. Nakano, *J. Health Sci.*, 2005, **51**, 220-223.
1527177. PSA, *Schematic diagram of the continuous-flow vapor/hydride generator. Millennium* 1528 *Excalibur, P. S. Analytical. Hydride generator users manual*, Issue 2.0. PS Analytical LTD, 1529 1998.
1530178. L.-N. Liang, G.-B. Jiang, J.-F. Liu and J.-T. Hu, *Anal. Chim. Acta*, 2003, **477**, 131-137.
1531179. USEPA, *Methyl mercury in water by distillation, aqueous ethylation, purge and trap, and* 1532 *CVAFS. Method 1630. EPA-821-R-01-020*, U.S. Environmental Protection Agency Office of 1533 Water Office of Science and Technology, Engineering and Analysis Division Pennsylvania 1534 Avenue NW, Washington, D.C., 2001.
1535180. M. Horvat, *Global and regional mercury cycles: Sources, fluxes and mass balances;* 1536 *Beayens, W., Ed.*, Kluwer Academic Publishers, The Netherlands, 1996.
1537181. H. Akagi and H. Nishimura, *Advances in Mercury Toxicology; Suzuki, T., Ed.*, Plenum 1538 Press, New York, U. S. A, 1991.
1539182. D. C. Baxter, I. Rodushkin, E. Engstrom, D. Klockare and H. Waara, *Clinical chemistry*, 1540 2007, **53**, 111-116.
1541183. B. Salih, R. Say, A. Denizli, Ö. Genç and E. Pişkin, *Anal. Chim. Acta*, 1998, **371**, 177-185.
1542184. P. Marsalek and Z. Svobodova, *Czech J. Food Sci.*, 2006, **24**, 138-142.
1543185. C. J. Cappon and J. C. Smith, *Anal. Chem.*, 1977, **49**, 365-369.
1544186. S. C. Hight, *J. Assoc. Off. Anal. Chem.*, 1987, **70**, 667-672.

1545187. R. A. Lorenzo, A. Carro, E. Rubí, C. Casais and C. R., *J. AOAC Int.*, 1993, **76**, 608-614.
1546188. J. E. O'Reilly, *J. Chromatogr. A*, 1982, **238**, 433-444.
1547189. USEPA, *Method 7472, Mercury in aqueous samples and extracts by anodic stripping voltammetry*, United States Environmental Protection Agency, 1548 <http://www.epa.gov/solidwaste/hazard/testmethods/sw846/pdfs/7472.pdf>, 1996.
1550190. PAR, *Fundamentals of stripping voltammetry*, Princeton Applied Research, S. Illinois 1551 Avenue, Oak Ridge, TN 37830, 1552 www.princetonappliedresearch.com/download.aspx?AttributeFileId=f1013b2b-413b-48db-8449-80bce19abbf0, 2015.
1554191. C. M. A. Brett and A. M. O. Brett, *Electrochemistry: principles, methods, and applications*, 1555 Oxford Science Pub, Oxford, UK., 1993.
1556192. G. E. Batley and T. M. Florence, *J. Electroanal. Chem.*, 1974, **55**, 23-43.
1557193. M. A. Augelli, R. A. A. Munoz, E. M. Richter, M. I. Cantagallo and L. Angnes, *Food Chem.*, 2007, **101**, 579-584.
1559194. B. Bouyssiére, F. Baco, L. Savary and R. Lobiński, *J. Chromatogr. A*, 2002, **976**, 431-439.
1560195. L. Liang, M. Horvat and P. Danilchik, *Sci. Total Environ.*, 1996, **187**, 57-64.
1561196. B. Bouyssiére, J. Szpunar and R. Lobinski, *Spectrochim. Acta, Part B*, 2002, **57**, 805-828.
1562197. J. H. Won, J. Y. Park and T. G. Lee, *Atmos. Environ.*, 2007, **41**, 7547-7552.
1563198. A. Shafawi, L. Ebdon, M. Foulkes, P. Stockwell and W. Corns, *Anal. Chim. Acta*, 2000, 1564 **415**, 21-32.
1565199. N. S. Poluektov, R. A. Vitkun and Y. V. Zelyukova, *Zh. Anal. Khim*, 1964, **19** 927-937.
1566200. N. S. Poluektov and R. A. Vitkun, *Zh. Anal. Khim.*, 1963, **18**, 37.
1567201. G. Vermeir, C. Vandecasteele and R. Dams, *Anal. Chim. Acta*, 1991, **242**, 203-208.
1568202. G. Lindstedt, *Analyst*, 1970, **95**, 264.
1569203. H. Morita, M. Sugimoto and S. Shimomura, *Anal. Sci.*, 1990, **6**, 91-95.
1570204. Y. Umezaki and I. K., *Jpn. Anal.*, 1971, **20**, 173.
1571205. T. Korenaga, E. Yamada, Y. Hara, H. Sakamoto, T. Chohji, C. Nakagawa, H. Ikatsu, M. 1572 Izawa and M. Goto, *Bunseki Kagaku*, 1987, **36**, 194-198.
1573206. T. Kimoto, H. Morimune, H. Morita, H. Sakurai and S. Shimomura, *Bunseki Kagaku* 1982, 1574 **31**, 637-641.
1575207. V. I. Muscat, T. J. Vickers and A. Andren, *Anal. Chem.*, 1972, **44**, 218-221.
1576208. M. J. Fishman, *Anal. Chem.*, 1970, **42**, 1462-1463.
1577209. L. A. Nelson, *Anal. Chem.*, 1979, **51**, 2289-2290.
1578210. L. Ebdon, J. R. Wilkinson and K. W. Jackson, *Analyst*, 1982, **107**, 269.
1579211. P. Cavalli and G. Rossi, *Analyst*, 1976, **101**, 272.
1580212. S. S. Bhattacharyya and A. K. Das, *At. spectrosc.*, 1988, **9**, 68-70.
1581213. R. Dumarey, R. Dams and J. Hoste, *Anal. Chem.*, 1985, **57**, 2638-2643.
1582214. S. E. Birnie, *The Journal of automatic chemistry*, 1988, **10**, 140-143.
1583215. H. Agemian and A. S. Y. Chau, *Anal. Chem.*, 1978, **50**, 13-16.
1584216. W. Pulfrey, *The geology and mineral resources of Kenya*, The government printer, Kenya, 1585 1947.
1586217. L. Siebert and T. Simkin, *Volcanoes of the World: an Illustrated Catalog of Holocene 1587 Volcanoes and their Eruptions*, Smithsonian Institution, 1588 http://gcmd.nasa.gov/records/GCMD_GVP_Holocene_Volcanoes.html, 2003.
1589218. J. S. Ogola, W. V. Mitullah and M. A. Omulo, *Environl. Geochem. and Health*, 2002, **24**, 1590 141-157.
1591219. UNEP, *Regional awareness raising workshop on mercury pollution, Pretoria, South Africa*, 1592 UNEP chemicals, Geneva, Switzerland, 2004.
1593220. W. Milka, *Exposure to mercury and other mine related hazards amongst gold mine 1594 workers in Migori gold belt, Kenya. Msc thesis*, Jomo Kenyatta University of Agriculture and 1595 Technology Kenya, 2006.
1596221. O. B. Odumo, A. O. Mustapha, J. P. Patel and H. K. Angeyo, *Bulletin of environmental 1597 contamination and toxicology*, 2011, **86**, 484-489.
1598222. M. Harada, S. Nakachi, T. Cheu, H. Hamada, Y. Ono, T. Tsuda, K. Yanagida, T. Kizuki 1599 and T. H. Ohno, *Mercury pollution in Tanzania: Relation between scalp hair, mercury level 1600 and health*, , Institute of Molecular Embryology and Genetics, Kumamoto, Japan, 1997.

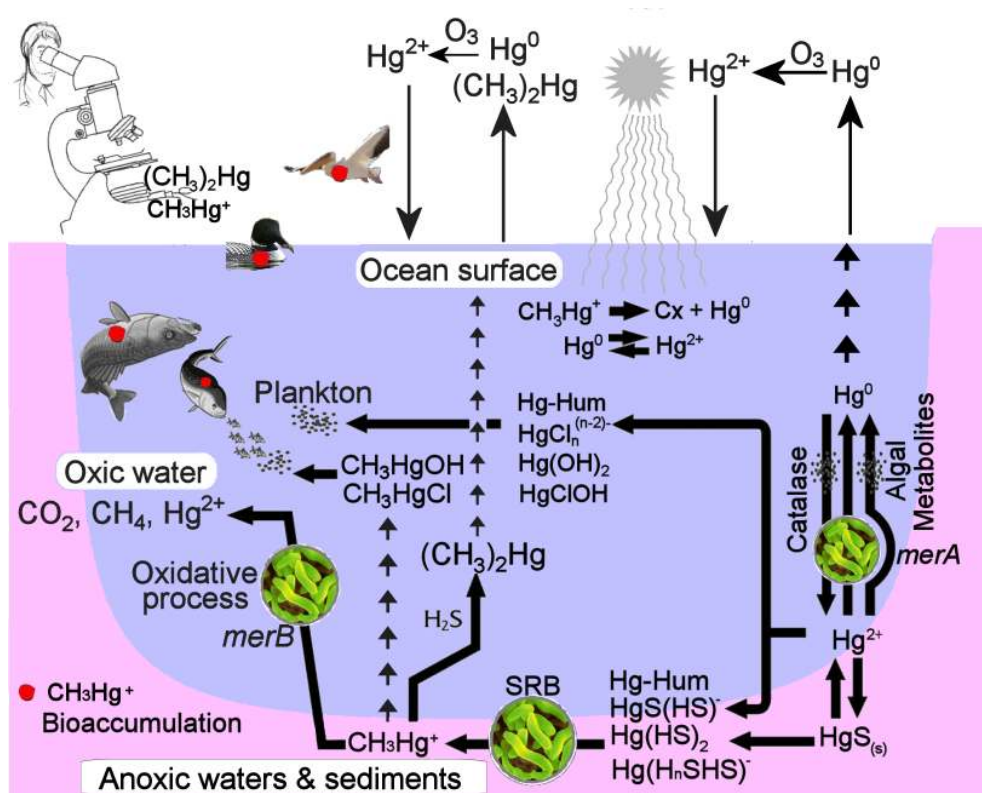
1601223. S. O. Jason, V. M. Winnie and A. O. Monica, *The socio-economic aspects of artisanal gold mining in Migori district, Kenya*. In: *The socio-economic impacts of artisanal and small-scale mining in developing countries: Gavin M. Hilson (Eds) Taylor & Francis, Netherlands, 2003.*
 1604224. M. S. Priscilla, *A failed eldorado: British trusteeship, Luyia land rights and the Kakamega gold rush, 1930-1952, Ph.D thesis, West Virginia University South Africa, 2000.*
 1606225. K. Kružiková, T. Randák, R. Kenšová, H. Kroupová, D. Leontovyčová and Z. Svobodová, *Acta Veterinaria Brno*, 2008, **77**, 637-643.
 1608226. M. Kongchum, I. Devai, R. D. DeLaune and A. Jugsujinda, *Chemosphere*, 2006, **63**, 1300-1303.
 1610227. S. M. Talebi, M. Moayed and I. Mohammadpour-Boltork, *Scientia Iranica*, 2008, **15**, 418-421.
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TOC

1615 Speciation of mercury in aquatic ecosystems, health effects and analytical techniques

1616 Omwoma S.,* Lagat S. C., Lalah J. O., Owuor P. O., and Schramm K.-W.

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16211. This review article outlines the speciation mechanisms of elemental mercury in aquatic

1622 ecosystems

16232. Health effects and biomonitoring mechanisms of different mercury congeners are discussed

16243. Analytical procedures employed in determining various mercury congeners are presented

16254. Kenyan gold mines are presented as amenable to mercury health effects