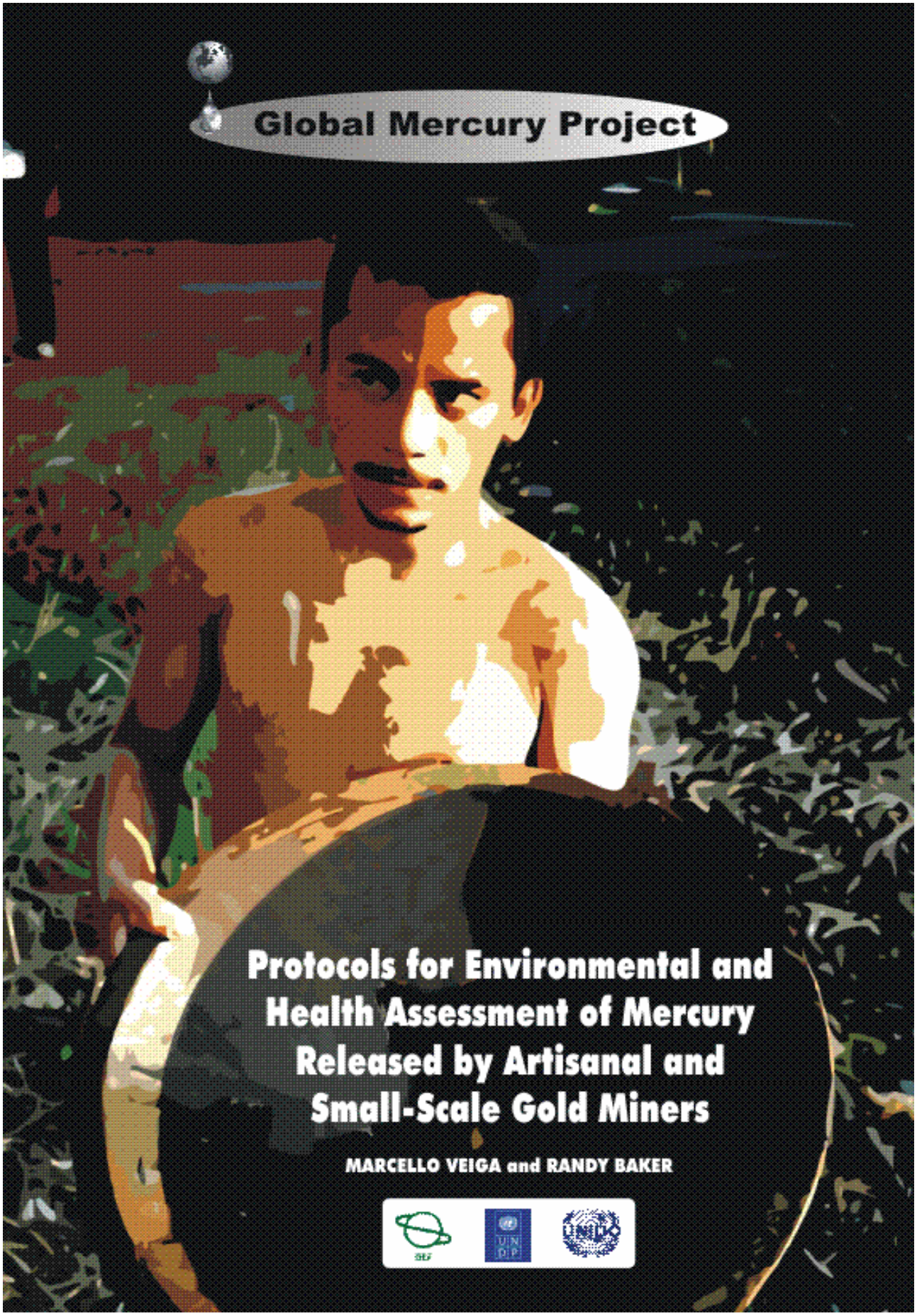
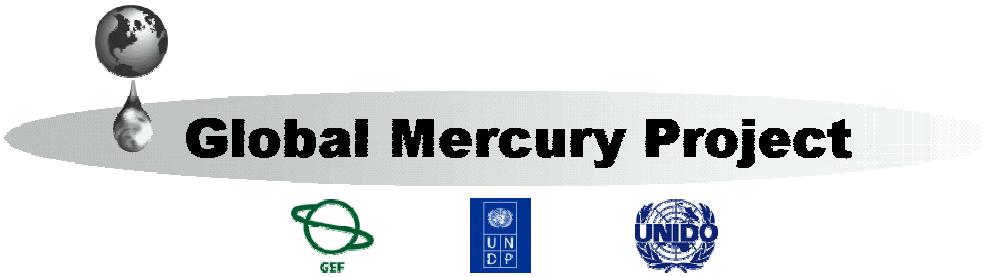

Global Mercury Project
Protocols for Environmental and Health Assessment of Mercury Released by Artisanal and Small-Scale Gold Miners
MARCELLO VEIGA and RANDY BAKER



Project EG/GLO/01/G34
Removal of Barriers to Introduction of Cleaner Artisanal Gold Mining and Extraction Technologies

Protocols for Environmental and Health Assessment of Mercury Released by Artisanal and Small-Scale Gold Miners

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Children and women are main victims of the misuse of mercury by artisanal gold miners

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Summary

Artisanal and small-scale gold mining (ASM) is a poverty-driven activity that provides an important source of livelihood for rural communities. As the price of gold has been increasing, the number of artisanal gold miners has risen to between 10 and 15 million people worldwide, producing from 500 to 800 tonnes of gold/a and emitting as much as 800-1000 tonnes/a of mercury (Hg). These activities are frequently accompanied by extensive environmental degradation and deplorable socio-economic conditions, both during operations and long after mining activities have ceased.

One option to determine the extent of environmental and human health effects of ASM Hg is to conduct an Environmental Assessment (EA). In general, EA is a tool used to identify, predict adverse effects and determine if mitigative actions are required.

However, the methodologies used in EA for large mining projects involving heavy metals are not ideally suited to assess the effects caused by Hg released by ASM in developing countries. This means that environmental assessments of ASM activities must be innovative and adapted to particular situations in the different countries where the activities take place. This is especially true when working with artisanal miners in impoverished and developing countries, where remote and difficult environments with little infrastructure and logistic support can pose many challenges.

Metallic mercury, which is the main form of Hg released by ASM, is capricious and difficult to work with. Because of the naturally volatile state of Hg and other confounding anthropogenic sources of Hg, this makes data interpretation difficult. Perhaps more importantly, the transformation of metallic Hg into its most toxic form, methylmercury (MeHg), is not clearly understood and there are no general rules governing this transformation. When Environmental and Health Assessments (E&HA) are conducted to determine Hg exposure, geochemical and biological samples should be carefully chosen to fulfill assessment objectives. In most cases, limitations of resources and time result in "short cuts" that can significantly impair data interpretation later on. Knowing that, the purpose of each monitoring step must be defined clearly before starting any field activities. Proper design of monitoring programs before entering the field is absolutely vital to establish the relevance and priorities for the sampling procedures.

Since 1995, UNIDO has been providing technical assistance to the small-scale mining sector in developing countries. Through numerous projects dealing with the introduction of cleaner technologies and mercury pollution abatement, the Organization has assessed the environmental and health impacts of Hg pollution caused by artisanal gold miners, *inter alia* in Venezuela, Ghana, and the Philippines. It is widely accepted that problems associated with artisanal gold mining in different developing countries are similar in nature. As such, solutions need a globally consistent and effectively coordinated approach in order to deal with these complex problems on a local level. The GMP (Global Mercury Project) was initiated by UNIDO in August 2002 to help demonstrate ways of overcoming barriers to the adoption of best practices, waste minimization strategies and pollution prevention measures that limit contamination of international waters. The Project, funded by GEF and co-funded by UNDP and UNIDO, is complemented by a suite of ongoing activities that are financed either through the participating countries' resources and/or bilateral programs.

The main goals of the GMP are to:

- reduce Hg pollution caused by artisanal miners on international waters;
- introduce cleaner technologies for gold extraction and teach miners how to use these technologies;
- develop capacity and regulatory mechanisms within local governments that will enable the sector to minimize Hg pollution;
- introduce environmental and health monitoring programs;
- build capacity in local laboratories to assess the extent and impact of Hg pollution.

The monitoring component of the Global Mercury Project (GMP) has specific goals, which are described in Objective 3 of the project proposal: "*identify hotspots in project demonstration sites, conduct geochemical and toxicological studies and other field investigations in order to assess the extent of environmental (mercury) pollution in surrounding water bodies and devise intervention measures.*"

One key feature of the monitoring program should be that it is designed to follow the evolution of Hg pollution in a mining area over time. To be successful, the program must focus on investigating bioavailability and bioaccumulation of Hg. Biota are the ultimate indicators providing direct evidence that Hg in the environment has become bioavailable and is being bioaccumulated. This document highlights the relevance of sampling aquatic biota and diminishes the importance of sampling water due to the low Hg levels in solution. Evidence of bioaccumulation must be obtained or predicted, in order to evaluate the appropriate course of action. If impacts to biota were not demonstrated at a contaminated site, then containment and long-term management would be more appropriate than other aggressive remediation measures. This, of course, is based on the acceptability to regulators.

As such, the monitoring program should establish **simple, replicable** and **sustainable operational** protocols for observing how Hg levels in different environmental and biological samples change over time. These protocols must then be transferred to

environmental agencies, mining-related institutions and researchers to ensure that these groups apply sound techniques for sampling, sample preservation, transportation, analysis, etc.

An important objective of the monitoring steps of the GMP is the identification of **mining hotspots**, which are sites with high concentration of metallic Hg. Often, mercury has been dumped by artisanal miners, into or near streams. There are also sites with the potential to become **environmental hotspots**; these are sites where Hg has been transformed into a more toxic and available form (i.e. methylmercury). **Hotspots** can have dimensions of a few square meters to hundreds of square meters and are the main sources of Hg dispersion into aquatic systems. Thus, they impact thousands of people who may or may not be involved with the mining activities. Identification and assessment of the risks posed by these hotspots should be a main objective of the Environmental Assessments.

Sampling procedures for such hotpots must be site-specific, taking into consideration the characteristics of the mining activity; the biodiversity of the region; the accessibility and availability of resources; risks; logistics; etc. Therefore, these Protocols, designed for the GMP, do not provide many details for such sampling. However, the Protocols do present the scientific rationale behind the decisions on what must be preferentially sampled and the methodologies. The researcher conducting E&HA must have a great deal of flexibility to adapt these concepts to the field conditions and the available budget. In EH&A, researchers must be careful not to create false expectations among local stakeholders related to solutions regarding Hg pollution. Environmental assessment is merely an initial step in addressing the issue by identifying problems and proposing solutions. This is frequently not understood by local stakeholders or by government regulators, who want to see procedures implemented and problems solved as quickly as possible.

In terms of technical solutions, when a situation with Hg vapour exposure is identified, such as when miners are burning amalgam in open pans, there are a number of quick and simple solutions that can be implemented immediately to reduce Hg exposure. These include the use of homemade retorts to recover Hg, removing women and children from the amalgamation area and discouraging the burning of amalgams in closed areas such as kitchens. These simple measures can be brought to the attention of miners and other individuals exposed to Hg easily, thus reducing the community's exposure to Hg significantly. To limit exposure of individuals or families to MeHg, the consumption of large amounts of carnivorous fish should be avoided. Instead, they should consume fish with lower MeHg concentrations or dilute their meals with vegetables, when these are available.

There is considerable controversy as to whether or not therapies should be discussed with Hg intoxicated people during a monitoring campaign. For ethical reasons, UNIDO has adopted the approach to inform the local and regional health care authorities when Hg intoxication problem is detected. UNIDO's mandate is to provide assistance in eradicating pollution sources, not to undertake active intervention. Although the organization understands that the health conditions of affected communities must be considered, medical intervention should only be undertaken by physicians operating within organizations better qualified for this task than UNIDO.

This document gathers information from many scientific publications and from the practical experience of the authors and their colleagues. Many "hints" provided here have been discovered through trial and error on projects not reported in scientific journals, for a number of international institutions. This information is offered to facilitate the implementation of the EH&A Protocols and to assist researchers who are operating in difficult field situations.

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Important Note

An Environmental and Health Assessment must be planned carefully. It is important to remember that, when dealing with humans, there are ethical issues that must be addressed. Also, when collecting and transporting samples (in or out of the country), permits are usually required. If samples are to be sent to another country for analysis, it is mandatory to obtain **in advance** all permits required to **send** and **receive** the samples. This has been one of the main problems in the monitoring programs conducted by UNIDO. In many cases the paperwork required by both countries (exporting and importing samples) is immense and can delay the entire analytical process. Some researchers obtain a permit to "export" geochemical and biological samples, but forget to obtain a permit to "import" the samples. It is not unusual to see samples in the Custom's refrigerator for more than one month or simply dumped at airports. The researcher must investigate the need for some or all of the following permits **before starting** the Environmental and Health Assessment:

- Permit from local Health Authority to collect biological samples (e.g. fish, snail, urine, blood, hair, etc.).
- Permit to transport geochemical and biological samples within the country.
- Permit to export samples.
- Permit to import samples

Some countries may also request fees from foreign professionals, in particular medical doctors, to work in their territory. This must be verified in advance.

PART 1 – ENVIRONMENTAL ASSESSMENT

Artisanal and small-scale gold mining (ASM) is an essential activity in many developing countries as it provides an important source of income, particularly in rural regions where economic alternatives are critically limited. The term **small mining** does not imply informal or rudimentary operations. There are many small mines in North and South America that use adequate technologies to extract gold from small primary gold deposits, while respecting legal and environmental regulations. **Artisanal mining** encompasses small, medium, informal, legal and illegal miners who use **rudimentary** processes to extract more than 30 different mineral substances worldwide (Priester *et al*, 1993). The choice of extraction technique is what differentiates conventional and artisanal mining. An artisanal miner is driven by the need to survive; to feed his/her family and pay bills. Such an operation is based on instinct; there is no previous "classical" geological exploration, drilling; proven reserves, ore tonnage establishments or engineering studies. Despite the small-scale and individual nature of the artisanal mining activities, the resulting environmental impacts can be surprising. When a large number of individuals excavate a single site, the resulting pit diameter can be as large as 2 km. This is the case of Serra Pelada, an infamous artisanal mining site in the Brazilian Amazon where, during the 1980's, more than 80,000 miners gathered to extract manually about 90 tonnes of gold from the same open pit.

The economic structure of artisanal mining is not different from any other capitalist activity. The concept of maximum profit with minimum investment is always present. Regardless of the size of the activity, this always creates some organization with hierarchy, duties and rules for all participants. The "boss" is the main investor who hires employees or, as preferred, shares part of his/her production with workers. As in any society, there are different types of people hidden behind professional categories. Some artisanal miners are trying to evolve, but some just think about the immediate benefits, regardless of the hazards to themselves and the environment (Veiga, 1997). Governments should be prepared to move beyond the establishment of legal frameworks or to identify deposits and areas amenable to artisanal miners. This may include: recognizing the constructive possibilities of working closely with local and international organizations; expanding the scope for local participation, including special attention to educational and gender issues and opportunities; establishing technical support; promoting local credit facilities and partnership arrangements with the domestic and foreign private sector; and facilitating environmental responsibility and occupational safety (Davidson, 1993). To achieve marked formalization in this poverty-inflicted and polluting activity, governments must simplify licensing procedures, provide more decentralized support and communicate more regularly and effectively with miners on the ground (Hilson, 2003).

The International Labour Organization (ILO, 1999) has estimated that the number of artisanal miners in 1998 was around 13 million in 55 countries and rising. This suggests that 80 to 100 million people worldwide have depended on this activity for their livelihood. Gold is easy to transport across borders and sell; it is also the main metal being extracted. As the price of gold has increased through 2003, the number of artisanal miners has also increased substantially. We estimate that the current number of artisanal gold miners is between 10 and 15 million people worldwide. The number of women and children directly employed in artisanal gold mining may be as much as 4.5 million and 300,000 respectively.

Since 1998, annual ASM gold production has constituted 20 to 30% of the global production, ranging from 500 to 800 tonnes (UNEP, 2002; MMSD, 2002). Assuming that miners lose between 1 and 2 grams of Hg per gram of gold produced, it is estimated that between 650 and 1000 tonnes of Hg are released annually to the environment, (using average Hg_{lost}:$Au_{produced}$~1.3). The predominant source of ASM Hg emission is from China (200 to 250 tonnes/a). We also estimate that Indonesia releases 100 to 150 tonnes/a, while Brazil, Colombia, Peru, Philippines, Venezuela and Zimbabwe each release from 10 to 30 tonnes/a of Hg (Gunson and Veiga, 2004; Shoko and Veiga, 2003, Veiga, 2003; Veiga and Hinton, 2002). Mercury emissions in Latin America are declining, as the most easily extractable ore has been depleted and operating costs have increased. However, the gradual increase in gold price in 2003 has been motivating miners to re-work abandoned ore deposits. There are reports identifying artisanal gold mining in more than 50 countries (Table 1.1).

Table 1.1. Countries with references on ASM
(artisanal & small-scale gold mining)

Country	Reference
Australia(*)	APLA (2004)
Benin	Yager *et al* (2002)
Bolivia	Maurice-Bourgoin *et al* (2000)
Brazil	Veiga (1997)
Burkina Faso	ILO (1999)
Burundi	Priester and Hentschel (1992)
Cambodia	Sotham (2001)
Canada(*)	Basque (1991)

Central African Republic	Yager *et al* (2002)
Chad	Mobbs (1996)
Chile	Silva Bruna (2001)
China	Gunson and Veiga (2004)
Colombia	Veiga (1997)
Costa Rica	Veiga (1997)
Democratic Republic of Congo	ILO (1999)
Dominican Republic	Veiga (1997)
Ecuador	Veiga (1997)
Ethiopia	Labonne (2002)
French Guyana	Fréry *et al* (2001)
Gabon	Priester and Hentschel (1992)
Gambia	Dolley (1996)
Ghana	Babut *et al* (2003)
Guinea	Labonne (2002)
Guinea-Bissau	Dolley (1996)
Guyana	Couture and Lambert, (2003)
Honduras	Veiga (1997)
India	Siddaiah (2001)
Indonesia	Veiga (2003)
Ivory Coast	Yager *et al* (2002)
Kenya	Yager (2002)
Kyrgyzstan	Appel *et al* (2003)
Lao PDR	Boungnaphalom (2003)
Leshotho	Coakley (2002)
Liberia	DLI (2003)
Madagascar	Rajaobelina (2003)
Malawi	Dreschler (2001)
Malaysia	Priester and Hentschel (1992)
Mali	MMSD (2002)
Mauritania	Mbendi (2004)
Mexico	Veiga (1997)
Myanmar	UNESCAP (2003)
Mongolia	Tumenbayar *et al* (2001)
Mozambique	Zacarias and Manuel (2003)
Nicaragua	Veiga (1997)
Niger	Alfa (2000)
Nigeria	Priester and Hentschel (1992)
Papua New Guinea	Crispin (2003)
Peru	Hruschka and Medina (2001)
Philippines	Drasch *et al* (2001)
Russia	Stepanov and Yusupov (2001)
Rwanda	Priester and Hentschel (1992)
Senegal	Bermudez-Lugo (2002)
South Africa	Mahlatsi and Guest (2003)
Sudan	Ibrahim (2003)
Suriname	Veiga (1997)
Tanzania	Kinabo (2003)
Togo	Yager *et al* (2002)
Uganda	World Bank (2003)
USA(*)	Weekend Prospector (2004)
Venezuela	UNIDO (2004)
Vietnam	Trung (2001)
Zambia	Dreschler (2001)
Zimbabwe	Shoko and Veiga (2003)

Note: (*) these artisanal miners are usually prospectors or weekend placer gold miners

Although the use of Hg in mineral processing is illegal in most countries, amalgamation is the preferred method employed by ASM. This is because it is easy to use, available, and inexpensive. Unfortunately, miners are seldom aware of, or chose to ignore the health risks associated with Hg handling.

Measurement designed to reduce Hg emissions must consider the above factors. The free trading of mercury from developing countries, in particular from Europe, makes mercury easily available at the mine sites. The amount of Hg

produced by mercury mining and gold mining (with Hg as a by-product) decreased from 6,066 tonnes in 1990 to 1,849 tonnes in 2000. However availability of recycled Hg from chlor-alkali plants and other sources has increased from 440 tonnes in 1990 to 910 tonnes in 2000, particularly in Europe and USA. Germany and the Netherlands also have significant Hg recycling facilities. This is relevant because the exports from Germany (and to some extent the Netherlands) to the three key ASM regions (Latin America, Africa and Asia) are explained partially by the fact that it is cheaper to recycle European Hg-bearing waste and sell the recovered Hg on the open market, than it is to dispose of Hg-bearing waste at a hazardous waste facility in Europe (Maxson, 2004). As a result, mostly in OECD countries, quantities of marketable recycled Hg are expected to continue increasing. The availability of Hg from decommissioned chlor-alkali plants has become an increasingly important contributor to global mercury supplies, and has dampened demand for virgin Hg. Chlor-alkali residual Hg has comprised 10-20% of global supplies in recent years, and is estimated to contribute 25-30% in the near future. It is possible to assume that by 2020, there will be an accumulated oversupply of 10,000 to 15,000 tonnes of Hg (Maxson, 2004). Developed countries should review their trading policies regarding mercury. In most cases, Hg enters developing countries through legal channels for legitimate use (e.g. dental fillings), but is then diverted to ASM operations. According to UN 1999 trade statistics, 96 tonnes of mercury entering Indonesia were registered from Spain and 33 tonnes from China. In 2000, Indonesia imported 28 tonnes of Hg from Spain, 17 tonnes from the Netherlands, 3 tonnes from Australia and 3 tonnes from Japan. Zimbabwe imports 20 tonnes of Hg/a from the Netherlands for ASM in Zimbabwe and Mozambique. In 2000, the Netherlands shipped 245 tonnes Hg to at least 18 countries, most of them in Latin American/Caribbean region; Spain shipped 774 tonnes, Germany 105 tonnes and UK 200 tonnes to countries outside EU, mainly in South Asia. Most of this Hg is low-priced and is suitable for ASM (Veiga *et al*, 2004).

Exposure to Hg by humans living in close proximity to mining sites is primarily via two pathways:

1. Occupational Hg vapour exposure from amalgam burning or gold melting,
2. Methylmercury (MeHg) from dietary sources, especially fish.

Inhalation of Hg vapour is the primary exposure pathway for miners, gold shop workers and people living near areas where Hg is handled. Residents in rural communities may be exposed to high MeHg concentrations in fish from waterways that are contaminated by Hg from mining sites. In many regions, fish are the main protein source for community residents. However, fish consumption can also result in the intake of greater amounts of MeHg than health authorities advise.

Before conducting an EH&A in gold mining regions, it is essential to understand the methods employed by miners to process gold ores and how Hg is being used (or was used). In the environmental literature, it is possible to observe a difference between Hg emissions and Hg releases. Because of its volatile characteristics, "mercury emission" usually refers to the portion of Hg emitted to the atmosphere. "Mercury release" refers to all forms of Hg discharged into all environmental media (air, soil, water). Relative proportions discharged to these media depend on the mining and processing methods. It is difficult to obtain reliable, quantitative data about Hg releases from active ASM, as miners do not freely provide information about the amount of Hg they use and their gold production is sporadic. At abandoned sites, the task is even more difficult. Analyses of geochemical materials surrounding mining sites only provide qualitative historical information about the amount of Hg released. Uncertainties associated with sampling processes prevent accurate determinations of the amount of Hg lost.

1.1. Amalgamation and Mercury Releases

Mercury (Hg), because of its normal liquid state, has a unique capacity to form an amalgam or bind with most metals except iron and platinum. The wetting of gold by mercury is not alloying, but a phenomenon of moderately deep sorption, involving some interpenetration of the two elements (Pryor, 1965). As the surface tension of mercury is greater than that of water, but less than that of gold, Hg adsorbs onto the surface of gold particles. In addition, mercury acts as a dense medium; gold sinks into the mercury while the lighter gangue material floats on top. When the resulting amalgam is heated, the mercury vaporizes, leaving gold *doré* (Gunson, 2004). Gold (Au), in particular, can combine with Hg to form a wide range of compounds from $AuHg_2$ to Au_8Hg. The three principal gold amalgams are: $AuHg_2$, Au_2Hg, and Au_3Hg. Mercury can also solubilize from 0.14% to 0.65% gold at room temperature and 100 °C respectively (Taggart, 1945; Sevryukov *et al*, 1950). Although this property has been recognized for more that 4000 years, the gold amalgamation process only became popular during the XIV century in Central Europe and was brought to the Americas in the XVI century by Portuguese and Spanish miners.

The amalgamation process was used widely by Canadian miners, from the 1860's until the 1890's, as observed in the reports of the Minister of Mines of British Columbia. Nuggets had a better price ($ 16.5/oz) than fine gold (MMBC, 1881) and Hg became a solution to extract fine gold from benches of the Fraser River extending from Hope to Lillooet. The text extracted from MMBC (1875) shows clearly this fact: *"...on the bars near the mouths of rivers, it is found in a fine impalpable dust, known as flour gold, and can only be collected by the aid of quicksilver."* Mercury was used in sluice boxes or in copper plates. It is reported that Chinese and native Indians were the best gold savers at that time (MMBC, 1881). Archives from British Columbia report the use of 25 lb of Hg/day/sluice by the old American and Canadian miners at Cariboo goldfields (1856) (Veiga and Meech, 1995a).

Amalgamation was also used extensively by gold miners in California from 1860s to early 1900s. Typically, 1.6 kg of Hg/m^2 was added to the riffles of the sluice boxes to amalgamate the entire placer ore. About 10 to 30% of this Hg was lost. It has been estimated that in California alone, 1,400 to 3,700 tonnes of Hg were lost to the environment (Alpers and Hunerlach, 2000). Elevated Hg concentrations have been detected in the sediments of the historic mines as well as in fish tissues. Extensive transport of remobilized placer sediments has been noticed and a positive correlation between Hg bioaccumulation and the intensity of hydraulic gravel mining has been demonstrated (Hunerlach, 1999). A similar situation occurs in Nevada. The gold mills located in Virginia City and surrounding towns adjacent to the Carson River discharged approximately 7,500 tonnes of Hg associated primarily with tailings into the drainage (USEPA, 2002a). Currently, amalgamation practices in North America are restricted to small gold placer prospectors who claim that they use Hg in a safe way.

As recently as 1889, gold amalgamation started to decline as an industrial process and was replaced with a cyanidation process (Ciminelli and Gomes, 2002). Today, nearly all organized mining companies have ceased the practice of amalgamation. Unfortunately, this is still the main process used by ASM in developing countries. The probability of having an alternative, environmentally sound process that remains simple and popular is not encouraging (Veiga, 1997).

1.1.1. Mercury Used by ASM

Amalgamating

Mercury is usually discharged with tailings and/or volatilized into the atmosphere. The magnitude of loss and means of Hg release from a specific site are defined by the Au-Hg separation procedures. A variety of amalgamation methods are used in artisanal mining operations; these must be primarily surveyed to establish a reliable environmental assessment. Typical amalgamation methods used by ASM are listed below (Veiga *et al*, 1995):

- Whole ore amalgamation: Hg is mixed with the whole ore in pump boxes; introduced in sluices during gravity concentration; added to the grinding circuit; or the whole ore is amalgamated using copper plates.
- Amalgamation of only gravity concentrates: mercury is mixed with concentrates in blenders or barrels; separation of amalgam from heavy minerals is accomplished by panning in water-boxes, in pools or at creek margins.

A common practice in many countries is to amalgamate the whole ore, either by spreading Hg on riffled concentration boxes or by using the old copper plate amalgamation method. When Hg loses its coalescence, it “flours” (i.e. forms a large number of small droplets that are carried away with tailings). Mercury contaminated tailings from sluices are usually discharged directly into a river. In a few places where hydraulic monitors are used, miners spread large amounts of Hg on the ground with the belief that the “quicksilver” will “magically” move on the dirt to collect all available gold. Amalgamation actually occurs later, when the riffled sluices retain Hg droplets and gold specks are pumped with the ore, giving the impression that gold is amalgamated on the ground. When this crude method is applied, losses can be higher than 3 parts of Hg to 1 part of gold produced and the opportunity to recover Hg is remote.

Copper-amalgamating plates have been used widely to amalgamate whole ore in most countries with ASM. Alluvial or ground ore flows over a copper plate covered with Hg and gold trapped as a solid amalgam is formed. The method works well for alluvial gold, but it has limited use for primary ore, as the gold is not completely liberated from the gangue minerals. As the ore pulp with 10 to 30% solids scratches the plate surface constantly, droplets of Hg are carried with the tailings. In Kadoma, Zimbabwe, the ore is ground in stamp mills and discharged onto copper-amalgam plates. This was a popular method used by American gold miners in California from the mid-1880s to the early 1900s (Alpers and Hunerlach, 2000). Zimbabwean miners have stated that they have to be very attentive and must rub the surface of the plates constantly to recover gold. Quite often, Hg (and gold) accumulates on a point of the plate surface and with the attrition of the ore pulp, both are lost. A miner stated: "*If I sleep and I do not spread the mercury on the plate, I lose mercury and my gold*" (Shoko and Veiga, 2003). Periodically the process is interrupted and amalgam is scraped off from the plates with a sharp piece of metal. At this stage, miners are exposed to high levels of Hg vapor. The amalgam recovered from the plate is squeezed to eliminate excess Hg and burned off, usually in bonfires.

In Venezuela, the use of copper plates in Bolivar State is very popular. At the Caroni River, Venezuela, miners used copper plates on board boats to amalgamate dredged ore. More than 5 tonnes of Hg were disposed into the river along with the tailings (Veiga, 1996). Acidic water may also cause yellow or green spots (copper oxidation) on the copper plate and can cause mercury to lose its amalgamation ability. Miners refer to these discolored areas as "sick". When this occurs, miners burn the plates to remove the mercury and clean the copper plate with cyanide to re-amalgamate it. Quite often, Venezuelan miners burn the amalgamation plates to "remove" fine gold trapped on the plate (Veiga and Gunson, 2004). In Kadoma, Zimbabwe, it is common to see miners handling a large tablet of sodium cyanide and rubbing it constantly on the copper plates during the operation, to remove the "yellow spots" (Cu-oxidation areas).

The worst situation occurs when Hg is added directly to the grinding circuit. In China, for instance, amalgamation using muller mills[1] leads to losses of 14 to 20 parts of Hg for one part of gold produced (Gunson and Veiga, 2004). In Peru, miners mix Hg with ore during the crushing step using the "quimbaliti" crusher/grinder, a round piece of rock revolved by the miner's feet (Hruschka and Medina, 2001). In North Sulawesi, Indonesia, it is common practice to add 1 kg of Hg into a steel-grinding mill (filled with balls, rods or cobbles) to grind 40 kg of ore per batch (usually 5 hours). Hg losses for this method are estimated to be around 50 g Hg/mill/day or 1.5 kg per unit (of 30 mills)/day. This makes the ratio Hg_{lost}: $Au_{produced}$ = 100, which is likely one of the highest in the world. The amount of Hg released by hundreds of operations spread across Indonesia can reach more than 100 tonnes (Veiga, 2003).

Some bad practices have been abandoned or are used less frequently, such as the use of "Jack pots", large Hg baths in which the entire ore passes through.

Nowadays, some miners are amalgamating only gravity concentrates. This is an important evolution in artisanal mining methods, resulting in significant decreases in Hg consumption and release. Using this method, approximately 14 grams of Hg are required to amalgamate 1 kg of concentrate (ratio Hg:concentrate ~ 1:70). Amalgamation is efficient for particles coarser than a 200 mesh (0.074 mm) and for liberated or partially liberated gold (Wenqian and Poling, 1983). With gold recoveries in excess of 90%, amalgamation can be improved when concentrates are processed in rolling barrels (Veiga and Fernandes, 1991). Two hours of operation provides good recovery but also increases the "flouring effect" as is the case in when amalgamation is performed in ball or rod mills. Some possible solutions to avoid Hg "flouring" are: the use of shorter amalgamation periods; the use of large rubber balls to promote contact between Hg and gold particle; and the application of oxidizing (e.g. $KMnO_4$) or complexing (chlorides) reagents to clean gold surfaces. Although chemical reagents can improve gold and silver recovery, they may promote metallic Hg dissolution and loss. Adding one gram of sodium hydroxide (NaOH) to every kilogram of concentrate amalgamated is an easy way to improve the effectiveness of the method. In many Latin American operations, amalgamation in rolling barrels takes place in 30 to 50 minutes with the addition of 1 part of Hg to 100 parts of concentrate (Veiga, 1997). Some miners introduce a large iron chain inside the barrels to promote adequate mixing of ore with Hg (Herman Wotruba, 2003 – Univ. Aachen, personal communication).

In Zimbabwe, amalgamation barrels run for too long (e.g. 4 hours) and with many iron balls inside, causing "flouring" and consequent loss of Hg to the tailings. This has been visually confirmed by examining amalgamation tailings. Many miners add sodium cyanide tablets into the amalgamation barrels to clean the gold surface and improve amalgamation. In many cases, the pH of the pulp is 7 or lower when sulphides are present in the ore. The possibility that HCN gas, which is extremely poisonous, will be produced is either ignored or unknown. The material discharged from amalgamation barrels is concentrated by panning in a plastic bowl and then passing the tailings through an amalgamating copper plate. The plates recover very little additional gold (Shoko and Veiga, 2003).

When gravity concentrates are amalgamated, the mineral portion is separated from the amalgam by panning in water boxes, in pools excavated in the ground or at creek margins. The heavy, mineral-rich amalgamation tailings frequently

[1] A heavy stone, concrete, or iron wheel that promotes the contact between mercury and gold particles while crushing the ore.

contain 200 to 500 ppm of residual mercury. These create **hotspots** when dumped into adjacent water bodies (Veiga, 1997). In an Environmental Assessment, the **fate of the amalgamation tailings is of great concern**. Some Zimbabwean miners take the amalgamation tailings home to re-grind. Sometimes they add more Hg, pan them in their backyards and roast them in kitchens. As the tailings are contaminated with Hg, this causes Hg to be dispersed throughout the region, as well as into urban areas. When the miners leave the amalgamation tailings at a concentration plant, millers leach them with cyanide together with the gravity concentration tailing (primary tailing).

In dredging operations in the Madeira River, Amazon region, Brazil, amalgamation of concentrates is done on board a boat using a blender. After amalgamation, tailings are dumped into the rivers, forming a large number of **hotspots** (Pfeiffer and Lacerda, 1988). A similar situation has been observed in the Kahayan River in Central Kalimatan, Indonesia. More than 3000 dredges are currently dredging sediments over 200 km of river. About 20 to 30 g Au/week is produced by 3-5 miners working on each raft. The ore is processed in sluice boxes lined with carpets and the concentrate is amalgamated manually on-board. The mercury contaminated tailing is then dumped into the river. In these cases, the rivers are so large and wide that the number of hotspots created in the river is widely dispersed, making them extremely abundant, very difficult to identify (Veiga, 2003) and almost impossible to amend.

Cyanidation of Tailings

Another practice that is gaining popularity with ASM is cyanidation. In many countries, such as Brazil, China, Ecuador, Indonesia, Peru, Philippines, Venezuela and Zimbabwe, miners have been observed extracting "free" gold with amalgamation and leaching Hg-contaminated tailings with cyanide. This leads to an additional problem. As those tailings have residual Hg, during the cyanidation process Hg becomes oxidized and is dissolved by cyanide along with gold. Although much of the Hg is leached with gold, a portion of Hg remains in the tailings. As Hg dissolves in cyanide at much slower rates than gold, the final tailings are richer in soluble Hg complexes. Typically, these tailings are discharged carelessly on land or in nearby streams.

In North Sulawesi, Indonesia, amalgamation tailings rich in Hg are submitted to cyanidation in 20 to 30 m^3 aerated tanks using 100 to 200 mg/L NaCN at pH 11 adjusted with lime and controlled once a day. There is a tank that pre-agitates the pulp (around 40% solids) and settles part of the residual Hg (300 to 500 g Hg recovered per batch of 20 tonnes). Between 100 and 150 kg of activated charcoal per 60 tonnes of material processed is added to the leaching tanks to adsorb Au and Hg cyanide complexes in solution. The Au-Hg-loaded charcoal is recovered by screening and burned in open drums to recover about 400 g of gold. The workers are not aware that Hg vapour is liberated when they burn the activated charcoal, exposing them to this hazard. Tailings, containing residual Hg-cyanide, are deposited in a large non-lined tailing pond or in wetlands (Veiga, 2003).

In North China, the muller mill tailings, ranging from 200 to 2,000 ppm Hg, are submitted to cyanidation in 10 tonne-leach vats using a solution with 2.5 to 5 kg of NaCN and 5 kg of lime / tonne of tailings per vat. No de-aeration takes place, and cyanide and lime are only added once at the start of the operation. The leach solution drains through the vats to a box of zinc strips, where gold and Hg is precipitated onto the strips through the Merrill-Crowe Process. After seven days of re-circulation, the strips are collected and smelted down in crucibles with borax in open drums. Coking coal and wood are burned to smelt the strips, with an electric blower at the bottom of the drum to increase the supply of oxygen. No effort is made to protect the miners from the Hg fumes emitted during this process. Tailings, containing residual Hg-cyanide, are deposited in non-lined piles beside waterways (Gunson and Veiga, 2004).

In the Kadoma region of Zimbabwe, miners take their ores to processing centers to be ground and concentrated by operators (known as millers) who charge about US$ 1.2 to $1.6/hour. Less than 30% of the gold is recovered by gravity concentration, which is followed by amalgamation. The primary tailings are left on the premises. This is an opportunity for the millers to apply vat-cyanidation to extract the remaining gold. Miners receive no compensation for the extra gold extracted by cyanidation. Most centers have 5 to 10 cyanidation tanks to extract residual gold from primary and amalgamation tailings (Hg-contaminated). About 20 to 70 tonnes of tailings are added to cement tanks to be leached with 18 kg NaCN/tank. There is no pH control. Operators add 50 kg of $Ca(OH)_2$/tank and wait an average of 6 days to end the leaching step. This practice is spreading and there are more than 70 processing-cyanidation centers in the Kadoma-Chakari region. The final cyanidation tailings, still rich with residual Hg-cyanide, are poorly are contained. Natural recovery of vegetation occurs rapidly when free cyanide degrades, leaving little evidence of an Hg hotspot (Shoko and Veiga, 2003).

An innovative solution to avoid Hg-cyanide formation was devised at Bald Mountain Gold Mine, a Placer Dome heap leaching operation in Nevada. As the ore is rich in natural Hg minerals, the operators had to eliminate Hg production to decrease cyanide consumption and reduce health and safety risks for workers, as well as environmental liability after mine closure. When commercial reagents (e.g. trithioic acid) are added to the cyanide solution, Hg precipitates as an insoluble organic sulfide that does not affect gold and silver production. This mine reduced Hg production from 172 to 2 lb/month (Wickens *et al*, 2001). Other inexpensive reagents must be investigated and brought to the attention of the ASM using cyanidation of amalgamation tailings. However, combining amalgamation with cyanidation should always

be avoided. When cyanidation facilities are available, miners should never use Hg to conform to the process used by all formal gold mining companies around the world.

Limited information is available about the environmental stability, reactivity and toxicity of Hg cyanide complexes. An additional mechanism may exacerbate these circumstances; the reaction of Hg (II) cyanide complexes with organic acids from cyanidation tailings is not known, but it seems to favor Hg (II) complexation with organics to produce methylmercury cyanide.

Removing Excess Hg

Miners tend to use excessive amounts of Hg to amalgamate concentrates or the whole ore. In many cases, when amalgamating concentrates, just 10% of the Hg added to an amalgamating barrel or to a pan (in the case of manual amalgamation) combines with gold to produce the amalgam. The rest is excess and must be removed and recycled.

The most common method to remove excess Hg from amalgams is to squeeze it through a piece of fabric manually. Metallic Hg is not strongly absorbed by the skin but direct contact must be avoided. A few cases of hypersensitivity or allergic dermatitis among dentists have been reported (WHO, 1991a). The amalgam usually consists of about 40% Hg. When the amalgam is centrifuged, as observed in an Amalgamation Center in Venezuela, the Hg content in the amalgam drops to 20-30% (Veiga, 1996).

A Zimbabwean equipment manufacturer (Small Scale Mining Ltd) developed a small centrifuge, using a food processor, to extract excess Hg from amalgams. This avoids manual contact with Hg and produces an amalgam with less Hg to be retorted.

Burning Amalgams

Once the amalgam is obtained, it is retorted or simply burned openly in pans. The main pathway of contamination by miners is inhalation of vapors. Retorts can be used to capture volatilized Hg and condense it, allowing the mercury to be recycled and reducing drastically occupational exposure to Hg vapors. Natural Hg levels in air in rural areas usually range from 0.001 to 0.004 μg/m³ and in urban areas from 0.01 to 0.17 μg/m³. Typically, Hg is found in air as elemental Hg but 1 to 25% can occur in the form of Hg (II), depending on the type of emission source (USEPA, 1993). The limit for public exposure is 1.0 μg/m³ (WHO, 2003). The recommended health-based exposure limit for metallic Hg is 25 μg/m³ for long-term exposure (TWA)[2] (WHO, 1991a). Table 1.2 compares the levels of Hg analyzed in air in different working places.

Table 1.2. Cases of occupational exposure to Hg vapors

Hg (μg/m³)	Workplace	Reference
60,000	amalgam burning in a mine site	Malm, 1991
12,000	dentist office (amalgam restorations)	Stopford, 1979
6,000	underground cinnabar mining	Stopford, 1979
3,000	police office - finger printing powder	Stopford, 1979
1,000	filling operation of fluorescent lamps	Stopford, 1979
300	gold dealer shop in Rondonia, Brazil	Malm *et al*, 1990
100	chlor-alkali plant and thermometer factory	Stopford, 1979
30	lighthouse in British Columbia	van Netten and Teschke, 1988

Malm (1991) measured up to 60,000 μg/m³ of Hg in air when amalgam was burned in open pans in an ASM operation. When retorts were used, this concentration dropped to as low as 10 μg Hg/m³. This is still high, probably because miners open the retorts while they are warm, but it is still lower than the 50 μg Hg/m³ limit for industrial exposure - TWA (BC-MEMPR, 1992). It is obvious that miners are exposed to unacceptably high levels of Hg when they burn amalgam in an open pan or shovel. The WHO (1991a) believes that an individual exposed to Hg levels in air above 80 μg/m³ has a high probability of developing symptoms of Hg intoxication.

Condensed Hg contains residual gold (0.1%) in solution, which is claimed to improve amalgamation (Taggart, 1947, Sevryukov *et al*, 1950). Retorts lead to more than 95% Hg recovery and reduce air pollution and occupational exposure significantly. There are many types of retorts. Some are made with stainless steel while others use inexpensive galvanized steel (water plumbing) (Veiga *et al*, 1995). The retorting efficiency depends on the type of connections or clamps used. Homemade retorts can also be made of steel tins or kitchen bowls (e.g. stainless steel or enamel bowls). An inexpensive option for retorting has been applied in Papua New Guinea and China. The Chinese two-bucket retort consists of a metallic bucket and a bowl filled with water. A larger bucket covers the first bucket that contains the amalgam. The PNG "tin-fish-tin" retort employs the same concept, but uses fish tins and wet sand instead of water. In

[2] TWA = Time Weighed Average means the time weighed average concentration for a normal 8 hour day and 40 hour workweek, to which nearly all workers can be repeatedly exposed without adverse effect.

both cases, the amalgam is heated using wood, charcoal or electric elements and Hg vapors condense on the cover-bucket walls (Hinton *et al*, 2003; Veiga and Gunson, 2004).

The usual practice to separate Hg from gold by burning the amalgam in a pan with a blowtorch results in very high direct losses of Hg to the atmosphere. This Hg is transported and condensed elsewhere, where it can be transformed into MeHg in aquatic environments. However, the main problem is the exposure of workers and surrounding communities to Hg vapor. In Central Kalimatan, Indonesia, amalgam is burned in open pans everywhere, including kitchens and restaurants. A similar situation was observed in the Mekong River in Lao PDR, where a large audience of women and children observed the "fascinating" color transformation when amalgam is burned and gold is obtained. In most African countries, amalgams are burned in small tins put into bonfires. Miners remove the *doré* from the fire when the surface of the amalgam becomes yellow. As the burning time and temperature are not enough to vaporize all Hg, the gold *doré* contains as much as 20% Hg. This is released in the gold buyer's shops or jewelries usually located in urban areas. A solution to make retorts viable is the use of air blower to increase the temperature of the bonfire (Veiga, 2004).

In places such as Lao PDR, where Hg is purchased in ASM areas for US$ 80/kg it makes sense to use the economic argument to convince miners to recycle Hg. In fact, miners put a short bamboo tube around the amalgam to collect Hg. When the bowl filled with amalgam is burned, Hg evaporates and condenses on walls in the interior of the fibrous bamboo tube. The condensed Hg is removed with a chicken feather to be re-used. Women and children typically carry out this process at home. Retorts are not used in the region (Boungnaphalom, 2003).

In Indonesia, South America and many African countries, the price of Hg ranges from US$ 10 to US$ 30/kg. Even though this is up to seven times higher than the international Hg price, this is still cheap, i.e. equivalent to 1 or 2 grams of gold. So, the economic argument should be replaced with other strategy. Despite the introduction of retorts through many programs (CETEM, UNIDO, Projekt-Consult GmbH, ITDG, Organization of American States, etc) and the obvious benefits that are associated with their use, artisanal miners are reluctant to use retorts, because of a lack of concern for environmental and health impacts. The most effective argument used to convince miners to use retorts is via social and cultural means. For example, in 1985, the Secretary of Mining of Goiás State, Brazil, started a campaign promoting retorts that included a brochure illustrating the effects of mercurialism. Impotence was stressed as one of the main symptoms. Although this is somewhat inaccurate and questionable from an ethical standpoint, it was effective in capturing the attention of miners (Hinton *et al*, 2003).

It is important to understand why miners do not use retorts. Engineers tend to look for the efficiency of the retorting process, when in many cases this is not the dominant factor for introducing cleaner technologies. The arguments are site-specific and sometimes fraught with misconception. However, in some cases there are good reasons why retorts are not being used and these must be analyzed carefully.

Some of the most common arguments used by ASM for not using retorts are:

Arguments	**Reasons**
• retorts are expensive	▪ miners do not know inexpensive options
• it takes time (sometimes miners become vulnerable to bandits)	▪ the retorting temperature is too low
• experience is needed to operate retorts	▪ the heating process must be uniform when using a blowtorch
• gold is lost during retorting	▪ with iron retorts, the amalgam is not visible so miners believe it has been lost
• gold sticks to the retort crucible	▪ this occurs when the temperature is too high or when the crucible is not lined with a thin layer of clay, talc or soot
• Hg loses coalescence	▪ sometimes condensed Hg disintegrates into fine droplets
• gold comes out brown from steel retorts	▪ the cause is not known; it is probably due to a superficial reaction with iron

All these factors must be taken into consideration when recommending the most appropriate type of retort for a specific mining region. In some cases, gold buyers use the miner's perception to lower the purchase price. This is common when miners sell brown retorted gold. Ways to avoid obtaining brown gold were not well studied yet, but some solutions being adopted elsewhere include:

- the use of oxidizing conditions
- the use stainless steel or enamel crucibles
- melting the gold

- hammering the gold *doré*

The first option has been adopted by the Gama Project in Peru. A cooperative built a large fume-hood where the artisanal miners can burn their own amalgams in their pans (as they traditionally do) with a blowtorch. The vapors are extracted and Hg is retained in condensers and filters. The efficiency of this process is around 85% (F. Hruschka, 2003 – Gama Project, Peru, personal communication). This raises an important point: cultural aspects must be carefully examined before suggesting any type of solution for ASM. Even a crude method of retorting, such as "baking" the amalgam in the scooped-out cavity of a potato, is better than nothing. This has been described in the "Gold Panner's Manual", a favorite of North American weekend prospectors (Basque, 1991). Readers are advised not to eat the potato after processing!

The best retorts are those made of local and easily accessible materials that are non-expensive and easy to demonstrate. Durability can be a factor, but as long as the retorts are cheap and accessible, this becomes less relevant for miners.

An example of an effective and creative solution has been applied in Venezuela, where Amalgamation Centers were constructed to increase gold recovery and reduce Hg releases. Miners bring their gravity concentrates to private or state-owned centers to be amalgamated, retorted and melted by specialized operators (Veiga and Beinhoff, 1997). Of course, this is a site-specific measure because transporting hundreds of kilograms of gold concentrates through the jungle can present a problem for some miners. However, even this solution has demonstrated weaknesses. Schulz-Garban (1995) found very high levels of Hg in air inside Amalgamation Centers in Venezuela. They discover that, under pressure from miners to rush the amalgamation process, employees of Amalgamation Centers were opening retorts while they were hot. Wearing inappropriate dust masks, they were exposed to residual Hg vapour in the warm retorts. At that moment, Hg in ambient air was as high as 250,000 $\mu g/m^3$, but reached background levels in 20 to 40 seconds. In this type of situation, dust masks can retain a small part of Hg vapor; contaminated masks must be discarded after use to avoid inhalation of Hg condensed on the mask. Appropriate respirators for Hg vapour must also be used. The accumulation of Hg on mask cartridges is rapid and can actually increase a worker's exposure when the mask is used repeatedly. Masks with activated charcoal cartridges are recommended with a restricted number of uses (Stopford, 1979).

Recycling Mercury

Recycled mercury or Hg recovered by retorting often does not have the same amalgamating properties as new Hg. A thin layer of "oxidation" is formed, probably by absorbed oxygen on the Hg drop surface. In this condition, Hg forms thousands of droplets and loses its amalgamation capacity (i.e. it flours). Facing this problem, many miners simply discharge the retorted Hg. The most efficient way to reactivate the surface of Hg is by using an ultrasonic bath, such those used by dentists, causing Hg droplets to coalesce in seconds (~US$200-400) (Hinton *et al*, 2003). Battery-operated-ultrasonic denture cleaners are more affordable (e.g. allSonix costs Euro 30) but not quite accessible to ASM. A much less expensive method involves electrolytic activation using table salt and a simple flashlight battery to clean the Hg surface (Pantoja and Alvarez, 2001). Some authors (Taggart, 1945) suggest the use of potassium permanganate to retrieve coalescence. A process to retain the contaminated water effluent (e.g. using lateritic material or activated charcoal with iodide) should accompany all activation methods. Even if the amount of effluent being discharged is small, some soluble Hg could be transformed into methylmercury after being released into the aquatic environment.

In some countries, miners recover Hg from amalgams through dissolution in nitric acid. Mercury can then be precipitated from solution using an aluminum or zinc wire. The major problem with this technique is that, after precipitation, the solution still contains residual Hg and must be treated before being disposed. Unfortunately, this never happens. In addition, mercuric nitrate fumes are highly toxic. Human beings have a tolerance of only 0.05 mg per m^3 of air for the prevailing compound in the process, mercury pernitrate - $Hg(NO_2)_2.H_2O$. A serious risk is also presented when mercury pernitrate contacts alcohol, resulting in the formation of fulminate ($Hg(CNO)_2$). This compound explodes readily when dry and is used in blasting caps and detonators. Currently, miners in some parts of the world, such as Colombia are not precipitating mercury from nitrate solution. They simply discharge all mercuric (Hg(II)) solution into creeks. This form of Hg is readily available to be biotically or abiotically methylated (Veiga, 1997).

Melting Gold

When the amalgam is retorted, a gold *doré* is obtained. This is then melted to rid the *doré* of some impurities. Melting occurs in village gold shops, jewelries, at home or at the mine site. In fact, the *doré* still contains about 20 g of Hg per kg of gold, which is released when gold is melted (CETEM, 1989). The retorted gold obtained in bonfires, as usual in African countries, can contain up to 20% Hg. At gold shops, the melting operation is usually carried out by gold buyers under the miner's supervision; Hg levels in the interior of these shops are very high. Malm (1991) measured a mean concentration of 83 $\mu g\ Hg/m^3$ over 2 hours when gold was not being melted. Fume hoods used to extract vapors are usually rudimentary, consisting only of a fan, which blows the Hg vapors out into the urban atmosphere. Exposure of innocent people living near gold shops to Hg vapour creates an extremely serious hazard. The video documentary "The Price of Gold" produced by the BBC in 1993 profiled the case of severe mercurialism in a 60 year-old citizen caused by

vapors emitted from a gold shop in the Amazon over a period of 10 years. This individual suffered from extreme muscle tremors and his neurological functions were dramatically reduced.

A local gold dealer in the town of Kereng-Pangi, Kalimantan, Indonesia buys about 3 kg of gold daily from about 10,000 ASM who extract gold from dry alluvial ore. When he melts the gold in the shop, Hg contaminated vapours go straight to a nearby elementary school. Assuming that the gold bullion contains 5% residual Hg, this shop alone releases approximately 55 kg of Hg annually. This is the same amount of Hg released annually by waste treatment plants all over Austria (Beinhoff, 2003).

The Hg_{lost}:$Au_{produced}$ ratio

In general, the amalgamation method defines the amount of Hg lost. When gravity concentrates are amalgamated properly and retorts are used, Hg losses are very low (Table 1.3).

Table 1.3. Mercury losses according to amalgamation method

Amalgamation Method	Hg_{lost} : $Au_{produced}$
Whole ore	>1
Concentrates, no retort	1
Concentrates, with retort	0.001

The ratio of Hg_{lost}:$Au_{produced}$ has been used as a parameter to quantify Hg releases. One of the common and confusing issues in reporting this ratio is that some authors report just the Hg_{used}:$Au_{produced}$ ratio, which does not necessarily reflect the amount of Hg lost. For example, Dreschler (2001) estimated the amount of Hg used by ASM to process one gram of gold in Manica, Mozambique. He reported that 53% of the miners use less than 1 g Hg/g of Au and 34% use from 1 to 5 g of Hg. In this case, he mentioned that 50% of the Hg used is recycled. In other cases, authors report the amount of Hg used, but not the amount of Hg recycled. Babut *et al* (2003) reported that the official mineral agency in Ghana has estimated that the Hg:Au ratio is around 4. This seems a very high ratio to represent the proportion of Hg lost when only gravity concentrates are amalgamated. It is unclear if this means Hg_{used} or Hg_{lost}. Maurice-Bourgoin *et al* (2000), assuming an Hg:Au of 5, estimated that 330 tonnes of Hg have been released into the Bolivian environment by ASM. Again, this is unclear. Farid *et al*. (1991) quantified the use of Hg in eight artisanal gold processing plants in Poconé, South of the Brazilian Amazon basin. Miners use either manual amalgamation or barrels to amalgamate gravity concentrates obtained from centrifuges and add 1 kg of Hg per 60 to 100 kg of concentrates. Figure 1.1 shows a simplified Hg-balance of these ASM operations. When retorts are not used, the Hg lost during the amalgam decomposition can be as high as 45% of the Hg used in the process. Significant Hg losses can also occur when retorts are used incorrectly or when miners open retorts while they are still warm, discontinuing the Hg condensing process. The main amount of Hg recovered in the process is when excess Hg is squeezed off with a piece of cloth. This Hg is recycled. The ratio of Hg_{lost}:$Au_{produced}$ varied from one operation to another and, when very little gold was produced, the ratio provided a false idea that a high amount of Hg was lost. It is clear that this ratio must be used regionally as a result of (monthly) production of several operations. Those processing plants that recover no or little gold should not be considered.

In all El Callao, Venezuela, miners excavate the ore using explosives. They then transport it in small trucks to the Processing Centers ("molinos"), each with 3 to 6 hammer mills. The miners pay 10% of the recovered gold to the "molinero" (Center owner). The material is crushed in jaw crushers and wet-ground below 1 mm in 25 HP hammer mills. The whole ore is amalgamated in copper plates. The hammer mills have a grinding capacity of 0.2 to 0.4 tonnes/hours, depending on the ore hardness. The hammers are changed according to the ore hardness. For soft material, a pair of hammers made of cast-iron that cost US$ 8 to 10 must be changed after grinding 10 tonnes of ore. For hard ores, the hammers are changed after grinding 1.5 to 2 tonnes of ore. This represents a major cost of the milling operation (as high as 65% of the operating costs); however, millers do not charge more for milling hard ores. The gold production in the region can reach 5 to 6 tonnes/a for all 80 to 90 Processing Centers. It was also observed that a Processing Center typically buys (and loses) 6 to 8 kg of Hg/month. Mercury is sold in the area at a price around US$ 20-25/kg. Millers provide mercury for the miners who add it to the plates during the operation. The ratio Hg_{lost}: $Au_{produced}$ is between 1.5 and 3. All over the entire El Callao region, Hg emission might be around 12 tonnes/a. Signs of serious intoxication and neurological damages were detected in a large majority of people who are directly involved in the amalgamation process, as well as in innocent people living near the Processing Centers (UNIDO, 2004).

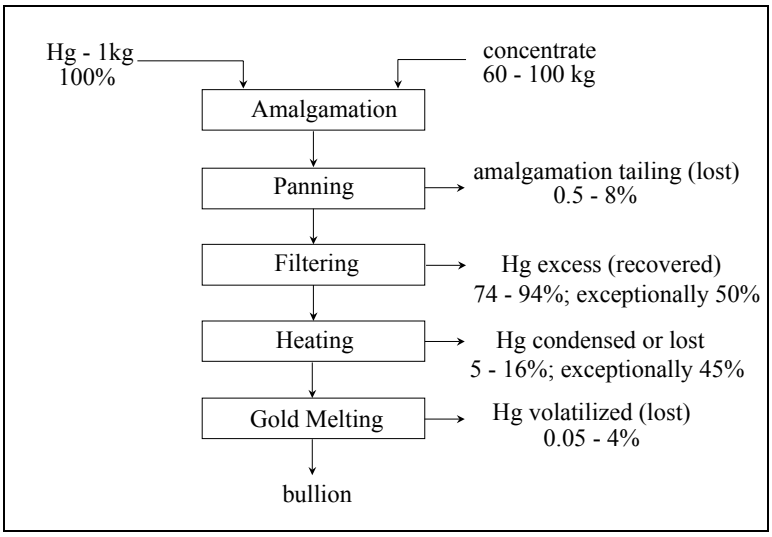


Fig. 1.1. Balance of Hg in the amalgamation steps
(adapted from Farid *et al*, 1991)

It is difficult to determine the amount of gold produced by artisanal miners and more difficult to determine the amount of Hg lost. For example, gold has been mined in Tanzania for more than 100 years. The gold production from ASM in Tanzania is not well established but Ikingura (1998) indicated that it might be around 4 tonnes Au/a. It is also difficult to estimate the exact number of people involved directly in gold mining activities in Tanzania, as miners migrate from one site to find easily exploitable gold. In 1999, van Straaten (2000a) estimated that there were 200,000-300,000 ASM in Tanzania, while D'Souza and Cliffe (2003) placed the number at 550,000. With this contingent of miners, it seems that 4 tonnes/a of gold is a very low estimate for gold production. A similar situation is found in Zimbabwe where it has been estimated that there are more than 200,000 ASM producing 5 tonnes Au/a (van Straaten, 2000a). Maponga and Ngorima (2003) estimated that ASM in Zimbabwe used 6 tonnes of Hg, of which about 3 tonnes were lost to the environment. However, when we interviewed one major mercury importer in Kadoma, Zimbabwe, he declared that he has been importing 20 tonnes of mercury/a, mainly from Netherlands, for "industrial and dental use". It seems reasonable to assume that more than 90% of this Hg is being diverted to artisanal gold mining operations. If this number is correct, and considering the ratio Hg_{lost}:$Au_{produced}$ = 1 to 2 as observed in the field, then gold production from ASM in Zimbabwe must be around 10 to 20 tonnes/a (Shoko and Veiga, 2003).

The numbers obtained from official sources are usually unreliable as gold production from ASM fluctuates substantially or miners do not declare actual production. For example, in 1991, when the Tanzanian Government was buying gold through the National Bank, the reported production was around 617 kg Au. This dropped to approximately 2 kg/a from 1993 to 1996 when the bank stopped buying gold. When a private company introduced a custom milling operation in 1997 and started buying gold in the region, the official gold production increased from 2 kg in 1996 to 420 kg in 1998. Recently, the company ended its activities and the reported gold production has declined again to 153 kg in 2001 and 14.1 kg in 2002. Currently, there are a number of individual gold buyers in the region and they do not report the exact amount of gold purchased from miners. As a result, the Government is not collecting adequate taxes and most gold is probably smuggled out of the country (Veiga, 2004).

At the Global Mercury Project site in Rwamagasa, Tanzania, just one concession has been producing constantly. Processing 10.5 tonnes of ore/week, they produce 3.6 kg Au/month. At this level of gold production, they buy 3 kg of Hg per month, which derives an Hg_{lost}:$Au_{produced}$ ratio of 0.8. Most operations in the Geita District use a similar process. Material extracted by miners is transported in 40-kg bags to the mills, where individuals are hired to process the ore. The cost for each individual ranges from US$ 0.012/kg for hand crushing and sluicing to US$ 0.10/kg for amalgamation. All grinding is conducted in dry ball mills. The dust comes out in pulses and it is stacked underneath the mill. The operators and all people around the mill inhale an incredible amount of dust. It is not clear why the miners use dry grinding, as water scarcity does not seem to be a problem in some places. It seems that dry grinding is a result of a work division scheme. Women carry ground ore to sluice boxes lined with sisal cloth that is set up at the riverbanks. After processing all bags, the sisal clothes are washed in metallic trays and mercury is added for manual amalgamation (Tesha, 2003). Individuals manually press mercury for at least 2 hours on the gravity concentrate (80% solids). Amalgam is then burned in bonfires. It is difficult to obtain an accurate Hg_{lost}:$Au_{produced}$ ratio of the region for a number of reasons: many operations are no longer producing; the gold production fluctuates considerably as a function of the ore grade; and/or production volumes have not been reported properly. Based on similar operations in Brazil, the Hg_{lost}:$Au_{produced}$ ratio is usually 1, when just concentrates are amalgamated carefully. It has been estimated by Appleton

et al (2004) that, on average, around 30 kg of Hg is released annually into the environment from Rwamagasa alone. The price of one kg of Hg in Rwamagasa is Tzsh 20,000 (US$20), which is five times the international mercury price.

In Brazil, two ASM sites have been assessed within the Global Mercury Project: São Chico and Crepurizinho, both in the Tapajós Region. In the Amazon, ASM activities are spread across an area of 236,000 km², corresponding to 4.34 % the total Brazilian Amazon area. In the Sate of Pará alone, the area reaches 150,000 km² making Tapajós the single largest artisanal gold mining area in the world (100,000 km²). Tapajós' historical official reported gold production is 180.6 tonnes. However, the estimated actual production since 1958 is estimated at 650 tonnes of gold (Villas Boas, 2003; Veiga *et al*, 2002; Silva, 2001). São Chico, a mining site that from 1999 to 2001 had 5,000 workers, now has less than 60 miners using hammer mills and copper amalgamating plates. Since 1963, about 3 tonnes of gold were produced and about 7.5 tonnes of Hg were released to the environment. Currently, with the use of Cu-plates, the Hg_{lost}:$Au_{produced}$ ratio is around 2.5. The introduction of cyanidation of tailings in São Chico is increasing Hg mobility and bioavailability (CETEM, 2003). Crepurizinho had its highest gold production between 1983 and 1990 with 4 to 5 tonnes/a when approximately 10,000 people were involved (Mathis, 2003). Currently, the site is producing 0.6 tonnes/a of gold using centrifuges that are copies of the Knelson[3] concentrator. The amalgamation of the gravity concentrates reduces the Hg losses. As miners do not use retorts, the Hg_{lost}:$Au_{produced}$ ratio is around 1 to 1.5 (Villas Boas, 2003).

Table 1.4. Estimated Mercury losses in the GMP sites

GMP Site	Hg_{lost}:$Au_{produced}$	Hg lost (tonnes/a)	Hg price (US$/kg)
Brazil (São Chico)	1.5 - 3	0.03 to 0.04	15 - 30
Brazil (Crepurizinho)	1 - 1.5	0.3 to 0.5	15 - 30
Indonesia (Galangan)	1 - 2.5	0.3 to 0.5	9 - 12
Indonesia (Talawaan)	60 - 90	20 to 30	10 - 15
Lao (Luang Prabang)	0.3 - 0.5	0.001 to 0.002	75 - 88
Sudan (Blue Nile)	1 - 1.5	0.3 to 0.4	25 - 30
Tanzania (Rwamagasa)	1 - 1.5	0.03 to 0.06	18 - 25
Zimbabwe (Kadoma)	1 - 3	3 to 5	12 - 25(*)

Note: (*) = price depends on the amount bought.

The Hg_{lost}:$Au_{produced}$ ratio and the amount of Hg lost/a in the mining sites studied within the Global Mercury Project (GMP) were estimated based on field observations and reports from the local project assistants and sub-contractors (Table 1.4).

Artisanal miners are certainly not exemplary taxpayers. The frequent border crossing motivated by better gold prices has contributed to the emergence of a black market within many Latin American and African countries. Unfortunately, in most cases, the "official" estimates of gold production are the only data available. Based on official gold production and assuming a ratio of Hg_{lost}:$Au_{produced}$ = 1, it is estimated that 5,000 tonnes of Hg were emitted to the Latin American environment by artisanal miners over the last 2 decades (Veiga, 1997). Based on a similar ratio, Lacerda and Marins (1997) estimated that the annual (1996) Hg emission from ASM activities in Brazil alone was around 78 tonnes. Using published estimates, Lacerda (1997) estimated that the amount of Hg emitted by ASM worldwide was around 460 tonnes in 1996 alone.

Other Important Aspects

When gathering information about ASM operations, it is important to understand **the labor relationship, the economics of the gold production** and **the technical capabilities of miners**. In order to introduce changes in the mining and processing procedures, the way in which miners think must also be understood. The working relationship can also influence the concern of miners towards Hg discharges. For example, in many parts of the world the "image" of the operation owner is well known. A specific mining activity requires investment for sluice boxes, pumps, camp, transport, etc. After deducting operating costs, the owner splits the profits of the gold extraction operation with his/her employees. This can range from 10 to 50% depending on the capital cost and degree of risk that the employees are willing to assume. If the employees that actually handle Hg are unhappy with their "boss" or if they are not local dwellers, they might have less concern about the fate of the mercury in the environment.

The **economic aspects** of the gold operation are also relevant in understanding why a miner is not recycling Hg. In this case, it is useful to evaluate the capital and operating costs of a mining/processing operation. When the price of Hg is low, miners do not attempt to reduce Hg losses. For example in Kereng Pangi, Indonesia, an alluvial mining operation using hydraulic monitors, miners use an excessive amount of Hg to amalgamate concentrates. To produce 1 kg Au per month, a miner loses 2.4 kg of Hg. At 2.5 times higher than the international price, mercury represents less than 1% of

[3] Knelson Concentrators are centrifuges with capacities ranging from lab scale to 100 tonnes/h of ore made in Langley, BC, Canada.

the miner's operating cost. Diesel for the pumps represents more than 90%. However, in El Callao, Venezuela, at a price of US$ 20-25/kg (5 to 6 times the international Hg price) Hg represents 14% to 33% of the operating cost of a Processing Center. The total operating cost ranges from 0.43 gAu/tonne of soft ore processed to 0.97 gAu/tonne of hard ore being processed. Hammers represent 65% of the operating cost when grinding hard rocks (Veiga and Gunson, 2004).

The **miners' skill** is another point to be considered. Sociological surveys usually have questions to evaluate the educational level, as well as skills of the miners. The acceptability of new ideas and techniques depends a great deal on this. When miners move from secondary to primary ore deposits, more sophisticated procedures are needed. Currently in Latin America, a significant number of miners are working with primary gold ores, which introduce additional problems to the operations. When artisanal miners start working with primary ores, (e.g. sulfide associated gold, often found at depth), they require substantial investment and greater technical capabilities. In places where sulfide-rich quartz veins are mined, such as Brazil, Peru, China, Sudan, Zimbabwe and Venezuela, the primitive underground work by ASM represents the main cause of fatal accidents in the mining sector. Silicosis is also a major killer. Primary ores must be ground to promote gold particle liberation from gangue minerals. Free or partially free gold particles can be concentrated. This is a fundamental principle of mineral processing; unfortunately, ASM do not know how finely they should grind the ore. They also do not know that gold can be locked in the sulfide particles and is extremely difficult to extract, even with lixiviants, without previous oxidizing processes. By examining tailings from ASM operations in quartz veins in Poconé, Brazil, it was possible to observe a large portion of gold still bound to quartz particles in the coarse fraction, with other portions lost in the very fine fraction. As a result, rudimentary gravity concentration methods are not able to recover fine gold particles (CETEM, 1989) and amalgamation cannot trap non-liberated gold particles. In the Philippines, it is known that artisanal miners leave up to 30 ppm of gold in the tailings (Murao *et al*, 2002). Amalgamation tailings in Venezuela contain more than 65 ppm of gold (UNIDO, 1996).

Although crushing and grinding (comminution) are relatively simple mechanical processes, they are also the most expensive unit operations in mineral processing. In ASM operations, comminution processes are severely limited by the availability of resources such as electric generators, fuel, steel rods or balls and drums, as well as spare parts and technical skills. Inevitably, when ASM work with primary ores, the production level is reduced because of heterogeneity of the ore body, difficulty with excavation and hauling and lack of gold liberation due to poor comminution processes. When this occurs, miners use greater quantities of Hg. This does not achieve the desired purpose, as gold is locked either in silicates or in sulfides, from which it is more difficult to extract. Facing these problems, ASM seek technical assistance. Normally, this support is not available. Engineering companies usually refuse to help ASM and hiring consultants is too costly. Local governments are not prepared to provide specialized personnel or appropriate technology, and research institutions offer only high-tech methods (Veiga and Hinton, 2002). As a result, when superficial deposits are depleted, miners migrate to other regions and often cross borders into neighboring countries, sometimes creating international conflicts (Veiga, 1997).

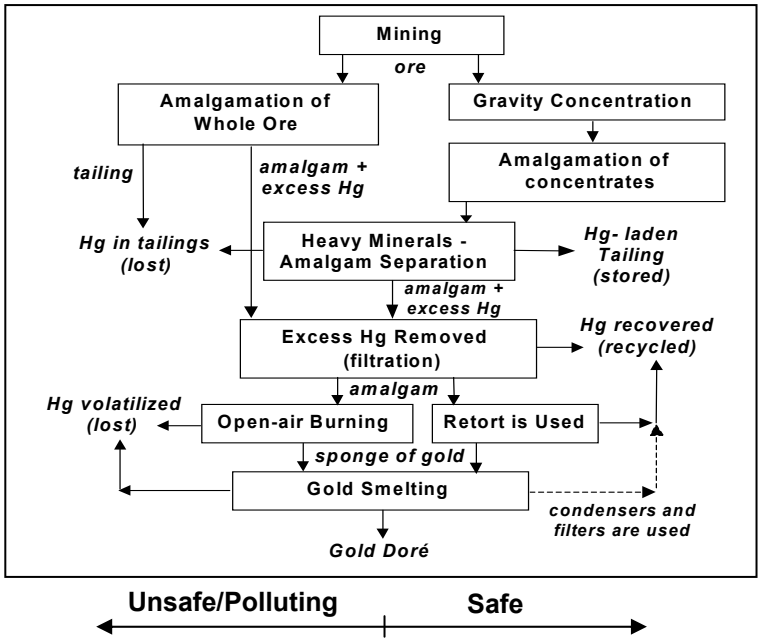


Fig. 1.2. Main steps in amalgamation by ASM (Veiga and Hinton, 2002).

In summary, Hg released by miners includes both the fraction lost to the atmosphere when the amalgam is inappropriately burned and the portion discharged with amalgamation tailings into aquatic environments (Fig. 1.2). When no retort is used, as much as 80% of the Hg initially introduced during amalgamation is lost to the atmosphere (CETEM, 1989). Additionally, the amount of Hg dumped with tailings is more significant when the whole ore is amalgamated. Amalgamation of the whole ore represents the main source of Hg losses. This can constitute as much as 50 parts of Hg lost for each part of gold produced. This should be the primary focus for attention of anyone interested in reducing Hg releases in ASM operations.

1.1.2. Evaluating Hg Releases

Monitoring programs based on soils or sediment analyses that estimate the quantity of Hg emitted from ASM are extremely costly and are **unlikely to yield reliable results**. Quantitative evaluations of Hg releases are more accurate when based on reliable surveys at gold processing plants.

It is important to use the Hg_{lost}:$Au_{produced}$ ratio carefully as an approximate and regional estimate of Hg release from various operations in an ASM region. It is advisable to use monthly or annual averages of gold production. A trustworthy relationship with miners is absolutely necessary to permit the researcher to have access to their mining and processing plants, so that s/he can obtain reliable figures regarding the amount of Hg lost and gold produced. It is natural that miners become suspicious when strangers are "inspecting" their activities. This is a time-consuming process, as a detailed survey about the amount of Hg entering and leaving each unit operation involves carefully weighing and analyzing Hg in products, such as recovered Hg, gold *doré* and amalgamation tailings. This can provide some indirect information about the data provided by miners on the amount of Hg they buy each month.

Sampling ASM Operations

In **active** operations, an interview with miners can result in an estimate of the quantity of mercury that is lost. The following hints are suggested to obtain the Hg_{lost}:$Au_{produced}$ ratio:

- Interview operation **owners**, who are in charge of supplying (acquiring) Hg as well other consumables.
- Obtain costs and amounts of all consumables such as diesel, carpet, soap, mercury, etc.; be sure to get the amount of Hg being **purchased** monthly or weekly.
- Interview as many owners (and miners) as possible and check for inconsistencies in data.
- Verify that the miner is providing correct information about the amount (and cost) of consumables per month, per unit or per group of unit. Similar information must be gathered when obtaining information about gold production.
- Obtain numbers of gold production in dry and rainy seasons.
- Obtain average estimates of gold production (miners exaggerate, giving production estimates only during "good days").
- If possible, ask permission to assess the processing operation and weigh all Hg being introduced and recovered.
- Sample amalgamation tailings and analyze Hg. Knowing the weight of amalgamation tailings being produced per month and Hg concentration, it is possible to calculate the Hg lost when tailings are discharged.
- If retorts are not used, weigh amalgam before burning and *doré*, after burning; melt the *doré*, if possible
- If retorts are used, weigh the amalgam and the mercury recovered, both before and after retorting. This can give some idea about the residual Hg in the *doré.*
- Check to see if the Hg balance through sampling is consistent with the data on Hg being provided by the miners.
- Repeat this procedure at as many mining operations as possible, to obtain average amounts of Au produced and Hg lost per month in a mining region.

Once the source of Hg release is characterized and the amount of Hg lost is quantified using historical gold production data, the fieldwork should concentrate on:

- Identifying "hotspots".
- Establishing the Hg pollution level of a region that is affected by artisanal gold mining.
- Assessing the risk of exposure to mercury for workers and the surrounding population.

1.2. Characterizing Contamination and Pollution

Before establishing levels of Hg in biological and geochemical materials, it is important to understand the difference between contamination and pollution, as this is useful to put into perspective the sampling procedure of environmental assessment work. According to Manahan (1994) a:

Pollutant *is a substance present in greater than natural concentration as a result of human activity and having a net detrimental effect upon its environment or upon something of value in that environment.*

Contaminants*, which are not classified as pollutants unless they have some detrimental effect, cause deviation from the normal composition of an environment.*

In other words, pollution implies a toxic situation in which bioaccumulation is proven. Soil, sediments, water and air analyses can characterize a contamination source, i.e. concentrations found in samples that are above the "natural" or "normal" background levels. This can also characterize a situation of risk if the receptors (biota) are in contact with the contaminated medium (stressor). Risk assessment is often used as the assessment of the probability of exposure (Fig. 1.3). A risk assessment can predict if Hg in any geochemical compartment is or can become bioavailable. In this case, regardless of geochemical material sampled, it is important to remember that **biota are the ultimate indicators, providing direct evidence that mercury in soil, sediments, water, or air has become bioavailable** and is being accumulated by organisms.

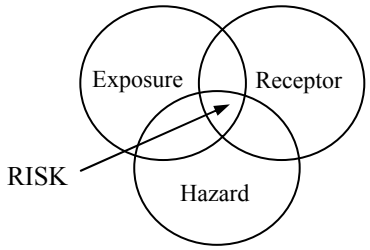


Fig. 1.3. Risk assessment

Risk is a result of the probability (or possibility) that a receptor (organism) has to be exposed to a hazardous substance

Evidence of bioaccumulation must be obtained or predicted to evaluate the appropriate course of action. If impacts to biota are not proven in a contaminated site, containment and long-term management are more appropriate than other aggressive remediation measures. This, of course, is based on the acceptability to regulators. If bioaccumulation is occurring, then remediation should be implemented.

As seen in Fig. 1.3, the **risk of exposure** is an important factor to be considered when selecting an area for the Environmental and Health Assessment (E&HA). The priorities for the E&HA must be established based on a risk assessment approach. In many cases, the selection of an area for monitoring does not involve a sophisticated process. A brief field visit to observe effects can be effective. Usually the main points to be evaluated in a preliminary field trip are:

- how many miners are there?
- how is (or was) Hg used and released?
- how much gold was produced in the area and how much Hg was released?
- what are the important environmental features (e.g. organic matter, pH, conductivity, etc.)?
- what are the possibilities of Hg mobility?
- are there resident biota?
- what are the risks?

There are many methodologies for sampling geochemical and biological materials for Environmental and Health Assessment. Depending on the purpose of the monitoring, one procedure may be more practical than another. Table 1.5 outlines the main purposes of Environmental and Health Assessments in ASM areas and the relevance of those objectives for the GEF/UNDP/UNIDO Global Mercury Project (GMP).

Table 1.5. Purpose of sampling in ASM areas

Subject	Purpose	Material to be sampled	Technique	Relevance for the GMP
Estimate Hg releases from ASM	estimate the relevance of Hg released by ASM in a region.	Hg introduced and recovered; Hg lost in amalgam, tailings, etc.	Hg_{lost}:$Au_{produced}$ ratio estimation through Hg balance in ASM operations	**high**: estimation of Hg lost is crucial
Identification of mining & environmental. hotspots	apply remedial procedures depending on bioavailability.	superficial soil and bottom sediments	Hg analysis (semi or quantitative) or panning for visual inspection	**high:** important for remedial measures
Hg methylation potential	acquire knowledge of kinetics of MeHg generation.	soil or bottom sediments	radiometric methylation rate or microbiological studies	**low**: complex and expensive
Hg bearing minerals	determine stability of Hg in sediments; bioavailability.	soil or bottom sediments	sequential or selective extraction or mineralogical studies	**low/medium**: explains why Hg is or not bioavailable
Hg leaching potential	determine ease with which Hg can be leached out from soil/sediments.	soil or bottom sediments	leaching tests; humidity cells	**low/medium**: indirect information about bioavailability
Hg size distribution	track transportation of fines with Hg.	soil or sediments	screening and centrifuging for -37 or -2µm fractions	**high**: predict transport of Hg associated to fines
Atmospheric Hg deposition over time	determine Hg accumulation on soils and sediments	lake core sediments	analysis of core slices and dating	**low**: indirect data about Hg emission; some confounders
Atmospheric Hg dispersion in a region	determine extent of contamination.	superficial soil analysis	total Hg analysis (semi or quantitative)	**low/medium**: hard to provide reliable quantitative data
Atmospheric Hg in workplace or public environment	determine exposure of public or workers to Hg vapor.	ambient air, ash, soot, house dust and tar in smelting huts	air analysis over many hours or with Hg sniffers; Hg analysis of dust	**medium/high**: direct data about Hg vapour exposure
Hg mobility in aquatic system	determine extent of contamination.	particulate matter	collect water and filter -0.45µm	**high**: main Hg mobility mechanism in drainages
Drinking water	determine quality to meet guidelines; resolve health issues.	filtered and unfiltered water	total Hg analysis	**low**: Hg is rarely detected; Hg in particulate >> Hg in water
Protection of aquatic life	determine contamination to meet guidelines.	filtered and unfiltered water	total Hg analysis	**low**: Hg is rarely detected; Hg in particulate >> Hg in water
Taxonomic Richness and Abundance	study the toxic effect on type and number of organisms.	invertebrates (or other aquatic organisms)	number and type of organisms	**low:** laborious and complex in tropical countries
Hg in aquatic biota and/or toxicity	obtain evidence of bioavailability.	fish (preferentially carnivorous)	total Hg and/or MeHg; standardized fish bioassays can be analyzed over time	**high**: bioavailability control
Hg in invertebrates and/or toxicity	obtain evidence of bioavailability.	invertebrates (e.g. worms, snails, etc.)	total Hg and/or MeHg; bioassays	**high**: simple to compare bioavailability. from site to site.
Hg in edible biota	mitigate health issues; meet guidelines.	edible biota (fish, etc.)	total Hg and/or MeHg	**high**: part of Health Assessment
Hg in edible vegetables and fruits	mitigate health issues; meet guidelines.	edible vegetables and fruits	total Hg and/or MeHg	**low**: usually low MeHg
Hg in hair	assess MeHg bioavailability; mitigate health issue.	human hair	total Hg or Hg in creatinine	**high**: MeHg exposure through fish ingestion
Hg in urine	determine undue short-term exposure to Hg vapours.	human urine	total Hg in urine and creatinine in urine	**high**: determine Hg vapour exposure
Hg in blood	determine long-term exposure to vapour or MeHg intake.	human blood	total Hg	**medium/high**: accumulates data on MeHg + Hg vapour exposure

1.3. Soils and Sediments

Depending on the Hg source and how it was released into the environment, mercury may be dispersed over extensive areas (km²) or concentrated in "hotspots" (100 m²). Once an initial survey of the amount of Hg used by miners has been conducted discrete sites should be sampled to verify the regional extent of the contamination or to locate hotspots.

Mercury methylation is the transformation of inorganic mercury (mercuric species) into the most toxic forms of mercury: monomethylmercury (CH_3Hg) and dimethylmercury ($(CH_3)_2Hg$)[4]. Methylation is related to the Hg(II) activity. The presence of hydrosulphide species in solution (H_2S or HS), even at very low concentrations, can precipitate HgS, reducing Hg availability to the methylating agents (Björnberg *et al*, 1988). Methylmercury (MeHg) poisoning was first identified in the early 1950s at Minamata Bay, Japan, when it was discovered that a plastic factory was discharging MeHg into the river and bay (see chapter 2).

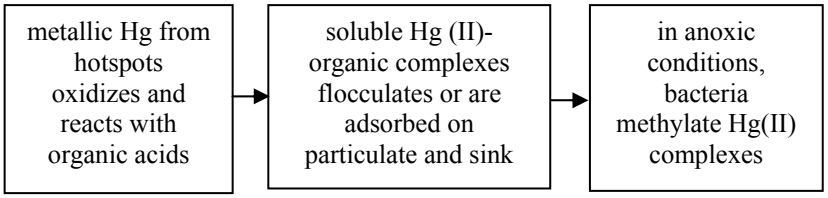
In 1967, a group of Swedish scientists proved that microbes living in bottom sediments could transform some inorganic species of mercury into methyl forms. Later on, knowledge about methylation processes increased, but some key steps about the chemical and biological mechanisms are still not well understood. Jensen and Jernelov (1969) provided the first indication of the biological formation of MeHg when they spiked sediments with $HgCl_2$. Cobalt is the active part of the methylcobalamin molecule to which the methyl group is attached. In the presence of Hg(II), cobalt is reduced to the 2+ oxidation state and methylation of mercury occurs. Any microorganism capable of synthesizing methylcobalamin is a potential MeHg producer (Gavis and Ferguson, 1972; Wood,1971). MeHg is produced by bacteria-mediated processes in aerobic and anaerobic environments. Because these pathways are shared by a large number of bacteria species, the capacity to methylate is not restricted to one or a few types of microorganisms but seems to be a widespread process associated with many bacteria (Hecky *et al*, 1987). MeHg production is significantly higher in anoxic than in aerobic environments (Porvari and Verta, 1995). MeHg concentration in soil and sediment occurs as a result of the balance between MeHg production and degradation of methylmercury into metallic Hg (i.e. demethylation). It has been recognized that methylmercury is produced mainly in sediments by methylating bacteria. It is then either released into the water column, where it is rapidly accumulated by biota (Jensen and Jenelov, 1969; D'Itri, 1972), or incorporated by benthic invertebrates at the base of the food web.

The availability of mercury species to aquatic organisms can be controlled by soil or sediment adsorption or precipitation mechanisms where hydrous Fe/Mn oxides and sulphides play a major role. However, MeHg does not bind as tightly with organic matter in sediments as do inorganic Hg compounds. Consequently, Me-Hg readily remobilizes from the stable and less reactive sediments into the overlying water. The rate of Me-Hg remobilization influences bioaccumulation in aquatic organisms, although the amount of Me-Hg can be small (<1%) relative to the total mercury concentration in the sediments (D'Itri, 1990).

Because Me-Hg is assimilated rapidly and is eliminated slowly, its synthesis in sediments does not have to be rapid to promote bioaccumulation.

Whatever the route of bioaccumulation, uptake of MeHg is much more efficient than inorganic Hg. The balance between Hg accumulation and excretion depends on the type of organism. Most Hg found in fish is in methylated form so it is easily transferred to man since the intestinal absorption of MeHg is extremely high.

Despite the uncertainties related to the transformation of metallic mercury into MeHg in ASM regions, a simplified sequence of reactions is suggested:



Not all sites with high Hg concentrations may be equally vulnerable to formation of MeHg via natural processes, but these sites are of **high risk**. Some soils and sediments have higher methylation potential than others. This depends on a large number of factors such as type of organic matter, type of bacteria, pH, dissolved oxygen, presence of sulfate, etc. Metallic mercury must first be oxidized and form soluble mercuric complexes before it is available to methylating bacteria.

As Hg in gold mining activities is released into the environment through amalgamation tailings and by amalgam burning, two different behaviours are predicted. With amalgamation tailings, the formation of **hotspots** is typical with low dispersion. The relatively low mobility of metallic Hg in natural watercourses creates points with high Hg concentrations. When Hg is emitted to the atmosphere, a large portion is precipitated nearby the source but a part is also **dispersed**. Whether Hg emitted by artisanal miners has significant global contribution is still controversial. However, Hg has obvious regional impact and this must be quantified.

[4] Dimethylmercury is one of the most potent neurotoxins known. In Aug 1996, Karen Wetterhahn, professor of the Dartmouth College, New Hampshire, spilled some drops of dimethylmencury in her latex gloves. In Jan. 1997, she had in the blood 4,000 µg/L of Hg and she died on June 8, 1997 (Cotton, 2003).

At this point, it is important to distinguish between "**mining hotspots**" and "**environmental hotspots**". A mining hotspot is characterized by relatively high concentrations of inorganic mercury (relative to background) in soils or sediments (i.e. up to 100x), indicating extensive use of **metallic mercury** for gold extraction. An **environmental hotspot** may be situated away from mining hotspots, in several locations, and is characterized by high organic Hg (e.g. MeHg) concentrations in sediments and/or aquatic biota in areas where inorganic Hg has been or has high likelihood of being methylated, and is bioavailable. Normally, MeHg concentrations in soils tend to be around 1% of total Hg, but in mining hotspots, this is not always true, as a large amount of metallic Hg is present.

Priorities must be established when searching for hotspots. For example, an environmental hotspot near a populated area or a water stream might be much more serious than an environmental hotspot that is isolated and not in contact with the drainage. The following factors must be considered when a hotspot is identified:

- Size of the hotspot
- Chemical and biological characteristics of the hotspot
- Contact with drainages
- Possibility of mercury mobility (e.g. with fine suspended sediments)
- Possibility of Hg being oxidized and reacting with organic matter
- Quality and quantity of biota being affected
- Risk of affecting biota and/or human beings

Soils and sediments are witnesses of a contamination process over the years but they **rarely provide quantitative data** on the absolute degree of contamination. Instead, soils and sediments can play a key role in ascertaining relative factor of heavy metals enrichment. As shown in Table 1.5, the use of soils and sediments in the environmental assessment may have distinct objectives, such as:

- **Identifying mining and environmental hotspots.**
- **Predicting and obtaining evidence concerning the transportation of Hg associated with fine particles to other areas.**
- Knowing how easily Hg can be leached out from soil and sediment components, as these also are indirect ways to determine bioavailability.
- Knowing how stable Hg is associated with soil and sediments components; this provides indirect hints about bioavailability.
- Obtaining information about atmospheric Hg dispersed over a region by analysis of superficial soils.
- Obtaining information on atmospheric Hg accumulation on soils and sediments, analyzing lake profile cores.
- Obtaining information about the kinetics of MeHg generation.

All these objectives are important and, in the majority of the cases, they are site-specific, i.e. depend on the type of environment. The list above is presented in an order of priority for the Global Mercury Project. The two first topics are of critical importance. In an active ASM operation, when miners dump Hg-contaminated amalgamation tailings into an aquatic environment, it is clear that this will form mining hotspots. The establishment of a sophisticated monitoring scheme is not necessary to prove this. A simple semi-quantitative analysis or even panning can, in many cases, identify sites with Hg levels. The main objective is to locate these hotspots to establish future remedial actions. Determining if the material from the hotspots can be transported to other sites, analysis of fines and knowledge of the hydrodynamics of the environment can provide hints about this process. In this case, analysis of screened fractions is recommended. Analysis of the particulate matter being transported by waters or found in depositional areas is the real evidence of mercury mobility.

1.3.1. Determining Soil and Sediment Background

In order to characterize anthropogenic mercury contamination, local background Hg concentrations (i.e. **reference conditions**) must be determined to provide a frame of reference for Hg contamination (even for mining and environmental hotspots). This is true, no matter what the objective of the soil and sediment monitoring program. It can be accomplished by locating areas upstream or upwind of mining sites, which are uncontaminated by Hg and have similar geological or physical characteristics in soil and sediment (e.g. grain size, lithology, mineralogical components, organic content). The degree of contamination of mining or environmental hotspots is compared relative to background soil and sediment.

Background Hg concentration in soil, sediments and rocks can be divided according to: a) natural, pre-industrial conditions and b) current conditions that reflect anthropogenic additions of Hg. Mercury background levels in surface soils and sediments have increased, coincident with global industrial activity (Lindqvist *et al*, 1984). Jonasson and Boyle (1979) showed a wide range of Hg concentration in igneous rocks, with an average Hg concentration of 0.028 ppm in basic and 0.062 ppm in acid rocks. The same authors showed a wide range of Hg concentration in sediments

ranging from 0.010 to 3.0 ppm. Andren and Nriagu (1979) suggest an average Hg concentration of 0.071 ppm for soils. Taylor (1964) reported a mean concentration of 0.080 ppm as the earth's crust background. Most of the artisanal-small-scale mining (ASM) areas are located in tropical regions of the globe, typically characterized by intense laterization. In lateritic soils and bottom sediments, Hg values range from 0.1 to 0.3 mg/kg (ppm) for the -200 mesh (<0.074 mm) fraction of these iron oxide-rich materials (CETEM, 1989; CETEM, 1992). Lacerda *et al* (1990) found Hg concentrations ranging from 0.05 to 1.2 ppm in bottom sediments of non-impacted Amazonian rivers for size fractions <0.063 mm. Greater values are related to higher organic content of the sediment, whereas intermediate numbers were observed for sediments rich in hydrous ferric oxides.

Indices to quantify soil and sediment contamination have been proposed (Håkanson, 1980). The Index of Geoaccumulation (I_{geo}), first proposed by G. Müller and described by Förstner *et al* (1990) as a quantitative measure of metal pollution in aquatic sediments, uses the relationship between concentration (C) of the element in the sediment (fraction <2 mm) and the background in a fossil argillaceous sediment (B):

$$I_{geo} = \frac{\log_2 C}{1.5 \cdot B}$$

Rodrigues-Filho (1994) applied this index to evaluate the -200 mesh fraction of sediments from artisanal gold mining sites in Poconé and Alta Floresta, Brazil and used the Hg concentration of the 0.074mm (-200 mesh fraction) of non-impacted creek sediments as the background level. Most sediments in Poconé showed I_{geo} between 0 and 2. An average index of 5 was observed in turbid rivers of Alta Floresta, which mirrors the capacity of the fine (ferruginous) sediment to transport adsorbed Hg. It is well known that fine sediments have higher levels of mercury than the coarse fractions. For this reason, "pre-concentrating" the sample by eliminating the coarse grain size fraction (Laird and Dowdy, 1994) is recommended. This will also introduce consistency into determining Hg content of soil and sediment.

When establishing background or collecting contaminated samples, most researchers screen samples in the field to remove debris and coarse fragments of silicates. Japanese protocols suggest wet screening in the field using a 2 mm sieve (JPHA, 2001). Malm *et al* (1990) analyzed the –0.074mm fraction of soils and sediments, obtained by wet screening, from the Madeira River, an Amazonian river extremely impacted by mining activities. Ikingura *et al* (1997), investigating Hg concentrations in ASM areas in Tanzania, dried samples at room temperature before sieving in 0.2 mm (around 65 Tyler mesh) screen. In geochemical prospecting, the sampling procedure for soils and sediments involves drying, desegregation and sieving below 0.177 mm (80 mesh) to separate the size fraction for analysis (Fletcher, 1981). In British Columbia, Canada, regulatory authorities have advised that all sediment and soil should be sieved (-2mm) before metals analysis (BC WLAP, 2001).

When analyzing soil and sediment, it is important to report the D80 (screen size in which 80% material mass passed through) or provide other indications of sample grain size. When comparing Hg levels in sediments from different locations, it is common for authors to omit this information. Without knowledge of mineralogy, composition and grain size, it is pointless to compare Hg from uncontaminated sediments from Africa with those from the Amazon or Philippines.

Another interesting aspect of preparing geological samples is the drying procedure. Koksoy *et al* (1967), cited in Fletcher (1981), mentioned lithogenic Hg losses up to 42% when geological samples were dried at 80 °C and that samples with high Hg can contaminate samples with low Hg contents when they are stored together. Fletcher (1981) suggested that drying temperatures should not exceed 65 °C and that preferentially natural drying, (i.e. in the sun or under an improvised tent), should be used whenever the work is conducted in hot climates.

Sampling Soils and Sediments for Background Determination

Regardless of the means by which soil or sediments are collected to determine background Hg concentrations, the following information must be collected from both reference and Hg contaminated sites:

1. Geological characteristics (mineralogical components).
2. Grain size distribution (less than 2 mm).
3. Sample preparation (sieving, handling, etc.).
4. Drying procedures and methods.
5. Packing and preservation methods.
6. Quality assurance/quality control procedures used.

The reference site(s) must be free of any possible anthropogenic point source contamination. Once identified, undisturbed surface sediment and soil, representative of ambient conditions, must be collected. Physical characteristics and sampling procedures used in reference areas must match conditions and procedures in contaminated locations sampled (PSEP, 1997).

Prior to sampling soils and sediments, a plan should be prepared to define requirements and designate specific procedures (i.e. sample labeling, preservation, and custody for sample analysis). Samples should be identified with a unique, non-repeating sample number for each sample, the date and time that this sample was collected, the initials of the person responsible for filling out each sample label and preparation of samples for analysis, and the method for analysis on each bottle. Health and safety issues that might occur during the sampling procedures should be discussed.

Selection of equipment to sample soils and sediment is another important concern, as in some cases a washing step for equipment decontamination might be needed. The sampling technique could consist of a variation of techniques. Subsurface sampling could consist of collecting soils near the top 6 inches of the ground level. Surface soil sampling equipment could consist of a shovel to dig through the top layer of undergrowth, a small metal spatula and clean 50 mL HDPE containers with screw caps. After sampling, the caps should be secured with Teflon tape and the containers should then be double bagged in plastic zipper bags for shipment (Paul Randall, 2003 – USEPA, personal communication). It is also important to study how the samples will be transported and refrigerated.

Samples must be (wet) screened in the field to remove debris and gravel larger than approximately 2 mm. If this cannot be done in the field, it can be done later on by the laboratory. The use of composite samples is encouraged as a means of reducing spatial heterogeneity, as well as the reducing cost of sampling at a particular site. Composite samples of surface soil (i.e. upper 5-10 cm) should be collected randomly within a known area (e.g. 25m^2 plots) and composited into a large container; then mixed, sieved and sub-sampled for grain size and Hg analysis. Properly used, composite samples can provide a means of assessing quickly Hg heterogeneity within an area and if further sampling is required. Analysis of finer (screened) fractions (e.g. –200 mesh) can remove the dilution effect of quartz and increase homogeneity of the sample when droplets of mercury are present.

The number of soil samples and mass of sample needed to establish background levels depend on the size of the area, grain size and mineralogical variation. By nature, background conditions will be much less variable or heterogeneous than mining affected areas, therefore the sample size required to define reference conditions will be much less. Approximately five composite samples of 500 to 1000 g are typically needed to adequately define reference conditions. At least two reference areas should be sampled to determine regional variability.

When sampling soils or stream sediments in the field, , the following basic information about the site must be obtained:

- Date and time of sampling and location (GPS and map sheet if possible).
- Color, consistency, organic content and depth of composite soil samples collected.
- Location within the stream, such as near shore, near middle, etc.
- Water depth at which sediment is collected.
- Stream flow condition (preferably with water velocity (m/s) and flow rate (m^3/s)).
- Other observations such as presence of emergent or bottom vegetation, proximity to other streams, marshes, wetlands, water color, transparency, etc.

Physico-chemical parameters of the soil and sediment at reference sites should be measured. This provides useful information to compare with the contaminated sites. Variables such as sediment (soil) Eh[5], pH, conductivity (µS/cm), total organic carbon (TOC), sulfate, etc. provide data about the "original conditions" in which the Hg contaminated material was deposited. Reference areas can also provide relevant information about background MeHg concentrations in soil and sediment, as well as methylation potential (Silva, 1997).

Stream or lake sediments should be collected using a standard grab sampler, such as a petite or standard Ponar grab or a similar device for stream environments; and an Ekman or Ponar grab, or similar device for lake environments. A sediment-coring device can also be used to collect superficial (upper 10 cm) sediment samples.

When sampling within a stream environment, anchor the boat to ensure that the sampling device is hauled up and down perpendicular to the bottom. One or two reconnaissance samples should be acquired before actual sample collection to determine ease of sampling, likelihood of collecting sediment, sediment grain size and composition and to assess grab penetration. If conditions for sampling are not adequate, an alternate sediment sampling location should be sought.

Once a station has been selected and described according to the above criteria, the following procedures can be followed in the field to collect a representative grab sample of bottom sediments for chemical analysis:

- Determine water depth.
- Slowly lower the grab to the bottom (by hand or by winch) at speeds not exceeding 0.3 m/s so that a bow wave is not formed in front of the grab to minimize disturbance of fine surface sediment.

[5] Eh, the Standard Hydrogen Electrode (SHE) potential, is actually hard to measure in the field. The most common electrode for monitoring is Ag coated with AgCl. When a saturated KCl electrolyte is used, the relation between readings obtained with this type of electrode and the SHE is: Eh = EAg/AgCl + 0.199 (in volts)

- Raise the grab to the surface and examine the sediment for acceptability criteria. Only those grab samples that meet the following criteria should retained for analysis: do not contain large foreign objects (e.g. roots, branches, rocks); have adequate penetration depth (i.e. >10 cm); are not overfilled (sediment surface not touching the top of sampler); do not leak (overlying water is present and there are no visible leaks); and are undisturbed (sediment surface is relatively flat). Grabs that do not satisfy these conditions should be retained and discarded once sampling at the station has been completed.
- Remove overlying water from acceptable grabs by decanting or siphoning gently.
- Describe and record sediment characteristics including: color, odor, grain size and the presence of other materials (e.g. organic debris, hydrocarbons, vegetation, biota).
- Remove the upper 4 – 5 cm of sediment from the surface of acceptable grab samples with a pre-cleaned stainless steel spoon and place in a stainless steel bowl.
- Repeat the above process from at least three separate areas within each station so that a minimum of three grab samples are collected and placed in the same bowl to form a composite sample.
- Using the spoons, mix the sediment composite sample until it has uniform color and consistency.
- Composite samples must be wet-screened in the field or the laboratory; first to –2mm (to remove debris), then to –80 mesh (0.177 mm) or –100 mesh (150 mesh) or –200 mesh (0.074 mm). The choice of the screen opening must be based on the existing field facilities, but the 200 mesh screen is preferred. The same procedure should be used for samples collected in contaminated sites. Finer fractions are more homogenous and richer in Hg than the –2 mm fraction.
- Analyses must only be performed on screened samples ("fines"), but some –2mm and + 80 or 100 or 200 mesh samples must be analyzed to compare with the samples from contaminated sites.
- Use the pre-cleaned stainless steel utensil to completely fill (i.e. no head space) 250 mL glass or PVC sample jars. Seal jars immediately and place in a cooler with ice or ice packs if available. Keep jars as cool as possible while in the field, during storage and during transport to the laboratory. Polyethylene bags should be used only for dry samples.
- Label the jar and lid with indelible ink with a unique sample locator number. Record in a field notebook and on chain-of-custody (COC) forms.
- At the end of the day, crosscheck COC forms with labeled jars.
- Measure physico-chemical parameters in the field as practical, such as sediment (soil) Eh, pH and conductivity (µS/cm). Composite samples can be split for analysis of mercury, grain size and total organic carbon and other parameters that can provide information about the potential of a mining hotspot to become an environmental hotspot.

The following information must be collected from sediment samples:

- Total Hg concentration (ppm dw).
- Sediment grain size (% sand, silt, clay in dw).
- Total organic carbon content (% dw).

Resident biota (e.g., invertebrates or small fish) should also be collected to provide baseline information for comparisons with Hg concentrations in biota from environmental hotspots. See Section 1.5.3 Sampling of Invertebrates.

When a local laboratory is available, the samples can be wet screened and dried, preferentially at room temperature, or at temperatures below 60 °C. Dried samples can be packed in glass or plastic jars or plastic bags and kept in a cool environment until transportation to the analytical laboratory. Sediment samples must be properly packaged and labeled and, if possible, placed on ice for transport to the laboratory. Ensure that COC forms are properly filled out and indicate whether sieving is required, if samples were not field sieved.

All USEPA methods for Hg analysis require that samples be refrigerated and analyzed within 28 days of collection.

All procedures used to collect, prepare and analyze samples to establish background levels must be applied to samples from contaminated sites.

1.3.2. Dispersed Contamination

The intensity of atmospheric Hg emissions as a result of burning or melting of amalgams and gold in open pans can be determined from soils analysis around the emission sources. Regular sampling of soil and sediment could provide quantitative data about Hg emitted and deposited near a mining site. However, this process is extremely expensive and would provide imprecise results, since the atmospheric deposition is very irregular.

Mercury can also be found dispersed in aquatic sediments as a result of erosion, which can spread highly-contaminated sediments. Ableson and Gustavson (1979) sampled surface sediments in a 3 km by 1 km regular grid from Pinchi Lake,

Canada, an old Hg mine, to determine the spatial distribution of Hg. The results provided information about how mercury is being spread in the lake from sites with high concentration of residual Hg sulfide and oxide in tailings from cinnabar roasting process.

Sampling soils and sediments, most researchers intend to establish the atmospheric Hg deposition rates. However, it is important to note that gold mining activities are not the only source of mercury emissions in ASM regions. Other sources of mercury are usually underestimated in tropical environments. Some other natural and man-made sources of Hg emission and/or mobilization in ASM regions are listed below:

- Geologic weathering and erosion.
- Evaporation from waters and soils.
- Run-off waters.
- Ancient gold and silver mining.
- Plant transpiration and decomposition.
- Waste incineration.
- Forest fires.
- Diffuse emissions.

Camourze *et al* (2001) highlighted the importance of erosion in transporting natural Hg bound to old, intensively-weathered soils to Amazonian aquatic systems. The authors stressed that this is a much more important source of Hg for the entire Amazonian environment than any other source, including ASM activities, which are more relevant locally near the mining sites. Roulet *et al* (2001) reinforce this point. The authors have stressed that the cumulative Hg deposition from gold mining activities in the Tapajós area, Brazil, represents less than 3% of the burden of Hg accumulated in the first 20 cm of soil. Then, the Hg waste from these activities has no effect on Hg concentrations measured in the water column 50 km downstream from the gold mining areas.

As most ASM activities occur in the jungle, the amount of Hg emitted by miners is usually confounded with Hg emitted from forest fires. Fire is the most primitive method of deforestation. It is also used to control agricultural pests. Forest fires mobilize Hg contained in biomass and redistribute it into the atmosphere, either as vapour or attached to particulates. Today, with the high rate of deforestation by fire in developing countries[6], Hg emissions derived from wood combustion are significant. The amount of Hg emitted annually by deforestation in the Amazon has been estimated at between 0.78 kg/km² and 1.76 kg/km² (Lacerda, 1995; Veiga *et al*, 1994). Estimates depend on vegetation biomass, the area burned and Hg levels in plants and organic matter (ranging from 0.02–0.3 mg/kg). Regardless of differences in emission estimates, the significance of the forest fire as a vector for Hg emissions in the Amazon region is indisputable. Concentrations as high as 1,000 mg/kg Hg were measured in smoke particles smaller than 2.5 µm in a forest fire in Amazon (Kaufman *et al*, 1992). Through analysis of aerosol particles, Artaxo *et al* (2000) estimated that about 30% of the Hg in atmospheric particles in the Amazon region might be associated with biomass burning and 63% from gold mining.

In a thorough review of available information on Hg in the Lake Victoria, Tanzania, Campbel *et al* (2003) concluded, "*it appears that gold mining is a relative minor regional source of total Hg to the entire lake*". The authors suggested that biomass burnings may deposit 6 to 18 tonnes/a of total Hg on the lake.

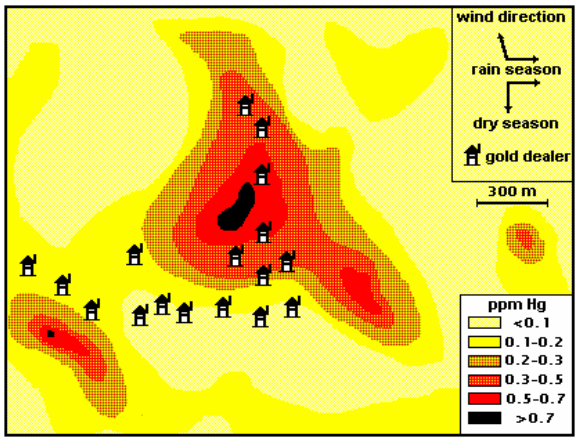


Fig. 1.4. Hg distribution in soils around gold shops of Alta Floresta, Amazon, Brazil (CETEM, 1992).

[6] Deforestation by fire in the Brazilian Amazon in 1995 = 29,059 km² of jungle.

Another issue under debate is, how far does ASM Hg travel? According to Marins *et al* (1991), the majority of Hg emitted from 32 gold smelting shops is deposited very near the emission source, within 1 km. In Alta Floresta, a town in the South of the Amazon Basin, neither air analyses nor soil samples analysed up to 600 m from gold shops showed significant elevations in Hg concentration (CETEM, 1992) (Fig. 1.4).

A simulation model of Hg emissions from gold shops in the same town concluded that Hg concentrations in air decrease quickly with distance (< 2km) from the source (Artarxo *et al*, 2000). Borochoff (2001), using mathematical simulation, estimated that the majority of Hg vapour emitted from gold mines and gold shops is not transported more than 2–3 km, mainly because it is at a relatively low temperature and controlled by lower, local wind currents.

The study conducted by Silva *et al* (1995) in Poconé, Brazil reinforces the idea of quick condensation and local deposition of Hg vapour. The authors monitored Hg vapour emissions from gold shops by analyzing house dust from 30 residences located within 400 m from gold shops. Collecting dust in hidden places, the authors claim that this procedure provides a reliable picture of the mercury deposition over time. They found levels in dust as high as 151,5 ppm Hg. The mercury levels were much lower in houses located in the outskirts of the town (500 m distant from gold shops). It was also found that individuals living near the gold shops had higher levels of Hg in urine (Câmara *et al*, 1998). This procedure also revealed residents who were burning amalgam inside their houses.

Hg "sniffers", or portable instruments that analyze Hg in the air (such as LUMEX, Jerome, Nippon, Genesis, etc.), can detect high levels of Hg in the houses of those who have been handling mercury. Murao *et al*. (2001) reported high Hg content, up to 15.7%, in dust, ash and soot in gold smelting and amalgamation huts. Much of the Hg emissions from ASM are likely to be in the form of elemental Hg. This can have longer residence time in dry atmosphere and can be transported greater distances. However, based on most of the work conducted around mine sites, most Hg is detectable no more than two kilometers downwind from the emission source.

Even when deposition is near the source, Hg from miners and gold shops can be re-emitted when a fire is ignited (Fig. 1.5). In his review on Hg in the Brazilian Amazon, Villas Bôas (2001) mentioned a simulation model that indicated that Hg from ASM can travel thousands of km when associated with aerosols.

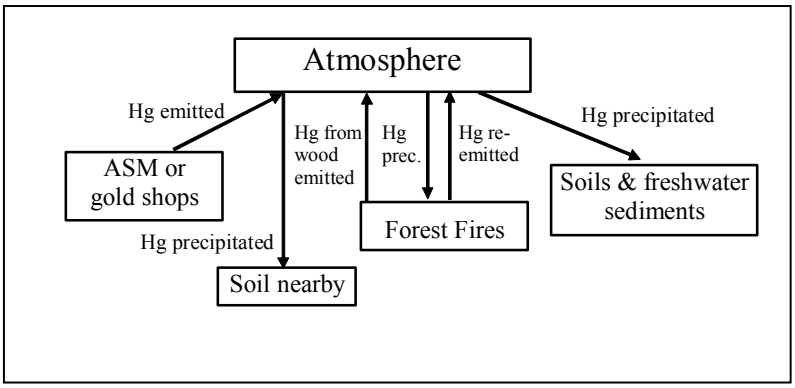


Fig. 1.5. Forest fires emit Hg from wood and redistribute Hg emitted by miners.

Deforestation, as a result of human occupation of the jungle environment, also exposes soils to rainwater, increasing the leaching and erosion processes that mobilize anthropogenic or natural mercury from soil surfaces to the aquatic systems (Carmouze *et al*, 2001). Mergler (2003) makes an interesting comment about the existence of other Hg sources in the jungle that can release even more Hg to the aquatic system than ASM activities:

The implication of this observation [existence of other Hg sources] for ecosystem management of mining activities is that mercury released through mining activities cannot be isolated from the other activities that were likewise increasing the mercury burden in this ecosystem. The argument that mining activities are only adding a small proportion to the total mercury load is inappropriate. Because the fragile ecosystem already has high levels of naturally occurring mercury, the addition of even very small amounts from any source can have very important effects on ecosystem disruption and human health. Adequate mitigation measures depend on a comprehensive assessment of the ecosystem, as opposed to a random grab bag of measurements.

Atmospheric Deposition Rates

On a global scale, the atmospheric mercury cycle is dominated by elemental mercury vapour (usually >95% of total airborne Hg). However, the speciation of Hg is determined by the source characteristics (Ebinghaus *et al*, 1999). In a comprehensive review, Porcella (1995) describes Hg emission and deposition rates in the northern and southern hemispheres. He believes that much of the background Hg emitted is elemental (Hg°), which evaporates from water surfaces, soils and vegetation. Conversely, forest fires and other high temperature emissions are likely to emit at least

partially oxidized Hg in particulate and gas-phase forms of Hg. Upon evaluation of data compiled from different sources, the author estimates that the annual global emission of Hg from all sources (natural and anthropogenic) is between 5,000 and 6,000 tonnes. Porcella also estimated that the Hg deposition rate in the northern hemisphere ranges from 11 to 14 µg/m²/a and in the southern hemisphere, where industrial activities are less intense, from 5 to 7 µg/m²/a. In wet conditions, such as in forested areas, Hg deposition rates can double. In central Brazil, von Tumpling *et al* (1996) estimated a deposition rate of 67 to 151 µg/m²/a from mining activities and grassland fires. Lacerda and Marins (1997) estimated an annual Hg deposition rate of 16 µg/m² in the Amazon, particularly near mining activities. Carmouze *et al* (2001), referring to a modeling study conducted by M. Roulet, indicated that the annual Hg deposition in the area between latitude 10°N and 10°S is around 13 µg/m².

Fosberg *et al* (1999) analyzed Hg in rainwater and estimated the annual deposition of Hg as 14.7 µg/m²/a in the Negro River basin, a region in the Brazilian Amazon with little mining influence. Assuming the average deposition rate from all sources of Hg emission in the Brazilian Amazon is between 10 and 16 µg/m²/a (Lacerda *et al*, 1999; Fosberg *et al*, 1999; Lacerda and Marins, 1997), in an area of 5 million km², the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from different natural and anthropogenic sources (Veiga *et al*, 1999).

The annual atmospheric Hg discharges from anthropogenic activities have been estimated as approximately 3,600 to 4,500 tonnes/a globally (Fitzgerald, 1995; Mason *et al*, 1994), which constitutes about 70% of total annual atmospheric discharges. About 60% of the Hg anthropogenic emissions are transported by long-range atmospheric processes, while the remaining 40% are deposited locally (Pilgrim *et al*, 1999). Since the beginning of the industrial revolution, the total amount of Hg in the atmosphere is believed to have increased 2 to 5 times (Boening, 2000).

An effective technique to estimate atmospheric Hg deposition rates is analysis of Hg in lake sediment cores (Lucotte *et al*, 1995). This controversial technique, sometimes known as "paleogeochemistry", has been applied together with a dating procedure that can be derived from analysis of stable isotopes such as lead-210 or cesium-137 or from evaluation of lake sedimentation rates. Atmospheric Hg adsorbed to mineralogical components are transported and deposited in depositional areas of lakes. Understanding the relationship between sedimentation rate and the vertical distribution of Hg concentration can provide estimates of the timing and magnitude of Hg emissions. Fitzgerald *et al* (1998) published a very comprehensive review about the usefulness of the lake sediment cores for evaluating anthropogenic Hg emission. They criticized the arguments against this technique, in which diagenetic processes may bring Hg up to the sediment surface. Lockhart *et al* (2000) reviewed three cases in Canada where Hg found in lake sediment cores would be a result of Hg emissions from a chlor-alkali plant, a gold mine and a mercury mine. In all three cases, the histories of Hg deposition derived from the cores agreed well with the known histories of inputs. A working group of experts on collection and analysis of Hg in lakes, peat and other cores has prepared a protocol to obtain, analyze and interpret patterns of Hg deposition. Based on these data from widely distributed sites, the researchers concluded that there has been a three-fold increase in Hg flux since the onset of the industrial era (Pilgrim *et al*, 1999)

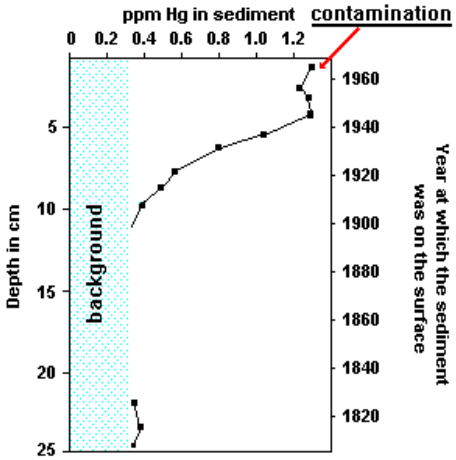


Fig. 1.6. Hypothetical example of evaluation of atmospheric Hg deposition rates using lake sediment.

A hypothetical example of this method is shown in Fig. 1.6. This methodology is not useful for soils because soil formation is different from lake sedimentation. As well, the erosion and weathering processes substantially alter the mineralogical profile. Researchers must be aware that other factors can skew the results. Lakes can be partially dried during some months of the year. In this case, interstitial waters (pore waters) are very reactive and have strong seasonal

effect, i.e. can migrate vertically. This is the environment in which most chemical and biological reactions occur. Small amounts of Hg in pore waters may be carried by capillary action towards the surface to be trapped by superficial organic matter or hydrous ferric oxides. In addition, vertical movements by benthic invertebrates can move contaminants up or down in the sediment layers.

There are bad and good examples of investigations where lake sediment cores have preserved the anthropogenic Hg atmospheric deposition. It is clear that the confidence of the method depends on the specific environment being investigated. Use of multiple cores is recommended to increase confidence (Fitzgerald *et al*, 1998).

Based on core samples from a floodplain of Jamari River, Rondonia, Brazil, Lechler *et al* (2000) observed increased Hg enrichment with depth. They concluded that atmospheric Hg deposition from ASM was not an important regional source of Hg to the Amazon soils, i.e. the impact is localized.

Rodrigues-Filho *et al* (2001) examined a sediment core from a lake in the vicinity of a road that serves an ASM area in Alta Floresta, Brazil. The core was composed of 10 cm of organic soil on top of ferruginous clayey sediments. It was noticed that Hg concentration increased from 50–70 ppm (lowest section) to 210 ppm in the top 10 cm. Estimating lake sedimentation rate, the authors were able to correlate Hg levels in the sediment profile with historical gold production in the region.

Atmospheric Deposition Forms

Most Hg emitted by miners is in the Hg° form and the majority of this is deposited near the source. It is difficult to predict if a small portion of gaseous Hg° travels long distances, as the rainfall in the mining areas of the tropical regions is seasonal. For example, in the ASM region in the Tapajós River, Brazilian Amazon, , monthly precipitation from November to March ranges from 200 to 400 mm and only 25 to 50 mm from June to August.

The recent discovery of water-soluble species of Hg in the atmosphere, named **reactive gaseous mercury** (RGM), has heightened concerns of toxicologists. Source measurements have indicated that RGM is formed by combustion processes (Lindberg, 1999; Ebinghaus *et al*, 1999). The nature of RGM is believed to consist of one or more simple Hg (II) compounds, such as $HgCl_2$. In Tennessee, the RGM represents 3 to 5 % of the total gaseous mercury in the atmosphere (Lindberg and Stratton, 1998). In Florida, this species represents the dominant form of total mercury in the atmosphere associated with dry deposition (S. Lindberg, 2001 – OAK Ridge Lab, personal communication). Lindberg and Stratton (1998) indicated that seasonal trends might exist in RGM concentrations; this variability was primarily associated with temperature, solar radiation, O_3, SO_2, and TGM. This research also suggested that vegetated areas may act as important sinks of RGM and, due to the high water solubility of the compounds, rainfall events are significant to RGM's removal from the atmosphere.

RGM might have a significant role in Hg deposition from emission sources such as forest fires or other diffuse forms. However, little is known about how Hg° emitted by miners can be transformed into RGM and what its relation is to fish contamination in areas with no influence from gold mining.

Mercury deposited on soils can be oxidized and complexed with organic acids to be carried to aquatic systems or bioaccumulated or methylated. Roulet *et al* (1998) studied the importance of soil erosion as a mode of transport of Hg from lithogenic and anthropogenic sources associated with particulate matter into the aquatic systems in Amazon. Similar results were found by Couture and Lambert (2003) studying areas in Guyana affected by ASM. They concluded that erosion of land sediments caused by storms or hydraulic monitors (used by gold miners on land) transports high amounts of Hg associated with fine particulates to the Potaro River. Sequential extractions indicated that organic matter is the main Hg-bearing phase.

When organic acids from the soils contact metallic Hg (e.g. deposited from the atmosphere) soluble complexes are formed. As oxygen is likely the main electron donor in the complex formation reaction, Hg oxidation is controlled by oxygen diffusion. Run-off waters can transport these contaminants easily to streams. The formation of Hg-organic complexes when reactive gaseous mercury (RGM) is deposited in soils or darkwater systems may be a significant mechanism worthy of detailed investigation. However, no information is available on this matter. Currently, most studies addressing interactions of metallic Hg with organic matter focus on understanding the chemistry and bioaccumulation of these Hg-organic complexes.

How these Hg-organic complexes transform into methylmercury is unclear. Approximately 1–3% of the total mercury in surface soil is already methylated and bound to organic matter. The other 97–99% of total Hg in soil can be considered largely Hg(II) complexes, although a small fraction of Hg in typical soils is elemental (Revis *et al*, 1990). Since fulvic acids are known to be methyl-group donors, methylation of these complexes seems to be feasible through either biotic or abiotic processes (Mannio *et al*, 1986; Verta *et al,* 1986). The soluble Hg complexes can also be adsorbed by colloidal organic matter, which serves as a substrate for methylating bacteria.

An intriguing aspect that deserves special attention is the potential for direct bioaccumulation of these Hg-organic complexes. Despite the lower toxicity of these complexes when compared with methylmercury or mercury chloride, they do bioaccumulate (Hinton, 2002).

Rowland *et al* (1977) showed that Hg (II) ingested as a chloride can be methylated in less than 20 hours by rat intestinal bacteria. They estimated that the total methylmercury synthesized from ingested inorganic mercury in humans is approximately 0.4 mg/day.

Hinton and Veiga (2002) used earthworms to study Hg bioavailability of Hg-organic complexes. Worms were exposed for 28 days to solutions prepared by dissolving metallic mercury in tannic acid solution. Total Hg and methylmercury were analyzed to assess whether methylation of Hg was occurring in the substrate, directly within the worms (e.g. in the intestines) or in the tannic acid-Hg solution. Results indicated that the ratio of MeHg : total Hg was up to 2400 times higher in worm tissues (32.2 ppb) than both the tannic acid-Hg solution (0.059 ppb) and the substrate (0.013 ppb). This result is particularly important as metallic Hg deposited in organic rich soils and in darkwater systems can react with natural organic acids.

Sampling Soils and Sediments with Dispersed Hg Contamination

There are two main categories of sampling designs: probability-based designs and judgmental designs. **Probability-based sampling designs (PBSD)** apply sampling theory and involve random selection of sampling units. An essential feature of a PBSD is that each member of the population from which the sample was taken has a known probability of selection. When a PBSD is used, statistical inferences may be made about the sampled population from the data obtained from the sampling units. PBSD provide: the ability to calculate uncertainty associated with estimates; reproducible results within uncertainty limits; the ability to make statistical inferences; and the ability to handle decision error criteria. The main disadvantages of this methodology include cost, difficulties in locating randomly contaminated sites, and the time consumed to establish an accurate, conceptual design. **Judgmental sampling designs** involve the selection of sampling units on the basis of expert knowledge or professional judgment, which can also be a disadvantage of the process. Nevertheless, it can be less expensive than probabilistic sampling and easier to implement, depending upon expert knowledge (USEPA, 2002b).

Sampling soils and sediments to establish the dispersion of Hg contamination is costly, as it can involve a large number of samples to be collected, preserved, transported, prepared and analyzed. As discussed above, information derived from soil and sediment analysis over a large area where Hg is or was dispersed can rarely be used to calculate the amount of mercury (vapour) emitted and deposited from ASM. Probably the most useful information that can be obtained is around gold shops or amalgamation sites, i.e. sites where Hg is being burned. In these cases, random sampling can provide better results than judgmental sampling. CETEM (1992) used a regular grid of 100 x 300 m to acquire 130 samples in an area of 3.4 km² around 17 gold shops in the town of Alta Floresta, Brazil. Each sample was a composite of 4 sub-samples, which were obtained from the top 10 cm of soil in an area of 100 m². This procedure indicated that most mercury was deposited within 600 m from the emission source.

It is important to determine the appropriate sample size, in order to distinguish quantitatively the magnitude of differences among areas and to select the number of sampling stations. Where background data are available, it is normal to use a detection limit based on $\pm$ 2 SD at a given level of power. The power analysis equation (Green, 1989) is as follows:

$$N = 2(t\alpha + t\beta)^2 \, (SD/ES)^2$$

Where n = sample size; $t\alpha$ = t value for α significance level; $t\beta$ = t value for β level significance; SD = standard deviation and ES = effect size (approximately 2 SD). Pre-calculated tables of n are available for a variety of α, β and SD values. If we assume that $\alpha = \beta = 0.1$ and ES = 2 SD, five (5) replicate stations are required for each area sampled. To increase confidence, if we assume that $\alpha = \beta = 0.5$, the sampling effort would increase to only eight (8) stations at each area sampled.

Where there is no background information on variation within or between stations, a minimum of 10 sampling stations is recommended from each area (Environment Canada, 2002).

Sampling procedures, sample preparation, packing and preservation must be identical to the procedures described above to establish Hg background at the reference sites.

It is stressed here that soil and sediment analysis is costly when Hg is dispersed. The best way to determine if Hg is becoming bioavailable within discrete areas is to analyze resident biota, such as invertebrates or small fish. The analysis of dispersed Hg has not been considered as a crucial objective of the Global Mercury Project.

1.3.3. Hotspots

An important objective of soil and sediment analysis in artisanal mining sites is the identification of **mining hotspots**, which are formed either by amalgamation tailings dumped into water streams or by active or abandoned sites excavated on the ground or near stream margins, which are used for the amalgamation of gravity concentrates. An **environmental hotspot** is characterized by the high risk (or evidence) of Hg bioavailability, which is usually associated with high methylation potential. For example, Baker and Allard (2002) observed a good relationship between MeHg and total Hg concentrations in mercury mining contaminated sediments from Pinchi Lake, Canada, although there were several orders of magnitude difference in concentration. However, not all sites with high inorganic Hg concentrations have high methylation potential or high MeHg concentrations.

Hotspots can have dimensions of a few square meters as in the case of amalgamation pools or hundreds of square meters when the entire ore is amalgamated in sluice boxes or copper plates. Whenever amalgamation takes place in an excavated pool, a water-box, beside a riverbed or in a sluice box, tailings are discharged into the environment, creating mining hotspots where the mercury concentration can reach hundreds of μg/g.

Whether a mining hotspot should be mitigated (e.g. dredged or capped) or monitored is a management decision based on an evaluation of the risk of bioavailability (to become an environmental hotspot), costs involved in the dredging operation and spoil treatment options. In some cases, the decision to remove contaminated soils or sediments is based exclusively on Hg concentrations in excess of numerical criteria. In Japan, for example, the decision to dredge sediments from Minamata Bay with Hg concentration above 25 ppm was based on many site-specific factors such as tidal range, sediment-to-water transfer rate and a safety factor of 100 in fishing zones (Kudo and Turner, 1999). In British Columbia, Canada, after the construction of a Convention Center on an old contaminated site, the Government established guidelines for Hg concentration in soils. The BC Ministry of Environment (1989) determined that soils or sediments with Hg concentration between 2 and 10 ppm require remediation to levels below 2 ppm if the land is to be used for residential and recreation purposes. For sites with concentrations above 10 ppm Hg, all uses of land are restricted, pending the application of appropriate remedial measures to reduce contaminant concentrations to less than 10 ppm.

The new Canadian Soil Quality Guidelines (1999) established the level of 6.6 ppm (μg/g) as the limit for soils with agricultural and residential/parkland use (Table 1.6) and 50 ppm for industrial use. Freshwater sediments have more restricted guidelines in Canada (0.49 ppm aquatic life protection).

Table 1.6. Some guidelines for total mercury in soil, sediment and water

	A/R	Com	Ind	ISQG	PEL	GV	CWQG
Soil (dw μg/g)	6.6	24	50				
Freshwater Sediment (dw μg/g)				0.17	0.49		
Drinking Water (μg/L)						1	
Water for Protection of Aquatic Life (μg/L)							0.1

NOTE: A/R = agricultural and residential/parkland use; Com = commercial use; Ind = industrial use; Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health, 1999. Canadian Council of Ministers of the Environment.
ISQG = Interim freshwater sediment quality guidelines; PEL = Probable effect levels. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: http://www.ec.gc.ca/ceqg-rcqe/English/Ceqg/Sediment/default.cfm
GV = Guideline Value (World Health Organization, 1996):
CWQG = Canadian Water Quality Guidelines for the Protection of Aquatic Life, 2001. Summary Table: http://www.ccme.ca/ceqg_rcqe/english/E1_06.pdf
dw = dry weight

It is clear that the simple soil or sediment analysis does not provide enough evidence to support remediation actions. A disposal site for tailings from a chlor-alkali operation in British Columbia, Canada, with 25,000 ppm of Hg was not removed but simply kept properly contained. It is evident that a risk-based approach must be applied.

Knowing the strong influence of organic matter in dissolving metallic mercury in oxic environments, the resulting compounds, soluble mercuric-organic complexes, can be either bioaccumulated or methylated by bacterial action (Veiga *et al*, 1999). So, when a mining hotspot exists in a shallow creek with considerable dissolved oxygen available and in contact with organic matter, the possibility of Hg-rich soluble complexes forming is high. This represents a high-risk situation that has the potential to become an environmental hotspot. When mining hotspots exist in deep aquatic sediments, available oxygen is likely to be extremely low and non-replenished (Meech *et al*, 1998), so the likelihood to become an environmental hotspot is low.

Sampling and Locating Mining Hotspots

The method used in the field to locate mining hotspots (i.e. sites with high Hg concentrations) depends on two basic aspects: First, if the mining and processing operation are active and second whether the hotspots are in dry terrestrial sites or underwater. The easiest way to locate hotspots, when a mine is active, is observing and asking the miners where they have conducted their amalgamation process. When a mine site is inactive, the process is more complicated, but it is still worth looking for old residents and former miners to obtain information about amalgamation sites.

In a project in the Poconé region, Brazilian researchers hired an old miner to help locate hotspots in an inactive mining area. He identified a 1 ha flooded area that had many amalgamation pools in the past. Researchers used panning as the quickest, most effective way to locate and demarcate mining hotspots (CETEM, 1989). Of course, other gravity concentration equipment such as a sluice box or a small centrifuge can be used, but manual panning is more practical and mobile and a large area can be covered quickly. Processing 50 kg of material, the concentrate can be visually inspected. If mercury droplets are visible in the concentrate after panning, the sediment might contain more than 3 µg/g (ppm) of Hg.

It is a difficult task to locate mining hotspots in large, wide rivers impacted by dredging operations. For example in Kahayan River in Indonesia, 3000 rafts are dredging over 200 km of a river 200 to 300 m wide. As the rafts are moving constantly from one site to another, the hotspots created by dumping Hg-contaminated amalgamation tailings into the river are dispersed along the riverbed. In these cases, mercury contamination is quite dispersed; therefore, it is advisable to focus on biota to evaluate bioavailability and avoid sediment sampling (Veiga, 2003).

The recent metallic Hg spill in Peru[7] illustrated the importance of considering the form of Hg present when conducting sampling programs. During the clean-up following this accident, a procedure used to analyze dispersed Hg was employed to detect metallic mining hotspots in dry sites along the roadsides. Although the procedure was accurately conducted, the size of the soil sample analyzed (1 gram) was too small to represent sufficiently the content of Hg in soils from a given location. Later the company in charge of cleaning up the spill employed a more effective procedure. Using a portable cold vapour atomic absorption analyzer, LUMEX RA915, able to detect 2 ng/m³ of mercury in air, an *in situ* analysis of soils was conducted using a flux-chamber. This chamber was made of a plastic tube with a diameter of 4" and length of 30 cm (distance from the soil). Mercury vapours that escaped from the contaminated soil passed through a 2.5 µm dust retention filter before entering the instrument. Readings were taken for a period of 2 minutes from each location. About 10,000 LUMEX readings were performed in total. Whenever readings exceeded 1,000 ng/m³, the site was marked for clean-up. After removal of the contaminated soil, samples were collected (about 60 grams) and sent to the lab for analysis by wet-procedures. Approximately 1,850 soil samples were collected and submitted for analysis. Once a correlation between Hg in the vapour from the chamber and Hg analyzed in the soil samples was established, the equipment was capable of analyzing Hg concentration on the ground along the road within a couple of minutes (Veiga and Hinton, 2000).

LUMEX also provides accessories to analyze waters (detection limit of 0.5 ng/L) and solids (soils, sediments, and eventually fish) with detection limit of 0.5 µg/kg (ppb). A pyrolysis chamber is used to release all mercury from solid samples to be analyzed by the instrument. The drawback of the procedure is the fact that it deals with very small amount of solids in the pyrolysis chamber (200 mg). When the Hg concentration in the sample is higher than 100 ppm, the amount of material to be analyzed must be reduced otherwise the equipment saturates with mercury, providing false readings. In these cases the flux-chamber, as used in Peru, is ideal for quick semi-quantitative analysis, which is sufficient for detecting mining hotspots.

Another effective procedure for detection of mining hotspots is a semi-quantitative method involving pyrolysis of 30g soil or sediment samples followed by a colorimetric analysis (CETEM, 1989). A wet sample of soil or sediment is heated in a Bunsen burner to temperature higher than 500 ^{o}C and the vapour is collected in an acidic permanganate solution. After reduction with a solution of 30% hydroxylamine chloride, the solution becomes colorless. Mercury is extracted from solution with 0.01% dithizone in chloroform solution. Shaking the organic phase with soda, an orange color is developed, confirming the presence of mercury in solution. By comparing results with other colorimetric standards, the procedure could analyze Hg concentrations up to 0.5 ppm in the sediment, which was enough to identify mining hotspots (Silva, 1996). The procedure is very simple and other variations of the method have been developed. Yallouz (2001) adapted the colorimetric method to screen Hg in fish muscle as well as in sediment. For sediment, the method uses 10 g of sample heated in the digestion flask for two hours with an acid mixture of 3 parts of HCl and one part of HNO_3. Following the extraction, 20 mL of deionized water were added and the mixture was filtered. After cooling, the solution was transferred to a determination flask (a sort of test tube), and 2 mL of the reducing solution ($SnCl_2$: HCl 50% m/v) were added. The solution was then aerated. Mercury from the sample was forced to pass through

[7] On June 2000, a truck transporting metallic mercury produced as a by-product of gold cyanidation of the Yanacocha Mine, spilled 151 kg of mercury along 42 km of road between a site above the town of San Juan and Magdalena, in the Peruvian Andes.

a paper containing cuprous iodide. Simultaneously, standard solutions were prepared with concentrations close to the expected ones, as defined in preliminary tests.

Another semi-quantitative process to detect the presence of metallic mercury in soils and sediments is to use special silver-based amalgamation plates, which is a new technology recently developed in Brazil by two manufacturers to remove Hg from contaminated sediments (Hinton *et al*, 2003). The equipment uses 16 plates placed in a sluice box in order to create a cascade effect. For monitoring purposes, just a small plate (30 cm x 15 cm) is enough to pass 30 to 50 kg of sediment quickly through its surface, which is activated with drops of vinegar. Qualitatively, the process is extremely efficient, as shining dots on the plate surface announce the presence of mercury in the sediment.

When released into the environment, metallic Hg often produces a "nugget effect", i.e. individual droplets increase the analyzed concentration at discrete locations, creating tremendous spatial heterogeneity. Consequently, large samples and composites are always needed to avoid sampling errors. In the screening stage, analytical precision of small, discrete samples is essentially irrelevant to the practical identification of "mining hotspots". For comparison, the appropriateness of sample sizes for gold sampling is shown in Table 1.7. As gold and Hg have similar specific gravity, it is reasonable to assume that the same relationship between sample mass and concentration can be applied for sampling of soils or sediments when looking for sediments contaminated with metallic Hg. In this case, to sample soil with 4 ppm Hg present as drops of 0.25mm, 1 kg of material is needed to be representative. Dry pulverization of the entire kilogram sample is required to reduce the size of Hg droplets and, in this case, smaller sub-samples can be used for chemical analysis (Hinton and Veiga, 2001). The homogeneity of the samples increases when working with finer grain sizes (e.g. 200 mesh or 0.074 mm).

Table 1.7. Required sample sizes
(adapted from Clifton *et al*, 1969)

Size of Gold Particle (mm)	**Kg of sample required**	
	4 ppm Au	**1 ppm Au**
2.0	400	1000
1.0	50	200
0.5	8	30
0.25	1	4
0.125	0.1	0.5
0.062	0.02	0.05
0.031	0.002	0.006
0.015	0.0002	0.002
0.008	0.00002	0.0001

When dealing with metallic Hg, sampling procedures involve other aspects than just sample size. As mercury released by ASM is in liquid form, it tends to pass through the sieve meshes to be accumulated in the finest fraction. Whenever samples with high concentrations of metallic Hg must be handled, CETEM (1989) recommends the following sequence:

1. Wet screening (this concentrates Hg in the finer fractions).
2. Drying of screened fractions (ambient temperature or <60 °C).
3. Dry homogenization and splitting of sieved samples.

Rodrigues-Filho and Maddock (1997), working with Hg-contaminated samples from mining hotspot, noticed that that wet screening is not 100% efficient to send all metallic Hg to the fine fractions but is definitely more efficient than dry screening.

As mercury amalgamates easily with copper and not with iron, it is not advisable to use copper screens for sieving. Nylon screens are ideal but usually not available. Despite the possibility of some amalgamation with Hg, stainless steel screen are better than copper screens.

To identify mining hotspots, random sampling is rarely suggested as it results in high costs and requires long periods in the field. CETEM (1989) employed random sampling to evaluate Hg levels in an abandoned tailing pond of 64,000 m² and thickness ranging from 0.1 to 9.3 m totaling 305,000 m³ of material. This material was suspected to be highly contaminated with Hg from ASM and has created many political issues in the region because of its proximity to the Pantanal, an ecological park in Brazil. Using a regular grid of 30 x 30 m, 89 Auger holes were performed collecting 10 kg of material from each core. Most samples showed Hg levels below 0.04 ppm Hg, the detection limit of the analytical method. When the samples were screened, the fraction –200 mesh showed consistent Hg levels above the detection limit, but still at the same magnitude as the geological background. Even by slicing every centimeter of 16 cores and analyzing the –200 mesh fraction, as well as using gravity concentration for all samples, it was not possible to detect

any anomalous Hg level that could be attributed to the metallic Hg released by the ASM. Although researchers knew that amalgamation of the whole ore was practiced in this region, they were unable to confirm the presence of significant Hg; either the random sampling process was not efficient enough to detect the hotspots or Hg was present as fine droplets diluted with the tailing material.

When amalgamation of the whole ore is used by the ASM, it is unlikely that Hg is concentrated in a specific spot. Then, dilution makes it difficult to notice a contrast between anomalous Hg concentrations and background levels. When mining hotspots were formed by discharging amalgamation tailings (i.e. amalgamation of gravity concentrates), it is always advisable to use judgmental sampling to locate them.

The following steps form a good basic procedure for locating mining hotspots:

- **ASK FIRST**: Find out about the history of Hg use in the mining region; ask former miners or residents, when the mine is not active.
- Look for specific sites where miners do or have done amalgamation.
- Look for the sites where the amalgamation tailings were or have been discharged.
- Screen the samples through a 2 mm screen to remove coarse debris and pebbles. Do not use copper screens.
- **TRY TO SEE Hg DROPLETS**: To delineate the hotspots, use a panning process or any other gravity concentration process to find quickly the areas with high metallic Hg content (visible droplets).
- **IF YOU DO NOT SEE, ANALYZE**: If Hg is not visible or the panning method is not efficient, collect some samples and use a semi-quantitative analytical method for the –2 mm fraction.
- Take some composite sediment samples to the lab to check the semi-quantitative analytical method used in the field; composites of 3 to 5 scoops taken from neighbor sites (within 10-30 m² depending on the size of the area being investigated) can be mixed, homogenized and split to obtain an aliquot of a specific site.
- **VERIFY THAT Hg IS ASSOCIATED WITH FINES**: Composite samples of the –2mm fractions must be wet screened in the field or lab to –80 mesh (0.177 mm) or –100 mesh (0.15 mm) or –200 mesh (0.74mm). The choice of the screen opening must be based on the existing field facilities, but the 200 mesh screen is preferred. The same screening procedure should be used to determine background levels. Do not use copper screens. Finer fractions are more homogeneous.
- Dry the fine (screened) fraction preferentially at ambient temperature using a tent. If this is not possible, dry samples at temperatures not exceeding 60 °C.
- The weight of the coarse fractions –2mm +80 (or 100 or 200) mesh and fine fractions must be registered.
- Occasionally, analyze Hg in the coarse fractions to obtain Hg distribution (in %), i.e. % of Hg in fines and % in coarse fractions.
- **OPTIONAL**: In the lab, analysis of total Hg in finer grain size fractions (e.g. 0.002 mm) can provide valuable information on the possibility of Hg being dispersed with fine particles.
- **PRESERVATION**: Pack the samples in glass or plastic jars or in double plastic bags and keep them stored in a cooler (NO ICE must be added). If a fridge is available, the samples can be kept inside until the time of transportation.
- **WHEN COLLECTING SAMPLES FROM HOTSPOTS**: Measure physico-chemical parameters such as sediment (or soil) Eh, pH, conductivity (µS/cm) and collect samples for analysis of Total Organic Carbon and other parameters that can provide information about the potential of a mining hotspot to become an environmental hotspot.

Sample preparation in the lab and chemical analysis involve the same procedures described for determining Hg background levels.

Sampling Air in Hotspots

In areas remote from industry, atmospheric levels of Hg are about 2–4 ng/m³, and in urban areas about 10 ng/m³. This means that the daily amount absorbed into the bloodstream from the atmosphere as a result of respiratory exposure is about 32–64 ng in remote areas, and about 160 ng in urban areas. A guideline for inorganic mercury vapour of 1 µg/m³ as an annual average has been established (WHO, 2000). In industrial environments where workers are subjected to long-term exposure to Hg vapour, WHO (2000) has mentioned that the LOAEL (lowest-observed-adverse-effect-level) might be around 15 - 30 µg/m³.

Sampling of Hg from ambient air in mining hotspot areas provides data about Hg vapour exposure levels to workers and surrounding population. Sampling can be conducted using Hg vapour analyzers, also known as Hg sniffers (e.g. Nippon or LUMEX or Jerome[8]), or vapour traps made of gold/silver (USEPA, 1999) or activated iodated charcoal

[8] Nippon detection limit is 0.1 ng/m³, LUMEX RA915 is able to detect 2 ng/m³ of mercury in air and Jerome Hg analyzer has detection limit around 3000 ng/m³.

(Bloom et al, 1995). Traps installed in strategic locations collect air samples over a long period of time. The traps are connected to a pump and to an air flow regulator that provides constant flow rates (e.g. ~400 mL/min). Mercury entrapped, after acid digestion, is analyzed by atomic absorption or fluorescence. Portable Hg sniffers usually provide a snapshot of the ambient air in workplaces and surroundings but some instruments can also analyze Hg in intervals of 10 to 60 seconds over many days and record data in a computer.

The preferred places to collect air samples are:

- In an area removed from mining sites (for background levels)
- Inside huts where miners sleep and cook
- In workplaces where ore is processed and amalgams are burned
- Inside gold shops where *doré* is melted and surroundings
- Inside houses near the mining sites or gold shops
- Outside, near mining hotspots

Sampling and Locating Environmental Hotspots

It is absolutely necessary to locate sites where Hg released from ASM has the potential to be transformed into methylmercury (MeHg), thereby creating an environmental hotspot. Processes to evaluate methylation potential can be costly and complex.

Carmouze *et al* (2001) observed that in the Amazon region, flooded forests, floating macrophytes, lakes with meanders, and anoxic places, etc. favour high MeHg production. Flooded areas have MeHg production rates that are at least ten times higher than those measured in sediments of flowing waters. The authors stressed that anoxic environments rich in organic matter are not only favorable to the methylating bacteria but are also acidic and thus promote desorption of Hg from organic-clayey material, increasing its bioavailability. So, it is clear that those organic-rich-flooded areas (e.g. wetlands) are the most favorable sites to produce MeHg.

Environmental hotspots can be identified using direct or indirect methods. Direct detection methods involve sampling resident biota (invertebrates and/or fish) for total Hg and MeHg analyses in areas suspected to be of high methylation potential. Comparisons of Hg in similar biota species in reference areas will identify the locations and relative importance of the hotspots. Indirect methods involve using *in-situ* methods to determine methylation capacity or MeHg production rate of soils or sediments.

Identifying environmental hotspots in the field (direct method) is perhaps the most critical, yet most difficult task to accomplish, especially in stream environments because of their large size and complex nature. Inorganic mercury can travel great distances between ASM activities and areas of deposition in sediment, where it may encounter favorable methylating conditions. Therefore, the spatial scope of the investigation may be large. There may be many downstream locations where inorganic Hg accumulates and becomes methylated, contributing to the problem. It is absolutely necessary to identify and quantify the extent and magnitude of MeHg production, as these are the entry points of this pollutant into the aquatic food chain and into humans, via fish consumption. Identifying environmental hotspots will help with decision making in the short-term, by identifying fish species with high Hg concentrations to ensure that these species are consumed less frequently; and in the long-term, to pursue remediation of these areas if possible.

It is important to dedicate sampling efforts to find upstream, uncontaminated environments and compare these conditions with representative, affected environments downstream of mining hotspots. Of course, it is not possible to sample all suspected environmental hotspot areas and field sampling must be stratified to identify those hotspots that are the greatest contributors of MeHg to the aquatic ecosystem. To accomplish this, it is necessary to seek out areas in the aquatic environment downstream from mining hotspots that have those physical and biological features that are most highly correlated with Hg methylation potential.

Identification of environmental hotspots by indirect methods involves the determination of the methylation capacity of a soil or sediment. The methylmercury production rates depend on: mercury complexing characteristics; microbial metabolic activity of the sediment; and total inorganic mercury concentration in the sediment (Bisogni and Lawrence, 1975). The availability of Hg (II) is generally regarded as the limiting factor to the methylation of mercury by biotic processes. There is no consensus among researchers that methylation rate increases at low pH. Acidic pH favours production of mercuric species Hg(II) that is easily methylated. Also, low pH favours proliferation of some microbes that may produce MeHg. These facts are not generic and the effect of pH in producing more MeHg in sediments is still unclear.

Kelly *et al* (1995), studying Canadian reservoirs, have concluded that the concentration of total Hg in sediments is **not** a good predictor of MeHg production and that certain environments enhance methylation rates relative to total Hg concentration. The rate and magnitude of biological methylation was determined primarily by the concentration and form of available Hg in the aquatic system as well as the methylating capacity of the microbes. Other environmental

factors that favour methylation include high DOC, high alkalinity, anoxia, high sediment sulfate concentrations, high sediment TOC (e.g. peat and humus; Porvari and Verta, 1995), high organic acid concentration and warm water temperatures. The physico-chemical and biological characteristics of aquatic systems also contribute to the methylation rate and subsequent bioaccumulation of MeHg in fish. Mercury biomethylation occurs mainly in sediments and its extent depends on their characteristics. In soils or the aquatic environment (sediments), only a small portion of the total Hg exists as MeHg, ranging from 0.1% to 1.4%.

Some scientists envisage that once the methylation potential of a soil or sediment is estimated, then priorities in terms of remediation actions can be established. However, as many different microorganisms have the ability to methylate Hg, the procedures to determine the methylation potential in a laboratory are not trivial.

A radiometric method, originally developed by Canadian scientists (Ramlal *et al,* 1986), was adapted to tropical conditions by Guimarães *et al* (1995) to determine the rate in which $^{203}HgCl_2$, as a source of Hg (II), is methylated in sediment or in other substrates, such as aquatic plant roots (Guimarães *et al*, 1998). Spiking sediments in the Amazon with radioactive traceable ^{203}Hg, scientists observed that higher methylation rates (10^{-2} $\%.g^{-1}.h^{-1}$) were found in rich, organic sediments in darkwater forest streams than in rivers with cloudy or clear waters. High methylation rates have commonly been associated with low pH characteristic of organic sediments and dark waters (Lacerda *et al*, 1995). Guimarães (reported in Silva, 1997) used this method to evaluate the methylation potential of soils and sediments from an ASM-impacted water stream, Rato River, in the Tapajós region. He found higher methylation rates in the most superficial layers of the sediment but did not observe a clear correlation with organic matter content in sediment. This is probably a good indication that the quality of the organic matter is more important in the methylation process than the quantity. Despite the efficiency of this procedure, it would be extremely expensive and labor intensive to use radiometric methods to select those sites (environmental hotspots) with highest methylation capacity. Ideally, the analysis of MeHg in soils could provide information about the risk associated with biological exposure. This analysis is also very expensive and would provide a static picture. Thus, frequent analyses are needed to evaluate the evolution of MeHg production in a contaminated soil or sediment over time. For screening purposes, simpler methods to evaluate the methylation potential of a soil/sediment should be developed.

Other studies have been dedicated to identifying bacteria capable of methylating Hg(II). then establishing a link with the methylation potential of a soil and sediment. Krabbenhoft *et al* (2001) highlighted that the methylation process in the Everglades, Florida, US is linked tightly to the sulfur cycle. Sulfide affects Hg bioavailability to methylating bacteria by precipitating dissolved Hg species, while sulfate enhances methylating bacteria activity. Dr. H. Bastardo (2001 - Univ. Central de Venezuela, personal communication) has been developing a procedure to evaluate the methylation capacity of sediments based on microbiological studies. Methylation is controlled by bioavailability of Hg(II) species to Hg-methylating sulfate reducing bacteria in the substrate and by the metabolic activity of these organisms (Benoit *et al*, 2001).

Sampling materials to determine if a site is an environmental hotspot is an expensive task. Deciding what to sample is a complex process that is based on several factors, such as budget, access to laboratory facilities and available personnel. Sample types can include soils and sediments or biota. Methylmercury concentrations in sediments determine the magnitude of methylation and provide an indirect measure of bioaccumulation potential. The same procedures must be applied to sampling suspected environmental hotspots as reference areas. To determine directly if an area represents an environmental hotspot, resident biota (small fish or invertebrates) can be measured for total Hg. Analysis for total Hg in biota is sufficient to provide information about the bioavailability of Hg in a specific site. Because MeHg comprises the majority of the total Hg concentration in fish tissue, analyzing for total Hg is less costly than for MeHg and therefore more samples can be analyzed for less cost. Note that biota samples must also be collected from reference areas, to establish the background and provide a benchmark to contrast with biota data from environmental hotspots.

Regardless of which procedure is used, the choice of the sampling sites is critical and must be decided carefully. Since mining hotspots are also sites with high risk (and potential) to become environmental hotspots, it is recommended that studies include an analysis of MeHg in soil or sediment samples collected on these sites, or total mercury in resident biota as described above.

If material from mining hotspots is being transported visibly by drainages to another site, this must be reported and samples from this new site must be collected to check if methylation is occurring. Methylation can also be occurring in a region due to different mercury sources not directly related to the mining hotspots (e.g. lithogenic Hg leached by run-off water, atmospheric Hg deposited from various sources including mining, etc.). In some cases, these sites produce much more MeHg than the mining hotspots. To select potential environmental hotspots, the following criteria should be used:

- Seek depositional areas in streams – areas with fine sediments (silt/clay) or fine sand; avoid eroding areas.

- Seek wetlands and marshes adjacent to or part of the stream, or stream areas that receive runoff from wetlands, marshes and bogs with low oxygen concentration.
- Seek sediments with visible organic material, indicating sediment nutrient sources and anoxic decomposition.
- If possible, use any of the techniques to identify rapidly sediments with high metallic Hg concentrations (mining hotspots). Although the correlation between high inorganic Hg in sediment and MeHg in sediments and biota is weak, this may be useful as a screening tool.
- Seek areas that are relatively easy to sample, with low flow, relatively shallow depth within the permanently flooded area of the stream.
- Seek areas where it is possible to collect benthic invertebrates, such as insect larvae and clams, or small minnow (fish) species for Hg analysis.

It is important that the technique used to collect sediment from all areas, including reference areas, is identical. Once the sites with the potential to be environmental hotspots are selected, the sampling and sample preparation procedures should follow the same methodology described above to establish Hg background levels. Analysis of fine particles is preferred. Again, measuring Hg in resident biota (e.g. invertebrates or small fish) is the best way to establish sites of methylation and bioaccumulation.

Sediments should be analyzed for **total Hg** and **MeHg** no more than 28 days after collection. Resident biota can be analyzed only for total Hg, but if MeHg analysis is available, this can provide useful information as well (see chapter on assessing bioavailability using invertebrates and using fish for further information). However, it is only necessary to analyze for MeHg in invertebrates, not in fish.

1.3.4. Mercury Mobility

Metallic mercury dumped by ASM is heavy and not easily transported in drainages. Natural or anthropogenic Hg from various sources, including ASM, when deposited on the ground, is transported to water streams in solution or suspension through run-off waters. Whether concentrated in "hotspots" or dispersed in sediments, Hg becomes mobile in terrestrial environments when Hg complexes (organic and inorganic compounds) adsorb on soil and sediment fine particles (main mechanism). Metallic Hg condensed from atmosphere can also impregnate fine soil particles to be transported. It is well known that fine soil and sediment particles usually have two or more times the Hg content of coarse fractions as a result of the interaction of Hg-oxidized complexes with soils and sediment components, in particular clay minerals and hydrous ferric manganese oxides (Ferreira and Veiga, 1995; Silva, 1997; Hylander *et al*, 2000).

Roulet *et al* (1998) studying the association of natural and anthropogenic Hg with soil weathering products in the central Amazon region, concluded that fine particles enriched in Fe-Al oxides and Hg have been eroded and transported. The erosion is a result of the fragility of the soil cover, deforestation and agriculture activities. This process can be responsible for up to 97% of the Hg burden to the Tapajós River, Brazil (an important tributary of the Amazon River).

Telmer *et al* (2003) analyzed 0.3 µg/L of dissolved Hg (filtered at 0.45µm filter) in acid mine drainages from an ASM tailing pond rich in sulfides. It is well known that sulfide oxidization produces sulfuric acid and that this poses an additional problem when the tailings are contaminated with residual metallic mercury. The result was that the mining effluent revealed Hg concentration 7,000 times higher than the one in non-impacted watercourses. However, within 100 meters from the tailing pond, the suspended solids adsorbed the dissolved Hg and the Hg concentration dropped 300 times. These authors suggested that Hg entering the Tapajós River is not just from ASM. Natural (lithogenic) Hg is the main source, but the mining activities, with poor sluicing, dredging and tailing disposal practices have substantially increased the amount of suspended solids and consequently the amount of Hg transported to the rivers.

An overlooked environmental impact caused by ASM is river siltation. Tailings are typically dispersed in a vast area or simply dumped into the water streams. The plumes of fines can be seen for kilometers in impacted rivers from the Tapajós region. Physical impacts on biota and their habitats are also evident. Mercury and other heavy metals become attached to the suspended particles that will be transported to remote areas, creating environmental hotspots.

In streams, transport of Hg is much more evident than in lakes. CETEM (1989) investigated the transport of Hg from an abandoned tailing pond formed by artisanal miners, a mining hotspot. As tailings were deposited into a drainage near an important Ecological Park in Brazil, "Pantanal Matogrossense," the project assessed the possibility that Hg would be transported and become bioavailable. The study concluded that particles released from the tailing pond did not travel long distances in small creeks and that the association of Hg with ferruginous clayey particles reduced Hg bioavailability. In another region, CETEM (1993), analyzing the –0.074mm fraction of bottom sediments from an affected river in the Tapajós basin, Brazil, also concluded that mercury-laden tailings were not carried too far.

Mercury mobility is also a result of formation of soluble Hg-organic complexes when Hg contacts organic acids (Meech *et al*, 1998). Fadini and Jardim (2001) concluded that Hg leached from soil is the major pathway of contamination for rivers in the Rio Negro area in the Brazilian Amazon, a region with no significant presence of artisanal miners. The climate seasonality in tropical rainforests definitely plays an important role in transporting Hg in solution or associated with particulate matter. For example, in the Tapajós region in the Brazilian Amazon, the rainy season lasts 5 to 7 months, with precipitation around 300 mm/month, as compared to around 50 mm/month in the dry season (INPE, 2003).

Trying to determine how Hg moves in aquatic systems by sampling superficial water, especially run-off water, can be a time-consuming, complex and uncertain process. Water sampling provides a brief snapshot of the current conditions. In addition, there are many confounding factors that can influence the results of Hg monitoring of surface water including differences in flow rate, time of year, upstream activity, location in the stream from where water is collected (i.e. near shore, near middle, near surface, near bottom, differences in water composition, etc.). These factors make it extremely difficult to determine the magnitude and range of Hg transport in aquatic systems.

The most appropriate method to evaluate Hg mobility is to sample depositional areas downstream. Sediments accumulate Hg over time. Methylation also occurs primarily in the sediments. Thus, by determining spatial distribution of inorganic and/or MeHg in sediments downstream of a mining activity, one can determine the source of Hg (mining hotspot) and determine where the majority of sediments are settling. The most effective way to identify potential environmental hotspots is to target sediments in depositional areas of streams and rivers, by sampling sediment and biota; not by sampling the water column.

Predicting Hg Mobility

Variables controlling Hg toxicity can be divided into two major categories: 1) parameters affecting the release of mercury into the aquatic environment (mobility) and 2) parameters affecting Hg accessibility to biota (bioavailability). Most laboratory and field techniques concentrate in assessing bioavailability by chemical or biological methods. However, few methods have been developed to **predict the mobility** of Hg either in suspension or in solution.

The simple analysis of fine particles obtained by screening (-0.074mm or 0.037mm) or centrifuging (-0.002mm) samples from mining or environmental hotspots, associated with information about the hydrodynamics of the aquatic system, can predict whether the material from the hotspots can be transported to other sites.

Baker and Allard (2002) applied a method to predict Hg mobility from erosion of "mining hotspots" in Pinchi Lake, an old Hg mine in British Columbia, Canada. The monitoring program was established to investigate the transport of Hg adhered to contaminated (up to 850 µg/g) surface sediments (<1cm) from locations where roasted cinnabar (HgS) ore (calcines) was deposited into the lake in the 1940s. Using Plexiglas core tubes to extract the top 0.5 to 1.0 cm of sediments, the authors observed that fine-grained material from the "hotspots" is occasionally re-suspended and transported to other areas as a result of wind and wave action. Low inorganic Hg concentration in surface sediments indicated that the main mechanism of Hg availability is the vertical mixing of historically-deposited calcine fines with recently deposited lake sediments, which gradually reduces surface sediment Hg concentrations over time.

Mercury mobility in solution can also be predicted by using the classical methods applied to predict Metal Leaching and Acid Rock Drainage (ML/ARD). Metal leaching (ML) problems can occur over the entire range of pH conditions, but are most commonly associated with acid rock drainage (ARD), i.e. when sulfides are oxidized by a biochemical process and release heavy metals. A series of prediction methods (Mills and Robertson, 1997) were developed to determine the timing and conditions under which metals from different geological materials, such as waste rock, tailings and mine walls can be leached. Those conditions mimic the situation in which materials will be exposed in the natural environment.

The assessment must also consider the effects of post-depositional processes such as weathering, erosion and sedimentation (BCMEM and BCMELP, 1998). There are two basic procedures: the **Static Methods**, which evaluate the solubility of trace elements and the neutralization-adsorption capacity of the rock-forming minerals, and the **Kinetic Tests**, which use humidity cells to speed up the weathering process. Ghomshei *et al* (1999) used humidity cells/columns associated with toxicity tests to predict the Hg mobility from amalgamation tailings with 27 ppm Hg from Brazilian ASM. The tailings were exposed to alternating weathering and leaching conditions. Organic acids were used to evaluate the Hg release from tailings. Test organisms (*Daphnia magna*) were exposed to the leachate. This supposes to simulate the reaction of natural organic acids with metallic Hg and subsequent bioaccumulation.

Sampling to Establish Mercury Mobility

Assuming that the investigation will focus on Hg mobility associated with solids in suspension, there are two main ways of sampling to establish if materials from a mining or environmental hotspot have been transported to other sites:

1. Analyzing sediment (and biota) in depositional areas in water courses.

2. Analyzing particulate matter in the water column.

In the first way, the purpose is to check if material from mining hotspots is being transported to areas where it can be methylated; or if material from sites already identified as environmental hotspots is being transported to other areas. In these cases, analysis of material from the hotspots (fines such as –200 or –400 mesh or clay fraction –0.002 mm) and also fine fractions of sediments from depositional sites, provides information about material being exported from hotspots. The sampling procedures are identical to those described above when environmental hotspots are sought. Analysis of the fine fractions is preferred. When looking for depositional areas in streams, it is important to avoid erosional areas, i.e. areas in which the contribution from soil erosion is significant.

In the second case, analysis of suspended particulate matter provides a snapshot of Hg transportation processes associated with fine particles. Sampling of suspended particles is a tedious process. Usually filtration through Milipore 0.7 and 0.45 μm filters is conducted in the field. At least 100 to 200 mg of particulate material must be filtered and collected to ensure sufficient quantities for analysis. This requires the filtration of many liters of water, which is time-consuming. Most of these small particles are negatively charged, which is the major reason for the stability of suspended soil particles. Particles that might otherwise settle are mutually repelled by these charges and remain in suspension. Coagulation is a chemical technique directed toward destabilization of particle suspension. The most commonly used coagulant is alum (aluminum sulfate). Coagulation is usually followed by flocculation, which is a slow mixing technique that promotes the aggregation of the destabilized (coagulated) particles (Willmitzer, 2000). Coagulation, followed by flocculation as an aid to sedimentation and filtration, has been practiced by CETEM (1993) and Silva *et al* (1993) to reduce the amount of water filtered in the field.

Sampling for suspended particles requires further precautions in order to avoid sample contamination. Centrifuges are now available to operate in the field to separate the 0.45 μm fraction at a flow rate up to 6 liters per minute (Chapman, 1996). It is difficult to determine a reliable relationship between the magnitude of Hg on suspended solids and the amount of Hg transport, especially without discharge information, which is usually seasonal and difficult to measure. In addition, the amount of material obtained by filtering or centrifuging is usually low and introduces difficulties and calculation errors into the analytical process.

Filter papers or centrifuged material can be dried at ambient temperature or at <60 °C and packed in plastic bags. Refrigeration is suggested for samples prior to and during transport to the analytical laboratory.

1.4. Water

Water samples usually do not provide useful information about Hg mobility or bioavailability. Analysis of Hg in water should only be conducted when there is a legal requirement to do so (e.g. to verify if guidelines are met) or for academic reasons (e.g. to study stability of Hg complexes). Water is not an easy geochemical material to be sampled and analyzed. As Hg usually occurs in very low concentrations in natural waters, a large volume must be analyzed or analytical instruments with very low detection limits must be available. Also, when Hg concentrations are low, the risk of contamination of sampling vessels is high. In addition, organisms accumulate MeHg quite quickly; therefore the concentration of this compound in water is usually very low (D'Itri, 1990), undetectable by common analytical methods. In seawater, the normal Hg concentration is around 0.05 µg/L Hg; in freshwater, the average concentration in world streams is around 0.07 µg/L Hg. Canadian freshwaters range from <0.005 to 0.24 µg/L Hg (CWQG, 1987). Typical detection limits of analytical instruments are approximately 1 to 2 ng/L for water samples (Sorensen *et al*., 1994). Mercury contamination of samples has been shown to be a significant problem in past studies. The use of ultra-clean sampling techniques is critical for the more precise measurements required for detection of low levels of mercury (USEPA, 1997).

Mercuric ion (Hg^{2+}) is not stable as a free ion in natural aquatic environments. Mercuric species are combined to form an inorganic or organic complex, such as $Hg(OH)_2°$ (aq) or Hg-fulvate complex. The use of Hg^{2+} to refer to mercuric species should be avoided as Hg(II) notation is more appropriate.

MeHg is stable in solution, but it is accumulated rapidly from the water by organisms. MeHg does not bind as tightly with organic matter in sediments as do inorganic Hg compounds. Consequently, MeHg readily remobilizes from the stable and less reactive sediments into the overlying water. The rate of MeHg remobilization influences bioaccumulation in aquatic organisms, although the amount of MeHg can be small (<1%), relative to total Hg concentration in sediment (D'Itri, 1990).

Analyses of pH and redox conditions (Eh) of freshwater sediments affected by ASM activities can provide useful information about Hg speciation. However, information obtained from Eh-pH diagrams with respect to natural systems must be used carefully. The theoretical values are applied to a system in equilibrium. In natural waters, it is common to find non-equilibrium conditions, as transformation rates to more stable compounds can be quite slow (Baeyens *et al*, 1979). The most toxic form of Hg, methylmercury is an example. It is thermodynamically less stable than inorganic species.

Assuming that metallic Hg is in equilibrium in a simple aquatic system, the predominant Hg species in solution would be undissociated mercury, Hg° (aq.) with solubility of 63 µg/L. If we consider the Amazon environment as an example, most freshwater environments have chloride concentrations between 2 and 3 ppm (pCl around 4) (Furch, 1984). Under these conditions, $Hg(OH)_2$ and $HgCl_2$ are the predominant inorganic species, depending on pH. The full lines of Fig. 1.7 represent the equilibrium of Hg° (aq) and $HgCl_2°$ (aq) and $Hg(OH)_2°$ (aq) respectively. The dotted lines represent conditions in which concentrations of uncharged species ($HgCl_2°$ or $Hg(OH)_2°$) are 1000 times lower than the Hg°(aq) concentrations, i.e. in this case the importance of these complexes in the formation of MeHg is considered insignificant as metallic Hg has to be oxidized to become more soluble, i.e. to form Hg(II) species or complexes, which are far more reactive. Mechanisms of MeHg formation are faster when Hg (II) compounds exist (Bisogni and Lawrence, 1975; Imura *et al*, 1971). The thermodynamic analysis based on Eh-pH diagrams (Fig. 1.7) suggests that metallic Hg emitted by ASM in an aquatic environment with a redox potential (Eh) below 0.4 V should be stable. However, the presence of soluble organic acids changes this conclusion.

It is well known that dissolved organic matter (e.g. fulvic acid) forms more stable and predominant complexes than any of the inorganic species (Ramammoorthy and Kushner, 1975; Duinker, 1980; Xu and Allard, 1991). The presence of fulvic acids (FA) is an important parameter that enhances solubility of organic matter and associated Hg. Schnitzer and Kerndorff (1981) have shown that, over a large range of pH (4 to 9) when more than 20 ppm of FA is added to solution, Hg becomes very soluble. The authors pointed out that Hg interacts with fulvic acid in partly hydrolyzed forms. Melamed *et al* (1999) demonstrated experimentally that humic acid solutions increase the solubility of metallic Hg; but the presence of calcium ions inhibits Hg solubilization.

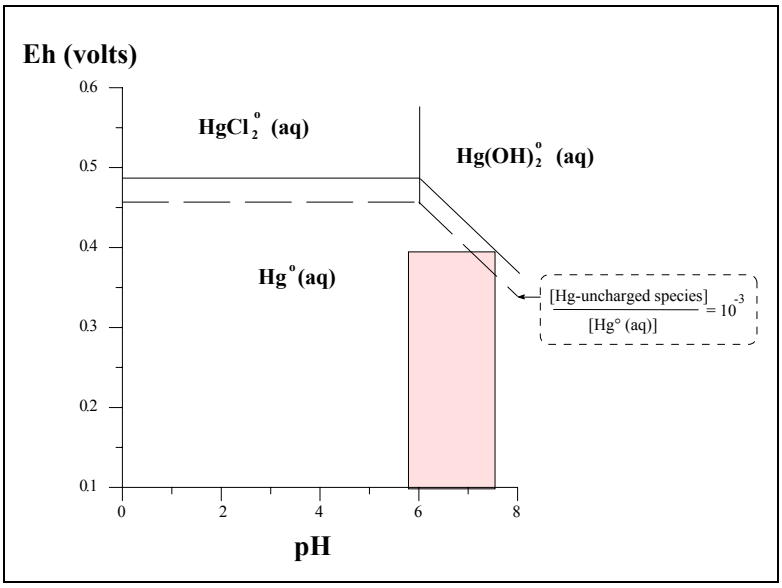


Fig. 1.7. Equilibrium boundaries of Hg^o(aq) and Hg-inorganic complexes
: Typical conditions of Eh and pH of Amazonian sediments

Tromans *et al* (1996) designed a Eh-pH diagram capable of predicting the stability of metallic Hg in contact with organic solutions (Fig. 1.8). It is observed that Hg-organic-soluble complexes are formed at lower Eh levels than those observed in the Eh-pH diagram for inorganic soluble Hg species. In the diagram, the organic ligand is represented as a diprotic acid H_2L. Two complexes are formed, HgL and $Hg(H_{-1}L)^-$. The upper line of the diagram represents equilibrium between complexes and Hg°(aq) in darkwaters, in which the dissolved organic matter concentration is 10^{-4} M (Walker, 1990). In this case, the redox potential of acidic waters must be above 0.48 V at pH 4 and above 0.38 V at pH 5.5 to favour Hg-organic complex formation. The higher the pH, the lower the potential required to form such complexes.

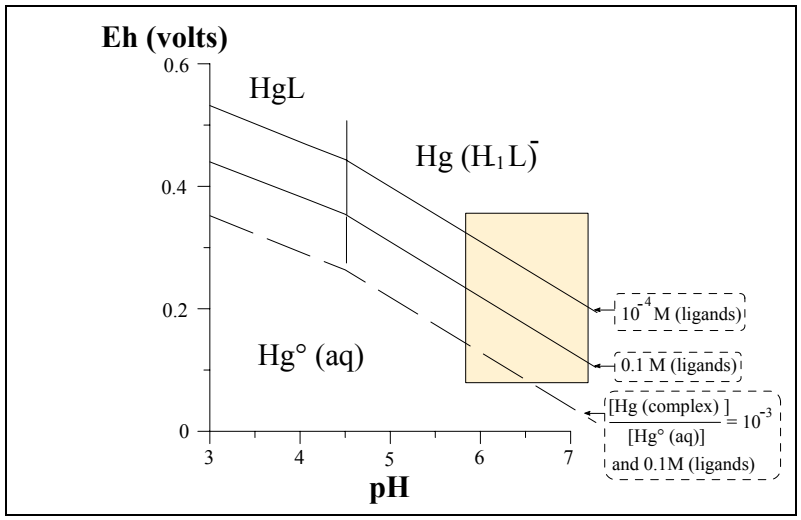


Fig. 1.8. Equilibrium boundaries of Hg°(aq) and Hg-organic complexes
: Typical conditions of Eh and pH of Amazonian sediments.

As the chemical composition of the organic acid that provides the complexing ligand is unknown, a molecular weight of 1,000 g has been assumed, with a concentration of 100 g/L (or 0.1 M) in the contaminated sediment. This condition is represented in Fig. 1.8 by the lower full line. The dotted lines represent a situation in which the Hg-complex concentration is 1,000 times lower than that of Hg°(aq). If we consider a Hg°(aq) concentration of 63 µg/L, then the Hg-complex concentration would be 0.063 mg/L. This level is close to background for natural waters. Under these conditions, Hg bioaccumulation or danger from these complexes is extremely unlikely, as no significant amount exists in solution.

The pH and Eh from various mining-impacted rivers in the Amazon Basin are plotted in Fig. 1.7 and 1.8. Since the early 1980s, miners have dumped Hg-contaminated tailings into these rivers and burned amalgams, on the barges or near river margins, usually without retorts. As observed in Fig. 1.8, the possibility of Hg-soluble complex formation is clear in the majority of the Amazonian rivers if organic acids are an important component of water and sediment. In several studies (CETEM, 1992; CETEM, 1993) fish from darkwater rivers, even those not directly affected by mining activities, have shown higher Hg levels than "white water" rivers (i.e. those with high amount of particulate matter (clay) in suspension).

Humic substances have also been shown to have a reductive capacity in aquatic systems and may account for as much as 70% of the volatile Hg released from some Hg contaminated streams (Allard and Arsenie, 1991). A study by Matthiessen (1998) stated that organic acids may facilitate Hg(II) reduction to volatile Hg° while complexed to or dissociated from humic substances. This study has determined that the amount of elemental Hg formed increases with pH. The role of humic and fulvic acids in reduction of Hg(II) seems to be further exacerbated in the presence of UV radiation. Allard and Arsenie (1991) observed a reducing capacity of 0.1 meq/h by fulvic acid and also found that Hg(II) reduction by humic substances is decreased by factors such as the presence of competing ions (e.g. Cl^-), air or methylation of carboxylic groups, and increased by light. Over a period of six hours of exposure to UV light, humic substances degrade by ~50% and remaining compounds decreased in molecular weight from 1,800 to 300 (Allard *et al*, 1994). In the process of photodegradation, adsorbed Hg is released and subject to transformations to lesser or more bioavailable species. Light penetration may also influence levels of MeHg in biota from darkwaters. As the rate of MeHg photodegradation has been demonstrated to be 350 times faster than degradation by microorganisms (CEQG, 1998), MeHg in areas with greater light penetration (i.e. clearwaters) may be more susceptible to degradation and transformation to biologically unavailable species. This is inconsistent with results from a study by Costa *et al* (2001), which demonstrated that Hg photoreduction in freshwater increases with dissolved organic carbon (DOC) concentrations (Hinton, 2002).

Sampling Water

When sampling water, a controversial issue is how to collect, analyze, and interpret results. Some researchers report dissolved Hg concentrations from filtered water (0.45 μm), while others report total concentrations from unfiltered samples. Most government guidelines for drinking water do not recommend filtering water samples. The lack of information about the filtration process can dramatically change the result, as Hg in solution is in the order of few ng/L, while on suspended particles concentrations can be in the order of hundreds of ng/g. Those in favour of analyzing unfiltered water argue that many people drink unfiltered water; however, mercury in the particulate matter (above 0.45 μm) is not necessarily bioavailable.

To collect water, great care must be taken to avoid contamination and proper quality/control samples must be taken. Collect water samples by pumping water from depth using weighted C-flex (food-grade silicone) tubing and a diaphragm pump. Ultraclean techniques (USEPA, 1996) should be employed to minimize contamination. Approach sampling stations from down-current to prevent possible contamination of the water column and sample from the bow of the boat without anchoring. Prime the pump and allow it to run for at least 5 minutes at sample depth to flush the tubing thoroughly and to ensure that there is no cross-contamination between stations before water samples are collected. After flushing, discharge the sample water directly into a rinsed sample vessel. Samples for Hg analysis can be preserved in the field immediately after collection by adding up to 0.1% HCl. Filtered samples, to measure dissolved Hg fractions, can be collected by pumping water through an inline filter unit (e.g. Gelman 0.45μm pore size). If a diaphragm pump and tubing are not available, water samples can be collected by hand, by submerging a sampling vessel beneath the water surface. Again, great care should be taken to approach the samplings station from a down-current direction to avoid disturbing and introducing sediment into the water column. Filtered and unfiltered water samples should be placed in ultraclean 250 mL glass bottles with Teflon lids. All samples must be stored in the dark and kept on ice immediately following collection, during shipping, and prior to analysis for total Hg and MeHg. Again, preserve water samples by adding HCl to 0.1% total volume. USEPA (2001b) suggests water sample preservation by adding 5 mL/L of pre-tested 12N HCl.

1.5. Assessing Mercury Bioavailability

Metallic mercury in the environment can become bioavailable by forming organic complexes in aerobic environments and ultimately, by transforming into methylmercury, usually in anaerobic conditions. The transformation and bioaccumulation depend on several environmental factors and bacterial activity that control chemical speciation of the metal (Carmouze *et al*, 2001). Sites with high Hg concentrations (mining hotspots) are not necessarily those with the greatest capacity to produce methylmercury (environmental hotspots) but they represent a situation of risk. To assess environmental hotspots, analysis for MeHg would provide the most accurate information. However, MeHg analysis is costly, has risk of sample contamination and demands special laboratory care. Therefore, there are other techniques that can be employed to assess Hg bioavailability when MeHg is not analyzed.

1.5.1. Using Selective Extraction to Assess Bioavailability

Sequential (or selective) chemical extraction procedures have been used to identify metal-bearing components in soils and sediments. Some procedures are very selective and can clearly distinguish between metals associated with clay minerals, hydrous ferric and manganese oxides, organic matter, sulfides, carbonates, and metal in the structure of silicates. The association of metals with mineralogical phases can either increase or reduce bioavailability. These chemical procedures give indirect information about the strength of the bond of a metal with the mineral surface. The stronger the bond, the lower the bioavailability.

Adsorption is the main mechanism to control availability of soluble Hg species to biota; but it is also responsible for transporting Hg from mining hotspots to other locations where methylation is more favorable. For many lakes, adsorption to particulate matter and consequent sedimentation of Hg compounds is expected to be the dominant process for removal of Hg from water (Sorensen *et al*, 1990; Fitzgerald *et al*, 1991). The mechanisms of adsorption depend on sediment grain size, composition and characteristics of aquatic systems. In fact, re-suspension of non-mercury polluted sediments has been suggested as a method to reduce bioavailability in the water column and to reduce concentration of Hg in the surface sediments of English Wabigoon River system, Canada (TCOSC, 1983).

Amorphous and poorly crystalline hydrous ferric and manganese oxides have an enormous capacity for fixation of heavy metal ions from solution (Chao and Theobald, 1976; Hem, 1974). Clay minerals also actively adsorb Hg from solution. Although the adsorption capacity of these minerals is quite high, the binding strength is usually weak and dependent on aquatic system variables such as pH, type of species in solution, Eh, conductivity, etc. In the case of Hg adsorption, the stable soluble species are not charged and little effect of pH is observed on $HgCl_2$ adsorption by clay minerals (Reimers and Krenkel, 1974). Clays may show an indirect effect in heavy metal adsorption due to their ability to act as nucleation centers for Fe/Mn oxides or organic matter. These materials are more effective for metal adsorption (Duinker, 1980). Despite the high adsorption capacity of clay minerals and hydrous ferric and manganese oxides, the inhibition of Hg adsorption is remarkable when high chloride levels are present in solutions (Lockwood and Chen, 1973; Reimers and Krenkel, 1974).

Organic matter is effective at adsorbing Hg species at all pH ranges, but is more effective in acidic conditions (pH<5). Breteler and Saska (1985) have shown that organic sediments are good scavengers of Hg but do not retain this metal very well. Mercury desorption was highest in the period immediately following the adsorption study. The functional groups on organic matter in which Hg is bound determine the strength of the bonding. The strongest bond occurs with sulfur (S) or thiols (-RSH) radicals. This explains the accumulation of Hg in organic-rich, upper soil horizons and the predominance of organic Hg-binding even in mineral horizons. As the presence of organic acids enhances solubility of mercuric species, it is not clear whether adsorption inhibits complexation or enhances it. The more organic acids present in the aquatic system, the more the metal becomes water-soluble as a complex. Hg (II) adsorption on organic matter can likely be a first step to promote reactions between humic substances and Hg. Unfortunately, little is known about these reactions between adsorbed Hg and organic matter. These organic substances are capable of forming complexes with many metal ions as a result of ligand groups present (-COOH, -OH, $-NH_2$, -RSH) (Lindqvist *et al*, 1991).

For environmental purposes, recognizing all Hg-bearing phases can provide information about the stability and mobility of Hg from the sediments into the water column (Table 1.8). In terms of risk assessment, it is common to consider only Hg-soluble and exchangeable fractions as the most bioavailable portion in which the heavy metal is found in sediments. The exchangeable fraction is the portion of Hg weakly-bound, usually to clay minerals in such a way that cations from the extracting solution can replace the heavy metal adsorbed on mineral surface.

$CaCl_2$ or MgCl have been applied frequently to determine the exchangeable portion of metals adsorbed on soils and clay minerals in particular. The classic and most frequently employed method of sequential extraction was developed by Tessier *et al* (1979), which provides information about forms of trace metals associated with soil and sediment components, such as clay minerals, hydrous ferric oxides or organic matter. The main criticism of selective extraction

procedures is the lack of uniformity between methods; therefore it is difficult to compare results (Quevauviller *et al*, 1997).

Table 1.8. Typical Hg bearing mineralogical components.

Mercury Bearing Material	Mechanism in which Hg was Incorporated into the Geochemical Material
Silicates or sulfides	in the structure of the main rock-forming minerals or sulfide minerals
Secondary hydroxides, sulfides, carbonates	secondary precipitation as a result of exceeding the solubility product
HFMO (hydrous ferric and manganese oxides)	co-precipitation or posterior adsorption (specific adsorption)
Organic matter (humic and non-humic substances)	adsorption followed by flocculation (colloid formation); strong adsorption on non-humic substances
Clayminerals	adsorption (weak forces, usually reversible)

Ferreira and Veiga (1995) developed a sequential extraction to study the Hg distribution in a contaminated ferruginous sediment (17% Fe_2O_3) from an ASM site in Poconé, Brazil. The material was collected in an abandoned amalgamation pool and the -200 mesh (0.074mm) fraction analyzed 1 ppm Hg. The clay fraction (-0.002 mm), extracted by centrifuging, had approximately 20 times more Hg than the 200 mesh fraction. As observed in Table 1.9, the procedure revealed that most Hg is associated with the hydrous ferric oxides (77.5%). The procedure used ammonium acetate (1M) to determine the labile fraction associated with clay minerals, followed by attack with oxygenated water (30% vol.) + 0.02 M nitric acid in the proportion of 5 H_2O_2 : 3 HNO_3 for five hours to evaluate the Hg portion associated with organic matter or in metallic state. Mercury associated with hydrous ferric oxides (mainly amorphous) was evaluated by attack with 50 mL HCl 0.5 M for 8 hours of agitation at room temperature. The residual Hg, i.e. that which is very tightly bound to the sediment, including the original lithogenic Hg, was determined by digestion with a strong triacid mixture: HF+$HClO_4$+HNO_3 (5mL+5mL+5mL) at 60 °C. One of the main drawbacks of this procedure is the lack of selectivity between metallic Hg and organic matter. However, the method was useful to indicate that Hg was associated with very fine particles, most likely to be transported in streams, and was not easily extracted and therefore less bioavailable.

Table 1.9. Sequential extraction of Hg from the clay fraction (-0.002 mm) of a ferruginous sediment from Poconé.

Hg-associated phase	% Hg extracted
Exchangeable	3.0
Organic matter + metallic Hg	2.0
Hydrous ferric oxides	77.5
Residual Hg	17.5
TOTAL	100

The adsorption capacity of ferruginous sediments revealed by the selective extraction procedure (Table 1.9) can also be responsible for the almost complete lack of incorporation of Hg in test organisms that were caged for 3 months in contact with heavily polluted sediments (CETEM, 1991).

Other studies have shown that organic acids are capable of removing Hg adsorbed by ferruginous particles (Valix *et al*, 2001; Lodenius *et al*, 1983; Bowles, 1988; Baker, 1973). Hinton (2002), using earthworms in laboratory experiments, noticed that comparatively low Hg bioaccumulation occurs when worms are in contact with clayey ferruginous sediment, suggesting that Hg associations with hydrous ferric oxides may be significant in reducing bioavailability. However, when organic acids were applied to a lateritic soil or amalgamation tailings, Hg bioaccumulation by earthworms was 2 to 28 times higher than in experiments where only water was applied.

Veiga and Veiga (2002) employed a sequential extraction procedure developed by Rodriguez *et al* (2000) to study the Hg-bearing phases in four samples of Hg contaminated sediments (concentration ranging from 92 to 857 ppm Hg) from Pinchi Lake, a former Hg mine in British Columbia, Canada (Baker and Allard, 2002). The exchangeable portion was considered negligible as no Hg was extracted with 1M sodium acetate. The procedure, outlined in Fig. 1.9, could not discriminate between Hg associated with organic matter and metallic Hg. Using 1M ammonium hydroxide, the Hg weakly associated with humic substances was extracted. Leaching the sediments with concentrated nitric acid for 16 hours, this solubilized the fraction of Hg likely in the form of metallic Hg, mercury oxide, and/or as Hg associated with

refractory organic matter. The residual Hg portion (i.e. not extracted by base or acid) was attributed to Hg bound to sulfides (cinnabar or metacinnabar) and/or associated with another mineralogical phase, such as silicates.

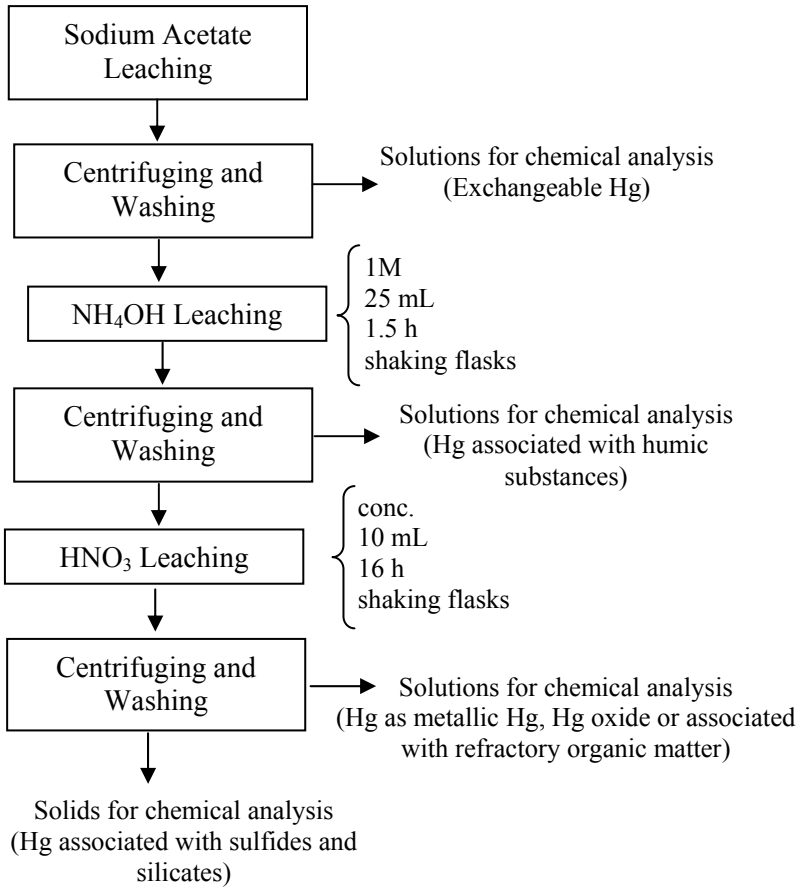


Fig. 1.9. Sequential extraction of Hg from Pinchi Lake sediments (British Columbia, Canada)

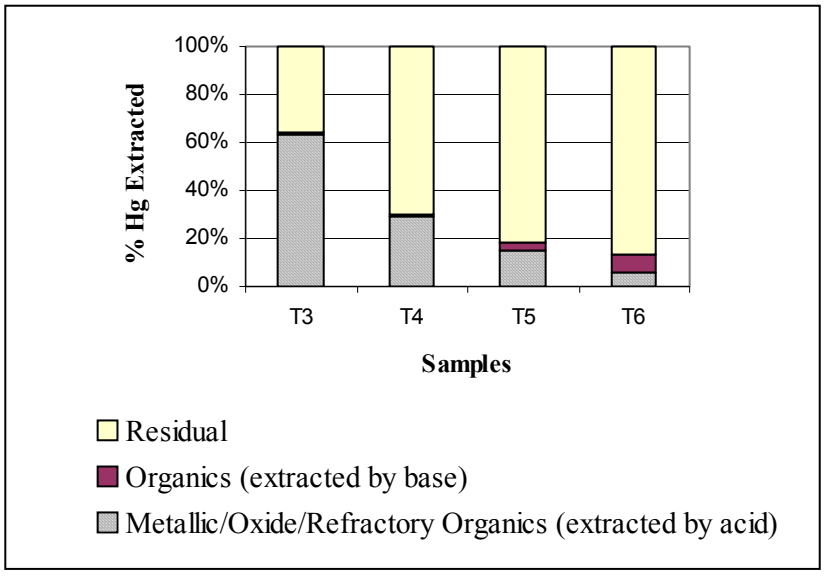


Fig. 1.10. Mercury partitioning in sediment samples from Pinchi Lake, B.C., Canada

This preliminary investigation revealed that Hg in contaminated Pinchi Lake sediments existed primarily as a non-exchangeable, non-labile form (Fig. 1.10). This suggests that the majority of Hg in these contaminated sediments was largely unavailable. However, the selective extraction studies indicated that the predominant binding phase of Hg differed significantly among samples. This might explain why Hg was more bioavailable at some stations than others (Baker and Allard, 2002).

1.5.2. Using Fish to Assess Bioavailability

Biota is the ultimate indicator of bioavailability of any form of Hg. Mercury, particularly MeHg, is highly biomagnified in the food web and reaches its highest concentrations in fish, especially fish-eating, carnivorous fish. Mercury concentration in fish is usually expressed on a wet weight basis as parts per million (ppm) which is equivalent to mg/kg or μg/g. The natural background in fish has been estimated to be between 0.05 to 0.3 ppm Hg and may be less than 0.01 ppm Hg in small, short-lived herbivorous species (Suckcharoen *et al*, 1978).

Methylmercury in fish is acquired almost exclusively via dietary sources and comprises at least 90% of total Hg concentrations in fish (Huckabee *et al*, 1979; Bloom, 1992). The remaining Hg is predominantly inorganic. Inside the cell, MeHg has a strong affinity for proteins. It binds to and affects the configuration of nucleic acids, inhibiting a large number of enzymes by blocking sulphydryl groups. The combination of the lipophilic properties and affinity for the sulphydryl groups of amino acid compounds results in rapid accumulation of MeHg in the muscles and fat tissues, until it is metabolized and excreted. The half-life of MeHg for a small fish (15g) has been reported as 60 days and 350 days for larger fish (100 g). A half-life of 700 days has been reported for northern pike, a large, long-lived carnivorous species (D'Itri, 1990). As MeHg is more slowly metabolized and eliminated than inorganic compounds, the overall result is a net concentration of MeHg by the organism over time (Armstrong, 1979).

Standardizing Approach

Many studies have shown that carnivorous (piscivorous) fish accumulate more Hg than other species. However, it is difficult to compare Hg levels in fish from different sites due to different migration habits and variable food sources. In the Amazon, black "piranhas" (*Serrasalmus rhombeus*) are an ideal bioindicator (Veiga, 1994): 80% of their diet is fish-based; they do not make long migrations; and they live mainly in quiet waters (Goulding, 1980). Unfortunately, black piranhas are not found in all areas of the Amazon or in other tropical countries. Roulet *et al* (1999) have found that some carnivorous fish from the Tapajós River, specifically "tucunaré" (*Cichla ocelaris*), "traíra" (*Hoplias malabaricus*) and "piranha" (*Serrasalmus nattereri*) show very good correlations between Hg content, weight and standard length. Consequently, these researchers believe it is possible to use some of these species as bioindicators of Hg contamination from different sites.

There is a well-known positive correlation between fish size (length and weight) and mercury concentration in muscle tissue (Scott and Armstrong, 1972; Bodaly, *et al*, 1984; Somers and Jackson, 1993). Larger fish generally have higher Hg concentrations. Therefore, the mean Hg concentration of a sample depends greatly on the size of the fish being measured. To eliminate the bias associated with differences in fish size, Hg concentrations in fish must be measured over a wide size range. Then, appropriate statistical procedures are used to determine the mean Hg concentration for a specific fish size, usually near the size most frequently captured by consumers. This is called the size-adjusted or "standardized" mean Hg concentration. When this is done for multiple lakes or years, comparisons of standardized mean Hg concentration can be made that are unbiased by differences in fish size (Baker, 2002).

Strange and Bodaly (1999) established a protocol that describes the sample size and range of fish size needed to derive a good statistical relationship between Hg concentration and fish size. Optimally, tissue from 25 – 35 fish is gathered from each species, ranging from small to large fish.

In the vast majority of historic studies, acquisition of tissue samples for Hg analysis required that the fish be sacrificed. With technological advances made in analytical techniques, reliable estimates of fish Hg concentrations can now be made with very small sample sizes (<100 mg) that do not require sacrificing fish. Baker *et al* (2004) have developed a non-lethal method of fish tissue sample extraction for Hg analysis. Using a Tru-cut^{tm} tissue biopsy needle (14 gauge x 7.6 cm cannula with a 20 mm notch), ~50 mg of tissue can be extracted from fish that have been anaesthetized with clove oil.

Using linear regression and analysis of covariance, quantitative comparisons of Hg levels can be made among populations and over time based on a standardized fish size.

The strength of the relationship between Hg concentration and fish size depends on sample size and the distribution of fish size over the size range being tested. Table 1.10 shows an example of the standard protocol for deriving standardized Hg concentrations. It describes the optimal sample size per size interval in British Columbia for mountain whitefish, rainbow trout and bull trout. These standardized sizes fall near the **middle of the size** range of fish collected

and have been used as standardized sizes elsewhere in B.C. and Canada, which facilitates comparisons between lakes and years for the same species (Fig. 1.11 and 1.12).

Table 1.10. Example of protocol to collect size-stratified fish samples for Hg analysis (Baker 2002)

LENGTH INTERVAL (mm)	WHITEFISH (n)	RAINBOW TROUT (n)	BULL TROUT (n)
100-199	7	6	
200-299	7	6	6
300-399	7	6	6
400-499	7	6	6
500-599		6	6
600-699		6	6
>700			6
Total	28	36	36
Standardized Length (mm)	300	350	550

Note : n = number of samples

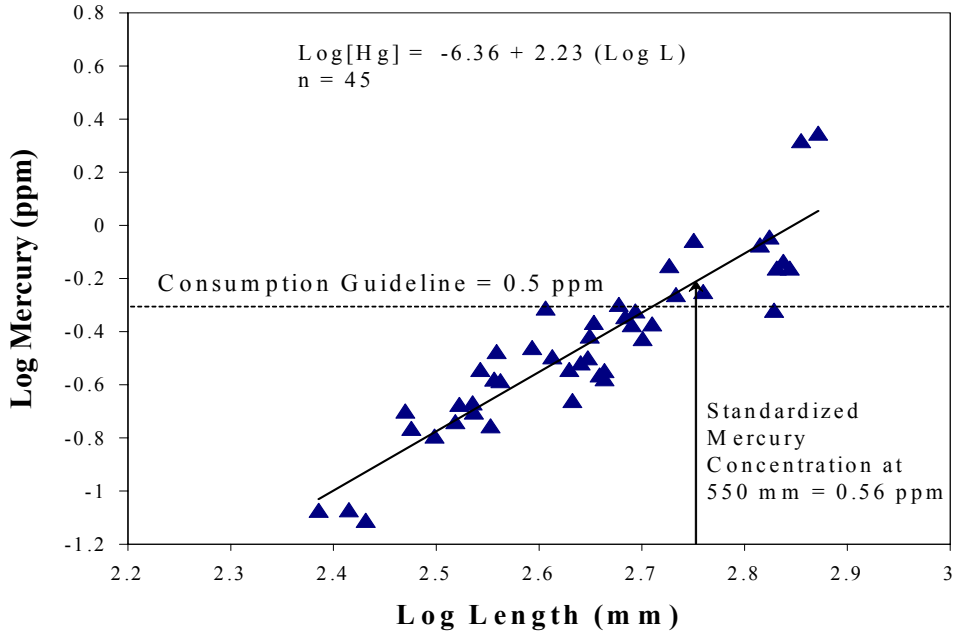


Fig. 1.11. Log fish length versus log Hg in bull trout tissue samples

Few methodologies have been adopted in regions impacted by artisanal mining to standardize the relationship between fish size and Hg. A large number of samples have been collected in the Amazon region and Hg data have been presented for different species, size and weight. However, the lack of a standardized procedure for sampling makes it hard to track the evolution of Hg over the years. A tentative standardizing procedure was applied in Venezuela. Rondon and Perez (1999) adopted the 250g carnivorous fish (*Hoplias malabaricus*) as a bioindicator while studying 15 dams. Incidentally, these researchers found high concentrations of Hg in fish from 7 lakes, 5 of which were not influenced by mining.

Maurice-Bourgoin *et al* (2000), working in ASM areas in Bolivia, did not observe a correlation between Hg content in some fish species and weight due to differences in diet among seasons, local fish migrations, and fish movement between lakes, (although Hg concentration in most individuals exceeded 0.5 ppm).

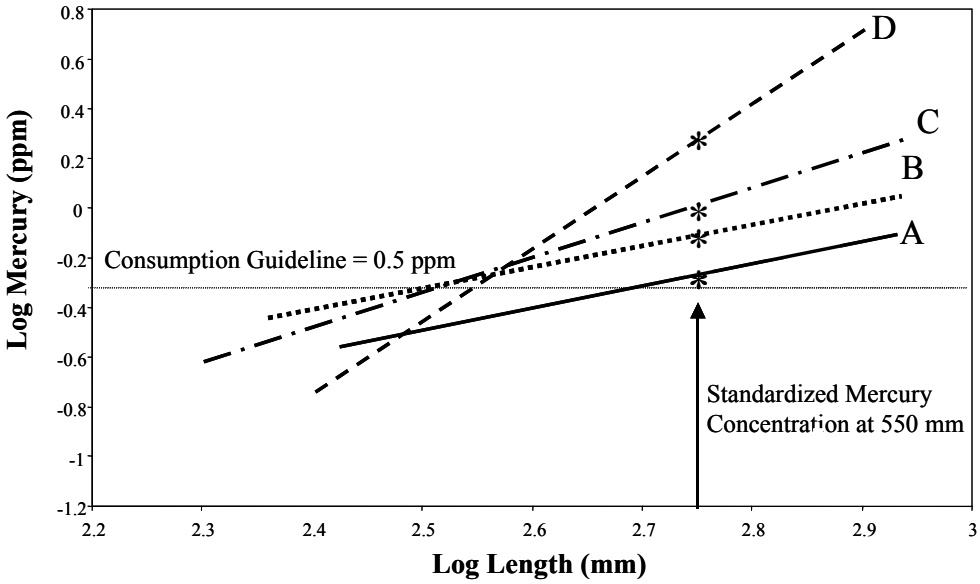


Fig. 1.12. Comparison of length – Hg relationships for different fish populations (Baker, 2001)

In an extensive study in the Tapajós River, Brazil, dos Santos *et al* (2000) documented a correlation between Hg concentration and fish weight in "surubim" (*Pseudoplatystoma sp*.) a large carnivorous fish, and found a negative correlation with "dourada" (*Brachyplaystoma flavicans*). They concluded that this type of correlation is not always observed in the Amazon, probably due to differences in diet and migratory behavior of different species. That is, if a particular fish species undertakes extensive movements, fish from uncontaminated areas can become mixed with fish from contaminated areas (and vice versa), thereby confounding the Hg – size relationship.

We recommend that length be used to generate a relationship with Hg for the following reasons. Depending on the time of year, a fish may weigh much more because of eggs or testes. For example, weight can differ considerably (20% or more) due to the accumulation of body fat or state of maturity (e.g. females with eggs) within a single season that is not reflected by differences in length. Depending on the season when fish are caught, this can significantly affect the Hg-weight relationship. Feeding also has a similar effect. For example, a large fish can consume up to 20 - 30% of its body weight in a single meal, so depending on the time of capture and whether the fish has eaten, this can also affect the Hg-weight relationship. Greater inherent variability in weight than length introduces variability that reduces accuracy and limits the quantitative ability to measure changes in Hg over time. Finally, length is easier to measure in the field than weight and an accurate scale is not required.

An interesting approach to compare Hg bioavailability from one site to another was adopted by Castilhos *et al* (2001). Using the "tucunaré" (*Cichla ocellaris*), the authors calculated the daily uptake of MeHg required to reach the Hg concentration observed in the tissue. The advantage of using the "tucunaré" is that it exhibits only small changes in weight over different seasons, has sedentary habits and has a carnivorous diet. Additionally, its meat is highly desirable in the Amazon region. This field dose-response approach was also used to compare the time needed for the "tucunaré" to reach Hg concentrations of 0.3, which is the US Fish Residue Criterion and 0.5 ppm, the Brazilian safety guideline. At sites distant from mining activities, the Hg concentration in the "tucunaré" reached 0.3 and 0.5 ppm in 4.1 and 6.8 years respectively, while in mining-impacted areas, this time was reduced to 1 and 2 years respectively. However, the highest Hg bioaccumulation rate was found in "tucunaré" from the Tucuruí hydroelectric reservoir in the Brazilian Amazon (Castilhos and Lima, 2001). Porvari (1995) also observed high Hg levels in the "tucunaré" from this Brazilian 2,430 km² hydroelectric reservoir. About 92% of samples of this carnivorous fish had mercury concentrations above 0.5 ppm. The average Hg concentration (n=33) was 1.2 ppm in a standardized 700g "tucunaré". Some authors (Aula *et al*, 1995) attribute Hg in this hydroelectric reservoir to Serra Pelada, one of the largest ASM site in the Brazilian Amazon, 250 km upstream the reservoir. However the prevailing wind direction is the opposite, blowing from Tucuruí to Serra Pelada.

Impoundment Effect

When conducting an environmental assessment in ASM regions, some confounding factors can introduce errors in the interpretation of the data. Volcanoes, soil erosion (Roulet *et al*, 1998), degassing, other industrial activities, forest fires

(Veiga *et al*, 1994), etc. can be responsible for high levels of Hg emissions that ultimately increase the levels of Hg in the aquatic biota. The researcher must be aware of these confounders and avoid sites with possible influence of other Hg sources than mining. Another possibility is to quantify the influence of these factors, but this is not easy. One of the main confounding factors is the existence of man-made water impoundments near the ASM areas. The reservoir impoundment effect is well known in increasing Hg concentration in fish after flooding (Kelly *et al*, 1997; Bodaly and Fudge, 1999). Airborne mercury of natural or anthropogenic origin is captured and incorporated in vegetation, deposited and ultimately retained in forest soils. Following impoundment and flooding, microbial degradation of the labile organic fraction leads to strongly reducing conditions, methane evasion and nutrient release. The major change in the Hg geochemistry of soils after impoundment is the gradual methylation of 10 to 30% of the Hg formerly present (Stoke and Wren, 1987; Lucotte *et al*, 1995). In many cases Hg sources are not identified but the influence of the submerged vegetation, type of organic matter and bacteria in flooded sediments are recognized as important factors in the magnitude of Hg response. Mercury bioavailability is related to quality and amount of flooded vegetation and humus.

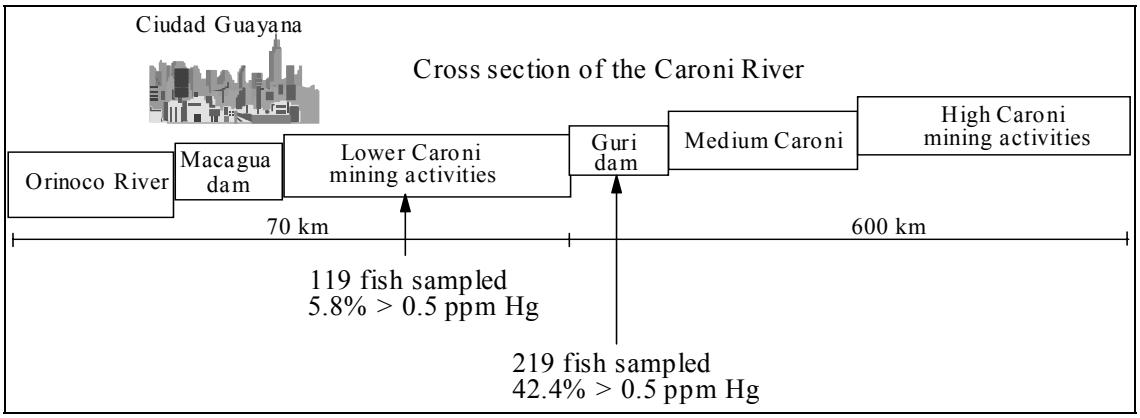


Fig. 1.13. Comparison of Hg levels in fish from Lower Caroni River and Guri Reservoir in Venezuela (Veiga, 1997)

The impoundment effect is exacerbated in darkwater systems, such as in the Guri reservoir, a 4,000 km^2 hydroelectric reservoir in southern Venezuela. The Caroni River, a 640 km long tributary of the Orinoco River, has four hydroelectric dams. The relatively favorable conditions for bioaccumulation in the river are indicated by natural variables such as slightly low Eh, slightly acidic pH, low conductivity, dark water color, low biomass productivity, and low amount of fine ferruginous particles in the sediment (Weibezahn, 1994; Bermudez *et al*, 1994). Two fish monitoring programs carried out in 1995 and 1998 revealed Hg in carnivorous fish as high as 8 ppm. It is interesting to note that despite the 5 tonnes of Hg dumped by miners over the years in the lower part of the river, a 35 km sector intensively impacted by ASM, fish have shown lower Hg levels than in the Guri reservoir (Veiga, 1997). This result suggests that Hg bioavailability in the Guri reservoir is strongly affected by flooding (Fig. 1.13), perhaps even more so than by ASM.

A similar situation is found in the GMP site in Ingessana Hills, Sudan. The Roseries Dam reservoir, a 410 km² hydroelectric impoundment built in 1965, is located some 50 km northeast of the mining area near El Damazin town. The drainage from the nearest mining activity, Gugub, to the reservoir is steep but seasonal. As such, the contribution of Hg in fish that comes from particulate matter transported to the reservoir from the mining area is small when compared with the "natural" degradation (impoundment) of the submerged organic matter and consequent Hg methylation. The way of establishing the influence of mining in the Hg pollution of the reservoir is to analyze the amount and bioavailability of Hg in the particulate matter (indirect methods).

Sampling Fish

The objective of the fish Hg assessment must be clear before establishing the sampling strategy. Fish capture programs should target two groups of fish: **1) fish species that are consumed by the local human population;** and **2) fish species that serve as indicators of mercury bioavailability in ASM and surrounding areas**. For a Health Assessment, for example, it is evident that the most important information is the dietary habits of local people. In this case, a battery of interviews about socio-economic and demographic aspects of the community must be applied. Visiting the local fish markets will also help identify those species most frequently purchased and/or consumed.

Regarding the location of hotspots, direct sampling of resident biota (invertebrates or small fish) provides an excellent indicator of Hg bioavailability in mining and environmental hotspots. Small fish, typically bottom feeding catfish species, can be excellent indicators. These indicator species forage within a relatively small area, do not range over long distances like large carnivorous species and integrate MeHg from sediments and lower trophic levels over time from

relatively discrete areas. The advantages of using fish instead of invertebrates to identify environmental hotspots are that they are relatively easy to capture, are well known taxonomically (i.e. easier to identify than most invertebrate species) and need only be analyzed for total mercury, not MeHg (less expensive).

Reference fish tissue data are important to put fish tissue Hg concentrations from hotspot areas into perspective with background levels; and to establish a baseline against which changes in fish Hg can be measured in future collections.

A strategic, quantitative approach to fish sampling must be adopted for determining Hg concentrations in each target species to determine Hg bioavailability around ASM sites. Because tissue Hg concentration is strongly affected by fish species and size (length, weight), these factors must be controlled or else the data will be too variable to be useful for long-term monitoring purposes. Muscle tissue samples acquired for Hg analysis from each target species should be collected according to a strict protocol, namely by stratifying the sample according to fish length (Table 1.10). For the reasons discussed above, developing a linear relationship between fish length (total length or fork length; i.e. the distance from the tip of the nose to the "v" in the fork of the tail) and Hg rather than total weight is advocated. The protocol for describing the relationship between Hg (ppm) and length (mm) is well known (Johnson, 1987; Bodaly *et al*, 1988; McMurtry *et al*, 1989) and is of the form:

$$\text{Log}_{10}[\text{Hg}] = a + b(\text{Log}_{10}[\text{Length}])$$

where "a" and "b" are the calculated intercept and slope respectively of the linear relationship. Size data are normally $\log_{10}$ transformed because growth of fish (irrespective of age, weight, or length) is curvilinear, not linear. Fish grow quickly when they are young, with growth rates declining with increasing size and age. Therefore, it is inappropriate to apply linear regression techniques against non-linear data without first transforming the data (Ricker, 1975; Sokal and Rohlf, 1981).

Mercury sampling of each species must be conducted independently because different species may have different Hg – length relationships.

There is an established fish-collecting protocol to attain an appropriate sample size and size range of fish, which enables the derivation of a statistical relationship between Hg concentration and length (Strange and Bodaly, 1998; Baker, 2001). Optimally, tissue from 28 – 36 fish per species is needed, ranging from small to large fish (Table 1.10). Typically, only muscle (i.e., skin and scales removed) should be analyzed for Hg, because the skin is not normally consumed. The effect of analyzing skin with the muscle might result in slightly lower total Hg concentrations than if the same fillet is analyzed without skin (Dellinger *et al*, 1995). For human health assessments, fillets should be analyzed without skin. For Hg bioavailability assessments using small fish, whole fish should be analyzed. Developing an Hg – length relationship is critical to selecting a "standardized size". The standardized size is the approximate size of fish consumed by local residents. By calculating the standardized Hg concentration from the standardized fish size, comparisons of Hg can be made between areas, regions or years without the bias of differences in fish size. In addition, by deriving a reliable length – Hg relationship for target species, the Hg concentration of a fish can be predicted accurately without knowing the empirical value. This can also be useful in estimating Hg exposure for the human Health Assessment if the species selected as "standard" is also highly consumed in a region.

Different species may have a different standardized length, depending on size (e.g. 60 cm for large species and 35 cm for smaller species). It is important that an appropriate standardized size is chosen and used consistently over time to ensure that comparisons in population Hg concentration are unbiased. The suggested criteria to select the fish species for monitoring purposes are:

1. A carnivorous species with high Hg concentration.
2. A species with sedentary habit (limited migratory habits).
3. Fish that are to catch and preferentially consumed by local residents.

Note that, if it is difficult to fill the required size ranges for an individual species, it is permissible to combine more than one target species to accomplish this, provided that the species being combined share a number of key characteristics. These include similarity in diet (e.g. carnivorous), size range, migration habits, maximum age, etc. This must be used as a last resort, as it is very important to understand the relationship between Hg and fish size for individual species. Long-term comparisons bring enormous benefits to support decisions.

Acquisition of fish tissue for Hg analysis from target species should follow the above protocol to ensure an equitable representation of Hg concentrations over the full size range of fish. It will be easier to fill certain size categories than others and every reasonable attempt should be made to acquire an adequate sample size within each size category. Investigators should attempt to acquire fish from local vendors and fish markets. If markets or vendors do not have fish of certain size groups (e.g. no small fish), then local fishermen should be hired to collect and fill the missing sample size intervals. Following this protocol will ensure that an optimal number of fish (i.e. not too few and not too many) are collected to derive a good statistical relationship without being wasteful of available resources in the field.

The following steps to collect fish tissue in the field should be adopted to ensure that a common protocol is followed for each collection program and to establish a quantitative approach to Hg monitoring over the long-term within each of the recipient countries.

- Identify the target species based on the criteria above and interviews with local people, fishermen, fish markets, and if possible from local or regional experts, such as a biologist.
- Identify sampling areas to collect fish including upstream, **reference** areas if possible, and an area(s) downstream of mining activities. **Sampling should target at least one upstream and two downstream areas to address geographic differences.** Sampling efforts to address health concerns should also coincide with geographic areas identified as "environmental hot spots" to address possible worst-case areas of methylation and accumulation by carnivorous species.
- Acquire individual fish among the target species according to the size protocol above. Fish can be acquired from markets or by accompanying or hiring local fishermen. Note that the geographic source of fish **must** be known. Every reasonable attempt should be made to collect tissue samples from each target fish species across the entire size range, from small to large.
- A minimum of 10 – 20 g sample of muscle tissue per fish is required. Muscle tissue can be collected from any part of the body of the fish, avoiding fatty or overly bony tissue.
- Tissue samples should be excised from the fish with a clean, stainless steel knife and placed in a labeled plastic bag such as Ziploc^{tm}, or Whirlpac^{tm} bag. Alternatively, tissue samples can be stored in small plastic vials (e.g. scintillation vials). Label sample containers with an indelible marker and record the information in a field booklet.
- At a minimum, fork length (cm) and the geographic location where the fish was captured must be recorded. Collect supplemental data, including fish weight (g), stomach contents, gender and maturity.
- As soon after sample collection as possible, put tissue samples on ice and/or freeze them. Samples must be frozen within three days of collection (see next section: Preservation of Fish Samples)
- Samples should be transported frozen and kept frozen until analyzed for total mercury concentration (ppm wet weight).
- Mercury concentration should be determined from tissue sub-samples using cold vapour atomic absorption or fluorescence spectrometry by an accredited laboratory. Remaining tissue should be stored frozen in the event that subsequent analyses are needed.
- Appropriate QA/QC procedures should be followed including 1) collection of duplicate, blind samples from 10% of all fish captured; 2) repeat analysis of tissue sub-samples to determine laboratory accuracy; and 3) analysis of standard, reference tissues with known Hg concentrations to determine laboratory precision.

Assuming that tissues from target species were collected within the size ranges list above, the following procedures should be followed with the data:

- Compile and enter the data into a spreadsheet and check for accuracy.
- Log_{10} transform the length (mm) and total Hg (ppm wet weight) data and plot the log (length) and log [Hg] relationship for each target species. Examine the plot for linearity and presence of outliers.
- Calculate linear regression equations for each relationship to determine the significance of the regression: *yes* ($p<0.05$) or *no* ($p>0.05$); intercept (a), slope (b) and the goodness of fit (r^2). An example of this procedure is illustrated in Figure 1.11, above. If the regression is **not** significant, then there is no relationship between mercury and fish size and it is not appropriate to apply any further statistical procedures. This is unlikely for carnivorous fish, especially in Hg contaminated environments. However, if this occurs, select another species.
- Graphical comparisons of the mercury data can be made where appropriate.
- The standardized Hg concentration can be determined from the linear relationship based on the standardized length.

If two or more data sets are gathered, analysis of covariance (ANCOVA) can be used to determine whether standardized Hg concentrations from upstream reference and downstream populations differ significantly from one another, or whether there are differences in standardized Hg concentrations among target species. ANCOVA allows for unbiased comparisons of concentrations to be made at a common size (i.e. the standardized length). The first test of covariance is for equality of slopes among groups. ANCOVA compares the linear regression relationships for log [Hg] and log [length] for a particular species between years or sites. If the slopes (i.e. the rate of Hg accumulation averaged over the entire size range of fish being tested) are ***not*** significantly different from each another ($p>0.05$), then one is justified in proceeding to the next step, which is to test whether differences in standardized mean Hg concentrations are significantly different between groups of fish being tested. An example of this procedure is illustrated in Fig. 1.14.

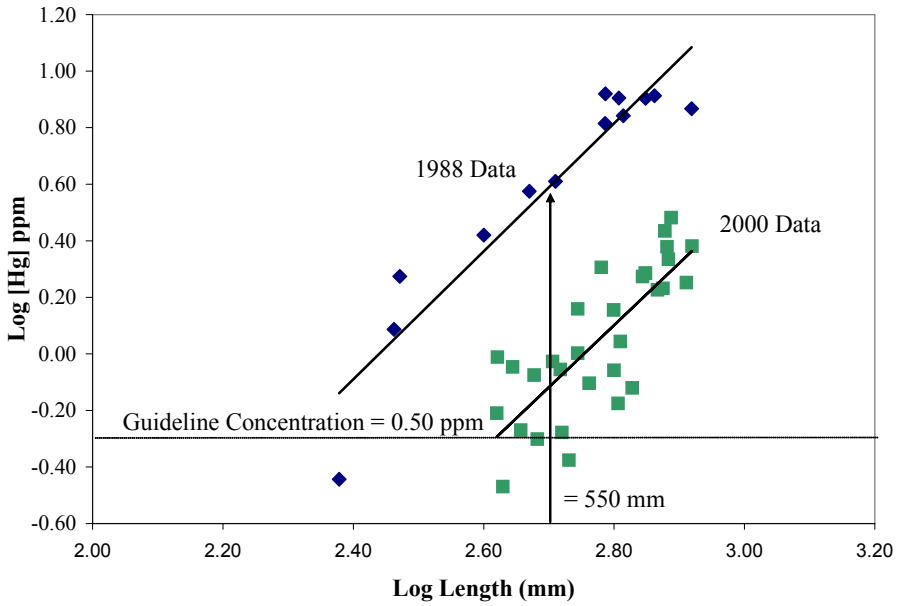


Fig. 1.14. Example of annual variation of Hg in fish of a standardized size.

In instances where standardized Hg concentrations are being compared among several areas or over time, a pair-wise comparison (e.g. Tukey's test) is used to determine whether Hg concentrations among the different sites being tested are significantly higher or lower.

If slopes are significantly different (p<0.05) from each other, then the relationship between the rate of Hg accumulation and fish size is **not** consistent between the two populations, and one is generally **not** justified in comparing differences in intercepts or standardized mean Hg concentrations. However, depending on the degree and nature of the slope differences, some qualified comparisons can be made. An example of how data from multiple populations, or in this case from different years, can be compared is illustrated in Fig. 1.14. By employing this simple data collection protocol and analytical procedure, differences in fish Hg concentrations can be accurately and quantitatively compared, unbiased by differences in fish size, either among geographic locations, over time, or between target-species of a similar size. This approach will also provide for precise human Health Assessments of Hg exposure.

It is important that the above protocol for deriving Hg-length relationships for all species is followed, regardless of whether the sampling is for the human Health Assessment, or for characterizing Hg bioavailability. The procedure is simple to follow and provides a quantitative, strategic approach to fish sampling. When sampling small fish for environmental hotspot identification, simply use smaller length ranges (e.g. by 50 mm size intervals) from which to collect fish, and to derive a smaller standardized size.

In instances where there are few large, carnivorous fish present (e.g., in small streams), but small fish <10 cm are abundant, fish of this size can be used to determine the magnitude of Hg bioavailability at suspected hotspots. Here, a similar, but simpler protocol can be followed. Small fish of the same or closely related species (i.e., with similar diet) should be sorted into two or three size categories (e.g., <4 cm; 4 – 7 cm; > 7cm). Quite often it is obvious where natural differences in size modes occur, which may be related to fish age (e.g., young-of-the-year, age 1, age 2). Within each size category, up to 5 fish should be composited to comprise a single sample. A minimum of 5 samples from each size class should be analyzed for total Hg from the suspected hotspot(s) area and reference area. Fish should be analyzed whole, without removing skin or body parts. Simple statistics (e.g. t-test or ANOVA) will indicate whether Hg concentrations in small fish from mining and environmental hotspots differ significantly from Hg concentrations of reference area fish, and if Hg biomagnification with increasing fish size is occurring.

It may be difficult to identify individual species in taxonomically rich or diverse streams such as in Brazil or Africa. Therefore, it is not absolutely necessary to sample the same species for human health or environmental hotspot assessment, provided that the diet of each species sampled is similar. For example, for top-level predator fish, if it is difficult to capture sufficient numbers from each species, different but closely-related species such as cichlids or piranha may be grouped together. However, it is very important that the grouped species have a very similar diet and life history strategy (e.g. small movements, similar growth and size). Ideally, a sufficient sample size should be collected from each target species according to the procedures described above.

Preserving Fish Samples

The most common procedure to preserve and transport samples of fish and other organisms is by freezing. Just after sampling, tissue samples should be placed on ice and/or directly frozen. An easy way to transport samples is adding dry ice to a thermal bottle or a cooler. If the samples must be transported by air, it is best to consult the airline first, as some companies do not allow the transportation of dry ice. An alternate procedure for preserving fish skeletal muscle samples collected in ASM impacted areas in French Guyana is described by A. Boudou and R. Maury-Brachet (2004 - Laboratory of Ecophysiology and Ecotoxicology of Aquatic Systems, Univ. Bordeaux 1/CNRS, personal communication). A 3x3 cm filet is taken from the fish at the middle-superior part of the body. Individual tissue samples are stored in plastic vials containing 30mL of a 10% formaldehyde solution. No refrigeration is required. These researchers compared the Hg results of fish (*Hoplias aimara*) tissues stored to -20°C with those preserved in formaldehyde. After 3 months of preservation and under different temperature conditions (20°C and 35°C), no Hg was detected in the formaldehyde solution and no significant difference was found between Hg concentration in frozen and formaldehyde preserved tissue samples ([Hg_{total}] dry weight). Caution should be taken when handling formaldehyde; inhalation and contact with skin should be avoided. Full strength formaldehyde cannot be transported by airlines because it is flammable and a hazardous substance.

Another possibility is to dry fish samples. Drying is easy and has been practiced for centuries. In the field, in tropical countries, leave the samples under the sun, protected from insects. Results of Hg content in fish should be according to dry weight and back-calculated to wet weight, based on moisture content. If the original moisture content of the species is not known, we advise using a moisture content of 78%, which is typical.

Bioassays

Bioassays, using fish as bioindicators, can also be used to assess bioavailability of a toxicant. Toxicity tests evaluate acute, sub-chronic and chronic exposures; and measure biological endpoints such as mortality, reproductive performance, growth and behavioral changes. Such tests can also assess the relative toxicity of a mixture of chemicals, by taking into account synergistic or antagonistic interactions among chemicals (SCOPE, 1995). Mortality is the most commonly used endpoint. The classical approach for evaluating acute toxicity is through the determination of the concentration of a specific substance at which 50% of the test-organism population dies (LC50). Although most protocols address metals and other substances in various aquatic systems, few methods were developed to evaluate bioavailability. The exception is the method developed by Environment Canada to establish guidelines for mining effluents using LC50 bioassay of rainbow trout. All methodologies, however, recognize that the toxicity of metals in aquatic systems is inherently linked with bioavailability, a factor that is controlled by metal speciation (Sandoval *et al*, 2001).

Bioassays can also provide information about bioaccumulation of pollutants over time. Comparatively few aquatic bioindicators have been developed to assess sub-lethal toxicity. Established protocols for the evaluation of toxicity of anthropogenic organic compounds using fresh water aquatic biota include methods for amphipods (*Hyalella azteca*) (USEPA, 1994-June) and inland silversides (*Menidia beryllina*) (USEPA, 1994-July), which use survival, growth and reproductive capacity as endpoints. African clawed frogs/FETAX (*Xenopus laevis*) (ASTM, 1997) are also used to evaluate developmental effects at early life stages. Considerably fewer protocols use bioaccumulation as an endpoint. Species like polychaetes (*Nereis virens)* (Chapman *et al*, 1995) and oligochaetes (*Lumbriculus variegates)* (USEPA, 1994-July), which are well known bioindicators that can be easily cultured, are not native to most environments. Established sub-lethal protocols have been developed for aquatic organisms, including brook trout *(Salvelinus fontinalis),* fathead and sheepshead minnow full life cycle, as well as *Daphnia magna, Ceriodaphnia dubia,* zebrafish *(Brachydanio rerio),* and mysid shrimp. Another protocol developed by Environment Canada (1992) suggests the use of early life stage salmonid fish to assess sub-acute toxicity.

Adverse effects can be generated even when sub-clinical symptoms are not evident. As many adverse effects are quite difficult to measure or quantify, few methodologies or protocols have been established. Moreover, it is becoming apparent that even at relatively low exposure levels, namely those at which organisms may show no apparent signs of stress or disease, a multitude of effects may develop long after the period of exposure and even in subsequent generations (Moore *et al*, 1997).

Laboratory methods to study bioavailability (non-lethal effects) of Hg from sediments or effluents to aquatic organisms are expensive and demand long and tedious work. For example, as aquatic organisms must be kept exposed to consistent conditions, aquarium water must be changed constantly and other permanent cares are needed. The duration of typical toxicity tests with aqueous samples ranges from four days for acute effects with an endpoint of mortality to 7 to 30 days for chronic and sub-chronic effects on survival, growth or reproduction. Life-cycle tests are also needed but are not frequently used because the duration is long and the cost is high. Test durations to assess sediment toxicity typically range from 10 days or less for acute effects to 30 days for chronic effects. Test endpoints may include survival, reproduction or emergence (SCOPE, 1995). Establishment of endpoints is a controversial issue. For

neurotoxins such as Hg compounds, animal research is problematic as it is hard to evaluate behavioral changes in the absence of visible damage to the animal's nervous system. Reliance on behavioral tests also makes it more difficult to correlate results from one study to another and translate those results to affects on human beings (ICME, 1994).

A more involved approach to study bioaccumulation is to conduct *in situ* bioassays such as stream cage studies. These studies typically involve fish or benthic macroinvertebrates enclosed by a cage, which is either attached to the substrate suspended in the pelagic zone or floating. These tests have the advantage of providing more "real-world" conditions; that is, contaminant accumulation proceeds at its normal rate (i.e. impacted by biotransformation and other fate processes) under normal temperature, light and other exposure parameters. However, the advantages to this type of study are also some of its disadvantages. Many variables cannot be controlled (e.g. pollution slugs, extremely high tides or flows, temperature, light, and food availability). These make the test a more reliable estimator of the real world, but also add covariates that in turn make the data more difficult to interpret. The potential for escape of test organisms or for a predator to enter the test enclosure somehow is also present. The logistics and costs of these studies also may be quite high (SCOPE, 1995)

A study of bioavailability was conducted by CETEM (1991) in Poconé, Brazil. Freshwater mollusks and fish from uncontaminated areas were moved to Hg-contaminated sediments containing approximately 2 ppm Hg. The organisms were subjected to the same natural conditions as the aquatic environments from where they were collected, except for the high Hg content in the sediments. After 60 days in cages, the organisms incorporated low or almost no Hg. The presence of iron oxides in these sediments likely explained the low bioavailability. The main criticism of caged fish studies is that these fish normally are subjected to temperature stress, dietary differences and other factors that would tend to bias the findings. Results of caged bioassays should only be compared with data obtained under similar circumstances from reference areas. However, *in situ* bioassays do provide more relevant data about the real impact of the effluent than laboratorial bioassays (Robertson, 1990).

The La Salle Foundation in Venezuela built a floating laboratory in the Macagua hydroelectric reservoir to study the effect of mercury-contaminated sediments from the Caroni River on detritivorous fish (*Geophagus* sp.). In 30 fiberglass water tanks of 200 L each, 20 kg of sediment spiked with 2 grams of metallic Hg were placed in each tank. Solar-powered pumps kept the dark water from the Caroni River circulating through the tanks at 2 L/min. Fifteen fish and fifteen aquatic snails were distributed in the tanks. After 60 days, they were sacrificed and Hg in their tissues was analyzed. Algae, grown on sediments, was used as a food source by the test animals. Most natural conditions were reproduced, except for the shallow depth. This scenario represents worst-case conditions, since aeration of the contaminated sediments enhances the reaction between soluble organic acids of the dark waters and metallic Hg from the sediments. This ingenious experiment was repeated, adding organic matter and iron oxides to observe variations in Hg bioavailability (Luis Perez, 2003 – Fundacion La Salle, personal communication).

When conducting a **health risk assessment**, the best bioindicator of MeHg exposure is fish acquired directly from local fishermen or in fish markets. In this case, the edible parts are analyzed. Then, a detailed questionnaire is administered about population diet, gender, age, weight, habits, type and quantities of fish consumed. These points are discussed in detail in the Health Assessment part of this protocol.

1.5.3. Using Invertebrates to Assess Bioavailability

Metals bioavailability in terrestrial and aquatic systems is dependent upon a number of geochemical and biological factors. These include organism physiology, internal solubilization capabilities, food quality and feeding behaviour. Studies using bioindicators of metal availability may be more revealing than geochemical methods alone. Invertebrates are useful bioindicators because they are simple, well-studied creatures that can provide indications of bioavailability in a short time frame at relatively low cost.

Baker and Allard (2002) examined total and MeHg concentrations and ratios in benthic invertebrates (chironomid larvae and bivalve clams) to determine environmental hotspots in Pinchi Lake, a mining Hg contaminated lake in northern British Columbia, Canada. Analyses of Hg and MeHg were conducted in invertebrates sieved from sediments from areas that had received Hg-rich roasted ore (calcines) and areas away from the calcine sediments that were suspected of being methylating environments. The objective was to determine the spatial pattern of MeHg production and bioaccumulation in benthic invertebrates to identify environmental hotspots and areas of potential remediation. The authors demonstrated that the calcine rich sediment was the greatest net producer of MeHg in benthic biota, relative to non-calcine sediments. These sediments were targeted for potential remediation to eliminate these environmental hotspots.

Mercury concentrations are higher in sediment feeders than in plant feeders. The contribution of MeHg to total Hg varies considerably more in invertebrates than in fish. Explanation of this variability lies in the relative paucity of data, analytical difficulties, effects of both surface and gut contamination when whole animals are analyzed, and the relative slow elimination rate of MeHg by fish (Huckabee *et al*, 1979). Tremblay (1999), analyzing aquatic insects from natural

boreal lakes in Canada, reported that the mean MeHg proportion to total Hg concentration depends on the feeding behavior of the animals, increasing from 35-45% in detritivorous insect larvae to 70-85% in predator insects.

Larvae of Ephemeroptera (*Hexagenia rigida*) assayed in aquaria showed an accumulation capacity of MeHg 50 to 80 times greater than for $HgCl_2$. Accumulation occurred rapidly. After one week of exposure to contaminated sediments, Hg accumulated reached a plateau in the organisms, with only small increments in Hg observed within longer time of exposure. When the water column was the Hg source, transfers of metal increased in acid conditions, for inorganic and organic forms. For the inorganic compound, 80% of Hg was localized in the intestines (Saouter *et al*, 1989). Crayfish were used as an efficient bioindicator. Results indicate that half-life[9] of Hg in crayfish muscles is about 2 days, demonstrating that Hg is more labile in invertebrates than in fish (Wright *et al*, 1991).

Substantial evidence indicates that earthworms accumulate heavy metals from polluted soils and other media (Ireland, 1983; Neuhauser *et al*, 1985; Goats and Edwards, 1988; Edwards, 1996). Earthworms are particularly suitable for the assessment of contaminant bioavailability for a number of reasons. They ingest large quantities of soil and are in full contact with the substrate they consume. They constitute up to 92% of the invertebrate biomass of soils and participate in many food chains, acting as a food source for a wide variety of organisms including birds, fish, insects, various mammals and reptiles (Ireland, 1983; ASTM, E1676-95). In addition, they are bred easily, have been studied extensively and are approved for use in toxicity testing by the USEPA, the European Union, and the Organization for Economic Cooperation and Development. Some studies have documented Hg concentrations in earthworm tissues (Martin and Coughtrey, 1982; Rhett *et al*, 1988; Braunschweiler, 1995).

Ideally, invertebrates found in the contaminated sites are good indicators of Hg bioavailability in sediment. However, it can be difficult to sample native invertebrates. A simple laboratory methodology developed by Sandoval *et al* (2001) uses "lab-grown" earthworms to evaluate the bioavailability of heavy metals from mining effluents. Bioavailability is based on tissue concentration, which is determined by exposing earthworms in a laboratory to contaminated soils, sediments or effluents for an established period. The methodology is summarized as follows and described in greater detail below:

- Mix 80g of tailings, sediments or clean sand (for solution assessments) with 20g of prepared "cellulose" and 80 mL of distilled water (in solids evaluation) or solution (effluent solution) and homogenize manually in a 900 mL glass jar (45% moisture content). This will form the substrate where the worms will live. It is important to control moisture (not too dry, not too wet).
- "Cellulose" can be prepared by shredding dry towel paper in a blender.
- Place 15 cleaned, weighed worms (*Eisenia foetida*) in the mixture for a period of 28 days.
- Duplicate or triplicate the jars for statistical evaluation of the results.
- Cover the jars with perforated paper or plastic. Do not open too many or too large holes whereby the worms can escape.
- Use a jar with worms, "cellulose" and clean sand as a "blank" reference jar.
- At the conclusion of the exposure period, remove and count the worms, clean and depurate them to void contents of the stomach and intestinal tract. Leave them in a jar with a mixture of 15g of clean "cellulose" and 50g of silica saturated with 50 mL of distilled water for a period of 5 days. The worms need clean silica to "crush" the "cellulose". These worms must starve to void their gut contents for 24 hours before analysis. Depuration is necessary to ensure that subsequent whole worm analysis is indicative of Hg levels in tissues and not residual particles retained in the intestinal tract.
- Wash depurated worms, weigh and digest in 20 mL nitric acid (0.7 M) for total Hg analysis or full metals scan.
- Analyze Hg (and also other metals if desired) concentrations in soil, clean sand and paper added to the jars for comparison with worm tissues and to obtain the Hg concentration in the substrate (knowing the proportion of soil and paper added). Comparison can also be done with Hg in tissues of worms from "blank" jars.
- Compare concentrations accumulated in tissues to earthworms in the media (substrate) in which they reside or consume.

Hinton (2002) applied this procedure to evaluate the bioavailability of Hg from a large area contaminated with amalgamation tailings in Cachoeira do Piriá, Pará State, Brazil. Once mining hotspots were identified based on Hg concentration in sediment, worms were exposed to the contaminated samples to establish bioavailability. Results (Fig. 1.15) indicated that there was no correlation between total Hg in contaminated sediment (jar substrate) and total Hg accumulated in worm tissues after 28 days of exposure. Using the jar procedure, areas with high Hg bioavailability (environmental hotspots) were indicated. Wetlands or densely vegetated areas (i.e. those with organic matter) showed greatest bioavailability. This study indicated that the earthworm method holds promise to confirm locations of

[9] Half life is the time required to eliminate 50% of the pollutant from an organism.

environmental hotspots. In spite of requiring total Hg analysis, the method is easy to operate, reproducible and appropriate in developing countries.

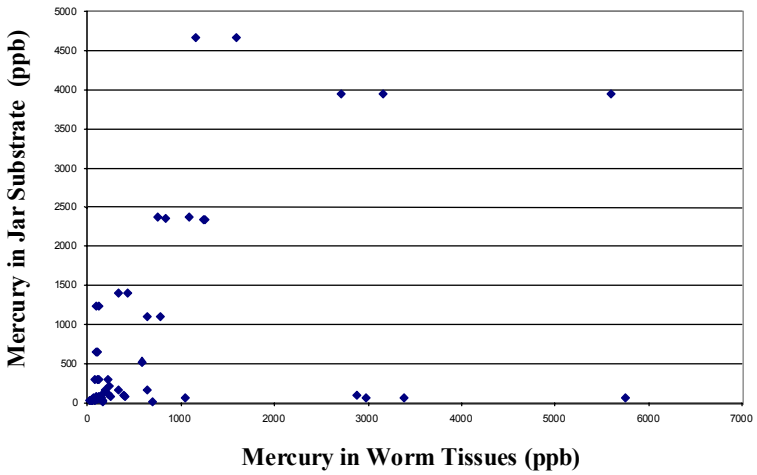


Fig. 1.15. Mercury in worm tissues
versus Hg in contaminated sediments (substrate) (Hinton, 2002).

Sampling Aquatic Invertebrates

In addition to the above, aquatic invertebrates can be sampled directly and analyzed for total and/or MeHg from suspected environmental hotspots. This will provide confirmation of areas suspected of being significant contributors of MeHg to biota at the base of the food web, which is transferred eventually to fish. However, it can be difficult and time-consuming to collect sufficient quantities of insects for analysis of mercury and in particular MeHg, despite the greater expense of MeHg analysis. In areas where clams are abundant, this group should be sampled because they are resident, long-lived and integrate Hg in water and in sediments over time. Notwithstanding the need for a biologist experienced with the collection methodologies and taxonomy during field sampling and additional cost of MeHg analysis, using benthic invertebrates as indicators of methylation and food chain bioaccumulation can provide extremely useful information.

Three major groups of aquatic invertebrates should be targeted for sampling: bivalve clams, gastropod snails and bottom dwelling larvae of aquatic insect groups (e.g. chironomids, stoneflies, mayflies, caddisflies). Bivalve clams are relatively large organisms that can be sampled easily, provided one knows where to look for them. Where possible, local people familiar with harvesting clams should be used to assist in the collection. Clams can be collected by digging in the sand along shorelines or by using a rake with a long handle. From the shoreline or a small boat, a rake can be dragged over the sediment surface until one or more clams are contacted and retained by the rake. Carefully pull the rake to the surface and place the clams in a bucket with site water. Approximately 5 to 10 clams per site are required for analysis. Depurate the clams in the bucket with clean water for at least 24 hours, or alternatively, remove the stomach containing sediment, as it may be contaminated with Hg.

To process the clams, the adductor muscle should be severed with a clean, stainless steel knife and the whole clam tissue excised from the shell. If the clam has not been depurated, remove the stomach with the knife and discard. Weigh the remaining tissue (g wet weight) and place in a small plastic bag. Label the bag on the outside and place a small label on the inside of the bag. Refrigerate or place on ice and freeze as soon as possible. Whole clam tissue should be homogenized in the laboratory and analyzed for total mercury and MeHg. The proportion of MeHg to total Hg and the magnitude of the MeHg concentration, relative to the reference or control area will provide an indication of the relative degree of mercury methylation within discrete areas or environmental hotspots.

Snails, if one knows where to find them, can simply be gathered from discrete areas. Several snails (5 – 7) should be composited to form a single sample. Record the location and weight (g) of snails collected from each composite sample. Keep them refrigerated and freeze them as soon as possible. Transport frozen. The entire snail from each composite should be homogenized and analyzed for Hg in the laboratory.

To collect benthic invertebrates, use a sediment grab sampler (e.g. Ponar, Ekman) to collect sediment from depositional areas in streams flowing out of suspected environmental hotspots. Remove the top 4 – 5 cm of sediment from the surface of the grab and sieve it through a 500 µm stainless steel sieve to remove fine sediments. The remaining sediment material and sieved benthos is placed in a glass sorting tray and individual invertebrates are picked from the tray using plastic or stainless steel tweezers and placed in clean water. A minimum of 100 mg of tissue is required for

mercury analysis. Organisms should be rinsed in clean water, placed in small-labeled plastic vials, placed on ice and frozen as quickly as possible (see fish preservation chapter above).

The same procedures to collect and process invertebrates should be used at suspected environmental hotspots as well as reference areas. If invertebrates cannot be collected then, small fish can also be used to identify environmental hotspots as described above in Section 1.5.2. Small, bottom-feeding fish typically reside within relatively small areas. They integrate sediment/dietary mercury and provide direct evidence of bioavailability. These fish are a step up in the food chain, are preyed upon by larger fish and are analytically advantageous because tissue samples need only be analyzed for total mercury. Consequently, environmental hotspots and mercury bioavailability can be assessed using resident benthic invertebrates and fish, provided that a sufficient number of invertebrate and fish samples are collected and compared to upstream or reference areas.

1.5.4. Taxonomic Richness and Abundance to Assess Bioavailability

There is no simple protocol for correlating taxonomic richness (i.e. species diversity) or abundance of benthic invertebrates with Hg bioavailability. In areas of high Hg methylation (and therefore, bioavailability), certain groups of carnivorous benthic invertebrates such as stoneflies, mayflies, certain chironomid species, and bivalve clams, will have higher total and MeHg concentrations than these same groups in sediments uncontaminated with Hg. These taxa can be used as indicators of methylation potential by identifying environmental hotspots, as discussed above. In addition, environments with greater species diversity and more complex and longer food chains will also result in greater MeHg concentrations in resident biota.

In theory, Hg-contaminated sediments might have a poorer benthic community or have a different community structure than uncontaminated sediments. Baker and Allard (2002) studied the effects of Hg on invertebrates living in highly contaminated "mining hotspots" in Pinchi Lake, Canada. Despite very high Hg (up to 850 μg/g) and MeHg concentrations (up to 0.05 μg/g) in sediment, the benthic community appeared healthy and community structure in contaminated sediments did not differ relative to community structure in uncontaminated sediments. The vast majority of Hg present in the sediments was not in an available chemical form (i.e. mostly cinnabar) that could be taken up by biota. Therefore, there was no correlation between sediment Hg concentration and degree of impact on taxonomic richness or abundance. Without intimate understanding of benthic community structure between suspected hotspots and reference areas or knowledge of the chemical form of Hg present in the sediments, determining hotspot location or magnitude of effects based on abundance and diversity of the benthic community would be extremely difficult.

1.5.5. Using Physico-chemical Variables to Assess Bioavailability

Many studies have attempted to find correlations between environmental factors and Hg bioaccumulation (Håkanson *et al*, 1988; Lindqvist *et al*, 1991). In fact, the search for parameters to predict bioaccumulation has always focused on finding a simple way to monitor and control Hg bioaccumulation. Unfortunately, exact equations are not obtained, even though the effect of each separate variable on Hg bioaccumulation is relatively well established. This suggests that there are too many "unknowns" and site-specific conditions to produce satisfactory models. Frequently these parameters do not directly correlate with bioaccumulation due to internal interactions between them, which result in synergistic and antagonistic effects. However, the effect of some natural variables on the bioaccumulation process is known, particularly those environmental parameters related to mercury speciation. Parameters such as environmental physico-chemical characteristics (pH, Eh, dissolved oxygen, etc), and the presence and abundance of competing ions and methylating agents significantly influence the speciation, and thus bioavailability of metals in natural waters, sediment, and soil (Parametrix, 1995).

Biota analysis is the ultimate evidence of Hg bioavailability. However, this analytical facility is not always available in all countries with ASM problems. For this reason, Veiga and Meech (1995b) developed a computer system HgEx to determine the likelihood of Hg bioaccumulation, based on observations and/or analysis of the physico-chemical variables such as humosity (water color), sediment color, pH, Eh, conductivity, biomass, etc. The influence of some of these parameters is discussed as follows.

Humosity

Several studies have shown that total mercury and MeHg concentration in water, sediment or fish is positively correlated with dissolved organic carbon (DOC) concentrations in water (Driscoll *et al*, 1994; Mierle and Ingram, 1991; Cid de Souza and Anjos, 2002). In surface waters, TOC concentrations are generally less than 10 mg/L, and in groundwater less than 2 mg/L, unless the water receives wastes or is highly-colored due to natural organic material (e.g. dark water rivers) (Moore, 1998). Mercury bioavailability increases as dissolved organic carbon increases in drainages (Nilsson and Håkanson, 1992; Driscoll *et al*, 1994; Mierle and Ingram, 1991). Wetlands are widely recognized as significant sources of MeHg to other water bodies (Rudd, 1995), in part due to high microbial activity (Kelly *et al*, 1995) and the presence of organic acids. Water color (brown) is well correlated with organic matter content in water.

Fish caught in darkwaters of the Amazon region are almost always higher in Hg than fish from whitewater rivers (CETEM, 1992; GEDEBAM, 1992).

Water Conductivity

Low water conductivity has been correlated with high Hg content in fish (Björnberg *et al*, 1988; Håkanson *et al*, 1988). As conductivity is related to calcium content in water, the influence of calcium is suggested. Low calcium waters increase the permeability of biological membranes (such as gills). Thus, in low conductivity waters, Hg species are more easily incorporated into fish via respiration than in high conductivity waters (Spry and Wiener, 1991). Boening (2000) report several studies where methylmercury uptake by fish was lower in hard water than in soft waters. Low conductivity (<20 μS/cm) is typical of darkwater rivers as evidenced in the Caroni River, Venezuela (Bermudez *et al*, 1994.).

Sediment pH

The effect of pH on Hg bioaccumulation is complex. Field and laboratory observations have shown more Hg accumulated into fish living in acidic waters (Beijer and Jernelov, 1979; Verta, 1986; Stokes and Wren, 1987; Lindqvist *et al*, 1991; Ponce and Bloom, 1991; Boening, 2000). A decrease in pH of one or two units doubled the amount of MeHg released from sediment into the overlying water (Miller and Akagi, 1979). Low pH can influence Hg uptake due to a) factors influencing bacterial processes and b) factors influencing geochemical processes (Richman *et al*, 1988).

Sediment Eh

Redox conditions of interstitial water are important in determining the stability of Hg° over Hg-organic complexes (i.e. whether metallic Hg can form complexes with organic matter with the influence of dissolved oxygen as an electron donor). Redox conditions also have a demonstrated effect on the generation of MeHg as this can be produced at the interface between oxic surface waters and the anoxic hypolimnetic water (cold, low oxygen bottom waters of lakes). Sulfate reduction and abundant microbial activity have also been identified at this boundary (Watras *et al*, 1998; Matilainen, 1995). Supply of Hg(II) species to SRB (sulfate reducing bacteria) is key for MeHg generation at the oxic/anoxic boundary (Hinton, 2002).

Biomass

Fish in more productive systems have been found to contain lesser amounts of Hg than in low productivity waters. Higher growth rates of individual fish dilute Hg concentrations in tissue (D'Itri, 1990; Mannio *et al*, 1986). Low biomass productivity is observed in darkwater bodies.

Temperature

There is some controversy about the influence of environmental temperature in Hg bioavailability and bioaccumulation. Temperature can increase the microbial production of MeHg as well as the metabolic rates of fish and Hg uptake. However, it was observed that the biological half-life of Hg in fish decreases with increasing temperature, i.e. excretion is accelerated. Therefore, fish from watercourses in which the temperature reaches 20°C can be expected to eliminate Hg approximately twice as fast as fish in water of about 10°C. (Spry and Wiener, 1991; D'Itri, 1990).

1.5.6. Using Humans to Assess Bioavailability

Intuitively, the best bioindicators for Hg bioavailability are humans. However, there are ethical issues associated with collecting biological samples from individuals. Alberto Rogerio B. Silva, former director of the Secretariat of Industry, Commerce and Mining in the State of Pará, gathered results of 8,333 samples of sediment, water and biological tissues from at least 30 research institutes around the world (Fig. 1.16). The Amazon region has been used as a living laboratory by academic researchers. In many monitoring programs, humans are seen merely as donors of hair, blood, or urine samples. In most cases, affected people never learn the results of the monitoring program. The goal of sampling biological materials should be explained clearly to participants. It is important to stress that the samples will not be used for anything else except mercury analysis. Volunteers must sign an agreement to donate samples. The application of **local ethical guidelines** and the requirement of recommendation by local ethical board should be considered.

There are three primary media taken from humans to gauge Hg exposure and bioaccumulation: urine, blood, and hair. Detailed descriptions of the value of each of these media in determining health effects are described below.

Mercury bioavailability in humans is monitored by examining:

- Hg in urine, especially from high intensity exposure, such as from Hg vapour exposure during amalgam burning.
- Hg and MeHg in hair, which is a useful indicator of long-term exposure to MeHg contamination, particularly from ingestion of Hg contaminated fish.

- Hg in blood as a further indicator of recent or current exposure, particularly from exposure to Hg vapours or high fish ingestion. While mercury in urine may correlate with long-term exposure (GEDEBAM, 1992; Wilhelm, 1996), blood analysis gives a combined picture of both metallic and MeHg contamination.

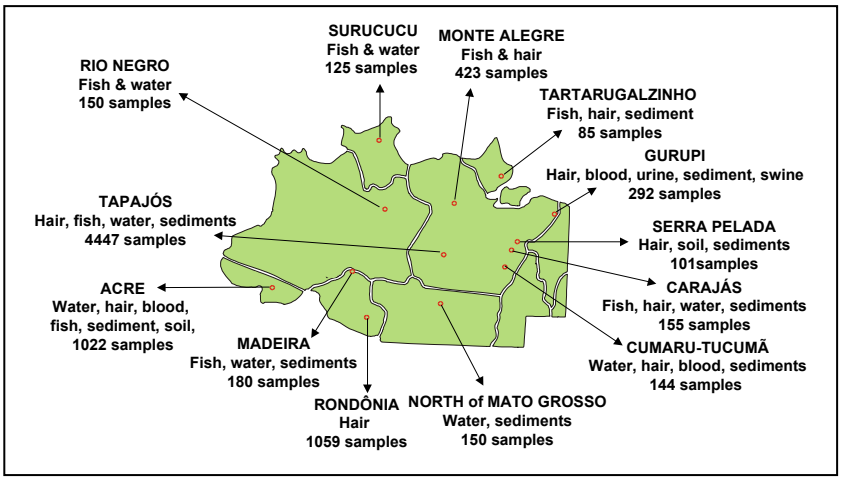


Fig. 1.16. Distribution of samples collected in the Amazon region for Hg monitoring (Silva, 2001).

Urine

Tsuji *et al* (2003) evaluated ten studies reporting paired air and urine Hg data and obtained a strong correlation between both media at medium and high concentrations. At air concentrations below 10 µg/m³, the authors concluded that the concentration of Hg in urine was indistinguishable from background levels. The WHO (1991a) described a relationship between Hg in air (A) in µg/m³ and in urine of exposed workers (U) expressed as µg/g creatinine: U = 10.2 + 1.01 A. Thus, a person exposed to about 40 µg/m³ of Hg in air should show levels of Hg in urine around 50 µg/g creatinine. This is the maximum urine Hg concentration recommended by WHO (1991a).

Drake *et al* (2001) found significant correlation between Hg in air from 0.1 to 6,315 µg/m³ and urine mercury levels from 2.5 to 912 µg/g of creatinine in gold miners from a Venezuelan mining site.

In order to compare Hg levels from different individuals, urine values should be corrected for grams of creatinine in the sample and should be expressed as µg Hg/gram creatinine. If urine is very dilute (relative density <1.010), interpretation of the results may be difficult. In persons not occupationally exposed to mercury, urine levels rarely exceed 5 µg Hg /g creatinine (Childress *et al*, 1996).

Miners who frequently burn amalgams in open pans show Hg levels in urine above 20 µg/L (Fig. 1.17). The WHO (1990) considers 4 µg/L as a normal Hg level in urine. Drasch *et al* (2002) consider the Hg level in urine of 5 µg/g creatinine an ***alert*** value and 20 µg/g creatinine as an ***action*** level (for health effects consult Chapter 2). Malm *et al* (1995a) analyzed values as high as 1,168 µg/L in urine of gold shop workers working in confined environments. CETEM (1992) analyzed urine of employees in gold shops at Alta Floresta, Mato Grosso, Brazil. The city had 32 gold shops where 1 tonne of gold was bought and melted per month in fume hoods with no filters. As mentioned earlier, gold bullion usually has between 2 and 5% Hg which is released when the *doré* is melted (Farid *et al*, 1991). The five most important shops were chosen and 17 workers were sampled. About 200 mL of the first urine of the day were collected. The results showed an occupational intoxication of at least 13 individuals (>20 µg/L Hg).

One of the highest levels of Hg in urine was analyzed in workers of milling centers in El Callao, Venezuela, where miners amalgamate the whole ore using Cu-plates and burn amalgams in shovels very close to their noses (for visual inspection). About 14.6% of the workers had shown levels ranging from 1221 to 3260 µg Hg/g creatinine (UNIDO, 2004).

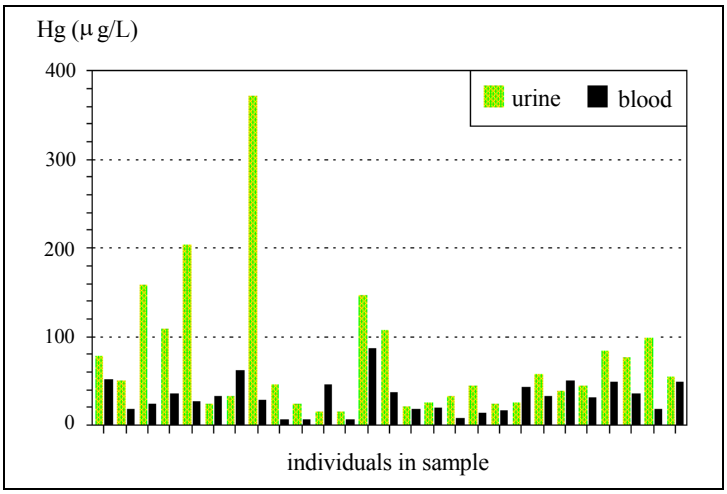


Fig. 1.17. Hg in blood and urine of workers burning amalgam daily (adapted from GEDEBAM, 1992).

A large number of studies have been dedicated to analyzing urine of miners burning or handling Hg. It seems obvious that somebody burning Hg in an open pan will accumulate Hg and exhibit high Hg concentration in urine. Studies dedicated to simply demonstrate this are pointless unless they are combined with medical (neurological) exams and solutions for miners to reduce Hg exposure and/or to remove Hg from their bodies. Urine analysis is more useful to evaluate Hg vapour exposure of those individuals not directly involved with the mining and amalgamation activities, such as employees and neighbors of gold-buying shops, as well as children and women living in mine sites, etc.

Sampling Urine

The ideal sample of urine is the first one in the morning. This analysis reflects Hg excreted by the body during the night. However, this collection is not always possible and spontaneous urine has been collected without dramatically affecting results (i.e. identification of undue exposure to Hg vapor; Drasch *et al*, 2001). Drinking large amounts of water a few hours before sample collection should be avoided, as this dilutes the urine samples.

When a high amount of cysteine or an oxidizing agent such as iodine (as used for radiological contrast) is present in urine, the Hg analysis can be difficult, especially reduction by stannous chloride (Nixon *et al*, 1999).

Drasch *et al* (2001) suggested acidifying the urine (sample of at least 10 mL) after sampling by adding several drops of 10% acetic acid. As a collecting recipient, the authors used small soft PVC-bottles (e.g. 20 to 50 mL) to reduce volume to be transported. The urine samples were stored cool at all times (around 4 °C) before being analyzed by CVAAS (cold vapour atomic absorption spectrometry) or CVAAS (cold vapour atomic fluorescence spectrometry). The authors described a method to analyze urine without pre-treatment. In this case, sodium-borohydride was used to reduce Hg in the analyzer. Nixon *et al* (1999) obtained compatible results of Hg in urine using CVAAS and ICP-MS (inductively coupled plasma mass spectrometry). They found that sample dilution with HCl and dichromate was effective at reducing ICP chamber contamination with Hg.

Creatinine in Urine

Mercury concentrations in urine should be corrected to the creatinine excretion. Creatinine is a breakdown product of creatine, which is an important constituent of muscle. By far, the most important source of energy inside cells is the high-energy phosphate bonds of the ATP molecule. The creatine molecule gradually degrades to creatinine with time. Creatinine is a waste product that cannot be used by cells for any constructive purpose. The daily production of creatine, and subsequently creatinine, depends on muscle mass, which fluctuates little in most normal people over long ranges of time. Creatinine is excreted from the body entirely by the kidneys. With normal kidney function, the serum (blood) creatinine level should remain constant and normal. Normal values are highly dependent on the age and lean body mass of the person the urine is being collected from. A healthy range for creatinine in spot urine is from 25 to 400 milligrams/deciliter (mg/dL) (Moran, 2003). Urine creatinine (24 hour sample) values may be quite variable and can range from 500 mg/day to 2,000 mg/day. The level of creatinine in a 24 h urine sample ranges from 8 to 22mg/dL/kg of body weight for children, from 11 to 20 mg/dL/kg b.w. for women and from 14 to 26 mg/dL/kg b.w. for men. So, a man weighing 70 kg has a normal level of 24-hour urine creatinine ranging from 980 mg/dL to 1,820 mg/dL. As the creatinine concentration is usually expressed in mg/dL, dividing the result by 100 the unit is transformed into g/L. The result of Hg in urine is usually expressed in µg Hg/L of urine. When this is divided by g/L of creatinine, the final result is expressed in µg Hg/g of creatinine.

There are many procedures to analyze creatinine, but the colorimetric procedure based on the Jaffé reaction is perhaps the most simple. An analytical kit is commercialized by Merck – Mercktest n. 3385 (Wilhelm *et al*, 1996). The color is analyzed in a spectrophotometer using the wavelength of 510 nm. The colorimetric analysis of creatinine is quite rudimentary when compared with mercury analysis. Some researchers do not consider results of creatinine below 0.3 mg/dL as this introduces large errors when the value of Hg concentration is divided by such a small number (Janice Yager, 2004 – EPRI, personal communication).

Proteinuria in Urine

Proteinuria is a condition in which urine contains an abnormal amount of protein. Normally, protein should not be detected in the urine. Proteinuria is a well-known symptom of an Hg-related effect in the kidneys. The test to detect it is inexpensive and can be carried out using a commercial kit (e.g. Teco diagnostics URS10). The test is based on the error-of-indicator principle. Test reagents are 0.3 % w/w tetrabromophenol blue, 99.7 % w/w buffer and non-reactive ingredients. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow ("Negative" reaction) to yellow-green and blue-green ("Positive" reaction). The test area is more sensitive to albumin than to globulin, hemoglobin, Bence-Jones proteins and mucoprotein. The test area is sensitive to 15 mg/dl albumin. The test strip is dipped into the native urine and the result is obtained after 1-2 minutes. The test is semi-quantitative. Possible results are 0, trace, 30, 100 and 300 mg Protein/dL of urine (S. Boese-O'Reilly, 2004 – Institute for Forensic Medicine, Ludwig-Maximilians University, Munich, personal communication)

Blood

Assessments of Hg concentrations in human blood and fish muscle suggest that a direct relationship exists between the two. Clarkson (1973) compiled results from several studies and showed that, for a 70 kg individual, Hg in blood (ppb) = 0.95 x Hg (mg) daily fish intake.

The impact of high Hg levels in fish to the riparian population in the Amazon region was studied in 1991-92 by an international team of Brazilian and British scientists who analyzed blood and urine of residents of Jacareacanga (Fig. 1.18), a community in the Tapajós River region (GEDEBAM, 1992). This area is situated 250 km upstream from ASM activities. Hg concentrations of blood considerably exceeded what is considered normal (6 to 12 µg/L; Krenkel, 1971). Theoretically, this poses a great health risk to indigenous people of this region. WHO (1991b) considers the normal mean concentration of total Hg in individuals with no consumption of fish with high concentrations of MeHg, between 5 to 10 µg/L.

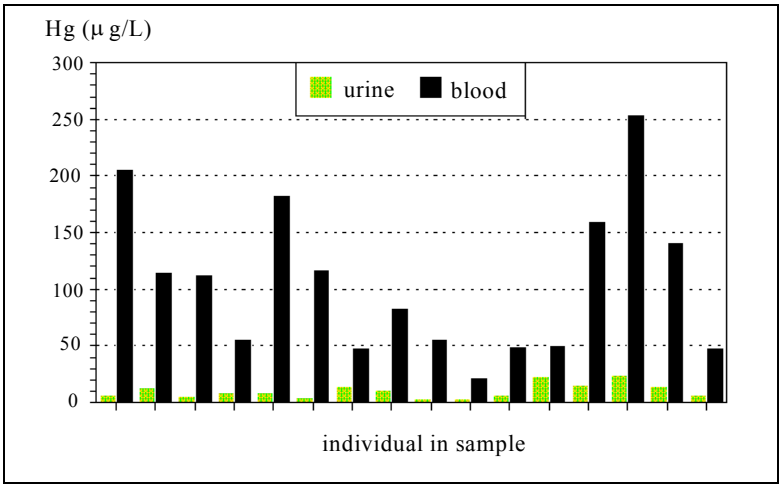


Fig. 1.18. Hg in blood and urine of fish-eating people from Jacareacanga (adapted from GEDEBAM, 1992).

As blood is difficult to sample, preserve, and transport, most researchers prefer to analyze hair in communities not directly exposed to Hg vapours (Brabo *et al*, 2000; Santos *et al*, 2000; Santos *et al*, 2002; Campos *et al*, 2002). In mining sites where individuals are exposed to both Hg vapours and MeHg from fish ingestion, blood analysis provides useful information (Drasch *et al*, 2001).

Sampling Blood

Drasch *et al* (2001) suggested that 10 mL of blood were enough for Hg analysis. In this case, the authors used EDTA-coated vials that were stored at 4 °C (NOT frozen) in a refrigerator. Sealed vials with blood samples can be stored under these conditions for months without a relevant change in Hg concentration. Other procedures include sampling of 7 mL of blood using Hg-free vacutainers containing sodium (or lithium) heparin as anticoagulant (Dolbec *et al*, 2000). Heparinized vacutainers are available commercially from most laboratory suppliers.

Hair

Mercury in hair from the scalp is a good indicator of MeHg exposure (Malm *et al*, 1995b). Hair grows about 1cm per month, excretes MeHg during its formation and shows a good correlation with blood Hg levels. Although hair analysis is affected by external factors, such as use of dyes and Hg° vapour exposure, the simplicity of sampling and analysis make it an amenable indicator for toxicological assessment of MeHg exposure (Malm, 1991).

Swedish individuals exhibit a direct relationship between Hg in blood and hair. The WHO (1990) derived several relationships between mg/kg (ppm) of mercury in hair (H) and µg/L (ppb) of mercury in blood (B) based on research from different regions of the world. Total Hg in hair is about 250 to 300 times higher than blood. Using the Japanese relationship of H = 0.25 B, a correlation between Hg in hair in ppm (H), mass of fish consumed daily in grams (W_f), and Hg concentration in fish in ppm (F) is approximately: $H = 0.2375 \times W_f \times F$. A relationship derived by a Japanese study of 765 people obtained somewhat different results: $H = 0.167 \times W_f \times F$ (Kojima and Araki, 1972). For example, in riparian populations of the Amazon, a 70-kg person was observed to consume an average of 200 g of "non-contaminated" fish on a daily basis. This fish contained an average of 0.3 ppm Hg (Barbosa *et al*, 1995; Castilhos and Bidone, 1999). As such, the person would be expected to have a daily intake of 0.86 µg of Hg per kilogram of body weight and show around 11 ppm of Hg in hair samples. This is almost 9 times the recent American Reference Dose[10] of 0.1 µg/kg bw (UNEP, 2002). This is clearly an approximation, since many site-specific variables must be taken into account.

In some ASM impacted communities, the burden of Hg in inhabitants is a mixture of contamination from vapour and from fish consumption. Using MeHg analysis, Akagi *et al* (2000), analyzing hair and blood of 162 schoolchildren from Apokon, Davao del Norte, Philippines, were able to differentiate between the influence of Hg vapour due to gold processing and MeHg from fish ingestion. The authors found that the portion of MeHg in hair ranged from 30 to 99%. Using the US Center for Disease Control guideline level of Hg in blood of 7.5 ppb (µg/L), the authors found that 6% of the sampled children have elevated Hg levels in blood. Boese-O'Reilly *et al* (2003) also studied a mining community in the Philippines using MeHg analysis of unwashed hair together with urine analysis to differentiate between these mercury pathways.

Several studies have noticed high levels of MeHg in hair of indigenous people in the Amazon region living distant from mining activities (Barbosa *et al*, 1998; Campos *et al*, 2002). This suggests that mining is not the only source of MeHg in fish. As discussed above, other natural and anthropogenic sources of emissions have been contributing mercury to the environment, which is ultimately deposited in remote areas of the rain forest.

Fernandes *et al* (1990) analyzed hair samples from fish consumers near Carajás, PA and observed Hg concentrations averaging 4.8 ppm. The normal level of Hg in hair is 1-2 ppm, (WHO, 1991b). These data illustrate that people consuming fish once or more per day will have Hg levels in hair exceeding 10 ppm (WHO, 1990).

Frèry *et al* (2001) also confirmed the high levels of Hg in hair from Amerindians in French Guyana who are not directly impacted by ASM activities. Results showed that 57% of the Amerindians had Hg levels above 10 µg/g (ppm) because of consumption of carnivorous fish with Hg levels up to 1.62 ppm.

An extensive monitoring program was conducted by Santos *et al* (2002) to investigate levels of Hg in human hair and fish in communities not impacted by gold mining where fish is extensively consumed. The following mean Hg levels in hair were observed:

- 4.33 µg/g (range 0.40 – 11.60 µg/g) in 321 individuals from Santana do Ituqui.
- 3.98 µg/g (range 0.40 – 11.76 µg/g) in 316 individuals from Aldeia do Lago Grande.
- 5.46 µg/g (range 0.37 – 49.85 µg/g) in 504 individuals from Vila do Tabatinga.
- 8.58 µg/g (range 0.61 – 45.59 µg/g) in 203 individuals from Caxiuanã.

Mean Hg concentrations in fish muscle from those locations ranged from 0.01 to 2.53 µg/g for carnivorous species and 0.001 to 0.87 µg/g for non-carnivorous species. This suggests that sources of Hg pollution other than ASM in the Amazon are contributing to elevated Hg levels in freshwater fish. An advisory has been developed to indicate to the public which species have the lowest Hg concentration.

Methylmercury usually comprises at least 70% of the total Hg analyzed in hair (Vanconcellos *et al*, 1999). Hair from the scalp of people with no direct contact with "garimpos" was collected in different sites along Tapajós River (Akagi *et al*, 1995). The study concluded that riparian communities, with a diet strongly based on fish, are the most affected. More than 85% of Hg analyzed in hair was methylated and a correlation with ingestion of large carnivorous fish was suggested. Akagi and Naganuma (2002) found a similar result when hair and blood were analyzed from fishing

[10] Reference Dose (RfD) is an estimate of the safe level for the daily intake of a substance that will not result in any adverse health effects over an average life-time.

villagers in the Amazon with no exposure to mercury vapour. In this case, nearly all (>90%) Hg in hair and blood was MeHg. By contrast, gold miners and gold shop workers had low levels of MeHg in hair, ranging from 13 to 43% of the total Hg (Akagi *et al,* 1995). The proportion of total mercury in hair from people living distant from gold mines, but to whom fish is an important dietary item, was greater than 90%.

Barbosa *et al* (1995) showed that Indians from the Madeira River region, Rondônia, Brazil, have more Hg in hair as well as blood (32 ppb) than miners (17 ppb), due to greater fish consumption. About 3% of fish-eating people showed MeHg concentration in hair ranging from 50 to 300 ppm. Malm *et al* (1995a) observed low concentration of Hg in hair (1.40 to 8.14 ppm) from Yanomami Indians, as their diet is quite diversified with consumption of mammals, birds, fruits and vegetables as well as fish. They also noticed that remote riparian communities along the Madeira River, 180 to 500 km from mining areas, have shown more Hg in hair than those in cities and mine sites. As gold miners and city dwellers have better economic conditions, they can afford to diversify their diet, eating beef several times a week. Maurice-Bourgoin *et al* (2000) found that people living by the Madeira River at the Bolivian side have less Hg in hair than in the Brazilian side. This was attributed to different species being consuming during different hydrological seasons. When the waters are too high to fish, people consume more fruits, eggs, and chicken.

Malm *et al* (1995) also investigated Hg in hair of inhabitants of Jacareacanga (upstream Tapajós River mining activities) and Brasilia Legal (downstream). They observed seasonal differences in Hg concentrations that can be attributed to many factors, but primarily because riparian communities eat more large carnivorous fish at the end of the rainy season (May-June) in the Brazilian Amazon. This is not quite in agreement with the findings of Dolbec *et al* (2001). Examining 24-cm strands of women hair in a village (Cametá) in the Tapajós River, they have observed that more Hg was accumulated in hair during the dry season when more carnivorous fish is consumed. About 72% of the interviewees responded that they prefer to eat herbivorous fish at the end of the rainy season and piscivorous fish at the end of the dry season.

Sampling Hair

Urine, hair, blood, and any other biological samples (e.g. nails) can be used for two purposes: 1) monitoring Hg exposure and bioaccumulation; and 2) obtaining information for the Health Assessment. As seen above, hair is an excellent biomonitoring material to evaluate MeHg exposure via food ingestion. Hair sampling is less complicated than blood as there is less risk of disease transmission in the sampling process and there are fewer cultural issues involved. This is not a universal rule as there are many superstitions around the use of hair in Africa (Ikingura and Akagi, 1996). Latin America is no different, as hair has been used for "black magic" purposes. In other cases, head hair sampling can pose some difficulties when men have short hair or are bald. In Africa, the use of whitening soaps that contain Hg poses additional problems for the evaluation of MeHg exposure (Glahder *et al*, 1999). Chemical speciation can differentiate the inorganic (mercury chloride) and methylated Hg compounds but the procedure is expensive.

Before sampling hair and blood a meticulous selection of the individuals (donors) must be conducted using a socio-economic-demographic questionnaire.

Lebel *et al* (1998) sampled hair stands close to the scalp taken from the occipital portion of the head to be stored in plastic bags with root ends stapled. Drasch *et al* (2001) also collected hair from the back part of the head but sampled strand by strand (from 150 to 250 mg). Afterwards the strands were bound together using cotton string (NOT adhesive tape) and stored at room temperature in paper envelopes. Ikingura and Akagi (1996) cut only 30 to 50 mg of head hair and stored the strands in a paper envelope, which they then kept in an airtight plastic bag. This seems an adequate procedure when working in hot and humid environments. The National Institute of Minamata Disease, Japan, recommends to cut at least 20 strands of hair, each one with about 10 cm, close to the root. The "proximal" portion of hair (hair near the root) is better than the "distal" part (hair tip) for analysis as the MeHg content can decrease during the hair growth under certain conditions, for example treatment with artificial hair waving procedures. So, if long hair strands are available (longer than 10 cm), the hair tips can be discarded.

Hair samples do not need to be frozen, but in hot environments it is advisable to keep samples refrigerated until they can be transported to the laboratory.

Drasch *et al* (2001) mentioned a study by Kijewski in 1993 where it was found that hair-washing procedures with different solvents cannot differentiate between airborne and internal Hg. The authors then used different washing methods before analyzing Hg in hair from ASM communities in Philippines and noticed that the chemical analysis results were inconsistent. Appleton *et al* (1999) have commented that many hair preparation methods are available but no differences could be detected when different wash methods were applied. In fact, no washing procedure was able to fully remove external Hg contaminants because this depends on the strength of the process (e.g. manual washing, ultra-sound bath, etc.). Malm *et al* (1990) suggested that a 0.01% EDTA solution could eliminate most of the dust and fatty substances that are responsible for external mercury contribution. Akagi *et al* (1995) washed hair samples with neutral detergent and water followed by acetone and followed by distilled water, but the discrimination between metallic Hg

and MeHg was done by chemical analysis. Santos *et al* (2000) recommended that at least 100 hair strands be sampled, cut 1cm from the scalp and washed with neutral detergent followed by acetone before mincing them.

Hair is not as good indicator of Hg vapour exposure as urine. It is clear that when chemical speciation is available, the difference between MeHg and (inorganic) Hg°, usually from external sources, can be determined and severe washing is not needed. However, MeHg analysis is an expensive procedure. In places where people are subjected to both types of exposure (i.e. Hg vapour and MeHg-contaminated fish), washing is a poor solution but sometimes **the only one available**. In these places, when hair is collected to examine MeHg exposure through fish ingestion and chemical speciation procedures are not available (i.e. MeHg analysis), it is advisable to use a washing procedure with neutral detergent, acetone and water (as suggested by Akagi *et al*, 1995) to eliminate at least part of the external contamination. A previous evaluation of the washing procedures (to establish how much MeHg is removed in each washing step) is strongly advised. When evaluating MeHg exposure in areas with no direct mining influence, a simple washing procedure with neutral detergent is sufficient to provide reliable results. In any case, a detailed questionnaire of the food habits and possibilities of exposure to Hg vapour can help estimate the contribution of MeHg.

In Guyana researchers interviewed and sampled hair from 108 people from eight mining and non-mining communities. The mean hair Hg content of these residents was 11.59 μg/g (standard deviation 10.01 μg/g). Individuals with fish as primary source of protein (12.14 μg/g) had significantly higher levels of Hg than those who consumed non-fish protein (chicken 6.50 μg/g) (Couture and Lambert, 2003).

Dolbec *et al* (2001) found Hg concentration in hair has seasonal variations. Thus, long hair can be used to observe these variations. If hair grows at a rate of 1 cm per month, then the analysis of 3-cm hair strands provides an average concentration of the last 3 months of an individual diet. Depending on the season (e.g. wet and dry), differences in seasonal exposure to MeHg can be derived from a single hair sample.

1.6. Analytical Procedures

This section describes recommended analytical procedures for analysis of total Hg and methylmercury (MeHg) in sediment, water, biota tissue (invertebrates and fish) and human health samples (urine, blood, hair). This document does not intend to describe in detail the analytical procedures, as they are fraught with specific methodologies that concern the analytical laboratories. A detailed and comprehensive description of Hg and MeHg analysis of environmental samples can be found in Pichet *et al* (1999). There are several acceptable analytical methods for each of these environmental media as promulgated in many of the publications reviewed, as well as accepted (e.g. USEPA) methodologies. Where reliable, established methods of Hg analysis are in place at the laboratories receiving environmental samples. These should be followed, including appropriate quality assurance/quality control measures (QA/QC), such as laboratory duplicates and testing of standard materials with known Hg quantities.

1.6.1. Water

Bloom and Crecelius (1993) propose a method of determining total Hg in water that has since been adopted (1996) by the USEPA as Method 1631 – *Mercury in water by oxidation, purge and trap and CVAFS* (USEPA, 2001a). The method prescribes that water samples are treated with a strong oxidant, BrCl and allowed to react for 8 hours. The residual BrCl is destroyed by addition of hydroxylamine hydrochloride. The digested sample is then placed into a sparging vessel and the reducing agent stannous chloride is added. Elemental mercury that is produced is bubbled off and collected on a gold trap. The gold trap is heated with an argon gas acting as a carrier, passing through it and releasing the mercury for quantification by atomic fluorescence spectroscopy. The detection limit for 100 mL analytical aliquots is about 0.1 ng/L.

Analysis of MeHg content in water can follow Liang *et al* (1994) method. Aliquots of surface waters, preserved with 0.2% clean HCl in the field are distilled (in 40 mL Teflon vials), ethylating the methylmercury to ethylmethyl mercury with sodium tetraethyl morate. The ethylmethyl mercury is purged onto a Tenax trap, drying the trap with nitrogen. The trap is then heated in an argon gas stream that sweeps the analyte onto a gas chromatograph (GC) column to separate the ethylmethyl mercury from other ethylated mercury compounds. The analytes are then passed through a pyrolyzer where the organic mercury is converted to Hg° before entering a cold vapour atomic florescence analyzer for detection (Horvat *et al*, 1993) using peak area for quantification. Matrix spikes and matrix spike duplicates, as well as process blanks should be included every 10 samples. The method detection limit is approximately 0.04 ng/L for a 40 mL sample.

1.6.2. Sediment

Sediment samples should be wet screened and dried, preferentially at room temperature, or at temperatures below 60°C. Dried samples (500 to 1000g) should then be homogenized using either a splitter of by coning and quartering on brown paper. Store half of the sample (250 to 500 g). The other half must be pulverized to at least –200 mesh (0.074 mm) using a ceramic or cast iron disk or a planetary pulverizer. Send half of the pulverized sample (125 to 250 g) to the analytical laboratory. Store the other half.

Digestion Procedures

There are a number of digestion procedures to solubilize mercury. USEPA Method 7471B (USEPA, 2003) describes a method in which 0.5 to 0.6 g of homogenized soil samples is digested for 30 minutes at 95°C in a hot block digester with 5 mL of *aqua regia* and 5 mL water in the presence of 15 mL of a potassium permanganate solution. The digestion oxidizes all forms of mercury to Hg(II). After adding 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate, the Hg(II) is reduced with stannous sulfate to elemental mercury, which is detected by atomic absorption.

Akagi *et al* (2000) described an analytical method to analyze total Hg in soils and sediments in which the digestion method is essentially the same as the one used for biological samples (fish, hair, blood, urine). About 0.5 g of finely pulverized sample is leached with 2 mL of nitric-perchloric acid (1+1). Then, 5 mL of sulphuric acid and 1 mL of water are added and heated to 230-250 °C on a hot plate for 20 minutes. After cooling, the digested sample is made up to 50 mL with mercury-free water. An aliquot of the solution is introduced into the analyzer (cold vapour or atomic fluorescence) where a solution of 10% stannous chloride[11] is used to reduce the mercury. Using air or nitrogen as a carrying gas, the sample is analyzed. The detection limit of the method is 1 ppb (ng/g) for 0.5 g of sample.

Sediment samples can also be analyzed following the method of Bloom and Crecelius (1993). Samples are homogenized with a clean stainless steel spatula and weight 1 mL sub-samples into acid cleaned test tubes. About 10 mL of a 1:2.5 nitric/sulphuric acid mixture is added and heated at 180^{o} C for 6 hr in an aluminum hot block. After

[11] USEPA Method 7471B: the reducing solution consists of 5% stannous chloride, 3% sodium chloride and 10% sulfuric acid.

cooling, 200 µL of bromine chloride (BrCl) are added and sample volume completed to 25 mL with low mercury deionized water. Aliquots (usually 100 or 200 µL) are analyzed and processed as for water samples. Matrix spikes/spike duplicates are performed as necessary to determine mercury recoveries. The average of these recoveries should be used to correct values. Sediment reference materials should also be concurrently digested and analyzed in duplicate. The detection limit is in the order of 1 ng Hg per gram (0.001 µg/g) wet sediment.

USEPA Method 245.7 (USEPA, 2001b) describes a method in which inorganic Hg compounds and organomercury species are oxidized by a potassium bromate/potassium bromide reagent. After oxidation, the sample is sequentially pre-reduced with NH_2OH-HCl to destroy the excess bromine, then the ionic Hg is reduced with $SnCl_2$ to convert Hg(II) to volatile Hg°. The Hg° carried by, high-purity argon, passes into an inert gas stream that carries the released Hg° into the cell of a cold-vapour atomic fluorescence spectrometer (CVAFS) for detection at 253.7 nm.

Cava-Montesinos *et al* (2004) describes a digestion method using an ultrasound water bath in the presence of 8% (v/v) aqua regia, 2% (v/v) antifoam A and 1% (m/v) hydroxilamine hydrochloride to analyze Hg in milk. After the ultra-sound, the solution is treated with 8 mmol l.1 KBr and 1.6 mmol l.1 $KBrO_3$ in a hydrochloric medium and Hg measurements obtained by cold vapour atomic fluorescence spectrometry.

In fact there are several methods for digesting and determining total Hg in solid and liquid samples. The main four methods used for measuring total mercury are: direct mercury analysis, cold vapour atomic absorption spectrometry (CVAAS), cold vapour atomic fluorescence spectrometry (CVAFS) and inductively coupled plasma mass spectrometry (ICP/MS). A brief summary of each technique is described below (P. Randall, 2003 – USEPA, personal communication).

Direct Mercury Analysis

Direct Mercury Analysis, USEPA Method 7473, Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation and Atomic Absorption Spectrophotometry. One of the significant advantages of this method is that instruments such as Milestone or LUMEX are available. The sample is analyzed directly, without the requirement of acid digestion to release the mercury into solution. This minimizes the time required per analysis and reduces the risks of volatile losses or contamination. With direct mercury analysis, less than 1 gram of sample is dried, then thermally decomposed. Some instruments use oxygen (Milestone) and others use air to carry the decomposition product. Milestone uses a gold trap in which the gaseous decomposition products pass through and mercury is preferentially trapped. The gold film is then heated to release the Hg, which is detected in a gas cell at 253.7 nm similar to CVAAS. The USEPA method 7473 that analyzes total Hg by direct pyrolisis using Milestone, has a Method Detection Limit (MDL) of 0.1 ng of Hg. Maximum sample capacity is 700 mg for solids (P. Randall, 2003 – USEPA, personal communication).

LUMEX uses a Zeeman process (Zeeman Atomic Absorption Spectrometry using High Frequency Modulation of Light Polarisation ZAAS-HFM) that eliminates interferences and eliminates the use of a gold trap. The detection limit reported by the manufacturer for sediments is 0.5 µg/kg (ppb) using 200 mg of sample in the pyrolisis chamber.

However, memory effects may be present when analyzing a sample of high Hg concentration before analyzing one of low mercury concentrations. A blank analysis with an extended decomposition time may be required following the analysis of a high concentration sample to minimize memory effects. These instruments were developed for solid samples but it was observed they could also work well for liquids when they are introduced into the pyrolisis chamber. In December 2003, a UNIDO team conducted a medical survey in El Callao, a traditional mining region in Venezuela, analyzed 300 urine samples in less than 12 hours using the LUMEX spectrometer with a pyrolisis chamber (UNIDO, 2004).

Measurement of total Hg using CVAAS

Atomic absorption and fluorescence spectroscopy are the most common techniques for quantitative analysis of Hg. They are based on electronic transitions of neutral atoms in the gas phase, mostly in the visible region, and rely on the fact that numerous transition frequencies for every element have been observed and catalogued. In atomic absorption, an incident beam of radiation is passed through the sample and the transmitted intensity is recorded at an appropriate wavelength (ApSci622, 1999)

The USEPA Method 7471B, Mercury in Solid or Semisolid Waste (Manual Cold Vapour Technique) describes a method for Hg analysis using cold vapour atomic absorption spectrometry (CVAAS). This is rapid, simple and sensitive. For CVAAS to perform well, however, Hg must be present in solution unbound as Hg^{2+} ion. The sample must not contain strong oxidants or ligands that could interfere with the reduction of Hg^{2+} to Hg°, or contain volatile organics that could interfere spectrally. These conditions require samples to be digested in strong acids. Once these conditions are achieved, a reducing agent, such as stannous chloride, is added to the digested solution to reduce the oxidized Hg to elemental Hg. The elemental Hg is bubbled from solution using an inert gas such as argon and the gas stream containing the Hg° is passed through a dessicant and then a spectrophotometric cell for detection at 253.7 nm. Common

limitations to this method are volatile losses or contamination from the acid digestion step and spectral interferences from organics, volatile halides or water vapour.

Measurement of total Hg using CVAFS

Atomic fluorescence spectroscopy is much more sensitive than atomic absorbance. An incident radiation (Hg lamp) is used to excite atoms in the gaseous current entering the analytical equipment and subsequently, the atoms re-radiate photons at a characteristic wavelength. Fluorescence radiation is generally measured at a 90° angle to lamp emission. Atomic fluorescence is extremely sensitive and usually used to analyze very low concentrations of Hg (e.g. in water). The sensitivity can increase, to reach sub-ppt (parts-per-trillion) levels, if Hg° vapour is pre-concentrated on a gold foil (WCAS, 2004). The main concern of this technique is the contamination of the samples during the digestion process as well as the laboratory environment. Measurement of total Hg using cold vapour atomic fluorescence spectrometry (CVAFS) is well described in USEPA (2001a and 2001b). The USEPA Method 1631 (USEPA 2001a) is for determination of Hg in the range of 0.5–100 ng/L. The detection limit using this method is usually dependent on the level of interference rather than instrumental limitations and has been determined to be 0.2 ng/L when no interferences are present. A Method Detection Limit (MDL) as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%) and extra caution in sample handling. The USEPA Method 245.7 (USEPA 2001b) is for determination of Hg in filtered and unfiltered water by CVAFS in surface and ground waters, marine water, and industrial and municipal wastewater. The highest MDL is 1.8 ng/L. This method may be used to determine Hg up to 200 ng/L and may be extended by dilution of the sample.

Measurement of total Hg using ICP/MS

The Inductively Coupled Plasma Mass Spectrometry (ICP/MS) is a precise and sophisticated method for Hg analysis and is described by USEPA Method 6020A. ICP/MS is a highly sensitive multi-element isotope detector. In ICP/MS, ions form an argon plasma and are extracted through a differentially pumped interface to be introduced into a mass spectrometer. There, they are separated according to their mass-to-charge ratio (m/z). The number of lines that can be observed for a singly ionized element corresponds to the number of stable isotopes of that element. Thus, if the entire periodic table was monitored, only a few hundred lines would be seen, which is simpler than the corresponding emission spectrum. ICP/MS is an ideal detector for Hg since ^{202}Hg is a perfectly clean mass with no isobaric or polyatomic interferences. ICP/MS detection can be superior to CVAAS since problematic spectral interferences from organics, water vapour or chlorine gas are not a problem in ICP/MS detection. However, the detection limits may not be as low as those achieved by CVAAS, because conventional sample introduction to the plasma leads to memory effects that are caused by the large surface area of the spray chamber. An advance in sample introduction to ICP/MS for high memory elements like Hg has been the direct injection nebulizer. In this technique the entire liquid sample, versus a fraction of the sample aerosol, is injected directly into the plasma. Using direct injection nebulization (DIN) ICP/MS, Hg can be organically bound or bound to strong ligands. The benefits of using DIN-ICP/MS are high sensitivity, low required sample volume, speed of analysis, simple sample preparation and the added benefit of being able to perform multiple metals analyses on the same solutions if desired. Limitations of ICP-MS, in general, are low tolerance for high dissolved solids and high salt matrices.

Analyzing MeHg in Soils and Sediments

A procedure to analyze MeHg in sediment is described by Horvat *et al* (1993). Sieved sediment samples of 1-2 g are placed in stills and the volume made up to 40 mL with deionized water and subsequently distilled as for water samples. Matrix spikes/matrix spike duplicates should be performed to determine recovery and to correct reported values. The method detection limit is about 0.05 ng methylmercury per gram dry weight of sediment (0.00005 μg/g). Extraction of MeHg can also be done by alkaline digestion. Sediment samples (0.2 to 0.5 g) are leached with 25% KOH in methanol solution at 75 °C for 3 hours; then 6 mL of CH_2Cl_2 and 1.5 mL of HCl are added. After shaking, the mixture is transferred to a separation funnel and the CH_2Cl_2 phase is collected. Adding 60 mL of Mili-Q water, the organic solvent is evaporated at 70 °C. The solution is purged with Argon to eliminate residual metyhylene chloride, then analyzed for methylmercury (MESL, 1987). The analysis of the aqueous solution can be done by ethylation with gas chromatography (GC) separation and CVAFS detection.

1.6.3. Biota Tissue

Total mercury analysis in biota tissue (invertebrates or fish) can follow the method described in Akagi *et al* (2000) as described above or can follow the standard USEPA Method 1631 *Total Mercury in Tissue, Sludge, Sediment and Soil by Acid Digestion and BrCl Oxidation*. Briefly, this method describes two sample preparation and digestion procedures for oxidation of total mercury and may be used in conjunction with Method 1631B *Mercury in Water by Oxidation, Purge and Trap, and Cold Vapour Atomic Fluorescence Spectrometry* (USEPA 2001a).

Before digestion and analysis, biota samples must be homogenized to a fine paste with a stainless steel mill or finely chopped with stainless steel tools on an acid-cleaned plastic cutting board. Note that samples can be stored frozen for at

least one year. The preferred digestion method for organic material such as tissue involves digestion of the sample with HNO_3/H_2SO_4. For tissue, weigh (nearest mg) approximately 0.2 – 0.4 gm of homogenized sample and add 10 mL of HNO_3/H_2SO_4. Place the digestion vessel in an acid fume hood and allow the sample to sit in the cold acid for at least four hours. After digesting at room temperature, place the vessel on a hot plate and bring to a gentle boil by increasing plate temperature over one hour. Reflux for 2 – 3 hours to fully oxidize the tissue sample. Once complete, make up sample volume to 40 mL with 0.2 N BrCl and mix thoroughly. Shake the BrCl/sample solution to homogenize and allow it to sit for at least 4 hours prior to analysis, to oxidize any remaining Hg.

Pipette 0.01 – 5.0 mL of diluted digestion solution directly into a bubbler containing approximately 100 mL of pre-purged stannous chloride ($SnCl_2$) containing water. Purge the solution onto a gold trap for 20 minutes. Change the $SnCl_2$ solution in the bubbler after a total of 10 mL of digestate has been added. For samples known or expected to contain high Hg concentrations, further dilute (by a factor of 100) an aliquot of the diluted digestate with 0.02 N BrCl solution and analyze a sub-aliquot. Because tissue samples have considerably more Hg than water or sediment samples, the sensitivity provided by a dual amalgam trap system and fluorescence detector may be too great and analysis using cold vapour atomic absorption spectrometry (CVAAS) may be sufficient.

1.6.4. Urine, Blood and Hair

For total mercury analysis in urine samples, Akagi (1994) has suggested to add 1 to 5 mL of urine drop wise, while stirring, into a 50 mL volumetric flask containing a mixture of 1 mL of nitric acid, 1 mL of perchloric acid, 5 mL of sulphuric acid and 1 mL of water. The mixture is heated to 230-250 °C for 20 minutes. After cooling, the solution is completed to 50 mL with water and taken to the spectrometer CVAAS or AFS.

There are many digestion and analytical procedures for hair and blood samples. Akagi (1994, 1997) and Ikingura and Akagi (1996) provide details regarding techniques to digest biological materials. For **total mercury** analysis, place 0.5 g of finely chopped fish muscle or 10 mg of finely cut hair or 5 mL of blood in a 50 mL volumetric flask. Add 2 mL of nitric-perchloric acid (1+1), 5 mL of sulphuric acid and 1 mL of water and heat to 230-250 °C on a hot plate for 20 minutes. After cooling the digested sample is made up to 50 mL with mercury free water and an aliquot of the solution is introduced into the analyzer (cold vapour or atomic fluorescence), where a solution of 10% stannous chloride is used to reduce the mercury. The sample is analyzed using air or nitrogen as a carrying gas. The detection limit of the method is 1 ppb (ng/g) for 0.5 g of sample.

For methylmercury analysis of hair, the "Akagi method" consists of placing 10 to 20 mg of finely cut hair in a test tube with crew cap. About 2 drops of ethanol are added to reduce surface tension; then 5 mL of 2N HCl is added and a small amount of cotton introduced to prevent the hair sample from floating. The test tube is capped and heated to 100 °C on a water bath for 5 minutes. After cooling, 1 mL of the extract is transferred to another 10 mL test tube and 4 mL of benzene is added. The benzene extract is analyzed by gas-liquid chromatography to detect MeHg concentration.

PIXE (proton induced X-ray emission) is a sophisticated analytical instrument that can be used to analyze very low levels of Hg in biological samples. Iwate Medical University, Japan, has applied PIXE to analyze hair without any sample preparation except cleaning the surface with acetone (Sera *et al*, 1999; Sera *et al*, 2002). This technique was used in a small-scale mining site in the Philippines (Murao *et al*., 2002). The methodology can also analyze Hg in urine and blood (30 μL of samples) in about 5 minutes.

1.6.5. Other Analytical Procedures

Analysis of TOC (Total Organic Carbon) in sediments is important to correlate with Hg to determine whether organic matter is the main Hg-bearing phase. One of the most frequently used procedures for total carbon is by using LECO induction furnace. The furnace control system allows temperature to range from 25 to 1100 °C. Multiple sources of carbon can often be differentiated by controlling temperature and furnace atmosphere. During analysis, all forms of carbon in the sample material oxidize into CO_2, (except some carbides like SiC). During oxidization organic samples also produce H_2O. The presence of organic carbon may be verified by finding coincident peaks in H_2O and CO_2. In an inert (N_2) atmosphere, both H_2O and CO_2 are detected, but organic carbon is not detected (LECO, 2003). When the soils and sediments do not contain graphite and there is no carbonate, one could probably assume that Total C determined by LECO will be the same as TOC. A preliminary leach with HCl and drying before LECO analysis would decompose any carbonate that might be in the samples (D. Appleton, 2003 – British Geological Survey, personal communication).

1.7. Quality Assurance/Quality Control

The objective of chemical sampling and analysis of data Quality Assurance/Quality Control (QA/QC) is to assure that chemical data collected are representative of the material being sampled, are of known quality, are properly documented and are legally and scientifically defensible. This requires sample collection using specified standardized procedures, analyzed samples at laboratories that have been certified for all applicable methods, and staffing the program with experienced samplers and analysts. Effective QA/QC is also achieved by implementing appropriate Data Quality Objectives (DQOs), particularly at the chemical analysis phase of the study. DQOs are numerically definable measures of analytical accuracy and analytical precision. Analytical accuracy and precision are ensured through the analysis of laboratory duplicates (MD), matrix spike duplicate (MSD), and certified reference materials (CRM). The effects of sample matrices on analytical accuracy are measured by the analysis of matrix spike samples (MS). In addition, field replicate samples should be collected at random intervals to provide an estimate of spatial heterogeneity in the sampling media, such as sediment, soil or fish tissue. The general DQOs for a typical field project are:

- Analytical Precision = $\pm$ 25% Relative Percent Difference (%RPD).
- Analytical Accuracy = 80 to 120% recovery of MS and CRM.

1.7.1. Precision

Precision measures the reproducibility of repetitive measurements and is usually expressed in terms of imprecision. Precision is strictly defined as the degree of mutual agreement among independent measurements as the result of repeated application of the same process under similar conditions. Analytical precision is a measurement of the variability associated with duplicate (i.e. two) analyses of the same sample in the laboratory and is determined by analysis of matrix spike duplicates or laboratory duplicates. These results were assessed using the Relative Percent Difference (RPD) between duplicate measurements. The equation used to calculate RPD is:

$$\mathrm{RPD}(\%) = \frac{(\mathrm{A}-\mathrm{B})}{((\mathrm{A}+\mathrm{B})/2)} \times 100$$

where: A = analytical result; B = duplicate result.

RPD values may be either positive or negative, and ideally should provide a mix of the two, clustered around zero. Consistently positive or negative values may indicate a bias. Large variations in RPD values are often observed between duplicate samples when the concentrations of analytes are very low and approaching the detection limit. The reason for this is apparent if one considers duplicate samples with concentrations of an analyte of 0.0005 and 0.0007 mg/L. In absolute terms, the concentration difference between the two is only 0.0002 mg/L, a very tiny amount; however the RPD value is 33.3%. This may lead to a belief that the level of precision is less than it actually is.

Precision measurements with three or more replicates are calculated as the Relative Standard Deviation (RSD):

$$\mathrm{RSD}(\%) = \frac{\mathrm{S}}{\mu} \times 100$$

where: S = standard deviation and μ = mean of replicate analyses

Note that replicate field samples should also be collected and analyzed to assess field precision and spatial heterogeneity.

1.7.2. Accuracy

Accuracy is a statistical measurement of correctness and includes components of random error (i.e. variability due to imprecision) and systematic error (i.e. bias). Therefore, accuracy reflects the total error associated with a measurement. A measurement is accurate when the value reported does not differ beyond acceptable limits from the true value or known concentration of a spike or standard. Analytical accuracy is typically measured by determining the percent recovery of mercury that was spiked into a sample before extraction at known concentrations. The accuracy of matrix spikes can be determined by:

$$\mathrm{RECOVERY}(\%) = \frac{(\mathrm{Spiked\ Sample} - \mathrm{Regular\ Sample})}{(\mathrm{Spike\ Added})} \times 100$$

The analysis of certified reference materials (CRM) also provides an indication of analytical accuracy. A variety of CRM for Hg is available for use, including Canada National Research Council (NRC) Sediment MESS-2 and NRC Tissue CRM DORM-2 for fish tissue.

Percent recovery is calculated as:

$$\text{RECOVERY}(\%) = \frac{A}{B} \times 100$$

where: A = obtained value; B = certified value.

Ideally, all percent recoveries should be 100%, however acceptable values vary widely dependent upon matrix and analyte. Generally, recoveries of 80 to 120% are set as the quality objective for accuracy. The exception is MeHg in sediments for which recoveries ranging from 75 to 125% are considered acceptable. Analytical accuracy is determined by calculating the percent recovery from the analysis of matrix spike samples and CRM containing known quantities of an analyte (e.g. sediment or fish tissue). Note that average spike recoveries for each analytical group should be used to correct sample results. If average recovery from a CRM is 90%, then values from test materials should be corrected to reflect the fact that lower than expected results were acquired. Proper conduct and reporting of field and laboratory QA/QC procedures and results is a critical component of the program and is a necessary reporting requirement.

1.7.3. Method Detection Limit

The Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific, and well-defined analytical method. It is essential to include all sample processing steps of the analytical method in the determination of the MDL. A **minimum of seven aliquots** of the sample should be used to calculate the MDL; each should be processed through the entire analytical method (USEPA, 2000). MDLs are matrix specific and must be calculated according to a specific procedure. The Method Detection Limit can be calculated by:

$$\text{MDL} = t_{(n-1,1-\alpha=0.99)} \times S$$

where: S = standard deviation of replicate analyses of matrix spikes with concentrations near the estimated MDL, and $t_{(n-1,\ 1-\alpha=0.99)}$ = the Students' t value appropriate for a 99% confidence level and alpha standard deviation estimate with n-1 degrees of freedom (see below).

Number of replicates	Degrees of freedom (n-1)	t (n-1, 0.99)
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821

Note: data extracted from USEPA, 2000

It is important that each laboratory finds its own MDL for mercury analysis using a specific instrument and methodology.

PART 2 – HEALTH ASSESSMENT

The World Health Organization (WHO, 1967) defines health as "*a state of complete physical, mental, and social well-being and not merely the absence of disease and infirmity*." Health is not "*an objective for living but a resource of everyday life*." Health encompasses social, economic, cultural, and psychological well being, as well as the ability to adapt to the stresses of daily life. Arduous work, combined with inexperience in mining and lack of knowledge about chemical exposures, can further exacerbate the potential for injury or illness, thereby perpetuating the cycle of ill health and poverty. The health and safety issues that plague artisanal miners can be attributed primarily to the informal and often illegal nature of artisanal mining; economic demands that result in inadequate equipment and neglect of safety measures; and a frequent lack of expertise and insufficient training (Hinton *et al*, 2003). Integration of Health Assessment and Environmental Assessment is important in order to identify the potential environmental effects that one activity might have on the biophysical and social environment, including any health issues that need to be assessed. The main reasons for this integration are outlined as follows (Health Canada, 1999):

a) address public concern;
b) minimize the need for separate Health and Environmental Assessment;
c) ensure cost effectiveness;
d) minimize the adverse and maximize the beneficial effects on health;
e) support the concept of sustainable development.

Mercury is widely recognized as one of the most toxic metals known to man. Mercury vapour released during amalgam decomposition poses a serious hazard to workers and surrounding communities. In many countries, gold decomposition takes place in the home (using the kitchen stove) or in small sheds adjacent to processing sites. Metallic Hg in contact with organic-rich soils becomes soluble and eventually converts into its most toxic species, methylmercury (MeHg), which is rapidly bioaccumulated. Communities that rely on fish, especially carnivorous species, as a primary food source may be particularly susceptible to ingestion of dangerous levels of MeHg. To assess human health, it is useful to assess exposure (environmental and biological monitoring), the body burden (human bio-monitoring such as urine, blood, hair) and medical/clinical assessment (effect monitoring).

The Health Assessment part of the Global Mercury Project was designed to complement the Environmental Assessment, providing indications of the level of Hg poisoning and its health effects on ASM communities by exposure to Hg vapors, by ingestion of contaminated food, (in particular fish as the most accessible protein in riparian rural communities), or both. Based on assessment of the pathways and bioavailability of mercury vapour and MeHg to the mining communities, the Health Assessment combines information from biological samples associated with medical exams to evaluate the level of impact that the pollutant caused or may cause to individuals residing in "mining and environmental hotspots". This is a basic procedure to establish risks and prioritize mitigation actions.

One of the most important points to be investigated in a Health Assessment is the pathway by which Hg is bioaccumulated in humans. The main ways are through metallic Hg vapour from amalgam burning (and gold melting) and ingestion of fish with high/moderate MeHg concentrations. Other pathways may include Hg evaporation from amalgamation tailings and dirt ingestion by children and women, such as seen in some African countries.

2.1. Health Effects Caused by Mercury Vapour

Inhalation of Hg vapour is more significant for mining and gold shop workers who are directly involved in handling metallic Hg; but it can also affect surrounding communities indirectly. Once in the lungs, Hg is oxidized, forming Hg (II) complexes, which are soluble in many body fluids. The ultimate effect of Hg and related compounds is the inhibition of enzyme action (Jones, 1971). Oxidized Hg can diffuse easily across the blood-brain barrier, which is a series of multiple systems regulating the exchange of metabolic material between brain and blood. Impairment of the blood-brain barrier, together with the possible inhibition by Hg of certain associated enzymes will certainly affect the metabolism of the nervous system (Chang, 1979).

Mercury vapour is completely absorbed through the alveolar membrane, then complexes in the blood and tissues, before reacting with biologically important sites (Mitra, 1986). The biological half-life of Hg in blood absorbed as vapour is about 2-4 days when 90% is excreted through urine and feces. This is followed by a second phase with a half-time of 15-30 days (Hacon, 1990; WHO, 1991a). The time interval between passage of elemental Hg through the alveolar membrane and complete oxidation is long enough to produce accumulation in the central nervous system (Mitra, 1986).

Symptoms of Hg Vapour Exposure

Mercury can damage the nervous system irreversibly. Kidneys are the most affected organs in exposures of moderate duration to considerable levels, while the brain is the dominant receptor in long-term exposure to moderate levels (Suzuki, 1979). Total mercury elimination through urine can take several years. Then, the **Hg levels in urine would not be expected to correlate with neurological findings** once exposure has stopped.

Several studies are in agreement that mild subclinical signs of central nervous system toxicity can be observed among people who have been exposed occupationally to elemental mercury at a concentration of 20 μg/m³ or above for several years. Extrapolating this to continuous exposure and applying an overall uncertainty factor of 30 (10 for interindividual variation and 3 for extrapolation from a lowest-observed-adverse-effect level, or LOAEL, with slight effects to a no-observed-adverse-effect level, or NOAEL), a tolerable concentration of 0.2 μg/m³ was derived. The limit for public exposure is 1.0 μg/m³ (WHO, 2003)

A **short-term exposure to high Hg levels** causes clinical symptoms that mainly involve the respiratory tract. Mercury levels in the urine of new workers should be lower than those of workers with a longer duration of exposure (Stopford, 1979). Symptoms typically associated with high, short-term exposure to Hg vapour (1,000 to 44,000 μg/m³), such as those miners are subjected to when they burn amalgams in open pans, are chest pains, dyspnoea, cough, haemoptysis, impairment of pulmonary function and interstitial pneumonitis (Stopford, 1979). **Acute** Hg poisoning, which can be fatal or can cause permanent damage to the nervous system, has resulted from inhalation of 1,200 to 8,500 μg/m³ of Hg (Jones, 1971). A few hours of exposure to high Hg levels of 1,000 to 2,000 μg/m³ may cause acute chemical bronchitis and pneumonitis. Two hours after exposure, lung injury appears as hyaline membrane formation, and finally, extensive pulmonary fibrosis occurs. Clinical findings correlate with the duration of exposure, the concentration of Hg and the survival time after exposure. There is no correlation between pathological findings and the concentration of Hg in the tissues. Necrosis of proximal convoluted tubules may be attributed to the disruption of the enzyme systems of Hg(II)-MT compound. When there is deposition of Hg in the brain, inorganic Hg causes less damage to neurons than organic Hg (Eto *et al*, 1999). In Japan, autopsies performed on 3 individuals who died due to acute high level Hg vapour exposure (2 weeks) revealed diffuse organized pneumonia, renal cortical necrosis, disseminated intravascular coagulopathy, and infarctions in the brain and kidneys. Drugs such as chelating agents and corticosteroids appear to effectively decrease the inflammation and delay pulmonary fibrosis (Asano *et al*, 2000).Experiments with animals indicate that continuous exposure to Hg above 0.3 μg/m³ of air may present a health hazard. **Long-term, low-level Hg vapour exposure** has been characterized by less pronounced symptoms of fatigue, irritability, loss of memory, vivid dreams and depression (WHO, 1991a). Occupational exposure of Hg has resulted in effects on the central nervous system. Acute exposure has caused delirium, hallucinations and suicidal tendency as well as erethism (exaggerated emotional response), excessive shyness, insomnia and, in some cases, muscular tremors. The latter symptom is associated with **long-term exposure to high levels of Hg vapor**. In milder cases, erethism and tremors regress slowly over a period of years, following removal from exposure pathways (WHO, 1991a). No specific lesion was found in the brain of two patients who worked in Hg mines in Japan for about 10 years. However, the assay and the histochemistry of Hg revealed that inorganic mercury was present in their brains (Eto *et al*, 1999). A person suffering from a mild case of Hg poisoning can be unaware because the symptoms are psycho-pathological. These ambiguous symptoms may result in an incorrect diagnosis (Cassidy and Furr, 1978).

The common manifestations of **chronic exposure to excessive levels of Hg vapour** are metallic taste and gum diseases such as gingivitis, ulcers and formation of a blue line at gum margins (Stopford, 1979). Since inorganic Hg poisoning affects liver and kidneys, high Hg levels in the urine can indicate undue exposure to Hg vapour. WHO (1991a)

collected a large amount of evidence to recommend 50 µg Hg/g creatinine as the maximum individual urine concentration. They concluded that a person with Hg level of 100 µg/g creatinine has a high probability of developing symptoms such as tremors and erethism (abnormal irritability). For Hg levels in urine between 30 and 100 µg/g creatinine, the incidence of certain subtle effects in psychomotor performance and impairment of the nerve conduction velocity can increase. The occurrence of several subjective symptoms such as fatigue, irritability, and loss of appetite can be observed. For Hg levels below 30 to 50 µg/g creatinine, mild effects can occur in sensitive individuals, but it seems more difficult to observe symptoms (WHO, 1991a).

Health Problems of ASM Exposure to Hg Vapour

In the Brazilian Amazon, gold shop workers with high levels of Hg in urine (average around 270 µg/L) exhibited some signs of mercurialism such as dizziness, headache, palpitations, tremors, pruritus and insomnia (Malm *et al*, 1995a). Schulz-Garban (1995) in a study of 20 amalgamation workers in Venezuela noticed that 8 individuals had high Hg levels in urine, exceeding 50 µg/L and 4 of them had symptoms of poisoning, such as stomach irritation, nausea, sexual dysfunction, headache and character alteration. Mercury levels in urine as high as 460 µg/L were observed.

Drasch *et al* (2002) examined the Hg threshold levels in urine and blood. The authors used two German indices, known as Human Biological Monitoring (HBM) values:

1. HBM I, which is comparable to the NOAEL (no observed adverse effect level): blood: 5µg/L and urine: 5 µg/ g creatinine[12];
2. HBM II comparable to LOAEL (lowest observed adverse effect level): blood: 15µg/L and urine: 20 µg/g creatinine[13].

Using data from an artisanal gold mining community in the Philippines, the authors concluded that mercury concentrations in blood and/or urine alone are not appropriate for the establishment of a toxicologically defined threshold limit like HBM values. A complex ranking, which includes medical parameters, must be associated with the blood and urine Hg levels to provide reliable diagnosis of intoxication.

Drasch *et al* (2001) studied the community of Mt. Diwata, Diwalwal region, Philippines, where 15,000 people derive their livelihood from gold mining. They found that the individuals not directly involved with Hg handling had higher Hg levels in urine (median = 4.1, max = 76.4 µ/L) than an outside control group (median = 1.7, max. = 7.6 µg/L). Miners with median levels of 11 and max of 294.2 µ/L showed classical symptoms of Hg intoxication such as tremors, ataxia, metallic taste, bluish line in the gums. The authors concluded that the main health problems of children in Diwalwal were tuberculosis, insufficient hygienic conditions, child labor, and high exposure to mercury vapours because the houses in which they live were also sites where amalgamation and amalgam burning were being carried out. The authors diagnosed a chronic Hg intoxication based on high Hg levels in blood/urine and/or hair together with abnormal medical examination results. Only when the chemical analyses were combined with a medical test score involving physical and neurological exams was a correct diagnosis of intoxication obtained. In further work in the same area, the authors administrated 400 mg/day of a chelating agent (DMPS – 2,3 Dimercapto-1-propane-sulfonic acid) to 95 intoxicated inhabitants of Mt. Diwata for 14 days. They observed that Hg that was previously distributed in other body tissues became concentrated in the kidneys, to be eliminated via urine (up to 86 times than before treatment). After a short treatment time, significant improvements in some symptoms were detected by medical examination. Through chemical speciation of Hg in hair, chronic intoxication in mining areas was due to inorganic Hg, however, downstream from mine sites, hair contained a higher percentage of MeHg, suggesting greater dietary exposure (Boese-O'Reilly *et al*, 2003). The authors confirmed that DMPS is an efficient chelating agent for MeHg. As Hg concentration in blood showed a relatively modest decrease, they concluded that the duration of the treatment should last more than 14 days; alternatively, the treatment must be performed in more than one cycle to guarantee the excretion of Hg from other tissues.

Mercury contamination in Tanzania due to ASM was reviewed recently by Mutakyahwa (2002). The author estimates that from 1991 to 1995, more than 20 tonnes of Hg were emitted into the environment, in particular around Lake Victoria and Lake Tanganyika. This is reflected in the high concentration of Hg in urine of miners burning amalgam in open crucibles (130-410 µg/L). It was found that 36% of the ASM around Lake Victoria, Tanzania have Hg in urine above the WHO limit of 50 µg/g creatinine (van Straaten, 2000b). The author stressed that, in this area, Hg vapour exposure is much more important than MeHg ingestion with fish. Harada *et al* (1999) clinically examined 118 gold miners working in the same area, around Lake Victoria, and found as subjective symptoms (in decreasing order of relevance of the findings): trembling, headache, numbness of extremities, disturbance in taste, chest pain, dyspnea, cough and sputum, palpitation, disturbance in smell, pain in limb extremities, sleepiness, vertigo and dizziness. As an

[12] this was considered around 7 µg/L of Hg in urine
[13] this was considered around 25 µg/L of Hg in urine

objective symptom, the authors found that 13.6% of the examined miners had gingivitis. Other objective symptoms found in this study were: sensory disturbances and tremors in 8.5% of the miners; a decrease in tendon reflex (in 5.1%); neurasthenia (in 3.4%); night blindness (in 3.4%); and hyperreflexia (in 2.5%).

One of the most serious cases of Hg vapour intoxication of gold mining workers and surrounding communities has been occurring in El Callao, Venezuela (UNIDO, 2004). The use of rudimentary copper-amalgamation plates associated with burning amalgam on shovels has resulted in almost 12 tonnes/a of Hg released in the region. A total of 209 samples of urine (66 from women, 62 from children, 48 from millers and 33 from miners) were collected and analyzed for Hg and creatinine using a portable atomic absorption spectrometer LUMEX. The overall average of total Hg concentration in urine was 104.59 µg Hg/g creatinine with standard deviation of 378.41 µg Hg/g creatinine. The findings showed that 61.7% of the sampled individuals had Hg levels in urine above the ***alert*** level of 5 µg/g creatinine in which about 38% of the individuals had Hg levels above the ***action*** level (20 µg/g creatinine). About 21% of the sampled individuals have Hg levels in urine above the ***maximum*** of 50 µg/g creatinine recommended by the World Health Organization (WHO, 1991a) and 15% above 100 µg/g creatinine, which is the level where neurological symptoms should be evident. The situation with millers is dramatic; 79% had Hg in urine above the ***action*** level and 52% above 100 µg/g creatinine. In addition, 14.6% of millers have shown extremely high Hg concentrations in urine, ranging from 1,221 to 3,260 µg Hg/g creatinine. As a result of indirect exposure of Hg vapours, it was observed that 27% of the women had Hg concentration in urine above the ***alert*** level and 21% were above the ***action*** level. About 53% of the 62 children sampled had Hg concentration in urine above the ***alert*** level and 14.5% above the **action** level. Almost 10% of the sampled children had Hg in urine above 100 µg/g creatinine. A mercury concentration of 320 µg Hg/g creatinine was identified in a 7 year-old boy. The neurological signs of Hg intoxication found in women, children, miners and millers were ataxia, trembling of the hands, trembling of eyes, incapability of performing the finger-nose test, dysdiadochokinesia, patellar and hyper-reflexia, and patellar and cubital hypo-reflexia. In 25% of women and children and 28% of the miners and millers, it was possible to identify some of these symptoms. When correlating the neuropsychological tests with levels of Hg in urine, it was possible to notice that individuals with concentrations above 50 µg Hg/g creatinine have shown difficulties in completing the WMS-Memory Test. A similar situation was observed in the Finger-Tapping Test. The Episodic Memory Test (Mini-Mental) was useful to show that the % of individuals who had no problems performing the test decreases when the Hg level in urine increases (Fig. 2.1). The lowest scores represent acceptable performance. The percentage of individuals with poor performance in this simple perception-memory test increases with the level of Hg in urine. About 27% of individuals who performed the specific neuropsychological tests had noticeable neurological problems detected in the clinical exams.

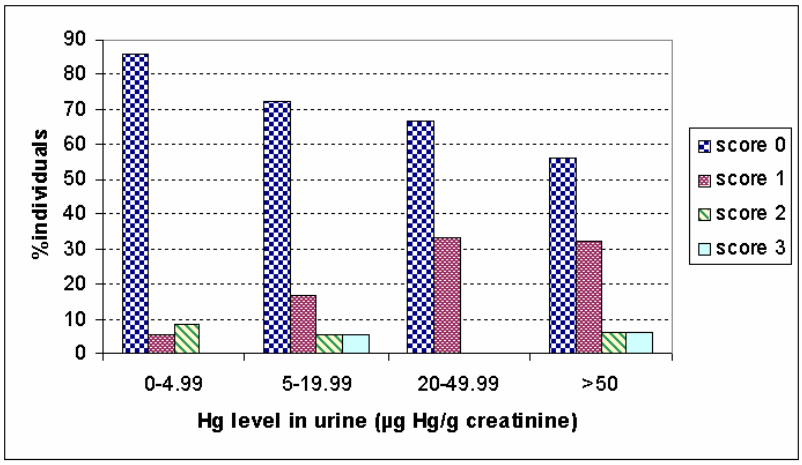


Fig. 2.1. Relationship between Hg in urine and Episodic Memory Test (Mini-Mental) in El Callao, Venezuela

2.2. Health Effects Caused by Methylmercury

In many rural communities, fish is abundant and inexpensive, making it the primary and often only source of animal protein. When contaminated fish is consumed, methylmercury (MeHg) is transferred to human beings. MeHg is easily bioaccumulated and biomagnified and becomes concentrated in fish, particularly in carnivorous fish.

Incidents of Hg poisoning through ingestion of contaminated food have been described since the incident in Japan where fish and shellfish, highly concentrated with methylmercury (MeHg), were ingested by thousands of people living around the Minamata Bay. This was the first and most controversial case related to organic mercury poisoning. However, the most tragic case took place in Iraq in the early 70s, when farmers ate Hg-treated seeds instead of planting them.

The effects of Hg released by ASM may not have the sudden impact of these two sad episodes, but the gradual MeHg contamination of fish-eating people in many parts of the world raises health concerns, in particular, concerning the long-term well-being of women and children. The effect of continuous ingestion of low-doses of MeHg is not yet well understood. Scientists and medical doctors around the world are trying to establish parameters to follow the evolution of the neurological problems and suggest dietary diversification for affected populations.

2.2.1. Iraq Tragedy

Incidents from eating alkylmercury-treated grains have been reported since 1940. The 1956 and 1960 epidemics in Iraq were confirmed as alkylmercury poisoning. However, knowledge of the two previous incidents did not prevent a third and more widespread epidemic in 1972 in Iraq. Farmers received around 95,000 tonnes of wheat and barley seeds to plant for crops. The seeds had been treated with organomercurials as fungicides. Instead of planting them, the farmers used the seeds to make bread (D'Itri and D'Itri, 1977). Flour made from this treated grain averaged between 8 and 9 ppm of alkylmercury. The Iraqi government broadcast warnings to avoid eating the grain. However, people in rural areas did not have radios, did not believe the warnings or chose to ignore them because they lacked other food.

Distribution of seeds began in September 1971. Between January and February 1972, around 6,500 patients were admitted to the hospitals (Bakir *et al*, 1973). The official number of fatal cases was 459, but Förstner and Wittman (1979) suggested that as many as 100,000 may have been permanently disabled. Sadly, many deaths could have been prevented, had the Iraqi government reacted differently to the outbreak. When it faced the initial disaster of the Hg-treated seed being distributed among the farmers, the government announced that any farmer possessing these seeds was liable to prosecution involving death. The peasants then disposed of the hazardous seeds in nearby rivers and lakes, spreading the contamination to even more remote regions.

2.2.2. The Minamata Outbreak

Minamata Disease (MD) (methylmercury-CH_3Hg poisoning) was discovered in 1956 around Minamata Bay, Kumamoto Prefecture, Japan. It is now well established that the source of the MeHg responsible for the Minamata outbreak was mercuric sulphate, a catalyst used between 1932 and 1968 for the industrial production of acetaldehyde. After 1995, the political problems related to MD were resolved in Japan and new facts have been gradually revealed. For example, it has been reported (Nishimura, 1998; Nishimura and Okamoto, 2001) that large amounts of MeHg were generated by the chemical processes of the Chisso acetaldehyde plant and later dumped directly into the Minamata Bay. This occurred due to technological changes introduced in the production process. In August 1951, manganese dioxide, initially used as a reaction promoter to maintain the activity of Hg catalyst, was changed to ferric sulphide. Ferrous iron was reduced in the reaction and then oxidized with nitric acid. As a result, the amount of MeHg generated in the process rapidly increased. The factory discharged about 82 tonnes of Hg (mostly as MeHg) into the river and bay (SSSGMD, 2001). In 1968, the plant stopped releasing wastewater into the bay. During 17 years of pollution, fish and shellfish accumulated MeHg through the gills and intestinal tracts. The amount of MeHg in the aquatic biota rose sharply in 1952, but dropped in 1968.

Since 1953, individuals living around Minamata Bay have shown neurological symptoms of MeHg intoxication. Thousands of cases of MeHg poisoning were documented in Minamata and Niigata in Japan. Adults who depended on fish for food exhibited severe neurological disorders and children who had been exposed *in utero* have shown signs of mental retardation even when mothers were asymptomatic. No new patients have been reported since 1976. However, cases of chronic MD are still being discovered, because of 17 years of contaminated fish ingestion between 1951 and 1968.

Before 1956, the reasons for the unusual behavior and disease of cats and humans were attributed to the atomic bomb in Nagasaki as well as pollution of many chemicals, such as Se, Tl, Mn, etc. In 1968, the Japanese Government recognized MeHg as a possible cause. Takeuchi *et al* (1959), Second Department of Pathology, Kumamoto University School of Medicine, reported on 14 July 1959 that organic Hg was the most probable cause of the MD. One week later, Hosokawa

et al. initiated an experiment in order to assess the toxicity of industrial waste from the acetaldehyde plant but the results were not published until 2001 (Eto *et al*, 2001b). As of March 1997, 17,000 people had applied to be recognized officially as victims of MD. Just 2,262 persons living in the Yatsushiro Sea area and 690 living in the Agano River basin were officially certified as having MD. They have been paid 144 billion yen as compensation. By March 1999, 11,235 people had been paid a lump sum of 2.6 million yen each by the polluting company (Osame and Takizawa, 2001). They were not certified for compensation as MD patients but it was recognized that they suffered some degree of MeHg poisoning. By 1998, 1,289 patients had died (Futatsuka *et al*, 2001).

2.2.3. MeHg Poisoning

Effects of MeHg on the Brain

Pathological changes caused by MeHg occur predominantly in selective areas of the cerebrum, including the calcarine region, the post-central and pre-central gyri and the temporal transverse gyrus. These areas are localized near the deep sulci, comprising the calcarine fissure, central sulci (Roland's fissure) and Sylvian's fissure. Ischaemia may be a result of the compression of arteries by edema of the adjacent tissues. Studies of acute MeHg poisoning in marmosets found an edema in the white matter of occipital lobes (Eto *et al*, 2001a). In acute cases of MeHg poisoning, neuron loss with gliosis was found in all layers of the cortex. The second and third layers of cortices are damaged in moderate or mild cases of poisoning. As a result of the location of the pathological changes, bilateral concentric contraction of the visual fields and impairment of visual acuity occur. Similarly, other effects are noticeable, such as sensory disturbance due to the damage of the sensory center (post-central gyrus), motor disturbance due to the damage of motor center (pre-central gyrus) and hearing impairment. Takeuchi and Eto (1999) have summarized all autopsy cases from 1956 to 1995 concerning MD.

Main Symptoms

The main symptoms of MeHg poisoning are sensory disturbance in the distal parts of the extremities followed by ataxia, disequilibria, concentric contraction of visual field, impairment of gait and speech, muscle weakness, tremor, abnormal eye movement and hearing impairment. These signs and symptoms are accompanied occasionally by disturbance of taste and olfactory sensation, and mental disorder (Tsubaki and Takahashi, 1986).

Muscular atrophy and mental disturbance are prominent in **acute** intoxication. Some cases of long-term effects of MeHg are reported. Forty-nine cases of people who lived in the Minamata area around 1956, but departed afterwards, are reported by Harada (1978). They had eaten contaminated fish for limited periods and the symptoms appeared many years after ingestion had been suspended. Studies on Iraqi and Japanese patients revealed the delayed appearance of neurological symptoms after a lapse of one year in persons who had elevated Hg levels in hair but could not confirm neurological symptoms at the first examination (Suzuki, 1979).

Tokuomi (1960), who was studying patients from Minamata, observed a number of typical symptoms of MD. His study reported sensory disturbance and contraction (reduction) of the visual field among 100% of individuals; coordination disturbance among 93.5%; dysarthria among 88.2%; hearing disturbance among 85.3%; and tremors among 75.8%. The symptoms of MeHg poisoning are variable as a combination of symptoms with various degrees can occur, depending on the individual and the level of poisoning.

One of the typical clinical signs of MD is **bilateral concentric contraction**[14] **of visual fields** that reduces the visual perception (e.g. lateral vision) of the patient. The pathological changes are more prominent in the anterior areas of the calcarine cortex, as compared to the posterior pole (Shiraki, 1979; Takeuchi, 1968). It is well known that the anterior area of the calcarine cortex relates to the peripheral parts, and the posterior areas to the central parts (Netter, 1975). Edema surrounding the anterior sulcus of the occipital lobe may compress the anterior calcarine cortex more than the posterior cortex, since the anterior sulcus is deeper than the posterior sulcus (Eto *et al*, 2001a).

MD patients have shown **cerebellar ataxia, disequilibria, impairment of gait and speech** (Shiraki, 1979; Takeuchi, 1968). Purkinje cells are well preserved compared with the smaller granule cell neurons. Granule cell loss beneath the Purkinje cell layer is typical. Tests with marmosets have revealed that cerebellar edema occurs in acute stages of MeHg poisoning. Cerebellar sulci were found compressed by edema, and Purkinje cells were lost in severe cases of MeHg poisoning (Eto *et al*, 2001a).

Typical signs and symptoms of the **sensory disturbance** occur initially in the distal parts of the extremities. The post-central cortex, the sensory center, is damaged. Therefore, it is very difficult to explain the glove-and-stocking[15] sensory disturbance due to damage of the sensory center alone. Studies with marmosets have shown axonal injury of the

[14] it is also known by some authors as visual constriction

[15] This is a sign of peripheral neuropathy, i.e. damage of the peripheral nerves (which go from the brain out to the body); symptoms include numbness and altered sense of feeling in the extremities, especially hands and feet.

peripheral nerves (Eto *et al*, 2002a), but this was not clear from autopsy of MD victims (Takeuchi, 1968). Marmosets treated for over 2.5 years with MeHg show regeneration of the axons and myelinated nerve fibers. Then the peripheral nerves might be regenerated after long time (Eto *et al*, 2002a). It is important to consider the period of MeHg pollution to understand sensory disturbance. Axonal changes are recognized in the initial stage of the degeneration of the peripheral nerves of MeHg poisoning. Sural nerve biopsy was performed in three patients of Minamata disease in 1976. Eto and Takeuchi (1977) described incomplete regeneration of the sural nerves, but within 10 years, patients of MD might show complete regeneration of these nerves.

Congenital Effects of MeHg

MeHg can penetrate into the placental barrier to be transferred to the fetus. It has been observed that when a female's intake of the poison is large and she becomes ill, sterility occurs. When the dosage is smaller, pregnancy can take place but the fetus may be aborted spontaneously or is stillborn. An even smaller dose permits conception and live birth, but the baby will have severe neurological symptoms. A dosage too small to cause noticeable neurological symptoms in the child may cause congenital mental deficiency. In all of these cases, the mother's symptoms are relatively mild. It was observed in Iraq that maternal milk contained 5 to 6% of the organic mercury concentration analyzed in the mother's blood (Harada, 1978; Bakir *et al*, 1973).

Approximately 64 cases of fetal MD have been identified in Japan and 13 of these patients have died (Harada M., unpublished data, 1999). The first cases of fetal MeHg poisoning in humans were reported by Takeuchi *et al* (1964). Fetuses were clinically diagnosed with cerebral palsy. Six autopsy cases of fetal MD were reported by the School of Medicine of Kumamoto University. Choi *et al* (1978) reported a fetus from Iraq damaged by MeHg. Hypoplastic changes were found in the cerebrum and cerebellum. Takeuchi *et al* (1964) and Matsumoto *et al* (1965) reported that the pathological changes in the cerebrum in fetal MD were different from those found in children and adults. In the latter ones, prediction sites of the pathological changes were relatively constant in the calcarine, post-central, pre-central and temporal transverse cortices; whereas, the changes in fetal cases were less localized to a specific site and showed hypoplasia or developmental arrest, rather than destruction of neurons. The cerebral gyri of fetuses were not so deep compared with those of children and adults. Electron microscopy has indicated that synapses between parallel fibers and Purkinje cells were well formed in the fetus cerebellum (Eto *et al*, 1991). Biopsy of the sural nerves in fetal cases revealed similar findings, characterized particularly by aplastic and hypoplastic myelination of small nerve fibers with abnormal, ovoid myelin degeneration, which are also characteristic of adult cases (Takeuchi *et al*, 1978). Effects of low-level exposure of MeHg to adults and fetuses are not yet well understood and have been discussed in many parts of the world, in particular in regions where fish is the main source of protein.

Maternal milk has also been used as a monitoring material to investigate transference of MeHg to infants (Boischio *et al*, 2003). The effect of fish ingestion on breast milk was studied in 47 mothers and their babies in remote areas of the Amazon region. The average Hg level in maternal milk was around 6 ppb with values as high as 24.8 ppb Hg. Correlation analysis revealed that Hg in hair was significantly affected by maternal MeHg ingestion during pregnancy, but not during the post-natal breast-feeding period. As well, the Hg levels in milk did not correlate with mother's or infant's hair (Barbosa and Dorea, 1998).

2.2.4. Low-level MeHg Exposure

The effect of MeHg on the human body varies according to the degree of contamination. Specifically, when large doses[16] of MeHg enter the body, there are symptoms of acute brain damage such as aberrations of consciousness, convulsions and paralysis, followed by death. When MeHg intake is lower, mild, atypical or incomplete symptoms may appear or another disease may be manifested. Previously, it was thought that the harmful effects of MeHg were confined to the nervous system; however it has become apparent that effects on other organs must also be considered (Harada, 1978).

At lower levels of exposure, as it occurs in ASM regions, continued interference with biochemical and cellular processes potentially causes neurophysiological and psychological functions to undergo slow alterations. This may be undetected in the early stages, due to nervous system plasticity and compensation. Neurobehavioral test batteries, designed to quantitatively evaluate small changes in performance, serve to identify motor, sensory, cognitive and emotional changes in exposed persons, with respect to control groups or to internal or external exposure parameters (Mergler, 2002).

Although MeHg concentrates in the hair and epidermis, these tissues have small excretory roles in relation to body burden. Variation in metabolism, detoxification and excretion of the different types of mercurials is considerable. Data

[16] Accumulation of 30 mg of MeHg in a 70 kg adult (0.43 μg/g of body) causes sensory disturbance and 100 mg (1.4 μg/g of body) causes all typical poisoning symptoms (Harada, 1984). Laboratory studies with cat and mice have shown that 30 μg of MeHg per gram of brain is likely the threshold level to manifest neurological symptoms followed by death (Nelson *et al*, 1971).

on excretion of MeHg compiled by Nelson *et al* (1971) show fecal excretion of about 4% in the first few days and then 1% per day thereafter. Only about 0.1% per day is lost in urine. In contrast, metallic Hg is poorly absorbed by the gastrointestinal tract, i.e. the majority is flushed out of the organism (WHO, 1991a).

Akagi *et al* (1995) reported that after clinical examination, no typical Minamata disease symptoms were identified in the ASM -affected areas of the Brazilian Amazon. However, many authors have described neurological effects.

Studying fish-eating inhabitants in the Tapajós River, Amazon region, Brazil, Grandjean *et al* (1999) noticed neuropsychological dysfunctions in children whose mothers had less than 10 ppm[17] Hg in hair. The authors also found that more than 80% of 246 children studied had Hg levels in hair above 10 ppm, which was believed to cause adverse effects on brain development. As mentioned before, it is important to stress that more than 90% of Hg found in hair of individuals from riparian communities in the Amazon is methylated (Akagi and Naganuma, 2002) and these communities are very distant from ASM activities. The excess fish consumption, especially carnivorous species, is the main cause of high levels of MeHg in blood and consequently in hair. Also, in the Tapajós region, Harada *et al* (2001) clinically examined 50 individuals who had more than 20 ppm Hg in hair. They detected as subjective symptoms: numbness of extremities in 34% of the individuals, vertigo and dizziness in 24%, headache in 24%, and reduction in vision, trembling, irritability, reduction in hearing, loss of memory, motor disturbance and insomnia in about 10% of the interviewed people. As objective symptoms, they detected sensory disturbance in 32% of the patients. They also found balance and coordination problems, tremors, hyperreflexia, and dysarthria (difficulty in articulating words) in about 10% of the examined individuals. The authors have described three individuals out of fifty as having symptoms of "mild Minamata Disease". These individuals consume large amounts of fish (as much as 1,000g per day). Their Hg hair concentrations range from 16 to 71.5 ppm. In addition, they failed some neurophysiological tests such as two-point discrimination, finger-to-nose, knee-to-knee and exhibited glove-and-stocking type sensory disturbance, tremors and numbness, among other objective symptoms.

Mergler (2002), in an excellent review of the neurobehavioral effects of MeHg due to intensive fish consumption, highlighted that in the Tapajós River, many authors observed a correlation between Hg levels in the hair and symptoms, such as loss of fine motor capacities, coordination, manual dexterity, and visual functions. Lebel *et al* (1998) observed that near vision contrast sensitivity and manual dexterity decreased significantly with increased Hg levels in hair. They confirmed previous remarks by WHO (1990) that 50 ppm of total Hg in hair is an adequate threshold to observe clinical effects.

Some researchers have used domestic animals instead of fish to monitor Hg bioavailability. Palheta (1993) and Palheta and Taylor (1995) collected blood and hair from pigs, cattle and sheep in Cachoeira, Brazil. High Hg concentrations (average 27 µg/L) were evident in the blood of pigs and levels in local animals were 30 to 45% higher than in control animals. Mercury concentrations in the blood and hair of sheep (average 1.82 µg/L and 0.24 µg/g, respectively) were lower than other animals sampled and were only slightly greater (10-20%) than control animals. The percentage of total Hg that was in organic form (i.e. MeHg) ranged from 37 to 99% in pigs and 32 to 99% in cattle. Mercury levels in animal hair were not correlated with blood. Because Hg concentration in tissue was not determined, the implications of these results with respect to human consumption were not ascertained. Based on this research, it is not clear by which pathway domestic animals are accumulating mercury (Hinton, 2002). Most likely it is from fish. In any case, it is clear that any monitoring program should be preceded by a survey on the dietary habits of community members to establish the relevance of sampling food for Hg analysis.

[17] A normal level of mercury in hair is 1 to 2 µg Hg/g hair (ppm)

2.3. Guidelines and Reference Doses

2.3.1. Hg in Urine

In persons not occupationally exposed to mercury, urine levels rarely exceed 5 µg Hg/g creatinine. This is considered the ***alert*** level (Drasch *et al*, 2002). The concentration of 20 µg Hg/g creatinine is considered an ***action*** level, i.e. the individual should be removed from the pollution source. The maximum level recommended by WHO (1991a) is 50 µg Hg/g creatinine. An individual with Hg levels in urine above 100 µg/g creatinine has a high probability of developing symptoms such as tremors and erethism (abnormal irritability).

2.3.2. Hg in Fish

The guideline[18] level of Hg content in fish varies among countries and is meant to provide guidance to fish consumers for edible parts of fish. Different guidelines have been adopted by different countries such as Canada and Brazil (0.5 ppm total Hg), Italy (0.7 ppm), Finland, Sweden and Japan (1 ppm) (Johansson *et al*, 1991; Hacon, 1990). The WHO (World Health Organization, 1991) adopted the safety guideline of 0.5 ppm of methylmercury for all fish except predatory fish, and 1 ppm for predatory fish. The WHO guideline highlights that *"where these guideline levels are exceeded, governments should decide whether and under what circumstances, the food should be distributed within their territory of jurisdiction and what recommendations, if any, should be given as regards restrictions on consumption, especially by vulnerable groups such as pregnant women."*

In Canada, the 0.5 ppm of total Hg guideline is enforced by the Canadian Food Inspection Agency (CFIA) and is intended to regulate commercial sales of fish. Canned tuna is consumed frequently and is subject to inspection and enforcement of the 0.5 ppm guideline (Health Canada, 2002). However, certain fish species sold in Canada such as shark, swordfish and fresh and frozen tuna contain mercury at levels that are known to exceed 0.5 ppm, (usually ranging from 0.5 to 1.5 ppm). Because these species are consumed only occasionally, they are exempt from this guideline. Therefore, another risk management strategy is followed, namely issuance of advisories recommending appropriate restrictions on amounts and frequency of fish consumption based on body weight, gender and age. This amount is termed the tolerable daily intake or "TDI". It is clear that the Hg guideline concentrations used to regulate Hg exposure in humans from fish do not target all kind of individuals. Rather, the actual amount of Hg ingested by individuals is the main concern of health authorities. In 1990, the World Health Organization (WHO, 1990) stressed that a tolerable daily intake (TDI) of 3 to 7 µg of MeHg/kg body weight would cause adverse effects of the nervous system, manifested as a 5% increase in the incidence of paraesthesia. Hair concentrations would be approximately 50 to 125 µg/g at this level of intake. The FAO/WHO Expert Committee on Food Additives (JECFA) concluded that Hg concentrations of 200 ppm in blood and 60 ppm in hair could be considered as the lowest observable adverse effect levels (LOAELs) in adults. When converted to dietary intake and combined with a 10-fold uncertainty factor, a TDI of MeHg of 0.47 µg/kg bw/day is obtained (Pilgrim *et al*, 1999). This is the TDI level adopted in Canada in 1998 for adults; for children and women of reproductive age, it is 0.2 µg/kg bw. The TDI for total mercury established by Health Canada is 0.71 µg/kg bw (Campbell *et al*, 2003). The MeHg Provisional Tolerable Weekly Intake (PTWI) was revised by FAO/WHO JECFA in June 2003. The level of 3.3 µg/kg bw/week was reduced to 1.6 µg/kg bw/week. For a 60-kg individual the guideline of 96 µg MeHg/week is recommended (FAO, 2003). The United States Food and Drug Administrations (FDA) action level for mercury in commercial fish is 1 ppm. The FDA established a tolerable daily intake (TDI) based on a weekly tolerance of 0.3 mg of total Hg per person, of which no more than 0.2 mg should be present as MeHg. In recognition of the developmental effects, FDA also warns pregnant women and women of child-bearing age to limit their consumption of fish known to have elevated levels of Hg (Pilgrim *et al*, 1999). In 1989, the USEPA had a daily MeHg reference dose of 0.3 µg/kg bw/day. This was revised in 1995 to 0.1 µg/kg bw/day (UNEP, 2002).

2.3.2. Hg in Hair

The normal Hg (mostly as MeHg) level in hair is less than 1-2 µg Hg/g of hair or ppm (WHO, 1991b). Hazardous effects to the fetus are likely when 20 ppm is analyzed in the hair of pregnant women (Krenkel, 1971; Malm, 1991). Levels of 10 ppm must be considered as the upper limit guideline for pregnant women (Skerfving, 1973). According to the WHO (1990), 50 ppm of total mercury in hair is an adequate threshold to observe clinical effects. Methylmercury level of 200 µg/L (ppb) in blood, corresponding to Hg concentration around 50 µg/g (ppm) in hair, is associated with a 5% risk of neurological damage to adults. The WHO (1990) reports that, based on statistical analyses, child-bearing women with Hg concentrations in hair above 70 µg/g (ppm) exhibit more than 30% risk of having neurological disorder in the offspring. Recent evaluation considers 5 ppm Hg in hair a safe guideline for pregnant women (Yagev, 2002).

[18] This level is established for an average ingestion of 400 g fish weekly.

2.4. Sampling Fish for Health Assessment

To quantify Hg exposure to local people and determine the potential for health effects, the following information must be known:

- The daily average quantity of fish consumed (grams), for different meals. Note that quantities may differ depending on the meal (i.e. breakfast, lunch, dinner).
- The number of meals per day or per week that fish are consumed.
- The relative proportion of different fish species consumed (i.e. the target species). Note that target species may differ depending on season (i.e. wet versus dry) in many countries, for example, Brazil.
- Size (length and weight) of the fish consumed.
- The tissue Hg concentration (ppm of total Hg in whole muscle in wet weight) of the target species consumed. Note that if more than one species comprises a major part of dietary fish consumption, Hg concentration must be determined for each target species.

Information on quantity and frequency of fish consumption of each target species can best be gained through interviews with the person responsible for preparing most of the meals, typically the women in the household (see example of medical questionnaire annexed). Alternatively, interviewing fishermen at the river banks or local fish shopkeepers will help identify the major species consumed and provide information on the relative abundance of the different species captured. These individuals will have a good idea of the type of fish most frequently available for sale and the relative amount of each species sold. Note that there may be different target species captured during wet and dry seasons; this information should also be solicited.

Carnivorous (fish-eating) fish are the ultimate aquatic receptor species of MeHg and represent the main pathway of MeHg exposure to humans via dietary sources. The proportion that MeHg comprises of total Hg concentration in carnivorous (piscivorous) species is at least 90% (Bloom, 1992). Akagi and Naganuma (2000) have also shown that the vast majority of Hg in herbivorous and detritivorous fish in the Amazon region is also in the form of MeHg. Therefore, fish tissue should be analyzed for total Hg only. Carnivorous fish are not always the most consumed species, due to either high prices or the difficulty in capturing them. In the Brazilian Amazon region, the frequency and type of fish consumed also vary according to season (Dolbec *et al*, 2001).

Identifying the target fish species is the most important step in establishing the sampling protocol for the human Health Assessment. It is important to know if the "most consumed fish" in a region can be used as a standard species. Note that a strategic sampling procedure for acquiring fish tissue for Hg analysis is described in Section 1.5.2. This procedure must be followed to select the appropriate species, derive a length-Hg relationship and evaluate data based on a standardized fish size. This relationship will provide the risk assessor with an empirical relationship between fish size and Hg concentration from which to estimate Hg exposure for Health Assessment purposes. Further, this will provide the health researcher with a methodology for following the evolution of Hg levels in fish over time as well as analyzing human biomonitoring materials (blood and hair).

Semi-quantitative Hg analysis for fish can also be used when analytical facilities are not available or for screening purposes (Yallouz, 2001). Yallouz *et al* (2004) applied this technique to analyze fish from the mining sites around Itaituba, Pará State, Brazil. About 10 g of fish sample are digested with an oxidant mixture, containing sulfuric acid, nitric acid and vanadium pentoxide. Ionic mercury in solution is reduced with an acidic solution of stannous chloride. The elemental Hg is purged out of the solution with air and contacts a paper containing cuprous iodide. The paper is compared with standards previously prepared. This alternative and simple analytical method was brought to the attention of laboratory technicians in the mining regions in Brazil and Indonesia. A preliminary diagnosis can be done rapidly to help the health assessment team. However, this procedure does not allow accurate evaluation of Hg concentration changes with time.

2.5. Medical Exam

2.5.1. Procedures

A Health Assessment is an epidemiological research project and therefore involves evaluation of the physical and mental conditions of individuals and possible influences of external factors that may or may not contribute to the aggravation of their health. Medical exams are usually designed to establish a relationship between biomonitoring materials (analysis of hair, urine and blood) and symptoms of poisoning, which in rough terms can be described as a dose-response procedure.

For neurotoxicants such as metallic Hg and MeHg, current epidemiological (and clinical) practices examine a continuum of responses by severity from subtle responses to very frank adverse outcomes. This becomes even more complex because most neurotoxicological tests deal with different neuro domains. For example, motor coordination is a different domain from memory (Wyzga and Yager, 2001). Symptoms can be very subjective, influenced by many confounding factors and are not always identified in a medical interview. Harada *et al* (2001) described subjective symptoms as those claimed by the patients in an interview. Some of them are: numbness, vertigo, dizziness, lassitude, pain in the extremities, back pain, reduction of vision, trembling, irritability, reduction of hearing, loss of memory, motor disturbance, insomnia and disturbance in taste (metallic taste). The authors listed those investigated in specific tests as more objective symptoms. Some of them are: sensory disturbance (glove-and-stocking type); disturbance in balance; disturbance in coordination; tremors; hyperreflexia; dysarthria; and gingivitis.

Before administering a battery of questions and tests, individuals must be carefully selected. This implies in a preliminary knowledge about socio-economic-demographic distribution and conditions of the individuals and their families. In this case, data such as family members (number and ages), hygiene, diet, education, occupation, income, expenditure, property, access to media, knowledge about mining, access to mercury, etc., must be noted. This aims at indicating, based on the routes of mercury exposure, the most susceptible and sensitive group of people in a community to be contaminated as well as about which groups of inhabitants can (or cannot) be selected as controls. This socio-economic-demographic study also provides information about the mining community demographic distribution. This is used in the Health Assessment to select groups of people that statistically represent the community. The study also provides valuable information for any kind of intervention on the site (technical, medical, environmental, economic). An example of this questionnaire is found in the appendix.

A medical exam consists of an initial questionnaire about the health history of the individuals, followed by physical and neurological examination. Questions related to health history are needed to exclude participants with severe diseases from the statistical evaluation (e.g. someone who has had a stroke might be excluded from the survey). Individuals are selected for a series of specific neurophysiological tests that are designed to detect effects of mercury poisoning. These are simple tests and local health care professionals must be trained to perform such a series of tests in local health offices. It is always advisable to use a local health post and involve local doctors and nurses in all steps of the Health Assessment.

Before starting the field assessment, questionnaires must be translated into the regional/national language and the consent from a local or regional Health Authority must be issued. It is important to have this ethical clearance from the Health Authorities and sometimes the questionnaires must be submitted to a review board or ethics committee. They must evaluate the ethical and cultural issues related to the type of questions to be applied. During fieldwork, each participant must be interviewed by national nurses, who should fill out the questionnaire. The medical experts must examine and test each participant. National doctors/nurses are the most appropriate people to take specimens of biomonitoring material. The volunteers, individuals selected by the socio-economic-demographic survey, must be informed about the entire project by the individuals (experts) interviewing them and informed as to how the data generated by the medical exam can help them and their community. Brochures are useful to provide this preliminary background to the volunteers. The brochures or pamphlets can also include some basic information about the hazards related to Hg exposure by vapour and a simplified diet advisory. Some formalities must be observed:

1. Consent to apply the proposed questionnaire must be obtained from a Health Authority (local or regional) or an ethics committee.
2. The volunteers must be informed that all personal information (especially identity) will be kept confidential.
3. They must be instructed about the goals of the assessment.
4. They must be informed that the Health Assessment will follow ethical procedures recommended by the Code of Conduct of the World Health Organization.
5. They will receive all results of the analyses of biomonitoring materials and will be informed about their health situation and any possibility of being intoxicated by mercury (this is a job to be conducted together with local health authorities).

6. When high certainty about mercury intoxication exists, they will be informed about available ways to reduce exposure.
7. In areas of high incidence of tropical diseases, malaria or tuberculosis incidence, it is suggested to test and recommend therapy for these treatable diseases.
8. The volunteers must sign a document in English and local language (example in Appendix) agreeing with the interviews, sample donation, physical exams and neuropsychological testing.

Wyzga and Yager (1999) stressed the importance of a well-designed Health Assessment. The authors compared two studies, one in Seychelles Islands and the other in the Faroe Islands, which were implemented to understand the health risks associated with fish consumption. Although both studies were well conducted, the Seychelles study could not find any statistically significant association between MeHg ingestion and several developmental tests. However, the Faroe Islands study demonstrated a clear relationship between neuropsychological test performance and *in utero* exposure to methylmercury. Wyzga and Yager (1999) speculated that the children examined in the Seychelles study were too young (6, 19, 29, and 66 months) and that effects might not be observed easily at this young age, when development is most rapid and it is difficult to distinguish adverse effects.

A Health Assessment must be designed for a specific purpose. There is no general rule for this. In some cases, the most evident exposed individuals may not be considered. As children and women of childbearing age are the groups most sensitive to mercury poisoning, the Health Assessment (including the questionnaires) can be designed to evaluate this specific group (Boischio and Cernichiari, 1998; Boischio and Henshel, 1996). When evaluating the effects of MeHg on a riparian community in the Amazon, Lebel *et al* (1998) selected a group for medical examination based on hair measurements, age (range of 10 years), gender and education level. It was observed that Hg in hair of men and women decreases with age, being higher in the group with ages from 15-24.

When children are part of the Health Assessment, a specific consent from the guardians must be obtained to interview them as well as to take blood, hair or urine samples.

The neurological and clinical tests must also take into consideration the existence of local infrastructure (e.g. electricity) as well as the level of education of the population (Mergler, 2002). Ideally, a specific questionnaire should be developed for children and other individuals with lower education.

A critical decision in Health Assessment is the number and type of samples (individuals) to be included in the study. As in the environmental assessment, the sampling process can be random or judgmental. Randomization assumes that all individuals in a community have the same chance of being exposed; no pre-conceived idea is imposed. It is definitely a more expensive and time-consuming process but provides broader picture of the public health than a selective (judgmental) sampling process. Using the judgmental approach, a questionnaire is applied to a large number of people in a community to select just the individuals at higher risk of being exposed to mercury vapour or MeHg by ingestion. A multistage sampling program can also be a useful strategy. In this case, clusters are selected, for example, specific groups of people in a neighborhood or site or family; then those clusters are compared to each other. This is a useful method when large variation on habits and living conditions occur within a community (IPCS, 2000). In the Global Mercury Project, UNIDO chose the random sampling approach. Using a socio-economic-demographic study based on interviews (questionnaire), it is possible to establish the characteristics of a mining community. Then the Health Assessment should follow similar societal distribution. In this case, all groups (young and senior miners, older and younger women, children, etc) are represented and sampled in a proportion that represents a specific community. A minimum of 200 individuals in a mining community are recommended to be sampled and 50 in the control area, i.e. a community with similar cohort of people but not impacted by ASM activities.

In order to obtain population distribution (census) figures to support the Health Assessment, a Government body should be consulted. If not available, teachers, health professionals, local religious or tribal leaders can also be consulted. Midwives, doctors or those who perform religious initiation ceremonies such as baptism, circumcision, etc. are usually knowledgeable about the approximate population growth and consequently the gender distribution.

In the Appendix, there is an example of a questionnaire and the content of a medical exam. The personal data is repeated in each chapter of the questionnaire, to allow the use of these chapters by different interviewers. The questionnaire is divided as follows:

1. General questions related to:
   - personal data
   - occupational exposure to mercury (routes of exposure)
   - confounding factors (to exclude candidates with other problems)
   - diet issues (frequency and type of food)
2. Questions related to Health Conditions and Subjective Symptoms (as described by the patient, e.g. metallic taste, salivation, fatigue, etc.).

3. Clinical and Neurological Examination (e.g. blood pressure, signs of gingivitis, ataxia, tremors, reflexes, etc.).
4. Specific Neuropsychological Tests (e.g. memory, coordination, etc.).
5. Sampling biomonitoring materials (urine, blood, hair).
6. Results of chemical analyses of biomonitoring materials.
7. Medical Score (how to combine pieces of information).
8. Decision on who is intoxicated with mercury.

Being more specific on the procedures to be adopted in a medical examination and neuropsychological testing, Drasch *et al* (2001) have suggested checking the following mercury poisoning indicators:

In the Clinical and Neurological Examination:

- Signs of bluish discoloration of gums.
- Ataxia.
- Tremor.
- Test of alternating movements or test for dysdiadochokinesia.
- Test of the field of vision.
- Reflexes: knee jerk reflex and biceps reflex.
- Pathological reflexes: Babinski reflex and labial reflex.
- Salivation and dysathria.
- Sensory examination.
- Proteinuria.

In the Neuropsychological Testing:

- Memory disturbances: digit span test (part of Wechsler Memory Scale) to test the short-term memory.
- Match box test of co-ordination, intentional tremor and concentration.
- Frostig score to test tremor and visual-motor capacities.
- Pencil tapping to test intentional tremor and coordination.

One important point highlighted by Dr. Boese O'Reilly, from University of Munich is the fact that local health care professionals must be trained to perform simple neuropsychological tests. These tests do not demand special equipment and, associated with analysis of biomonitoring materials, can provide an accurate picture of degree of mercury intoxication.

Analysis of biomaterials may impose further difficulties in collection, preservation and transportation of samples to a local laboratory. This is even more complex when samples must leave the country. Some *in situ* analyses of total Hg in biomaterial samples (e.g. using LUMEX or colorimetric procedures) can be very useful for a preliminary screening and rapid diagnosis.

2.5.2. Confounding Factors

Confounding factors must be investigated to exclude from the statistical analysis other explanations for any symptom found. There are many factors that derive symptoms such as fatigue, dizziness, and tremors which introduce false diagnosis to the clinical examination and neuropsychological tests. Some of the confounding factors extracted from several authors (Mergler, 2002; Crompton *et al*, 2002; Campos *et al*, 2002; Drasch *et al*, 2001; Dolbec *et al*, 2000; Grandjean *et al*, 1999; Wyzga and Yager, 1999; Lebel *et al*, 1998; Akagi *et al*, 1995; Veiga, 1994) are listed as follows:

- Alcohol consumption
- Use of drugs
- Smoking
- Malaria and other tropical diseases
- Tuberculosis
- Parasitosis
- Constant handling of gasoline and kerosene
- Handling of pesticides
- History of neurological disorders (epilepsy, stroke, Parkinson, etc.)
- History of health problems (kidneys, high blood pressure, lungs, etc.)
- History of stress
- Allergies
- Number of dental amalgam fillings
- Ingestion of selenium (from fish or nuts)

- Cumulative effect with exposure to other pollutants (e.g. PCB)
- Use of Hg-containing soaps and creams for skin lightening

One of the main confounding factors is alcoholism. Regions with high concentration of artisanal miners usually have high alcohol consumption (in particular in South America). Alcohol can influence or bias results of medical and neurophysiological tests. It is hard to obtain reliable information about the amount of alcohol ingested by an individual. There are possibilities for toxico-kinetic as well as toxico-dynamic interactions between alcohol and Hg. Ethanol as an inhibitor of the enzyme catalase, reduces oxidation of Hg vapour into ionic Hg in the blood (Yoshida *et al*, 1997). Magos and Webb (1979) showed evidence of increasing Hg exhalation and decreasing Hg deposition in lung, blood, heart and brain when alcohol pre-treatment was applied to mice. Mercury concentration in the liver increased, however. Satoh (1994) examined exhalation of Hg after alcohol ingestion in an ex-Hg miner of Itomuka, Japan. This miner worked for 24 years until mining activities were discontinued in the early 1970s. Mercury levels in his blood and urine were in the normal range of non-exposed people. After 30 minutes of ingestion of 20 g of ethanol in form of beer or "sake", Hg concentrations in expired air peaked, decreasing after 120 min. The author concluded that even years after cessation of exposure, Hg still remained deposited presumably in the kidneys.

Alcohol can increase the concentration of MeHg in the liver, the kidney and the brain, while inorganic Hg is lowered in the liver and kidneys. In rats, it has been shown that ethanol, in combination with MeHg, enhances the retention of mercury in the kidney and increases the nephrotoxicity while it has no effect on the neurotoxicity of MeHg (McNeil *et al*, 1988). Beside these toxico-kinetic interactions, chronic alcoholism may cause several adverse neurological effects.

An interesting confounding factor was noticed when Harada *et al* (1999) analyzed hair of ASM in Tanzania. They found a low MeHg:total Hg ratio, because most gold miners and residents are subjected to high burdens of Hg vapour from inadequate amalgam burning procedures. However, the highest level of (inorganic) Hg of 48.2 ppm was detected in the hair of six females who used soap containing Hg. Kinabo (2002) observed very high levels of total Hg (7 to 880 ppm) in hair from Tanzanian women using soaps with up to 0.87% of inorganic mercury salts. The author cites that previous work has analyzed levels up to 100 µg/L of Hg in urine of women using Hg-creams and soaps. Most of these Hg-skin-whitening soaps and creams are produced in Europe and exported to African countries.

2.5.3. Data Management

Data management consists of two basic goals: 1) to represent the data quantitatively and 2) to accumulate knowledge on health effects caused by mercury poisoning.

Once the information is obtained from the health questionnaires and chemical analyses, statistical methods must be employed to validate the findings. IPCS (2000) published a comprehensive book on Human Exposure Assessment where statistical considerations for data management are discussed extensively. When analyzing Hg in biomonitoring media such as urine, blood and hair, it is important to report the median (midpoint where 50% of individuals occur above a certain value), the mean (arithmetic average of the results), the minimum and maximum values, standard deviation, and confidence levels. The concept of percentile is an important aspect as it describes the percentage of the investigated group that is at or above a certain value. A percentile is first determined by ordering (ranking) the values from the lowest to highest. Then the p% is the percentage of the data at or below a specific value. Histograms of frequency and box plots are useful ways to illustrate the analytical results.

Correlations between Hg poisoning symptoms and Hg in biomonitoring materials have been a classical approach to identify health problems in exposed individuals. Drasch *et al* (2001) highlights that, in a community in Philippines, only some of the clinical data characteristics for Hg intoxication (e.g. tremor, loss of memory, bluish gum discoloration, etc.) correlate with Hg in blood or urine, but not with Hg in hair. The medical score sum correlates only with Hg in urine. The poor correlation between the Hg concentration in the biomaterials and classic clinical signs of chronic Hg intoxication may be explained by several factors; however the main point is that Hg in blood, urine, and hair do not adequately reflect the Hg burden of the target tissues, especially the brain. Memory tests correlated with Hg in blood and urine. Metallic taste, labial reflex and frequency of proteinuria are correlated with Hg in urine and the Frostig test with Hg in blood. In studies with Amazonian riparian communities living distant from mining operations, Hg in hair correlated with neuropsychological findings (Lebel *et al*, 1998; Grandjean *et al*, 1999; Mergler, 2001; Dolbec *et al*, 2001; Harada *et al*, 2001). The most frequent reported sign of MeHg intoxication was alteration of visual functions (Lebel *et al*, 1998).

Scoring or ranking procedures have been used in Health Assessments. But in many cases, all pieces of information were ranked with the same weight (importance). Drasch *et al* (2001) have concluded that diagnosis of **Hg intoxication cannot be done based on Hg concentrations in biomonitoring materials alone, but by a balanced combination of these Hg values and the medical score sum.** Results of the physical and neurological exams were scored in two levels (0 = no; 1 = yes). Some neuropsychological tests were arranged by three levels of ranges. Each range received a score (score 0 for best performers and 2 for worst performers). All scores were summed obtaining a final medical test score

and then the correlation with biomonitoring materials was checked. In the Appendix I, the doctors from the University of Munich suggest a Medical Score using just part of the answers of the medical questionnaires.

Correlations between pieces of information from the questionnaire with results of chemical analyses of the biomaterials are useful to visualize sources of Hg intoxication. For example, at the end of each questionnaire in Appendix I, it is possible to obtain a % by dividing the Total Points Obtained in each chapter of the questionnaire by the Maximum Points Allowed. In this case the Maximum Points Allowed considers just the questions/tests applied to the volunteer. In other words, the interviewer can select/skip the questions. Low % represents low likelihood of having problems related to Hg. The % can be used to obtain correlations between scores and the chemical results of biomaterials (urine, blood and hair).

When considering ranking or scoring methods, many procedures do not prioritize one observation over another. For example, is *discoloration of the gums* more significant as a symptom of undue exposure to Hg vapour than a *chronic headache* or do both have the same weight? Some knowledge accumulation methods establish a threshold, for example 80% of certainty (or any kind of score), in which all pieces of information such as symptoms, physical evidence, analyses of biomonitoring materials carrying a certainty factor (relevance) are combined by addition. When the threshold is reached or passed, a certainty factor, which is a degree of belief in a conclusion, is issued (Meech and Kumar, 1992). In this case, the level and frequency of the symptom are only considered in specific rules created for this end.

The knowledge accumulation process used in social and medical science is an intriguing process since the inputs are fraught with uncertainties and subjectivities. It is not a trivial process to use procedures to gather all relevant pieces of information and conclude that Hg poisoning is uncertain, likely, or definitely occurring. Usually, using a scoring process, the knowledge accumulation is simplified but unfortunately in many cases intermediate assumptions or degree of intensity of a symptom are not taken into consideration. For example, if an individual has a headache or a lack of energy, the symptom must be ranked based on the frequency and intensity.

Forsyth (1984) described methodologies to accumulate knowledge, especially in medical science where very subjective pieces of information must be considered. The Fuzzy Logic technique devised by Zadeh (1965) is another interesting process that employs human analysis to provide an approximate and effective way to describe behaviour in situations that are too complex or ill-defined to allow precise mathematical analysis. One of the classical methods to accumulate knowledge in medical exams is the famous Perceptron expert system, adopted by Rosenblatt in 1957 (Minsky and Papert, 1969) to diagnosis hospital infection. The method uses a basic neural equation that propagates weighted evidence to a conclusion. This method, so-called "Weighted Inference Method" was used by Veiga and Meech (1995b) to develop an Expert System (HgEx) to estimate possibilities of mercury bioaccumulation and human poisoning in ASM sites. In the Health Assessment part of the software, each piece of information related to diet, working method, symptoms, etc. have an importance (weight) established by experts in mercury poisoning. The Weighted Inference Method derives a Degree of Belief ($DoB_{conclusion}$) in a conclusion (e.g. Hg poisoning is occurring) combining the importance of each factor (W_i) with the Degree of Belief (DoB_i), given by the interviewer (e.g. nurse or doctor), on the intensity or frequency that the factor (e.g. symptom) is occurring. :

$$DoB_{conclusion} = MIN\ (100,\ \sum_{i=1}^{n}\ DoB_i \times W_i\ )$$

This procedure is transparent and easily adapted. The method was also designed to accommodate cultural, socio-economic and political differences from one region to another (Veiga and Meech, 1994). The $DoB_{conclusion}$ (**Hg intoxication is occurring**) can be a summation of different Degrees of Belief with different weights (importance). For example, the belief in Hg intoxication can be a combination of many **factors,** such as *symptoms* (objective and subjective), results of *specific* (neurological) *tests*, *occupational exposure* (observation about how the individual handles mercury), *life style* (where the individual lives – near a mine or gold shop, if the individual lives with a miner who keeps Hg-contaminated clothes at home; etc.), with the *results of Hg analyses of biological materials*. The importance (Wi) of each **factor** is established by the experts. For example, for workers (miners handling Hg), the following equation can be suggested:

$DoB_{conclusion}$ (Hg intoxication) = W_1 x DoB (symptoms) + W_2 x DoB (specific tests) + W_3 x DoB (occupational exposure) + W_4 x DoB (life style) + W_5 x DoB (Hg in biomaterials), where: W_1....W_5 are the weights (importance) of each **factor** to the final conclusion (Hg intoxication is occurring)

An example is shown in Table 2.1. This process allows enormous flexibility as the experts can change the weights (importance) of the **factors** depending on site-specific conditions. If the individual is not a miner, the weight related to the component of occupational exposure (handling Hg) must be distributed among other factors. In the example of Table 2.1, it is clear that the results of analysis of biological materials (urine and hair for example) have higher importance than any other piece of evidence.

Table 2.1. Example of importance of factors associated with Hg intoxication (given by experts)

Factors	**Weights (importance)**
symptoms of mercurialism	$W_1 = 0.2$
results of specific (neurological) tests	$W_2 = 0.2$
occupational exposure	$W_3 = 0.1$
life style	$W_4 = 0.1$
Hg analyzed in biological materials	$W_5 = 0.4$
Total	1

Table 2.2 shows a hypothetical example of results of a medical evaluation and the Degree of Belief in the symptoms. In this hypothetical example, the **experts**, in consensus before starting the Health Assessment, have agreed with the importance (W_i) of all factors associated with symptoms of mercurialism from –1 (confounding factor) to 1 (very important factor) being evaluated. For example, *tongue tremor* was considered very important and *recent malaria* (within 3 months) was considered a reasonable, but not very strong, confounding factor ($W_i = -0.5$). During the medical examination, the patient told the nurse that he was victim of a strong malarial outbreak 3 months ago. The nurse accepted the information but with restrictions, as she did not have evidence of the fact and she did not know the severity of the outbreak. Therefore, she assigned a certainty value (DoB) of 70% (see Table 2.2) to this piece of evidence (i). The nurse also noticed a slight evidence of gum discoloration, but she was not 100% certain about this and therefore assigned a certainty value of 50% to this factor. At the end of the medical exam/questionnaire, the conclusion on symptoms related to mercurialism has 80% of certainty. A similar process must be done for the other factors such as specific tests, occupational exposure, etc. At the end of the medical exam, all pieces of evidence are combined to conclude about Hg intoxication. When the $DoB_i \times W_i$ passes 100% then this value is assigned as a result.

Table 2.2. Hypothetical example of knowledge accumulation on "symptoms" associated with mercurialism using weighted inference method.

Factor/evidence	**Intensity or frequency of the evidence (DoB_i) (%)**	**Importance of the evidence (W_i) from -1 to 1**	**$DoB_i \times W_i$**
discoloration of gums	50	0.5	25
salivation	80	0.2	16
hearing problems	40	0.3	12
visual constriction	20	0.6	12
tongue tremor	50	1	50
recent malaria	70	-0.5	-35
Total ($DoB_{symptoms} \times W_1$)			**80**

For life style for example, some pieces of information can be used to obtain a DoB in the conclusion about how life style contributes to Hg intoxication. Some examples of components influencing the "life style factor" are given in Table 2.3. Based on the results of Table 2.3, it is clear that a person under these living conditions will have chances (91%) to be intoxicated by Hg.

To input the DoB associated with biological materials, a range of importance must be developed. Using the WHO (1990 and 1991a) values as well as the German HBM (Human Biological Monitoring) values it is possible to establish a scale. The values of Hg in blood may combine levels of inorganic Hg and MeHg. This makes difficult to establish ranges. By assigning a weight (W_5) for each biomaterial (urine and hair), it is possible to obtain the $DoB_{biomaterial} \times W_5$.

Table 2.3. Hypothetical example of knowledge accumulation on "life style" associated with Hg exposure using weighted inference method

Factor/evidence	**Intensity or frequency of the evidence (DoB_i) (%)**	**Importance of the evidence (W_i)**	**$DoB_i \times W_i$**
keep work clothes at home	100	0.3	30
had Hg spill at home	50	0.3	15
store Hg in open vials at home	80	0.2	16
live near a gold shop	100	0.3	30
Total ($DoB_{life\ style} \times W_4$)			**91**

For example a child living in a house near a gold shop has her urine analyzed and a level of 15 µg Hg/g creatinine was obtained. Then the $DoB_{biomaterials} \times W_5$ would be 30% according to Table 2.4.

It is important to notice that the weights shown in Table 2.4 are hypothetical and must be adapted by experts.

Table 2.4. Levels of Hg in urine and hair used to accumulate knowledge on "biological materials"

Human Sample	Normal Level	Alert Level	Action Level	Very High Level
urine (µg Hg/ g creatinine)	<5	5 - 20	20 to 100*	>100**
hair (µg Hg/g)	<10	10[#] - 50	50[@] - 100	>100
W_5 (importance)	0	0.3	0.7	1

** high probability of developing subtle symptoms in psychomotor performance.*

*** high probability of developing visible neurological symptoms.*

[@] threshold to observe clinical effects: 5% risk of neurological damage to adults; >70µg/g more than 30% risk of neurological disorder in the offspring.

[#] recent evaluation considers 5 ppm Hg in hair a safety guideline for pregnant women.

2.6. Public Information

It is important to inform the public about the results obtained in the E&HA. Be aware, however, that "immediate advice" to local people, especially related to fish consumption, heightens the likelihood of mistakes. Fish consumption advice should be based on reliable facts about routes of exposure. Such advice should also include recommendations on what types of local fish may be consumed because they either are not contaminated or contain Hg levels which do not adversely affect human health. Local people should not be discouraged from eating fish. They should also know that MeHg is not unique to their fish but rather a phenomenon occurring in fish around the planet. Fish consumption advisories must not be holistically developed based solely on mercury concentrations. They must consider dietary options and health benefits of eating fish (Frank Anscombe, 2003 – USEPA, personal communication).

In many regions, especially in the Amazon, there are other sources that can release even more Hg to the aquatic system than ASM activities (e.g. erosion) (Roulet *et al*., 1998). Most people tend to blame miners for all Hg pollution of the ecosystem. In many cases, communities living hundreds of kilometers distant from mining activities have blamed mining as the sole polluting source. When the mining activity diminishes, they believe that fish will be adequate for consumption again. It is important to demonstrate that there are other sources of Hg and that carnivorous fish will likely not suddenly be clean of Hg because mining stopped.

Health effects of Hg vapour must be brought to the public attention using all arguments to show the benefits to use retorts. It is important that any initiative related to public awareness or distribution of retorts should be accompanied by a strong educational campaign. Information packages must consider issues such as illiteracy rates, which can be high in many regions, and other social and cultural aspects. Unfortunately, the amount of money and effort spent on education of miners is considerably lower than those of other approaches, such as enforcement and monitoring. This must be changed.

2.7. A Suggested Sequence of Actions

Some steps of a Health Assessment are costly and must be carefully evaluated at the beginning of the process. Priorities of actions must be established. For example, the neuropsychological tests are conducted by medical experts and therefore are costly (e.g. traveling to the field, living expenses, physical space to work, presence of nurses, etc.). Analyses of blood, urine and hair samples are also expensive, involving hospital material, cooling methods, transportation, etc. and must be prioritized according to previous information. The following criteria are suggested:

1. Recognize the main Hg exposure pathways to humans (environmental assessment).
2. Obtain the population distribution by applying socio-economic-demographic questionnaire; use similar group distribution in the Health Assessment.
3. Select volunteers for the study following the cohorts obtained in the socio-economic-demographic study.
4. Obtain consent from Health authorities to apply the questionnaire.
5. Inform volunteers about the purpose of the assessment and instruct them.
6. Apply criteria to form groups or clusters (age, gender, education, occupation, fish consumption, proximity of the Hg source, etc.).
7. Apply general (work, diet, health history and possible confounders) questionnaire.
8. Apply specific health questionnaires related to Hg poisoning.
9. Apply physical neurological (medical) exam.
10. Select volunteers with suspicion of MeHg or Hg vapour exposure.
11. Apply neuropsychological tests (memory test, Match Box test, Finger Tapping, Frostig test, Visual Field test, etc.).
12. Collect biomonitoring samples: hair, urine and/or blood.
13. Apply knowledge accumulation process (scoring) using clinical + biomonitoring sample results.
14. Re-examine those individuals with high scoring.
15. Suggest simple and easy to implement remedial measures (technical improvements such as use of retorts, filters, amalgamation of concentrates, safe disposal of amalgamation tailings, removal from source, diet advisory, etc.).
16. Use a control group (non-exposed group, distant from the site) to collect biomonitoring materials and to apply the same clinical examination and neuropsychological tests. It is suggested that one avoid control groups with history of high ingestion rates of fish.

2.8. Disclaimer

UNIDO recognizes that it is an ethical obligation to treat individuals once mercury intoxication is identified. Whether or not therapies should be discussed with Hg intoxicated people is very controversial. UNIDO has adopted the approach to inform the local and regional health care authorities when a mercury intoxication problem is detected. UNIDO's mandate is to provide assistance in eradicating pollution sources, not to undertake active intervention. Although the organization understands that the health conditions of affected communities must be considered, medical intervention should only be undertaken by physicians operating within organizations better qualified for this task than UNIDO.

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Appendix 1: Example of a Health Assessment Survey

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United Nations Industrial Development Organization (UNIDO)
Global Environment Facility (GEF)
United Nations Development Programme (UNDP)

Name: _____

I hereby declare that I want to take part in the UNIDO project. I will be questioned about my living circumstances and health problems related to mercury. I will be medically examined including neurological examination. Blood, urine and a small amount of hair will be taken. The …..... will inform me after the laboratory analysis about my personal results. The UNIDO and the …..... will get the results in a form where my name can not be identified. The assessment is done respecting the "Recommendation for Conduct of Clinical Research" (World Health Organization Declaration of Helsinki).

>>translation<<

Local and Date: _____

Signature: _____
(in case of children signature of parents/guardian)

Witnesses (if needed):

_____ and _____
(Name): (Name):

1. General Questionnaire

1. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Date of interview: _____

Name of the interviewer: _____Code of the interviewer _____

1.2. Work Exposure

Occupation (Detailed description of the job)
____ Miner
____ Mineral processor (in charge of amalgamation)
____ Gold smelter (gold buyer)
____ Worker at a cyanidation plant
____ Farmer
____ Office Job
____ Driver
____ Student (not working)
____ Other job: ._____

Do you live near a mining operation or gold shop?
0 ____ No
1 ____ Yes, Which one? _____ for _____ years

Have you ever worked in the _____ area?
0 ____ No
1 ____ Yes, for _____year(s)

Have you ever worked as a miner in direct contact with mercury?
0 ____ No
1 ____ Yes, from when to when: _____

Have you ever worked burning amalgam in open pans or melting gold in inadequate fume hoods?
0 ____ No
1 ____ Yes, from when to when: _____

Have you (or a family member living with you) ever burned amalgam at home?
0 ____ No
1 ____ Yes

Have you ever used a retort?
0 ____ Yes, when_____ and which type:_____
1 ____ No

Have you (or a family member living with you) ever stored mercury containers or flasks?
0 ____ Never
1 ____ At work
2 ____ At home

Have you (or a family member living with you) ever had a mercury spill?
0 ____ Never
1 ____ At work
2 ____ At home

Have you (or a family member living with you) kept your dirty working clothes at home?
0 ____ No
1 ____ Yes, where? _____

For how many years have you been working with mercury?
0 ____ not applicable (have not working directly with mercury)
1 ____ year(s)

TOTAL POINTS OF Work Exposure: _____

MAXIMUM NUMBER OF POINTS ALLOWED: _____

(The Maximum Number of Points was created to allow the interviewer to select/skip questions. Divide the Total Points by the Maximum Number of Points Allowed and obtain a %. Low % represents low likelihood of having problems related to Hg. The same applies to the following sections).

1.3. Confounders

Have you ever had any neurological disorders (epilepsy, stroke, Parkinsons, etc.) or mental disorders (schizophrenia, bi-polar disorder, etc.)?
0 ____ No
1 ____ Yes
Which disease (problem)? _____

Have you ever had malaria?
0 ____ No
1 ____ Yes
If yes, how long ago was your last incidence?
_____ (days or months or weeks)

Do you have fever at the moment?
0 ____ No
1 ____ Yes

Do you handle gasoline and kerosene regularly? (*this can cause tremors*)
0 ____ No
1 ____ Yes
If yes, how long have you been doing this? _____ (years)

Do you handle insecticides or pesticides regularly?
0 ____ No
1 ____ Yes
If yes, how many years you have been doing this? _____ (years)

Do you smoke?
0 ____ Never
1 ____ Rarely (0-10 cigarettes per day)
2 ____ Moderate (10-20 cigarettes per day)
3 ____ Excessive (more then 20 cigarettes per day)

Do you drink alcohol?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Do you have HIV/AIDS?
0 ____ No
1 ____ Yes
When did this happen? _____ (days or weeks or months or years) ago

Did (or Do) you suffer from Leprosy?
0 ____ No
1 ____ Yes

Have you been using whitening soap/bleach (for lightening the skin)?
0 ____ No
1 ____ Yes

Have you ever had hepatitis or any other hepatic disorder?
0 ____ No
1 ____ Yes
Which disease (problem)? _____

Did (or Do) you ever have tuberculosis?
0 ____ No
1 ____ Yes
When did this happen? _____ (days or weeks or months or years) ago

Have you ever had any other major infectious disease?
0 ____ No
1 ____ Yes
Which disease (problem)? _____

Have you had any serious accidents (to go to hospital)?
0 ____ No
1 ____ Yes, but not severe
2 ____ Yes, and it was severe (more then 1 hour unconsciousness)
When did this happen? _____ (days or weeks or months or years) ago

How is your current financial situation?
0 ____ ☺ (above average)
1 ____ 😐 (average)
2 ____ ☹ (below average)

How is your current social life? (friends, family, hobby activities, etc.)
0 ____ ☺ (OK)
1 ____ 😐 (medium)
2 ____ ☹ (bad)

(To be completed by the project doctor).
Exclusion criteria from statistical evaluation
Severe neurological disease such as Parkinsons, stroke, severe accident (brain injury), birth trauma, tetanus, polio, hyperthyroidism, epilepsy, malaria or any acute severe disease, etc. may introduce factors that confound with Hg intoxication symptoms. Based on the confounders above, should this individual be ***excluded*** *from the Health Assessment?*
____ No
____ Yes

Why? _____

_____

1.4. Diet Issues

How frequently do you eat fish?
0 ____ Never
1 ____ At least once a **month**
2. ____ At least once a **week**
3. ____ At least once a **day**

The interviewer should ask about the size of the portion of fish consumed. Based on the portion, the interviewer can estimate the approximate mass of fish consumed:

_____ grams (_____ times per ☐ **day** or per ☐ **week**).

Name the fish you consume regularly (if possible indicate if the fish **species** is **c** = carnivorous, **o** = omnivorous, **d** = detritivorous, **h** = herbivorous). If possible, list from the most to the least consumed species (*try to obtain a % of each species consumed in each season*).

Fish Name	Species	% (dry season)	% (wet season)

Do you know where the fish come from?
0 _____ don't know the origin of the fish (buy in the market)
1 _____ from areas distant from mining
2 _____ from areas affected by mining

Can you name the river and place where most of the fish you eat come from?
____ No
____ Yes, the river (or lake or pool) is _____

Does this river (or water body) have dark water (Coca-cola color)?
0 _____ don't know the origin of the fish (buy in the market)
0 _____ No
1 _____ Yes, mild
2 _____ Yes, very dark

Name the place where you obtain drinking water: _____

Do you consume local chicken, ducks or eggs?
0 ____ Never
1 ____ At least once a **month**
2 ____ At least once a **week**
3 ____ At least once a **day**

Do you consume local beef, pork, etc.?
0 ____ Never
1 ____ At least once a **month**
2 ____ At least once a **week**
3 ____ At least once a **day**

Do you consume local vegetables and fruits?
0 ____ Never
1 ____ At least once a **month**
2 ____ At least once a **week**
3 ____ At least once a **day**

TOTAL POINTS OF Diet Issues: _____

MAXIMUM NUMBER OF POINTS ALLOWED: _____

2. Health Questionnaire

2. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Date of interview: _____

Name of the interviewer: _____Code of the interviewer _____

2. 2. Physical Condition and Subjective Symptoms

Do you ever have a kind of a metallic taste in your mouth?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Do you suffer from excessive salivation?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

How is your appetite?
0 ____ ☺ (OK)
1 ____ 😐 (medium)
2 ____ ☹ (bad)

Have you lost weight within the last year?
0 ____ No
1 ____ Yes

Have you lost hair within the last year?
0 ____ No or only rarely
1 ____ Yes, slight to moderate
2 ____ Yes, marked to severe

Have you had a cough within the last year that lasted more than for 3 months?
0 ____ No
1 ____ Yes

Have you ever had kidney disease (other than a urinary tract infection)?
0 ____ No
1 ____ Yes
Which disease (problem)? _____

Have you ever had severe respiratory problems (asthma, pneumonia)?
0 ____ No
1 ____ Yes
Which disease (problem)? _____

Are you healthy now?
0 ____ Yes
1 ____ No
Why not? _____

Has the health problem worsened since exposure to mercury?
0 ____ No mercury exposure
1 ____ Yes, somehow
2 ____ Yes, definitely

for females:
Are you pregnant?: _____(Yes/No)
Breastfeeding: _____(Yes/No)
Can provide sample of breast milk? : _____(Yes/No)

TREMORS

Have you had any problems with tremors (shaking)?
(Clinical Tremor Rating Scale)
0 ____ I have no tremors or tremors do not interfere with my job.
1 ____ I can work, but I need to be more careful than the average person.
2 ____ I am able to do everything, but with errors; poorer than usual performance because of tremors.
3 ____ I am unable to do a regular job, I may have changed to a different job. due to tremors; it limits some housework, such as ironing.
4 ____ I am unable to do any outside job; housework very limited.

SLEEP DISTURBANCES

How do you feel after a usual night of sleep?
0 ____ ☺ (OK)
1 ____ 😐 (medium)
2 ____ ☹ (bad)

FATIGUE

Score to estimate fatigue (Wessely S, Powell R: Fatigue syndrome)

Do you get tired easily?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you need to rest more?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you feel sleepy or drowsy?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have trouble starting things?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you lack energy?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have less muscle strength?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you feel weak?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Can you start things without difficulties, but get weak as you go on?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Physical fatigue sum: _____ points

MENTAL FATIGUE

Do you have problems concentrating?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have problems thinking clearly?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have problems finding the right words when you speak?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have problems with eye strain?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have problems with memory?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Mental fatigue sum: _____ points

WELL BEING

Do you feel nervous?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Do you feel sad?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

How is your current sexual life? (for men)
0 ____ ☺ (OK)
1 ____ 😐 (average)
2 ____ ☹ (bad)

Do you have palpitations? (*Feeling the heart beating)*
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Do you get headaches?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Do you have nausea?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Do you feel numbness, prickling or aching anywhere on your body?
Mainly perioral dysesthesia and sensory impairment of the glove and-stocking type
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3 ____ at least once a **day**

Well being sum: _____ points

TOTAL POINTS OF
Physical Condition and Subjective Symptoms: _____

MAXIMUM NUMBER OF POINTS ALLOWED: _____

3. Clinical-Neurological Examination

3. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Date of interview: _____

Name of the interviewer: _____Code of the interviewer _____

Blood pressure:_____/_____ mmHg

3. 2. Clinical Exam

MOUTH AND TEETH CONDITIONS

Clinical signs of stomatitis
0 ____ No
1 ____ Yes

Clinical signs of gingivitis
0 ____ No
1 ____ Yes

Bluish discoloration of the gums
0 ____ No
1 ____ Slight
2 ____ Yes, obvious

How many teeth with dental fillings (Amalgam)?
0 ____ None
1 ___ One or more → how many _____

Examination of the eyes:
0 _____ No changes
1 _____ Bluish-colored iris ring
2 _____ Kayser-Fleischer ring

WALKING
Person is asked to walk up and down, first with eyes open, then with eyes closed.

Ataxia of gait (walking)
Examiner is watching for signs of ataxia (*Klockgether Score*)
0 ___ Absent
1 ___ Slight (ataxia only visible when walking on tandem or without visual feedback)
2 ___ Moderate (ataxia visible in normal walking; difficulties, when walking on tandem)
3 ___ Marked (broad-based, staggering gait; unable to walk on tandem)
4 ___ Severe (unable to walk without support; wheelchair bound)
5 ___ Most severe (bedridden)

Rigidity of gait (walking)
Examiner is watching the gait, the swing of the arms, general posture.
0 ____ Normal
1 ____ Mild diminution in swing while the patient is walking
2 ____ Obvious diminution in swing suggesting shoulder rigidity
3 ____ Stiff gait with little or no arm swinging noticeable
4 ____ Rigid gait with arms slightly pronated; this would also include stopped-shuffling gait with propulsion and retropulsion

STANDING

Tremor - finger to nose test
Person is asked to stand still, legs together, arms outstretched. Eyes closed. Finger tip should touch the nose. Examiner is watching and rates the ***tremor*** (*modified Clinical Tremor Rating Scale*)
0 ____ None
1 ____ Slight to moderate (amplitude < 0,5 cm – 1cm); may be intermittent,
2 ____ Marked amplitude (1-2 cm)
3 ____ Severe amplitude (> 2 cm)

Dysmetria - finger to nose test
Person is asked to stand still, legs together, arms outstretched, eyes closed. Finger tip should touch the nose. Examiner is watching and rates the dysmetria
0 ____ Normal
1 ____ Moderate pathologic
2 ____ Severe pathologic

Dysdiadochokinesis
Person is asked to twist hands very quickly (alternating movements of the wrists (Klockgether Score)
0 ____ Absent
1 ____ Slight (minimal slowness of alternating movements)
2 ____ Moderate (marked slowness of alternating movements)
3 ____ Severe (severe irregularity of alternating movements)
4 ____ Most severe (inability to perform alternating movements)

Tremor – eye lid
Eyes closed. Examiner is watching and rates the ***tremor*** *(Davao Pool score)*
0 ____ None
1 ____ Slight
2 ____ Marked

LYING
Person is asked to lie on the examination bench.

Mentolabial reflex
0 ____ Negative
1 ____ Positive

Babinski reflex
0 ____ Negative
1 ____ Positive

Hoffmann reflex
0 ____ Negative
1 ____ Positive

Sucking reflex
0 ____ Negative
1 ____ Positive

Grasp
0 ____ Negative
1 ____ Positive

TSR (Tricipital reflex) *(still being tested, then no score is given)*
A ____ No reflex
B ____ Hyporeflexia
C ____ Normal
D ____ Hyperreflexia
E ____ Clonus

BSR (bizeps brachii reflex)
0 ____ Normal
1 ____ Hyporeflexia
1 ____ Slight hyperreflexia
2 ____ No reflex
2 ____ Very brisk or reflex zone enlarged or clonus

AR - Achillean tendon reflex, ankle jerk
0 ____ Normal
1 ____ Hyporeflexia
1 ____ Slight hyperreflexia
2 ____ No reflex
2 ____ Very brisk or reflex zone enlarged or clonus

LYING – OTHER TESTS

Intentional Tremor - heel-to-shin test
Person is asked to touch with his/her heel the knee of the other leg. Then , she/he should move the heel along the shin to the foot. Repeat and do it with both sides. Eyes first open, then closed. Rate tremor during heel-to-shin test (Klockgether Score)
0 ____ Absent
1 ____ Slight (slight terminal tremor)
2 ____ Moderate (marked terminal tremor)
3 ____ Marked (kinetic tremor throughout intended movements)
4 ____ Severe (severe kinetic tremor heavily interfering with everyday life)
5 ____ Most severe (maximal form of kinetic tremor making intended movements impossible)

Ataxia - heel-to-shin test
Rate ataxia (Klockgether Score)
0 ____ Absent
1 ____ Slight (slight hypermetria in heel-to-shin test)
2 ____ Moderate (hypermetria and slight ataxic performance of the test)
3 ____ Marked (marked swaying: unable to stand with feet together)
4 ____ Severe (pronounced ataxia in performing heel-to-shin test)
5 ____ Most severe (unable to perform heel-to-shin test)

Sensory disturbances
Sensory disturbances such as sensory impairment of the glove-and-stocking type
0 ____ Absent
1 ____ Present
Comments_____

Bradykinesis
Rate your observation whether there was any sign of bradykinesis during the examination (slower active movements, absent or altered synkinesis of upper extremities during gait).
0 ____ Absent
1 ____ Present

Hypo-mimia
Rate your observation whether you have observed an hypo mimic expression of the face during the examination).
0 ____ Absent
1 ____ Present

TOTAL POINTS OF Clinical Exam: _____

MAXIMUM NUMBER OF POINTS ALLOWED: _____

4. Specific (Neuropsychological) Tests

4. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Date of interview: _____

Name of the interviewer: _____Code of the interviewer _____

4. 2. Tests

MEMORY DISTURBANCES: *(different memory tests can be used)*
Forward *digit span test (part of Wechsler Memory Scale):*
Repeat each column of numbers. Score the longest series correctly repeated

Obtained	Score	Test
	4	6-4-3-9
	4	7-2-8-6
	3	4-2-7-3-1
	3	7-5-8-3-6
	2	6-1-9-4-7-3
	2	3-9-2-4-8-7
	1	5-9-1-7-4-2-3
	1	4-1-7-9-3-8-6
	0	5-8-1-9-2-6-4-7
	0	3-8-2-9-5-1-7-4
Total:		

Memory test score: _____
Score 2 for total score in the table above = 0, Score 1 for total score = 1 to 2, Score 0 for total score >3

EPISODIC MEMORY TESTS (OPTIONAL):
Mini-Mental State Examination - MMSE

Orientation to time - season:
0 ____ correct answer
1 ____ incorrect answer

Orientation to time - part of the day:
0 ____ correct answer
1 ____ incorrect answer

Orientation to place - name of the village
0 ____ correct answer
1 ____ incorrect answer

Orientation to place - name of the country:
0 ____ correct answer
1 ____ incorrect answer

Registration of 3 words: *Tell the patient to memorize 3 words, for example: Fish, Ball and Tree. After 5 to 10 minutes, ask the patient to repeat the words you told him. After 5 to 10 min. do the same.*
0 ____ Registered all 3
1 ____ Registered just 2
2 ____ Registered just 1
3 ____ Registered none

Testing attention: *Ask the patient to respond to your command and to do something in 3 steps: For example**:** Grab a piece of paper, fold it in half and put it on the floor.*
0 ____ correct action
1 ____ incorrect action

DEXTERITY AND COORDINATION

Match Box Test (from *MOT*)
*Put **20 matches** on a table, half of each on one side of an open matchbox, approx. 15 cm away. Take the time until all matches are put into the box. Use left and right hand alternatively.*

_____ seconds Score: _____

Matchbox test score: 2 = 21 seconds or more,
1 = 16-20 seconds,
0 = 0-15 seconds

Finger Tapping Test (from *MOT*)
*Sitting at a table. Elbows should be placed on the table. Try to do as many points as possible on a piece of paper with a pencil. Count the amount of points within **10 seconds**.*

Score: _____

Tapping test score: 2 = 0-53 dots,
1= 54-64 dots,
0 = 65 or more dots

FROSTIG SCORE
Draw a line from one symbol to the other (USE A WIDER PAPER SHEET, this is just an example). Do not interrupt while drawing. Do not touch the borders. Please try to stay within the lines.

F1  

Point: _____ 0 = good; 1 = bad; 2 = very bad

F2  

Point: _____ 0 = good; 1 = bad; 2 = very bad

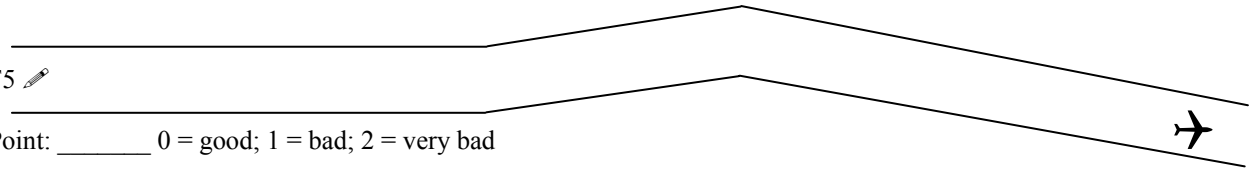
F3  

Point: _____ 0 = good; 1 = bad; 2 = very bad

F4  

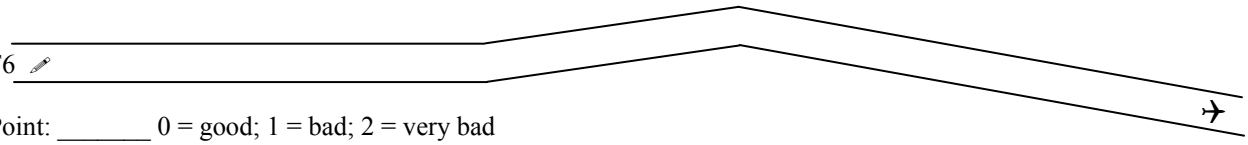
Point: _____ 0 = good; 1 = bad

F5



Point: _____ 0 = good; 1 = bad; 2 = very bad

F6



Point: _____ 0 = good; 1 = bad; 2 = very bad

Please connect the symbols with a straight line.

F7

Point: _____ 0 = good; 1 = bad; 2 = very bad

F8

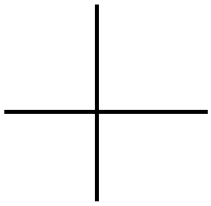
Point: _____ 0 = good; 1 = bad

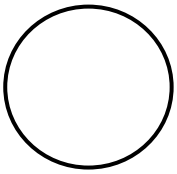
TOTAL FROSTIG SCORE: _____

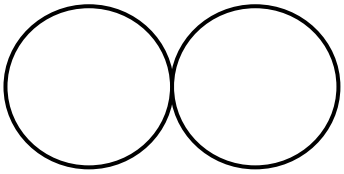
Frostig test score: 2 = 0-9 points,
1 = 10-12 points,
0 = 13-14 points.

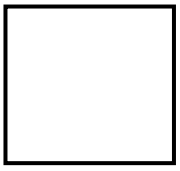
COPYING FIGURES

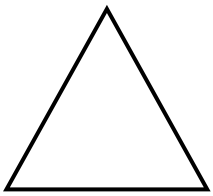
(Select some simple and more complex figures according to the degree of instruction of the patient; based on the quality, time and difficulty to perform the test, make your score for each figure). For each figure you can use the score: 0 = good; 1 = bad; 2 = very bad.

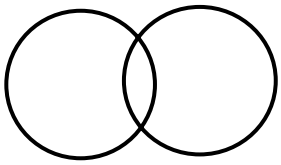


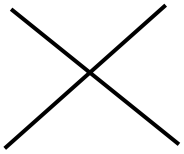


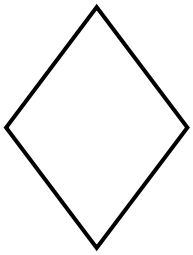


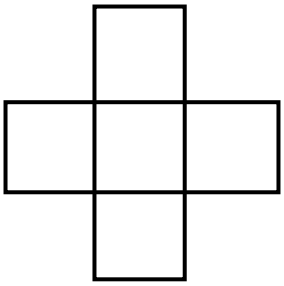


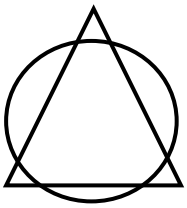


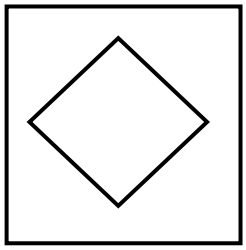


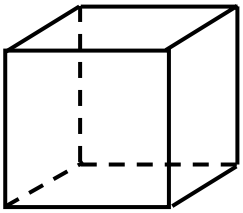


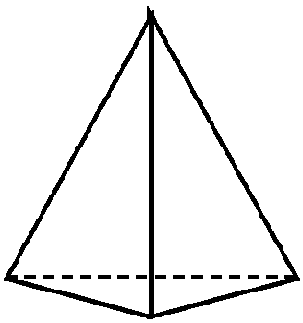


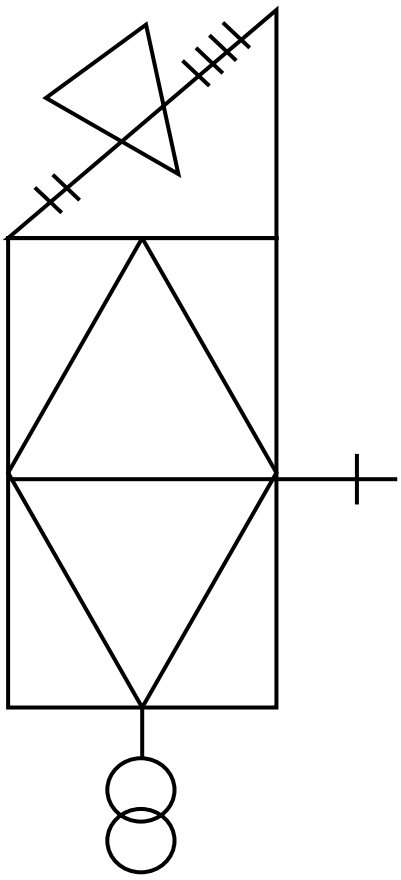


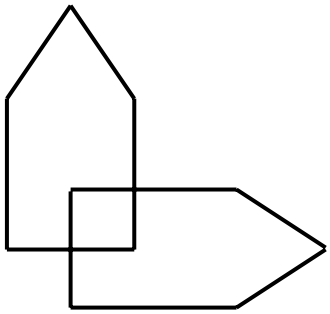












TOTAL POINTS OF Neuropsychological Tests: _____

MAXIMUM NUMBER OF POINTS ALLOWED: _____

5. Specimens

5. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Date and Time of the specimen sampling: _____

Name of the specimen taker: _____ Code _____

Blood (EDTA-blood 10 mL)
____ Yes
____ No

Malaria smear (only, if high prevalence of malaria in the area)
____ Negative
____ Positive

Urine (spontaneous urine sample 10 mL)
____ Yes
____ No

Urine total mercury (field test) (additional)

Result: _____ unit: _____

Proteinuria? (same test should be used)
0 ____ negative
1 ____ trace
2 ____ +
3 ____ ++
4 ____ +++
5 ____ ++++

Hair
____ Yes, sample collected
____ No

Hair total mercury (field test) (additional): Result: _____ unit: _____

6. Laboratory Analysis Results

6. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Material/test	Result	Unit
Blood		
Total mercury		
Methylmercury		
Selenium		
Urine		
Creatinine		
Proteinuria		
Total mercury		
Methyl mercury		
Hair		
Total mercury		
Methyl mercury		
Others (saliva, nails, breast milk, feces, etc...)		

Comments:

7. Medical Score Sum

7. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Selected Data for the Medical Score according to Dr. S. Boese-O'Reilly

Test	Score Points	Results
Anamnestic data		
Metallic taste	0/1	
Excessive salivation	0/1	
Tremors at work	0/1	
Sleeping problems at night	0/1	
Health problems worsened since Hg exposed	0/1	
Clinical data		
Bluish coloration of gums	0/1	
Ataxia of gait	0/1	
Finger to nose tremors	0/1	
Dysdiadochokinesis	0/1	
Heel to knee ataxia	0/1	
Heel to knee tremors	0/1	
Mento labial reflex	0/1	
Proteinuria [19]	0/1	
Neuropsychological tests		
Memory test [20]	0/1/2	
Matchbox test [21]	0/1/2	
Frostig test [22]	0/1/2	
Tapping test [23]	0/1/2	
Points obtained	max 21	

[19] The test is semi-quantitative. Possible results are 0, trace, 30, 100 and 300 mg Protein/dL of urine. When the result of Proteinuria is 0 or trace, the medical score is 0; when the result of Proteinuria is 30 or higher the score is 1.
[20] Memory test: 2 = score 0; 1 = score 1-2; 0 = score >3
[21] Matchbox test: 2 = 21 seconds or more; 1 = 16-20 seconds; 0 = 0-15 seconds
[22] Frostig test: 2 = 0-9 correct answers; 1 = 10-12 correct answers; 0 = 13-16 correct answers
[23] Tapping test: 2 = 0-53 dots; 1= 54-64 dots; 0 = 65 or more dots

8. Decision for the diagnosis of "chronic Hg intoxication"

8. 1. Personal Data

Participant ID Number: _____

Family Name: _____ First Name: _____

Date of Birth: _____Age: _____(years) Education level:_____

Gender: ____ Female ____ Male

Weight: _____kg Height: _____ m

Address:_____

Any telephone for contact: _____

Threshold limits for mercury

	Hg-blood (µg/L)	Hg-urine (µg/L)	Hg-urine (µg/g creatinine)	Hg-hair (µg/g)
HBM I	5	7	5	
HBM II	15	25	20	5 (by analogy)
WHO			50	7
BAT for metallic and inorganic Hg	25	100		
BAT for organic Hg	100			
BEI (Biological exposure index)	15 (after working)		35 (before working)	

Note: Toxicologically established threshold limits for mercury in blood, urine and hair (HBM = Human Bio-Monitoring; BAT = Biologischer Arbeitsstoff-Toleranzwert; BEI = Biological Exposure Indices). The BAT value is the maximum. allowable concentration of a substance or its metabolites in body fluids. It should guarantee that the health of healthy people is not affected when being exposed 8 hours a day or 40 hours a week.

Decision for the diagnosis of "chronic mercury intoxication"

		Medical Score Sum (%)		
		0 – 19	20 – 45	45 - 100
Hg in all biomonitors	< HBM I	–	–	–
Hg at least in one biomonitor	> HBM I	–	–	+
	> HBM II	–	+	+
	> BAT	+	+	+

Intoxication: _____ No _____ Yes

Appendix 2: Example of a Socio-Economic Questionnaire

by Mrs. Susan Wagner, Dar Es Salaam, Tanzania

STRUCTURED QUESTIONNAIRE FOR COMMUNITY MEMBERS ON REMOVAL OF BARRIERS TO THE INTRODUCTION OF CLEANER ARTISANAL GOLD MINING AND EXTRACTION TECHNOLOGIES

Village _____Ward _____ District _____ Region _____

Date: _____ Name of Enumerator: _____

Phone or Address for contact: _____

Introduction and Informed Consent
(Introduce yourself, explain the purpose of the interview and request consent to be interviewed)

A. DEMOGRAPHIC INFORMATION (BIODATA)

Q1. Who is the head of household?
a). Male
b) Female

Q2. Age of the respondent:
a) 10 – 19 years old
b) 20 – 29 years old
c) 30 – 39 years old
d) 40 – 49 years old
e) 50 and above

Q3. Marital status of the respondent:
a) Single
b) Married
c) Widow
d) Widower
e) Separated

Q4. Number of children:

Sex	**F**	**M**
Number		
Living		

Q5. What is the highest level of education achieved by --?

Father	Mother	Respondent	Siblings
			1. 2. 3. 4. 5. 6. 7. 8.

1. Illiterate, 2. Primary education, 3. Secondary Level, 4. University, 5. other, specify

Q6. How long have you being here? _____ years, from where? _____

and which tribe? _____

B. HOUSEHOLD STRUCTURE:

Q7 How far do you live from the mine? _____ m.

Q8. Uses of the house:
1. Residential/commercial
2. Residential only
3. Other (specify): _____

Q9. How many people in the household? men: _____, women: _____, children: _____

Q10. Hygiene and sanitation:
a) Toilet
b) Pit latrine
c) Using the bush
d) Other (specify): _____

C. SOCIO-ECONOMIC LIFE OF THE RESPONDENT

Q11. What type of work do you do?
_____

Q12. On average, what is your monthly income (Tz Sh)?
a) Below 50,000
b) 51,000 – 100,000
c) 101,000 – 200,000
d) 201,000 – 400,000
e) 401,000 – 800,000
f) 801,000 – 1,000,000
g) Above 1,000,000

Q13. On average, how much do you spend on the following per month?

a) Food	
b) Water	
c) Rent	
d) Health	
e) School fees	
f) Clothing	
g) Transport	
h) Energy	
i) Servants	
j) Others (specify)	
Total expenditure	

Q14. What is the source of your water?
a) Rain water
b) Ponds
c) River
d) Boreholes
e) Shallow wells
f) Tap water
g) Other (specify): _____

Where do you get water for the following activities?

a) Domestic use	
b) Washing/bathing	
c) Mining/sluicing	
d) Irrigation	
e) Livestock	
f) Others, specify	

Q15. How is the quality of water?
a) Good
b) Muddy
c) Hard water
d) Unsafe
e) Other (Specify): _____
How do you treat water before using for domestic use? _____

Q16. Who fetches water?
a) Women
b) Men
c) Boys
d) Girls
e) Other (specify): _____

Walking distance to the water source:
a) <0.5 h b) 0.5- 1 h c) 1-2 h d) 3-4 h e) > 4 h

Q17. How many times per week do you eat each of the following foods?
a) Meat
b) Fish
c) Chicken
d) Eggs
e) Milk
f) Beans
g) Vegetables
h) Fruits
i) Other (specify): _____

Q18. Source of energy:
a) Company
b) Generator
c) Fuel
d) Wood
e) Other (specify: _____

Q19. Source of information and communication:
a) Radio
b) Newspaper
c) TV
d) Local leaders
e) Other (specify): _____

D. ARTISANAL MINING INFORMATION

Q20. How many hours per day do you spend on mining activities, on the average? _____ hours

Q21. When did you start (year)? _____

Q22. How did you get involved in mining? Who provided assistance at the beginning and in what form?
a) Self
b) Husband
c) Relative loan
d) Government Loan
e) NGOs Loan
e) Others (Specify) _____.

Q23. Are you a member of any Mining Association? a) Yes b) No

If the answer is yes, which association?: _____

If the answer is no, why not? _____

Q24. What kind of support you are getting from the association?

_____

Q25. Who in your family is employed/working in this business?

Self	**Spouse**	**Children**		**Other dependants**	
		M	F	M	F

Note: M = Male, F = Female

Casual Employment		**Permanent Employment**		**Seasonal Employment**	
M	F	M	F	M	F

Indicate in the boxes above how much they are paid in cash? In kind? Both?

E. EQUIPMENT AND INPUTS (mercury, tools, dynamite etc)

Q26. Where do you get mercury for amalgamation and other inputs?
a) Gold Dealers
b) Spouse
c) Relative
d) Others (specify)

Q27. Do you have any problems handling the overall process of gold production?
a) Yes, Explain:

_____

_____

_____

b) No. Explain:

_____

_____

_____

Q28. Are you aware of any environmental or health hazards that may be caused by the use of mercury in gold mining?
a) Yes
b) No

Q29. If yes, what are the hazards?

_____

_____

_____

Q30. How did you learn about this?

_____

_____

_____

F. PROPERTY OWNERSHIP

Q31. Indicate kinds of properties you use and control. (This means those you own outright).

Properties	Access	Value	Control	Self	Spouse	Both	Others
Gold pit							
Livestock							
House							
Farm Equipment							
Milling Equipment							
Vehicle							
Others (specify)							

G. DECISION MAKING ON INCOME AND EXPENDITURE

Q32. Who manages the income and expenditure in your household?
a) Self
b) Spouse
c) Both

Q 33. Who decides how to spend the money obtained from your business?
a) Self
b) Spouse
c) Both

H. MARKET

Q34. Where do you sell your gold? _____

Q35. What difficulties do you encounter while selling your gold?

_____

_____

_____

Q36. Do you have plans of changing your occupation? a) Yes b) No

Explain: _____

Q37. How is your present situation financially, compared to what it was before you started the business?
a) Increased
b) The same
c) Worse

I. TRAINING

Q38. Have you received any training regarding your mining activities?
a) Yes b) No

Where? _____

What type of training? _____

Who facilitated the training? _____

Q39. Has the training helped in your activities? a) Yes b) No

Q40. How? _____

_____

Q41. Do you have any recommendations for improving training.

_____

Q42. What are your comments on the following?
Licensing/taxation/ Hygiene and sanitation/ Pollution etc.

_____

_____

_____

J. ATTITUDES THAT MAY INFLUENCE ADOPTION OF IMPROVED MINING TECHNOLOGIES

(The interviewer should read a short description of the improved mining technology and explain it if necessary)

Q43. What do you think about introducing improved mining and processing technology?

_____

_____

_____

Q44. Would you be willing to learn this technology?
a) Yes
b) No
c) Uncertain

Q45. What form of training do you think you will need in order to learn it?
a) Short course
b) Demonstration
c) Tour
d) Other

Q46. What difficulties might you encounter during the change-over?

_____

_____

_____

Thanks for your cooperation, do you have any questions?

Appendix 3: User's Guide

Introduction

This User's Guide was developed to present important information for those planning to establish Environmental and Health Assessments (E&HA) in areas impacted by mercury (Hg) released by artisanal and small-scale mining (ASM). This document summarizes information from the detailed document that contains the scientific basis for the questions and answers described here. The format used is in the form of ***Frequently Asked Questions*** to provide the reader with simplified information about critical issues. It is important to highlight that mercury pollution in ASM regions is a complex issue, as mercury from miners is not the only source of Hg. Natural and global industrial sources may also play a significant role. Mercury from these sources are much more difficult to control and eradicate than ASM operations. Environmental and Health Assessments are important first steps to characterize the magnitude of the problem of Hg from ASM. However, this program must be followed by the introduction of concrete solutions to reduce Hg emissions, stop human exposure and mitigate critical situations.

What does one have to do before starting an E&HA?

A: An Environmental and Health Assessment must be planned carefully. It is important to remember that when dealing with human beings, there are **ethical issues** that must be previously addressed. When collecting and transporting samples (in or out of the country), permits are needed. If samples will be sent to another country to be analyzed, it is mandatory to obtain **in advance** all papers required to **send** and **receive** the samples. For orientation of the researcher, the following papers might be obtained **before starting** the Environmental and Health Assessment:

- Permit from local Health Authority to collect human and other biological samples, if required (e.g. fish, snail, urine, blood, hair, etc.).
- Permit to transport geochemical and biological samples within the country.
- Permit to export samples.
- Permit to import samples

Some countries request fees for foreign professionals, in particular medical doctors, to work in their territory. This must be checked in advance.

How does one assess mercury releases from ASM?

A: By its volatile characteristics, "mercury emission" is a term usually adopted to refer to the portion of Hg sent out to the atmospheric environment. The term "**mercury release**" refers to all kinds of Hg discharged into all environmental media (air, soil, water) and this depends fundamentally on the mining and processing methods. It is difficult to obtain reliable, quantitative data about Hg releases from active ASM, as miners do not freely provide information about the amount of Hg they use and their gold production is sporadic. However, this is the best way to obtain data. In abandoned sites, the task is even more difficult. Analyses of geochemical materials surrounding the mining site can provide only qualitative historical information about the level of Hg released and uncertainties associated with sampling processes prevent accurate determinations of the amount of mercury lost. In order to obtain reliable figures about the amount of Hg lost and gold produced, it is necessary to have a trustworthy relationship with miners to allow the researcher to access their mining and processing plant. It is natural that miners become suspicious when strangers are "inspecting" their activities. This is a time-consuming process, as a detailed survey about the amount of Hg entering and leaving each unit operation must be obtained carefully through weighing and analyzing Hg in end products, such as amalgamation tailings.

In **active** operations, an interview with miners can result in an estimate of the quantity of mercury that is lost. The following steps are suggested:

- Interview operation **owners**, who are in charge of supplying (acquiring) Hg as well other consumables.
- Obtain costs and amounts of all consumables such as diesel, carpet, soap, mercury, etc.; be sure to have the amount of Hg being monthly or weekly **purchased**.
- Interview as many owners as possible and check for inconsistencies in data.
- Verify that the miner is providing correct information about the amount (and cost) of consumables per month, per unit or per group of unit. Similar information must be gathered when obtaining information about gold production.
- Obtain numbers of gold production in dry and rainy seasons.
- Obtain average estimates of gold production (miners exaggerate giving production estimates only during "good days").
- If possible, ask permission to assess the processing operation and weigh all Hg being introduced and recovered.

- Sample amalgamation tailing and analyze Hg. By knowing the weight of amalgamation tailings being produced per month and Hg concentration, it is possible to calculate the Hg lost when tailings are discharged.
- If retorts are not used, weigh amalgam before burning and *doré*, after burning; melt the *doré*, if possible
- If retorts are used, weigh amalgam before retorting and after, as well as the mercury recovered; this can give some idea about the residual Hg in the *doré.*
- Check if the Hg balance through sampling is consistent with the data on Hg being provided by the miners.
- Repeat this procedure in as many mining operations as possible to obtain average amounts of Au produced and Hg lost per month in a mining region.

Is it possible to estimate Hg releases from ASM based on soil/sediment analyses?

A: Monitoring programs to estimate the quantity of Hg released from ASM based on soil or sediment analyses are extremely costly and are unlikely to yield reliable results. Quantitative evaluations of Hg releases are more accurate when based on reliable surveys at gold processing plants.

How can the Hg:Au ratio be used to estimate Hg emissions?

A: The ratio of Hg_{lost}:$Au_{produced}$ has been used as a parameter to quantify mercury releases. One of the most common and confusing problems with this ratio is that some authors report only the Hg_{used}:$Au_{produced}$ ratio, which does not necessarily reflect the amount of Hg lost. In many cases, the amount of Hg recycled is not reported. It is important to use the Hg_{lost}:$Au_{produced}$ ratio carefully as an **approximate** and **regional** estimate of mercury emission from **various operations** in an ASM region. The ratio of Hg_{lost}:$Au_{produced}$ varies from one operation to another and, when very little gold is produced, the ratio gives a false impression that a high amount of mercury has been lost. The amalgamation method defines the amount of Hg lost. When the whole ore is amalgamated, losses can be higher than 1 part of mercury to 1 part of gold produced. When gravity concentrates are amalgamated properly and retorts are used, mercury releases are very low.

Is it possible to analyze the levels of Hg in air?

A: Yes, in areas remote from industry, atmospheric levels of Hg are about 2–4 ng/m³, and in urban areas about 10 ng/m³. A guideline for inorganic mercury vapour of 1 µg/m³ as an annual average has been established by the World Health Organization - WHO. The recommended health-based exposure limit for metallic Hg is 25 µg/m³ for long-term exposure.

Sampling of Hg from ambient air in mining hotspot areas provides data about Hg vapour exposure levels to workers and surrounding population. Sampling can be conducted using Hg vapour analyzers, also known as Hg sniffers (e.g. Nippon or LUMEX or Jerome), or vapour traps made of gold/silver or activated iodated charcoal. Traps installed in strategic locations collect air samples over a long period of time. The traps are connected to a pump and to an air flow regulator that provides constant flow rates. Mercury entrapped, after acid digestion, is analyzed. Portable Hg sniffers usually provide a snapshot of the ambient air in workplaces and surroundings but some instruments can also analyze Hg in intervals of 10 to 60 seconds over many days and record data in a computer.

The preferred places to collect air samples are:

- In an area removed from mining sites (for background levels)
- Inside huts where miners sleep and cook
- In workplaces where ore is processed and amalgams are burned
- Inside gold shops where *doré* is melted and surroundings
- Inside houses near the mining sites or gold shops
- Outside, near mining hotspots

How can one determine if the Hg pollution source is dispersed or concentrated?

A: This depends on how the miners operated. If they amalgamated the whole ore or worked in rafts in a large river, dumping the amalgamation tailings into the river, the mercury would likely be dispersed over a very wide area. Much of the Hg emissions from open pan burning practice are likely to be elemental and distributed. Local Hg deposition is generally detected within two kilometers downwind from the emission source. If the miners only amalgamate concentrates in pools, Hg tends to be concentrated within a relatively small area, forming a "mining hotspot."

What are the main pathways of Hg to humans?

A: Exposure to Hg by humans living in close proximity to ASM sites is primarily via **main** two pathways:

1. Inhalation of Hg vapour from amalgam burning and gold melting, and

2. Ingestion of methylmercury (formula CH_3Hg^+; abbreviation: MeHg) from dietary sources, especially fish.

What is the difference between Hg contamination and pollution?

A: A Hg-contaminated site is identified by the high level of Hg in geochemical materials such as soil and stream sediment. In this case, the Hg concentration is higher than at a reference site (background). An Hg-polluted area can also be characterized by the bioavailability of Hg; i.e. Hg is in a form that organisms can easily incorporate (usually MeHg). Not all sites with high Hg concentrations may be equally vulnerable to formation of MeHg compounds via natural processes, but these sites are of **high risk**.

How is pollution characterized?

A: Biota are the ultimate indicators of Hg pollution, as they provide direct evidence that Hg in soil, sediments, water or air has become bioavailable and is being bioaccumulated by the organism, especially in aquatic environments.

What is a mining hotspot?

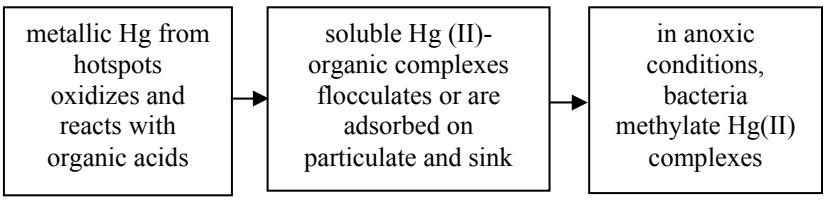
A: A **mining hotspot** is characterized by relatively high concentrations of metallic Hg relative to background levels in soils or sediments, indicating extensive use of Hg for gold extraction. Usually mining hotspots are formed either when amalgamation tailings are dumped into watercourses, at abandoned (or active) sites excavated on the ground or near stream margins used for amalgamation of gravity concentrates.

What is an environmental hotspot?

A: An **environmental hotspot** differs from a mining hotspot in that it is a site where MeHg is being produced or has high likelihood of being produced. An environmental hotspot may be situated away from mining hotspots, in several locations. It is characterized by high MeHg concentrations in sediments and/or aquatic biota in areas where Hg has been methylated and become bioavailable.

How does methylation of metallic Hg occur?

A: MeHg in sediment is produced primarily by methylating bacteria and is either released into the water column where it is rapidly accumulated by biota, or incorporated by benthic invertebrates at the base of the food chain. Metallic Hg must first be **oxidized** and form soluble mercuric complexes to be available for methylation by bacteria. Usually these complexes are formed by reaction of metallic Hg with soluble organic acids such as fulvic, tannic and humic acids in aerobic conditions. MeHg can be produced by bacteria-mediated processes in aerobic and anaerobic sediment but its production is significantly higher in anoxic environments. MeHg concentration in soil and sediment occurs as a result of the balance between MeHg production and degradation of MeHg into metallic Hg (i.e. demethylation). Despite the uncertainties related to the transformation of metallic Hg into MeHg in ASM regions, a simplified sequence of reactions is suggested:



How does one determine if Hg dispersed over large areas is bioavailable?

A: Analyzing aquatic biota, in particular fish, is the most effective means. Organisms are the best indicators of Hg bioavailability, particularly MeHg. MeHg is biomagnified in the food chain and reaches its highest concentrations in fish, especially fish-eating, carnivorous fish.

What are the most favorable environments to produce methylmercury?

A: Flooded forests, floating macrophytes, lakes with meanders and anoxic environments favour high MeHg production rates. For example, flooded areas have MeHg production rates that are at least ten times higher than those measured in sediment of flowing waters. Anoxic environments rich in organic matter are also favorable to methylating bacteria. Thus, organic-rich-flooded areas (e.g. wetlands) are the favored sites for MeHg production.

What information is obtained when one analyzes Hg in soils/sediments?

A: Soils and sediments are witnesses of a contamination process over the years but they **rarely provide quantitative data** on the absolute degree of contamination. Instead, soils and sediments can play a key role in ascertaining relative factor of heavy metals enrichment when this is compared with a reference site (uncontaminated). Analyses of soils and sediments in an environmental assessment may have distinct objectives:

- **Identify mining and environmental hotspots.**
- **Predict and obtain evidence whether Hg associated with fine particles can be transported to other areas.**
- Learn how easily Hg can be leached out from soil and sediment components; these also are indirect ways to determine bioavailability
- Learn how stable Hg is associated with soil and sediments components; this provides indirect hints about bioavailability.
- Obtain information about atmospheric Hg dispersed over a region by analysis of superficial soils.
- Obtain information on atmospheric Hg accumulation on soils and sediments, analyzing lake profile cores.
- Obtain information about kinetics of MeHg generation

All of these objectives are important and, in the majority of the cases, they are site-specific, i.e. depend on the type of environment being studied. The two first objectives are of critical importance for an environmental assessment. In an active ASM operation, when miners dump Hg-contaminated amalgamation tailings into an aquatic environment, it is clear that this will form mining hotspots. The establishment of a sophisticated monitoring scheme is not necessary to prove this. A simple semi-quantitative analysis or even panning can, in many cases, identify sites with Hg levels in sediments. The main objective is to locate these (mining) hotspots to establish future remedial actions. Analysis of fines and knowledge of the hydrodynamics of the environment can provide hints as to whether or not the material from the hotspots can be transported to other sites. In this case, analysis of screened fractions is recommended. Analysis of the particulate matter being transported by waters or found in depositional areas is the real evidence of mercury mobility.

What is the best way to establish reference Hg levels in soil/sediment?

A: In order to characterize Hg-contaminated sites (mining or environmental hotspots) it is important to establish background levels (i.e., **reference conditions**), no matter what the objective of the soil and sediment monitoring program is. Data from reference areas will provide a context for understanding environmental contamination from of ASM activities. The site(s) must be free of other anthropogenic point source contamination. Once identified, undisturbed surface sediment and soil samples, representative of ambient conditions, must be collected. Physical characteristics and sampling procedures used in reference areas must match conditions and procedures in Hg contaminated locations sampled. Regardless of the means by which soil or sediment is collected to determine background Hg concentrations, the following information must be collected from both reference and Hg contaminated sites:

- Geological characteristics (mineralogical components).
- Grain size distribution (less than 2 mm).
- Sample preparation (sieving, handling, etc.).
- Drying procedures and methods.
- Packing and preservation methods.
- Quality assurance/quality control procedures used.

Samples must be (wet) screened in the field to remove debris and gravels larger than approximately 2 mm. If this cannot be done in the field, this must be done later in a laboratory. The use of composite samples is encouraged as a means of reducing spatial heterogeneity as well as reducing the cost of sampling at a particular site. Composite samples of surface soil (i.e. upper 5–10 cm) should be collected randomly within a known area (e.g. 25m^2 plots) and composited into a large container, mixed, sieved and sub-sampled for grain size and Hg analysis. Properly used, composite samples can provide a means of quickly assessing Hg heterogeneity in an area and determining whether further sampling is required. Analysis of finer (screened) fractions can remove the dilution effect of quartz and increase homogeneity of the sample when droplets of Hg are present.

The number of sediment samples and mass of sample needed to describe local condition levels depends on the size of the area, grain size and mineralogical variation. For example, background conditions will be much less variable or heterogeneous than mining affected areas; therefore, the sample size required to define reference conditions will be much smaller. At least two reference areas should be sampled to determine regional variability.

When sampling stream sediments in the field, the following basic information about the site must be gathered:

- Date and time of sampling and location (GPS and map location if possible).

- Color, consistency, organic content, depth of composite soil samples collected.
- Location within the stream, such as near shore, near middle, etc.
- Water depth at which sediment is collected.
- Stream flow condition (preferably with water velocity (m/s) and flow rate (m^3/s)).
- Other observations such as presence of emergent or bottom vegetation, proximity to other streams, marshes, wetlands, water color, transparency, etc.

The following information must be collected from sediment samples:

- Total Hg concentration (ppm dw).
- Sediment grain size (% sand, silt, clay in dw).
- Total organic carbon content (% dw).

Physico–chemical parameters of the soil and sediment at reference sites should be measured. This provides useful information to compare with the contaminated site. Variables such as sediment (soil) Eh[24], pH, conductivity (µS/cm), total organic carbon (TOC), sulphate, etc., provide data about the "original conditions" in which the Hg contaminated material was deposited. Reference areas can also provide relevant information about background MeHg concentrations in soil and sediment, as well as methylation potential.

Stream or lake sediments should be collected using a standard grab sampler such as a petite or standard Ponar grab or a similar device in stream environments, and an Ekman or Ponar grab, or similar device in lake environments. A sediment coring device can also be used to collect superficial (upper 10 cm) sediment samples.

Once a sampling station has been selected and described according to the above criteria, the following procedures can be followed in the field to collect a representative grab sample of bottom sediments for chemical analysis:

- Determine water depth.
- Slowly lower the grab to the bottom (by hand or by winch) at speeds not exceeding 0.3 m/s so that a bow wave is not formed in front of the grab to minimize disturbance of fine surface sediment.
- Raise the grab to the surface and examine the sediment for acceptability criteria. Only those grab samples that meet the following criteria should be retained for analysis: do not contain large foreign objects (e.g. roots, branches, rocks); have adequate penetration depth (i.e., >10 cm); are not overfilled (sediment surface not touching the top of sampler); do not leak (overlying water is present and there are no visible leaks); and is undisturbed (sediment surface was relatively flat). Grabs that do not satisfy these conditions should be retained and discarded once sampling at the station has been completed.
- Remove overlying water from acceptable grabs by gently decanting or siphoning.
- Describe and record sediment characteristics including: color, odor, grain size, and the presence of other materials (e.g. organic debris, hydrocarbons, vegetation, biota).
- Remove the upper 4 – 5 cm of sediment from the surface of acceptable grab samples with a pre-cleaned stainless steel spoon and place in a stainless steel bowl.
- Repeat the above process from at least three separate areas within each station so that a minimum of three grab samples are collected and placed in the same bowl to form a composite sample.
- Using the spoons, mix the sediment composite sample until it has uniform color and consistency.
- Composite samples must be wet-screened, in the field or the laboratory to –80 mesh (0.177 mm) or –100 mesh (150 mesh) or –200 mesh (0.074mm). The choice of the screen opening must be based on the existing field facilities, but the 200 mesh screen is preferred; the same screening procedure should be used to prepare samples collected in contaminated sites. The finer fractions are more homogenous and richer in Hg than the –2 mm fraction.
- Analyses must only be performed on screened samples ("fines"), but some –2mm and + 80 or 100 or 200 mesh samples must be analyzed to compare with the samples from contaminated sites.
- Use the pre-cleaned stainless steel utensil to completely fill (i.e. no head space) 250 mL glass or PVC sample jars. Seal jars immediately and place in a cooler with ice or ice packs if available. Keep jars as cool as possible while in the field, during storage, and during transport to the laboratory. Polyethylene bags should be used just for dry samples.
- Label the jar and lid with indelible ink with a unique sample locator number. Record in a field notebook and on chain-of-custody (COC) forms.
- At the end of the day, crosscheck COC forms with labeled jars.

[24] Eh, the Standard Hydrogen Electrode (SHE) potential, is actually hard to measure in the field. The most common electrode for monitoring is Ag coated with AgCl. When a saturated KCl electrolyte is used, the relation between readings obtained with this type of electrode and the SHE is: Eh = EAg/AgCl + 0.199 (in volts).

All procedures used to collect, prepare and analyze samples to establish background levels must also be applied to samples from contaminated sites.

When a local laboratory is available, the samples can be wet screened and dried, preferentially at room temperature, or at temperatures below 60 °C. Dried samples can be packed in glass or plastic jars or plastic bags and kept in a cool environment until analyzed.

How does one characterize and locate Hg mining hotspots?

A: Mining hotspots can have dimensions of few square meters, as in the case of amalgamation pools, or hundreds of square meters when the whole ore is amalgamated in sluice boxes or copper plates or ball mills (while grinding the ore). Whenever amalgamation takes place in an excavated pool, a water-box, beside a riverbed, or in a sluice box, tailings are discharged into the environment creating mining hotspots where the mercury concentration can reach hundreds of µg/g.

The method used in the field to locate mining hotspots (i.e. sites with high Hg concentrations) depends on two basic aspects: First, if the mining and processing operation is active and second whether the hotspots are in dry terrestrial sites, but near water. The easiest way to locate hotspots in active mines is by observing and asking miners where they have conducted their amalgamation processes. At inactive mine sites, the process is more complicated, but it is still worthwhile searching for old residents and former miners to obtain information about amalgamation sites.

The main steps suggested to locate **mining hotspots** are:

- **ASK FIRST**: Find out about the history of Hg use in the mining region and specific sites; ask former miners or residents, when the mining is not active.
- Look for specific sites where the miners do or have done amalgamation.
- Look for the sites where the amalgamation tailings were discharged.
- **TRY TO SEE Hg DROPLETS**: To delineate the hotspots, use a panning process or any other gravity concentration process to quickly find the areas with high metallic Hg content (visible droplets).
- **IF YOU DO NOT SEE, ANALYZE**: If mercury is not visible or the panning method is not efficient, collect some samples and use a semi-quantitative analytical method for the –2 mm fraction.
- Screen the samples through a 2 mm screen to remove coarse debris and pebbles. Do not use copper screens.
- Take some composite sediment samples to the lab to check the semi-quantitative analytical method; composite of 3 to 5 scoops taken from neighbor sites (within 10-30 m² depending on the size of the area being investigated) can be mixed, homogenized and split to obtain an aliquot of a specific site.
- **DETERMINE IF Hg IS ASSOCIATED WITH FINES**: Composite samples of the –2 mm fractions must be wet screened in the field or lab to –80 mesh (0.177 mm) or –100 mesh (0.15 mm) or –200 mesh (0.74mm). The choice of the screen opening must be based on the existing field facilities, but the 200 mesh screen is preferred; the same screening procedure should be used to determine background levels. Do not use copper screens. Finer fractions are more homogeneous.
- Dry the fine (screened) fraction preferentially at ambient temperature using a tent. If this is not possible, dry samples at temperatures not exceeding 60 °C.
- The weight of the coarse fractions –2 mm +80 (or 100 or 200) mesh and fine fractions must be registered.
- Occasionally analyze Hg in the coarse fractions to obtain Hg distribution (in %), i.e. % of Hg in fines and % in coarse fractions.
- **OPTIONAL**: In the lab, analysis of total Hg in finer grain size fractions (e.g. 0.002 mm) can provide valuable information of the possibility of Hg being dispersed with fine particles.
- **PRESERVATION**: Pack the samples in glass or plastic jars or in double plastic bags and keep them stored in a cooler (NO ICE must be added). If a fridge is available, the samples can be kept inside until the time of transportation.
- **WHEN COLLECTING SAMPLES FROM HOTSPOTS**: Measure physico-chemical parameters such as sediment (or soil) Eh, pH, conductivity (µS/cm) and collect samples for analysis of Total Organic Carbon, and other parameters that can provide information about the possibilities of a mining hotspot to become an environmental hotspot.

Sample preparation in the lab and chemical analysis involve the same procedures described for determining Hg background levels.

Can Hg from mining hotspots be dispersed (mobilized) in solution?

A: Metallic Hg dumped by ASM is heavy and not easily transported in drainages. Metallic Hg, when oxidized, can be adsorbed on soil and sediment particles and be introduced into streams, usually through run-off waters. Metallic Hg condensed from the atmosphere can also impregnate fine soil particles to be transported. It is well known that fine soil

and sediment particles usually have twice as much Hg than coarse fractions because of the interaction of Hg-oxidized complexes with soils and sediment components, in particular clay minerals and hydrous ferric manganese oxides.

How does one characterize and locate an environmental hotspot?

A: It is not easy to locate sites where Hg released by ASM activities has high potential to be transformed into MeHg and bioaccumulated by aquatic biota to become an environmental hotspot. Processes to evaluate methylation potential of different natural environments can be costly and complex. Sampling geochemical materials such as soil and sediments to determine if a site is an environmental hotspot is an expensive task and the decision on what will be sampled must be carefully examined and determined based on laboratory facilities, budget, personnel, etc. MeHg concentration of soil and sediment determines the magnitude of methylation and provides an indirect measure of bioaccumulation potential. MeHg concentration in sediment or soil must be compared with at least two reference areas in order to provide some understanding of natural variation in MeHg concentrations from uncontaminated areas.

Locating environmental hotspots can be achieved using ***direct*** or ***indirect*** methods. To directly determine if an area represents an environmental hotspot, ***resident biota*** (small fish or invertebrates such as clams or snails) can be measured for total Hg. Analysis for total Hg concentration is sufficient to provide information about the bioavailability of Hg in a specific site. Because MeHg comprises the majority of the total Hg concentration in fish tissue, analyzing for total Hg is less costly than for MeHg and therefore more samples can be analyzed. Note that biota samples must also be collected from reference areas to establish the background and provide a benchmark against which to contrast with biota data from environmental hotspots.

Indirect methods involve methods to determine methylation capacity or the methylmercury production rate of soil or sediment. This can be accomplished by applying bioassays, for example by using earthworms.

Mercury can travel great distances from ASM Hg release sites. The place where it is deposited in sediment and encounters favorable methylating conditions may be far away from its point of origin. Therefore, the spatial scope of the investigation may be large. There may be many downstream locations where inorganic Hg will accumulate and become methylated, contributing to the problem. For example, in large riparian environments, where active sluicing of sediments is abundant and widespread, it may be impractical or impossible to locate and quantify even the most important methylation environments using sediment assessment methods. In these circumstances, determining the magnitude of Hg methylation and bioaccumulation is accomplished best by using small, non-migratory fish species. Because fish integrate Hg well over space and time, these are the best biomonitoring organisms to use, rather than undertaking widespread sediment assessments or bioassays.

Is it important to analyze soil/sediment around mining impacted areas?

A: Sometimes, but not always. In general, when Hg is dispersed over a large area, it is difficult and costly to characterize Hg-contamination merely by analyzing sediment and soil. Biota analysis can provide better information about Hg bioavailability.

How does one determine if Hg from hotspots is being dispersed?

A: There are two main ways of establishing if Hg from hotspots are being dispersed, and transported to other sites:

1. Analyzing sediment (and biota) in depositional areas in watercourses
2. Analyzing particulate matter in the water column

In the first instance, the purpose of the investigation is to determine if material from mining hotspots is being transported to areas where it can be methylated (to create and environmental hotspot) or if material from sites already identified as environmental hotspots are being transported to other areas. In these cases, analysis of material from the hotspots (fines such as –200 or –400 mesh or clay fraction –0.002 mm) and fine fractions of sediments from depositional sites (slow or static flow) provide information about material being exporting from hotspots. The sampling procedures are identical to those described above when environmental hotspots are sought. Analysis of the fine fractions is preferred. If material from mining hotspots is visibly being transported by drainages to other sites, samples from this new site must be collected to determine if methylation is occurring. Methylation can also occur in a region due to different Hg sources not directly related to the mining hotspots (e.g. lithogenic Hg leached by run-off water, atmospheric Hg deposited from various sources including mining, inputs from upstream reservoirs, etc.). In some cases, these sites produce more MeHg than mining hotspots. To prioritize potential environmental hotspot areas downstream of ASM activities, the following criteria should be used to determine whether or not to conduct sampling:

- Seek depositional areas in streams – areas with fine sediments (silt/clay) or fine sand; avoid areas of erosion.

- Seek wetlands and marshes adjacent to or part of the stream, or stream areas that receive runoff from wetlands, marshes and bogs with low oxygen concentration.
- Seek sediments with visible organic material indicating sediment nutrient sources and anoxic decomposition.
- Use reliable techniques to identify sediments with high metallic Hg concentrations (mining hotspots) rapidly. Although the correlation between high inorganic Hg in sediment and MeHg in sediments and biota is weak, this may be useful as a screening tool.
- Seek areas that are relatively easy to sample, with low flow, relatively shallow depth within the permanently flooded area of the stream.
- Seek areas where it is possible to collect benthic invertebrates, such as insect larvae and clams, or small minnow (fish) species for Hg analysis.

It is difficult to sample all suspected environmental hotspot areas. Field sampling must be stratified to identify those hotspots that are the greatest contributors of methylmercury to aquatic systems. To accomplish this, it is necessary to seek out areas in the aquatic environment downstream from mining hotspots that have those physical and biological features that are most highly correlated with Hg methylation potential.

In the second instance, analysis of suspended particulate matter provides a snapshot of the Hg transportation process associated with fine particles suspended in the water column. Sampling of suspended particles is a tedious process that is subject to contamination. Usually filtering water through 0.7 and 0.45 μm Milipore filters is conducted in the field. At least 100 to 200 mg of particulate material must be filtered and collected, to ensure sufficient solid sample for analysis. This requires the filtration of many of liters of water, which is time-consuming. Coagulation followed by flocculation as an aid to sedimentation and filtration is helpful to reduce the amount of water filtered in the field. Filter papers or centrifuged material can be dried at ambient temperature or at <60 °C and packed in small plastic bags or vials. Refrigeration of samples is recommended prior to and during transport to the analytical laboratory.

How can it be determined if mining hotspots are also environmental hotspots?

A: Analysis of MeHg in soils and sediments is costly and provides indirect information about the bioavailability of Hg. Direct sampling of resident biota (invertebrates or small fish), provides an excellent indicator of Hg bioavailability in mining. Small fish, typically bottom feeding catfish species can be excellent indicators. These indicator species forage within a relatively small area, do not range over long distances like large, carnivorous species, and integrate MeHg from sediments and lower trophic levels over time from relatively discrete areas.

When should a mining hotspot be mitigated?

A: Whether a mining hotspot should be mitigated (e.g. dredged or capped) or monitored is a management decision based on an **evaluation of the risk of bioavailability** (to become an environmental hotspot), costs involved in the dredging operation, and spoil treatment options. In some cases, the decision to remove contaminated soils or sediments is based exclusively on Hg concentrations in excess of numerical criteria. In Japan, for example, the decision to dredge sediments from Minamata Bay with Hg concentration above 25 ppm was based on many site-specific factors such as tidal range, sediment-to-water transfer rate, and a safety factor of 100 in fishing zones. In British Columbia, Canada, the BC Ministry of Environment (1989) determined that soils or sediments with Hg concentration between 2 and 10 ppm require remediation to levels below 2 ppm if the land is to be used for residential and recreation purposes. For sites with concentrations above 10 ppm Hg, all uses of land are restricted pending the application of appropriate remedial measures that reduce contaminant concentrations to less than 10 ppm. The Canadian Soil Quality Guidelines (1999) established the level of 6.6 ppm (μg/g) as the limit for soils with agricultural and residential/parkland use and 50 ppm for industrial use.

What is the purpose of analyzing Hg in fish?

A: The objective of the fish Hg assessment must be clear before establishing the sampling strategy. Fish capture programs should target two groups of fish: 1) fish species that are consumed by the local human population; and 2) fish species that serve as indicators of mercury bioavailability in ASM and surrounding areas. In any case, **reference** fish tissue data are important to put fish tissue Hg concentrations from hotspot areas into perspective with background levels; and to establish a baseline against which reductions in fish Hg can be measured in future collections. Regarding Health Assessment, the most important information to acquire is the dietary habits of local people. In this case, interviews about socio-economic and demographic aspects of the community must be applied. Visiting local fish markets will also help identify those species most frequently purchased and/or consumed by communities, as well as other nearby villages. Fish markets can also provide samples of fish for analysis as well.

Is it possible to determine if Hg bioavailability is increasing/reducing over time?

A: Yes, by measuring Hg in the same species of fish from the same standardized fish size to avoid the bias of different sizes. Fish and invertebrates are the ultimate indicators of bioavailability of Hg, especially MeHg. MeHg is most concentrated in fish-eating, carnivorous fish. Because MeHg is acquired almost exclusively via dietary sources and comprises at least 90% of total Hg concentrations in fish, tissue samples should only be analyzed for total Hg concentration and reported in units of ppm wet weight. Piscivorous fish accumulate more Hg than other species and frequently have a positive correlation between increasing fish size and increasing mercury concentration. Therefore, the size of fish will influence mercury concentration. There is a well-known positive correlation between fish size (length and weight) and mercury concentration in muscle tissue. Therefore, the mean mercury concentration of a sample depends greatly on the size of fish being measured, with larger fish having generally higher Hg concentrations. To eliminate the bias associated with differences in fish size, mercury concentrations must be measured over a wide size range. Then, appropriate statistical procedures can be used to determine the mean mercury concentration for a specific fish size, usually near the size most frequently captured by consumers. This is called the size-adjusted or **standardized mean mercury concentration**.

When this is done for multiple sites or years, comparisons of standardized mean mercury concentration can be made that are unbiased by differences in fish size. Optimally, tissue from 25 – 35 fish is gathered from each species, ranging from small to large fish. The strength of the relationship between mercury concentration and fish size depends on sample size and the distribution of fish size over the size range being tested.

Very few studies in regions impacted by artisanal mining have attempted to standardize the relationship between fish size and Hg. Although there is a large data set for Hg, especially in the Amazon region, it is hard to track the evolution of Hg over time because of the lack of standardized procedures.

The correlation between fish length and Hg is generally more reliable than with weight for many reasons. Depending on the time of year, a fish may weigh much more because of eggs or testes. For example, weight can differ considerably (20% or more) due to the accumulation of body fat or state of maturity (e.g. females with eggs) within a single season that is not reflected by differences in length. Depending on when a fish is caught, the Hg-weight relationship can be significantly different. For example, a large fish can consume up to 20 – 30% of its body weight. Depending on whether or not the fish has eaten recently, this can affect the Hg-weight relationship dramatically. In addition, differences in length tend to be less variable than with weight because of the smaller range over which both extend. When measuring Hg-size relationships for large fish, length typically ranges from 20 to 70 or 80 cm. Over the same length range, body weight ranges over a much greater range, from 200 g to 8 or 10 kg, reducing precision of the relationship. This greater variability within the weight range than the length range introduces variability that reduces accuracy and limits the quantitative ability to measure changes in Hg over time. Finally, length is easier to measure in the field than weight and no special equipment is required.

By calculating the **standardized Hg concentration** from the standardized fish size, comparisons of Hg can be made between areas, regions or years without the bias of differences in fish size. Also, by deriving a reliable length – Hg relationship for target species, the Hg concentration of a fish can be accurately predicted, without knowing the empirical value. This can also be useful in estimating Hg exposure for the human Health Assessment if the species selected as "standard" is also highly consumed in a region. Assuming that tissues from target species were collected within different size ranges, the following procedures should be followed with the data:

- Compile the data and enter the data into a spreadsheet and check for accuracy.
- Log_{10} transform the length (mm) and total Hg (ppm wet weight) data and plot the log (length) and log [Hg] relationship for each target species and examine for linearity and presence of outliers.
- Calculate linear regression equations for each relationship to determine the significance of the regression: yes ($p<0.05$) or no ($p>0.05$); intercept (a), slope (b) and the goodness of fit (r^2). An example of this procedure is illustrated in Figure 1, above. If the regression is **not** significant, then there is no relationship between mercury and fish size and it is not appropriate to apply any further statistical procedures. This is very unlikely for carnivorous fish, especially in Hg contaminated environments. If this occurs, select another species.
- Graphical comparisons of the mercury data can be made where appropriate.
- The standardized Hg concentration can be determined from the linear relationship based on the standardized length.

It is important that the above protocol for deriving Hg-length relationships for all species is followed, regardless of whether for the human Health Assessment, or for characterizing Hg bioavailability. The procedure is simple to follow and provides a quantitative, strategic approach to fish sampling. When sampling small fish for environmental hotspot identification, simply use smaller length ranges (e.g. by 50 mm size intervals) from which to collect fish and to derive a smaller standardized size.

It may be difficult to identify an individual species (**bioindicator**) in taxonomically rich or diverse streams such as in tropical countries. Therefore, it is not absolutely necessary to sample the same species for human health or environmental hotspot assessment, as long as the diet of each species sampled is very similar. For example, for the top-level predator fish, if there are closely related species of cichlids or piranha and it is difficult to capture sufficient numbers from each species, different species can be grouped together. However, it is very important that the grouped species have a very similar diet and life history strategy (e.g. small movements, similar growth and size). Ideally, a sufficient sample size should be collected from each target species according to the procedures described above.

What is the most appropriate fish species to be chosen as bioindicator?

A: The specific species selected to be the bioindicator of an area or site should consider the following criteria.

1. A carnivorous species with high Hg concentration.
2. A species with sedentary habit (no migration habits).
3. Easy to catch and preferentially consumed by local residents.

If it is difficult to fill the required size ranges for an individual species, it is permissible to combine more than one target species to accomplish this, provided that the species being combined share a number of key characteristics. These include similarity in diet (e.g. carnivorous), size range, migration habits, maximum age, etc. This must be used as a last resort, as it is very important to understand the relationship between Hg and fish size. Long-term comparisons bring enormous benefits to support decisions. Acquisition of fish tissue for Hg analysis from target species should follow the above protocol to ensure an equitable representation of Hg concentrations over the full size range of fish. It will be easier to fill certain size categories than others. However, every reasonable attempt should be made to acquire an adequate sample size within each size category. Investigators should attempt to acquire fish from local vendors and fish markets. If markets or vendors do not have fish of certain size groups (e.g. lack of small fish), then local fishermen should be hired to collect and fill the missing sample size intervals. Following this protocol will ensure that an optimal number of fish (i.e. not too few and not too many) are collected to derive a good statistical relationship without being wasteful of available resources in the field.

If the ideal bioindicator is not edible, is it still useful to be sampled?

A: Yes, a non-edible fish can be used as an indicator of bioavailability (bioindicator) in a site or region, applying the standardized method to follow the evolution of Hg concentration over time.

What is the procedure to sample fish for Hg analysis?

A: The following steps to collect fish tissue in the field should be adopted to ensure that a common protocol is followed for each collection program and to establish a quantitative approach to Hg monitoring over the long-term within each of the recipient countries.

- Identify the target species based on the criteria above and interviews with local people, fishermen, fish markets, and if possible from local or regional experts, such as a biologist.
- Identify sampling areas to collect fish including upstream, **reference** areas if possible, and an area(s) downstream of mining activities. **Sampling should target at least one upstream and two downstream areas to address geographic differences.** Sampling efforts to address health concerns should also coincide with geographic areas identified as "environmental hot spots" to address possible worst-case areas of methylation and accumulation by carnivorous species.
- Acquire individual fish among the target species according to the size protocol above. Fish can be acquired from markets or by accompanying or hiring local fishermen. Note that the geographic source of fish **must** be known. Every reasonable attempt should be made to collect tissue samples from each target fish species across the entire size range, from small to large.
- A minimum of 10 – 20 g sample of muscle tissue per fish is required. Muscle tissue can be collected from any part of the body of the fish, avoiding fatty or overly bony tissue.
- Tissue samples should be excised from the fish with a clean, stainless steel knife and placed in a labeled plastic bag such as Ziploc^{tm}, or Whirlpac^{tm} bag. Alternatively, tissue samples can be stored in small plastic vials (e.g. scintillation vials). Label sample containers with an indelible marker and record the information in a field booklet.
- At a minimum, fork length and the geographic location where the fish was captured must be recorded. Collect supplemental data including fish weight (g), stomach contents, gender, and maturity.
- Tissue samples should be placed on ice and/or frozen as soon as possible after collection. Samples must be frozen within three days of collection.

- Samples should be transported frozen and kept frozen until analyzed for total mercury concentration (ppm wet weight).
- Mercury concentration should be determined from tissue sub-samples using cold vapour atomic absorption or fluorescence spectrometry by an accredited laboratory. Remaining tissue should be stored frozen in the event that subsequent analyses are needed.
- Appropriate QA/QC procedures should be followed including 1) collection of duplicate, blind samples from 10% of all fish captured; 2) repeat analysis of tissue sub-samples to determine laboratory accuracy; and 3) analysis of standard, reference tissues with known Hg concentrations to determine laboratory precision.

Is there another method to characterize Hg bioavailability?

A: There are **indirect** methods to **predict** bioavailability. Sequential (or selective) chemical extraction procedures, for example, have been used to identify metal-bearing components in soils and sediments. Some procedures are very selective and can clearly distinguish between metals associated with clay minerals, hydrous ferric and manganese oxides, organic matter, sulfides, carbonates and metal in the structure of silicates. The association of metals with mineralogical phases can either increase or reduce bioavailability. These chemical procedures give indirect information about the strength of the bond of a metal with a mineral surface. The stronger the bond, the lower the bioavailability. However, the selectivity of these procedures for Hg species is not very good. Nevertheless, some methods can provide useful information about the amount of Hg associated to clay minerals (exchangeable), humic substances, hydrous ferric oxides, metallic Hg + refractory organics, sulphides and silicates.

Another method to predict Hg bioavailability is based on the physico-chemical conditions of the environment. Many studies have attempted to find correlations between environmental factors and mercury bioaccumulation. In fact, the search for parameters to predict bioaccumulation has always focused on finding a simple way to monitor and control Hg bioaccumulation. Unfortunately, exact equations have not been obtained, even though the effect of each separate variable on Hg bioaccumulation has been well established. This suggests that there are too many "unknowns" and site-specific conditions to produce satisfactory models. Frequently these parameters do not directly correlate with bioaccumulation due to internal interactions between them, which result in synergistic and antagonistic effects. However, the effect of some natural variables on the bioaccumulation process is known, particularly those environmental parameters related to mercury speciation. Parameters such as environmental physico-chemical characteristics (pH, Eh, dissolved oxygen, humosity, dissolved organic matter, etc.), and the presence and abundance of competing ions and methylating agents significantly influence the speciation and thus bioavailability of Hg in natural waters, sediment and soil. Studies using bioindicators of metal availability may be more revealing than geochemical methods alone.

Is it possible to sample other animals instead of fish to characterize Hg bioavailability?

A: Yes, it is possible. Invertebrates are useful bioindicators because they are simple, well-studied creatures that can provide indications of bioavailability in a short time frame at relatively low cost. However, it can be difficult and time-consuming to collect sufficient quantities of invertebrates for analysis of Hg and in particular MeHg, despite the greater expense of MeHg analysis. A problem with invertebrates is that MeHg proportion to total Hg concentration is very variable depending on the feeding behavior of the animals. This can range from less than 30% to 85%.

Which invertebrates are useful to indicate mercury bioavailability?

A: Ideally, invertebrates found in the contaminated sites are good indicators of Hg bioavailability in soils and sediments. Aquatic invertebrates can be directly sampled and analyzed for total Hg and/or MeHg from suspected environmental hotspots and this will provide confirmation of areas suspected of being significant contributors of MeHg to biota at the base of the food chain. In aquatic systems, three major groups of invertebrates should be targeted for sampling: bivalve clams; gastropod snails; or bottom dwelling larvae of aquatic insect groups such as chironomids, stoneflies, mayflies, or caddisflies. Bivalve clams and snails are relatively large organisms, resident, long lived, and integrate Hg in water and in sediments over time. They can be easily sampled, as long as one knows where to look for them. Where possible, local people familiar with harvesting these organisms should be used to assist in the collection.

In dry environments, particularly those with high Hg concentration, resident earthworms can also be good indicators of Hg bioavailability.

What is the procedure to sample aquatic invertebrates?

A: Clams can be collected by digging in the sand along shorelines or by using a rake with a long handle. From the shoreline or from a small boat, a rake can be dragged over the sediment surface until one or more clams are contacted and retained by the rake. Carefully pull the rake to the surface and place the clams in a bucket with site water.

Approximately 5 to 10 clams per site are required for analysis. You should either depurate clams in the bucket with clean water for at least 24 hours or remove the stomach containing sediment (possibly contaminated with Hg) from the clam tissue. Notwithstanding the need for a biologist experienced with the collection methodologies and taxonomy during field sampling and additional cost of MeHg analysis, using benthic invertebrates as indicators of methylation and food chain bioaccumulation can provide extremely useful information.

To process the clams, the adductor muscle should be severed with a clean, stainless steel knife and the whole clam tissue excised from the shell. If the clam has not been depurated, remove the stomach with the knife and discard. Weigh the remaining tissue (g wet weight) and place in a small plastic bag. Label the bag on the outside and place a small label on the inside of the bag. Refrigerate or place on ice and freeze as soon as possible. Whole clam tissue should be homogenized in the laboratory and analyzed for total Hg and MeHg. The proportion of MeHg to total Hg and the magnitude of the MeHg concentration, relative to the reference or control area will provide an indication of the relative degree of mercury methylation within discrete areas or environmental hotspots.

To collect benthic invertebrates, a sediment grab sampler (e.g. Ponar, Ekman) should be used to collect sediment from depositional areas in streams from suspected environmental hotspots. The top 4 – 5 cm of sediment is removed from the surface of the grab and sieved through a 500 µm stainless steel sieve to remove fine sediments. The remaining sediment material and sieved benthos is placed in a glass sorting tray and individual invertebrates are picked from the tray using plastic or stainless steel tweezers and placed in clean water. A minimum of 100 mg of tissue is required for mercury analysis. Organisms should be rinsed in clean water, placed in small labeled plastic vials, placed on ice and frozen as quickly as possible.

Snails, if one knows where to look, can simply be gathered from discrete areas. Several snails (5 – 7) should be composited to form a single sample. Record the location and weight (g) of snails collected from each composite sample and refrigerate, then freeze them as soon as possible after collection. Transport frozen. The entire snail from each composite should be homogenized and analyzed for Hg in the laboratory.

The same procedures should be used to collect and process invertebrates at suspected environmental hotspots, as well as reference areas. If invertebrates cannot be collected, then small fish can also be used to identify environmental hotspots.

Are fish better than invertebrates to characterize Hg bioavailability?

A: The advantages of using fish instead of invertebrates to characterize Hg bioavailability are that they are relatively easy to capture, are well know taxonomically (i.e., easier to identify than most invertebrate species), are easy to sample and need only be analyzed for total mercury, not MeHg (less expensive).

Is there any laboratory method to predict bioavailability using animals?

A: Bioassays, using fish as bioindicators, can also be used to assess bioavailability and provide information about bioaccumulation of mercury over time. However, laboratory methods to study bioavailability (non-lethal effects) of mercury from sediments or effluents to aquatic organisms are expensive and demand long and tedious work. For example, as aquatic organisms must be kept exposed to consistent conditions, aquarium water must be changed constantly and other permanent cares are needed. The duration of typical toxicity tests with aqueous samples ranges from four days for acute effects with an endpoint of mortality to 7 to 30 days for chronic and sub-chronic effects on survival, growth, or reproduction.

A more involved approach to study bioaccumulation is to conduct *in situ* bioassays such as stream cage studies. These studies typically involve fish, snails or bivalve clams enclosed within a cage that is either attached to the substrate suspended in the pelagic zone or floating. These tests have the advantage of providing more "real-world" conditions; that is, contaminant accumulation proceeds at its normal rate (i.e., impacted by biotransformation and other fate processes) under normal temperature, light, and other exposure parameters. However, the advantages to this type of study are also some of its disadvantages. Many variables cannot be controlled (e.g. pollution slugs, extremely high tides or flows, temperature, light, and food availability). These make the test a more reliable estimator of the real world, but also add additional covariates that make the data more difficult to interpret. The potential for escape of test organisms, predation, or tampering can also occur. The logistics and costs of these studies also may be quite high

A simple laboratory methodology using "lab-grown" earthworms can be used to evaluate the bioavailability of mercury from mining and environmental hotspots. The results of bioavailability are based on tissue concentration, which is determined by exposing earthworms in laboratory to contaminated soils, sediments or liquid effluents for an established period of time. The methodology is summarized as follows and described in greater detail below:

- Mix 80g of tailings, sediments or clean sand (for solution assessments) with 20g of prepared "cellulose" and 80 mL of distilled water (in solids evaluation) or solution (effluent solution) and manually homogenize in a 900 mL

glass jar (45% moisture content). This will form the substrate where the worms will live. It is important to control moisture (not too dry, not too wet).

- "Cellulose" can be prepared by shredding dry towel paper in a blender.
- Place 15 cleaned, weighed worms (*Eisenia foetida*) in the mixture for a period of 28 days.
- Duplicate or triplicate the jars for statistical evaluation of the results.
- Cover the jars with perforated paper or plastic. Do not open too many or too large holes whereby the worms can escape.
- Use a jar with worms, "cellulose" and clean sand as a "blank" reference jar.
- At the conclusion of the exposure period, remove and count the worms, clean and depurate them to void contents of the stomach and intestinal tract. Leave them in a jar with a mixture of 15g of clean "cellulose" and 50g of silica saturated with 50 mL of distilled water for a period of 5 days. The worms need clean silica to "crush" the "cellulose". These worms must starve to void gut contents for 24 hours before analysis. Depuration is necessary to ensure that subsequent whole worm analysis is indicative of Hg levels in tissues and not residual particles retained in the intestinal tract.
- Wash depurated worms, weigh and digest in 20 mL nitric acid (0.7 M) for total Hg analysis or full metals scan.
- Analyze Hg (and also other metals if desired) concentrations in soil, clean sand and paper added to the jars for comparison with worm tissues and to obtain the Hg concentration in the substrate (knowing the proportion of soil and paper added). Comparison can also be done with Hg in tissues of worms from "blank" jars.
- Compare concentrations accumulated in tissues to earthworms in the media (substrate) in which they reside or consume.

Can people be contaminated by Hg from drinking water?

A: This is very unlikely. MeHg is stable in solution but its residence time in natural aquatic environments is short as organisms accumulate MeHg from the water column very quickly. Hg-organic complexes, which are much less toxic than MeHg, can stay longer in solution but their concentration is also very low. The Canadian guidelines for total Hg in drinking water is 1 μg/L and for concentration for the protection of aquatic life is 0.1 μg/L. Usually, water is filtered (0.45 μm) before analysis. If people are drinking unfiltered water full of suspended particles, it seems that Hg is the least health problem related to this.

When must water be sampled?

A: Usually water samples do not provide useful information about Hg mobility or bioavailability. Water must be analyzed just when a legal requirement exists (e.g. to verify if guidelines are being met) or for academic reasons (e.g. study of the stability of Hg complexes). Water is not an easy geochemical material to be sampled and analyzed. As mercury usually occurs in very low concentration in natural waters, a large volume must be analyzed or analytical instruments with very low detection limit must be available. In seawater, the normal mercury concentration is around 0.05 μg/L Hg and in freshwater the average concentration in streams is around 0.07 μg/L Hg. Mercuric ion (Hg^{2+}) is not stable as a free ion in natural aquatic environments. Mercuric species are combined to form a inorganic or organic complex, such as $Hg(OH)_2°$ (aq) or Hg-fulvate complex. MeHg does not bind as tightly with organic matter in sediments as do inorganic Hg compounds. Consequently, MeHg remobilizes readily from the stable and less reactive sediments into the overlying water. As organisms accumulate MeHg very quickly, so that the concentration of this compound analyzed in water is very low or often, undetected by analytical methods, even in contaminated environments. Typical detection limits of analytical instruments are on the order of 1 to 2 ng/L for water samples.

One of the controversial points in water analysis is how to collect, analyze and interpret the results. Some researchers report dissolved Hg concentrations from **filtered** water (0.45 μm), while others report total concentrations from **unfiltered** samples. Most government guidelines for drinking water do not recommend filtering water samples. The lack of information about the filtration process can dramatically alter the result, as Hg in solution is in the order of few ng/L, while on suspended particles concentrations can be in the order of hundreds of ng/g. Those in favour of analyzing unfiltered water argue that many people drink unfiltered water; however mercury in the particulate matter (above 0.45 μm) is not necessarily bioavailable.

Can humans be used to assess Hg bioavailability in a site or region?

A: Intuitively, the best bioindicators for Hg bioavailability are human beings. However, there are ethical issues associated with collecting biological samples from individuals. Affected people **must** learn the results of the monitoring programme. Examining the following biomonitoring materials can monitor mercury bioavailability in humans:

- Hg in urine, especially from high intensity exposure, such as from Hg vapour exposure during amalgam burning

- Hg and MeHg in hair, which is a useful indicator from long-term exposure to MeHg contamination, particularly from ingestion of Hg contaminated fish.
- Hg in blood as a further indicator of recent or current exposure, particularly from exposure to Hg vapors or high fish ingestion. While mercury in urine may correlate with long-term exposure, blood analysis gives a combined picture of both metallic and MeHg contamination.

Urine, hair, blood and any other biological samples (e.g. nails) can be used for two purposes: 1) monitoring Hg exposure and bioaccumulation; and 2) obtaining information for the Health Assessment. Before sampling biomaterials, a meticulous selection of the individuals (donors) must be conducted using a socio-economic-demographic questionnaire.

What are the health effects of inhaling metallic Hg vapor?

A: Hg vapour is absorbed completely by the lungs through the alveolar membrane and complexes in the blood and tissues before reacting with biologically important sites. The biological half-life of Hg in blood from vapour is about 2–4 days. Ninety % is excreted through urine and feces. This is followed by a second phase with a half–time of 15–30 days. The time interval between passage of elemental Hg through the alveolar membrane and complete oxidation is long enough to produce accumulation in the central nervous system. Mercury can irreversibly damage the nervous system. Kidneys are the most affected organs in exposures of moderate duration to considerable levels, while the brain is the dominant receptor in long-term exposure to moderate levels. Total mercury elimination through urine can take several years. Hg levels in urine would then not be expected to correlate with neurological findings once exposure has stopped. A short-term exposure to high levels causes clinical symptoms, which mainly involve the respiratory tract. Mercury levels in the urine of new workers should be lower than those of workers with a longer duration of exposure.

Symptoms typically associated with high, short-term exposure to Hg vapour (1,000 to 44,000 μg/m³), such as those miners are subjected when they burn amalgams in open pans, are chest pains, dyspnoea, cough, haemoptysis, impairment of pulmonary function and interstitial pneumonitis. The common manifestation of chronic exposure to excessive levels of Hg vapour is metallic taste and gum diseases, such as gingivitis, ulcers, and formation of a blue line at gum margins. Long-term, low-level Hg vapour exposure has been characterized by less pronounced symptoms of fatigue, irritability, loss of memory, vivid dreams, and depression. Occupational exposure of mercury has resulted on effects on the central nervous system. Acute exposure has caused delirium, hallucinations and suicidal tendency as well as erethism (exaggerated emotional response), excessive shyness, insomnia, and in some cases muscular tremors. The latter symptoms are associated with long-term exposure to high levels of Hg vapor. In milder cases, erethism and tremors regress slowly over a period of years following removal from exposure pathways. A person suffering from a mild case of Hg poisoning can be unaware because the symptoms are psycho-pathological. These ambiguous symptoms may result in an incorrect diagnosis.

Since inorganic Hg poisoning affects liver and kidneys, high Hg levels in the urine can indicate undue exposure to Hg vapor.

What are the normal levels of Hg in urine?

A: In order to compare Hg levels from different individuals, urine values should be corrected for grams of creatinine in the sample, and should be expressed as μg Hg/gram of creatinine. If urine is very dilute (relative density <1.010), interpretation of the result may be difficult. In persons not occupationally exposed to mercury, urine levels rarely exceed 5 μg Hg/g creatinine. This is considered the *alert* level. The concentration of 20 μg Hg/g creatinine is considered an *action* level, i.e. the individual should be removed from the intoxication source. The maximum level recommended by WHO is 50 μg Hg/g creatinine. An individual with Hg levels in urine above 100 μg/g creatinine has a high probability of developing symptoms such as tremors and erethism (abnormal irritability).

How is urine sampled?

A: The ideal sample of urine is first one in the morning. This analysis reflects the mercury excreted by the body during the night. However this is not always possible and spontaneous urine has been collected without dramatically affecting results; i.e., identification of undue exposure to Hg vapor. Subjects should avoid drinking large amounts of water a few hours before sample collection, as this dilutes the urine sample. Mercury concentrations in urine should be corrected to the creatinine excretion.

What is creatinine?

A: Creatinine is a breakdown product of creatine, which is an important constituent of muscle. The creatine molecule gradually degrades to creatinine with time. The daily production of creatine, and subsequently creatinine, depends on muscle mass, which fluctuates little in most normal people over long ranges of time. Creatinine is excreted from the

body entirely by the kidneys. With normal kidney function, the serum (blood) creatinine level should remain constant and normal. Normal values are highly dependent on the age and lean body mass of the person from whom the urine is being collected. A healthy range for creatinine in spot urine is from 25 to 400 milligrams/deciliter (mg/dL). Urine creatinine (24 hour sample) values may be quite variable and can range from 500 mg/day to 2000 mg/day. The level of creatinine in a 24 h urine sample ranges from 8 to 22mg/dL/kg of body weight for children, from 11 to 20 mg/dL/kg b.w. for women and from 14 to 26 mg/dL/kg b.w. for men. So, a man weighing 70 kg has a normal level of 24-hour urine creatinine ranging from 980 mg/dL to 1820 mg/dL. As the creatinine concentration is usually expressed in mg/dL, dividing the result by 100 the unit is transformed into g/L. As the result of Hg in urine is usually expressed in µg Hg/L of urine, then dividing this by g/L of creatinine. Therefore, the final result is expressed in µg Hg/g of creatinine, which is the usual unit.

There are many procedures to analyze creatinine, but the colorimetric procedure based on the Jaffé reaction is very simple. An analytical kit is commercialized by Merck – Mercktest n. 3385. The color is analyzed in a spectrophotometer using the wavelength of 510 nm. The colorimetric analysis of creatinine is very rudimentary when compared with mercury analysis. Some researchers do not consider results of creatinine below 0.3 mg/dL as this introduces large errors when the value of Hg concentration is divided by such a small number.

What is proteinuria?

A: Proteinuria is a condition in which urine contains an abnormal amount of protein. Normally, protein should not be detected in the urine. Proteinuria is a well-known symptom of an Hg-related effect in the kidneys. The test to detect it is inexpensive and can be carried out using a commercial kit.

How is methylmercury transferred to humans?

A: In many rural communities, fish is the primary and frequently the only source of animal protein. The main exposure pathway of MeHg by humans is from fish consumption. MeHg is bioaccumulated and biomagnified easily and becomes concentrated in fish particularly carnivorous fish. These species are usually the preferred species of consumption by most people and should therefore be the focus of most attention. The amount of MeHg exposure is directly related to the amount, frequency, and type of fish consumed. Different people have different tolerances and sensitivities to mercury. Pregnant women, children, and women of child-bearing age should consume less carnivorous fish than adult males per unit of body weight.

What are the main effects of methylmercury in humans?

A: The effect of MeHg on the human body in terms of the degree of contamination is thought to be as follows: when very large doses[25] of MeHg enter the body, there are symptoms of acute brain damage such as aberrations of consciousness, convulsions, and paralysis, followed by death. When MeHg intake is lower, mild, atypical or incomplete symptoms may appear, or another disease may be manifested. Previously, it was thought that the harmful effects of MeHg were confined to the nervous system. However, it has become apparent that effects on other organs, such as kidney, must also be considered.

Is it possible to assess MeHg exposure?

A: Mercury in hair from the scalp is a good indicator of MeHg exposure from food. Hair grows about 1cm per month. During its formation, it excretes MeHg and shows a good correlation with blood Hg levels. Total Hg in hair is about 250 to 300 times higher than blood.

How is it possible to know whether Hg in hair is from food or from external sources?

A: In places with no influence of ASM (i.e., no Hg vapour), more than 85% of Hg analyzed in hair is methylated and a correlation with fish ingestion can be found. In some ASM impacted sites, the burden of Hg on inhabitants is a mixture of contamination from Hg vapour and from fish consumption. In this case, hair-washing procedures with different solvents cannot differentiate between airborne and internal MeHg. Some chemists have suggested that washing the hair strands with neutral detergent and water followed by acetone and followed by distilled water can eliminate most of the dust and fatty substances that are responsible for external Hg contribution. However the best method to discriminate between metallic Hg and MeHg is by chemical analysis, i.e. analyzing for MeHg. Unfortunately, this is expensive.

[25] Accumulation of 30 mg of MeHg in a 70 kg adult (0.43 µg/g of body) causes sensory disturbance and 100 mg (1.4 µg/g of body) causes all typical poisoning symptoms. Laboratory studies on cats and mice have shown that 30 µg of MeHg per gram of brain is likely the threshold level to manifest neurological symptoms followed by death.

How should hair be sampled?

A: Cut hair stands (from 150 to 250 mg) close to the scalp from near the occipital portion (back part) of the head. Store in paper envelopes or plastic bags with the root ends stapled. Bind the strands together using cotton string (NOT adhesive tape) and store at room temperature (in hot environments is advisable to keep samples refrigerated until they can be transported to the analytical lab).

How should blood be sampled?

A: Acquire 10 mL of blood and place in EDTA-coated vials and store at 4 °C (NOT frozen) in a refrigerator. Sealed vials with blood samples can be stored under these conditions for months without a relevant change in Hg concentration. Other procedures include sampling of 7 mL of blood using Hg-free vacutainers containing sodium (or lithium) heparin as an anticoagulant. Heparinized vacutainers are available commercially from most laboratory suppliers.

What are the normal levels of Hg in blood and hair?

A: In blood, the normal concentration of total Hg ranges from 5 – 10 µg/L or ppb (in individuals with no consumption of fish with high concentrations of MeHg). The normal Hg level in hair is less than 1–2 ppm (µg/g). Hazardous effects to the fetus are likely when 20 ppm is revealed in the hair of pregnant women. Levels of 10 ppm must be considered as the upper limit guideline for pregnant women. Recent evaluation considers 5 ppm Hg in hair a safety guideline for pregnant women. The WHO reports that, based on statistical analyses, child-bearing women with Hg concentrations in hair above 70 µg/g (ppm) exhibit more than a 30% risk of showing a neurological disorder in the offspring. A MeHg level of 200 µg/L in blood, corresponding to Hg concentration around 50 µg/ g (ppm) in hair, is associated with a 5% risk of neurological damage to adults.

What are the guidelines and reference dose of Hg in fish?

A: The guideline[26] level of Hg content in fish varies among countries and is meant to provide guidance to fish consumers for ***edible*** parts of fish. Different guidelines have been adopted by different countries, including Canada and Brazil (0.5 ppm ***total Hg***), Italy (0.7 ppm), Finland, Sweden, and Japan (1 ppm). The WHO (World Health Organization) adopted the safety guideline of 0.5 ppm of ***methylmercury*** for all fish except predatory fish and 1 ppm for predatory fish. The WHO guideline highlights that *"where these guideline levels are exceeded, governments should decide whether and under what circumstances, the food should be distributed within their territory of jurisdiction and what recommendations, if any, should be given as regards restrictions on consumption, especially by vulnerable groups such as pregnant women."*

It is clear that the Hg guideline concentrations used to regulate Hg exposure in humans from fish do not target all kind of individuals. Rather, the actual amount of Hg ingested by individuals is the main concern of health authorities. In 1990, the World Health Organization stressed that a tolerable daily intake (TDI) of 3–7 µg of MeHg/kg body weight would cause adverse effects of the nervous system, manifested as a 5% increase in the incidence of paraesthesia. Hair concentrations would be approximately 50–125 µg/g at this level of intake. The revision of the MeHg Provisional Tolerable Weekly Intake (PTWI) by FAO/WHO JECFA occurred in June 2003. The level of 3.3 µg/kg bw/week was reduced to 1.6 µg/kg bw/week. For a 60-kg individual the guideline of 96 µg MeHg/week is recommended. The TDI level established in Canada in 1998 is 0.47 µg/kg body weight for adults and 0.2 µg/kg bw for women of reproductive age and children. In 1989, the USEPA had a daily methylmercury reference dose of 0.3 µg/kg bw and in 1995 this was revised to 0.1 µg/kg bw.

How are fish sampled for Health Assessment?

A: To quantify Hg exposure to local people and determine the potential for health effects, the following information must be known:

- The daily average quantity of fish consumed (grams), for different meals. Note that quantities may differ depending on the meal (i.e. breakfast, lunch, dinner).
- The frequency (number of meals per day, per week) that fish is consumed.
- The relative proportion of different fish species consumed (i.e., the target species). Note that target species may differ depending on season (i.e. wet versus dry) in many countries.
- Size (length and weight) of the fish consumed.

[26] This level is established for an average ingestion of 400 g fish weekly.

- The tissue Hg concentration (ppm whole muscle in wet weight) of the target species consumed. Note that if more than one species comprises a major part of dietary fish consumption, Hg concentration must be determined for each target species.

Information on quantity and frequency of fish consumption of each target species can best be gained through interviews with the person responsible for preparing most of the meals, typically the women in the household. Alternatively, interviewing fishermen at the river banks or the local fish market will help identify the major species sought after and consumed and provide information on the relative abundance of the different species captured. These individuals will have a good idea of the type of fish most frequently available for sale and the relative amount of each species sold. Note that there may be different target species captured during wet and dry seasons; this information should also be solicited.

Identifying the target fish species is the most important step in establishing the sampling protocol for the human Health Assessment. It is important to know if the "most consumed fish" in a region can be used as a standard species. Note that a strategic sampling procedure for acquiring fish tissue for Hg analysis is described above. This procedure must be followed to select the appropriate species, derive a length – Hg relationship and evaluate data based on a standardized fish size. This relationship will provide the risk assessor with an empirical relationship between fish size and Hg concentration from which to estimate Hg exposure for Health Assessment purposes. Further, this will provide the health researcher with a methodology to follow the evolution of Hg levels in fish over time as well as analyzing human biomonitoring materials (blood and hair).

Why are medical exams important?

A: A Health Assessment is an epidemiological research project and therefore involves evaluation of the physical and mental conditions of individuals and possible influences of external factors that may or may not contribute to the aggravation of their health. Medical exams are usually designed to establish a relationship between biomonitoring materials (analysis of hair, urine and blood) and symptoms of poisoning, which in rough terms, can be described as a dose-response procedure.

A medical exam consists of an initial questionnaire about the health history of the individuals followed by physical and neurological examination. Questions related to health history are needed to exclude participants with severe diseases from the statistical evaluation (e.g. someone who has had a stroke might be excluded from the survey). Individuals are selected for a series of specific neurophysiological tests designed to detect effects of mercury poisoning. These are simple tests and local health care professionals must be trained to perform such series of tests in local health offices.

Why is socio-demographic evaluation important?

A: Before administering a battery of questions and tests, individuals must be carefully selected. This implies in a preliminary knowledge about socio-economic-demographic distribution and conditions of the individuals and their families. In this case, data such as family members, hygiene, diet, education, occupation, income, expenditure, property, access to media, knowledge about mining, access to mercury, etc., should be found. This aims at indicating, based on the routes of Hg exposure, the most susceptible and sensitive group of people in a community to be contaminated as well as about which groups of inhabitants can (or cannot) be selected as controls. This socio-economic-demographic study also provides information about the mining community demographic distribution. This is used in the Health Assessment to select groups of people that statistically represent the community. The study also provides valuable information for any kind of intervention on the site (technical, medical, environmental, economic).

How many individuals should be selected for medical examination?

A: A critical decision in Health Assessment is the number and type of samples (individuals) to be included in the study. As in the environmental assessment, the sampling process can be random or judgmental. Randomization assumes that all individuals in a community have the same chance of being exposed; no pre-conceived idea is imposed. It is definitely a more expensive and time-consuming process but provides a broader picture of the public health than a selective (judgmental) sampling process. Using the judgmental approach, a questionnaire should be previously applied to a large number of people in a community to select just the individuals at higher risk of being exposed to mercury vapour or MeHg by ingestion.

In the Global Mercury Project, the random sampling approach has been used. It is possible to establish the characteristics of a mining community, by using a socio-economic-demographic study based on interviews (questionnaires). The Health Assessment should then follow similar societal distribution. In this case, all groups (young and senior miners, older and younger women, children, etc.) are represented and sampled in a proportion that is representative of a specific community. Minimum of 200 individuals in a mining community are recommended to be sampled and 50 in the control area, i.e. a community with similar cohort of people but not impacted by ASM activities. In order to obtain population distribution (census) figures to support the Health Assessment, a Government body should

be consulted. If not available, teachers, health professionals, local religious or tribal leaders can also be consulted. Midwives, doctors or those who perform religious initiation ceremonies such as baptism, circumcision, etc. are usually very knowledgeable about the approximate population growth and consequently the gender distribution.

What formalities must be observed during the medical examination?

A: Before starting the field assessment, questionnaires must be translated into the regional/national language and the consent from a local or regional Health Authority must be obtained. It is important to have this Ethical Clearance from the Health Authorities and sometimes the questionnaires must be submitted to a review board or ethics committee. They must evaluate the ethical and cultural issues related to the type of questions to be applied. During field work, each participant must be interviewed by national nurses and the nurses need to fill out the questionnaire. The medical experts must examine and test each participant. National doctors/nurses are the most appropriate people to take specimens of biomonitoring material. The volunteers, individuals selected by the socio-economic-demographic survey, must be informed about the entire project by the individuals (experts) interviewing them and informed as to how the data generated by the medical exam can help them and their community. Brochures are very useful to provide this preliminary background to the volunteers. The brochures or pamphlets can also include some basic information about the hazards related to Hg exposure by vapour and a simplified diet advisory. Some formalities must be observed:

- Consent to apply the proposed questionnaire must be obtained from a Health Authority (local or regional) or an ethics committee.
- The volunteers must be informed that all personal information (especially identity) will be kept confidential.
- They must be instructed about the goals of the assessment.
- They must be informed that the Health Assessment will follow ethical procedures recommended by the Code of Conduct of the World Health Organization.
- They will receive all results of the analyses of biomonitoring materials and will be informed about their health situation and any possibility of being intoxicated by mercury (this is a job to be conducted together with local health authorities).
- When high certainty about mercury intoxication exists, they will be informed about available ways to reduce exposure.
- In areas of high incidence of tropical diseases, malaria or tuberculosis incidence, it is suggested to test and recommend therapy for these treatable diseases.
- The volunteers must sign a document in English and local language (example in Appendix) agreeing with the interviews, sample donation, physical exams and neuropsychological testing.

What is a typical medical examination questionnaire?

A: Usually a health questionnaire is divided into the following parts:

1. General questions related to:
   - personal data
   - occupational exposure to mercury (routes of exposure)
   - confounding factors (to exclude candidates with other problems)
   - diet issues (frequency and type of food)
2. Questions related to Health Conditions and Subjective Symptoms (as described by the patient, e.g. metallic taste, salivation, fatigue, etc.).
3. Clinical and Neurological Examination (e.g. blood pressure, signs of gingivitis, ataxia, tremors, reflexes, etc.).
4. Specific Neuropsychological Tests (e.g. memory, coordination, etc.).
5. Sampling biomonitoring materials (urine, blood, hair).
6. Results of chemical analyses of biomonitoring materials.
7. Medical Score (how to combine pieces of information).
8. Decision on who is intoxicated with mercury.

What is checked in a physical and neurological examination?

A: Usually:

- Signs of bluish discoloration of gums.
- Ataxia.
- Tremor.
- Test of alternating movements or test for dysdiadochokinesia.
- Test of the field of vision.
- Reflexes: knee jerk reflex and biceps reflex.

- Pathological reflexes: Babinski reflex and labial reflex.
- Salivation and dysathria.
- Sensory examination.
- Proteinuria.

What is checked in a neuropsychological testing?

A: The following symptoms are usually checked:

- memory disturbances.
- coordination, intentional tremor and concentration.
- tremor and visual-motor capacities.

These tests do not demand special equipment and, associated with analysis of biomonitoring materials, can provide an accurate picture of degree of mercury intoxication.

What are some of the confounding factors during the medical examination?

A: Confounding factors must be investigated to exclude from the statistical analysis other explanations for any symptom found. There are many factors that derive symptoms such as fatigue, dizziness and tremors which introduce false diagnosis to the clinical examination and neuropsychological and tests. Some of the confounding factors are:

- Alcohol consumption.
- Drug use.
- Smoking.
- Malaria and other tropical diseases.
- Tuberculosis.
- Parasitosis.
- Constant handling of gasoline and kerosene.
- Handling of pesticides.
- History of neurological disorders (epilepsy, stroke, Parkinson, etc.).
- History of health problems (kidneys, high blood pressure, lungs, etc.).
- History of stress.
- Allergies.
- Arthritis.
- Diabetes.
- Venereal disease.
- Number of dental amalgam fillings.
- Ingestion of selenium.
- Cumulative effect with exposure to other pollutants (e.g. PCBs).
- Use of soaps and creams that contain Hg for skin lightening.

Are the medical exams enough to indicate Hg intoxication?

A: No, they have to be combined together with analyses of biomaterials. Correlations between Hg poisoning symptoms and Hg in biomonitoring materials have been a classical approach to identify health problems in exposed individuals, but in many cases a clear correlations cannot be established. This may be explained by several factors. However, the main point is that Hg in blood, urine, and hair do not adequately monitor the Hg burden of the target tissues, especially the brain. Scoring or ranking procedures have been used in Health Assessments to combine all pieces of information (symptoms, physical conditions, test results, biomaterial analyses, etc) to derive a conclusion on intoxication level.

What is a sequence of actions to be used in Health Assessments?

A: Priorities of actions must be established. For example, the neuro-psychological tests are conducted by medical experts, which make them costly. Analyses of blood, urine and hair samples are also expensive, and must be prioritized. The following criteria are suggested:

1. Recognize the main Hg exposure pathways to humans (environmental assessment).
2. Obtain the population distribution by applying socio-economic-demographic questionnaire; use similar group distribution in the Health Assessment.
3. Select volunteers for the study following the cohorts obtained in the socio-economic-demographic study.
4. Obtain consent from Health authorities to apply the questionnaire.

5. Inform volunteers about the purpose of the assessment and instruct them.
6. Apply criteria to form groups or clusters (age, gender, education, occupation, fish consumption, proximity of the Hg source, etc.).
7. Apply general (work, diet, health history and possible confounders) questionnaire.
8. Apply specific health questionnaires related to Hg poisoning.
9. Apply physical neurological (medical) exam.
10. Select volunteers with suspicion of MeHg or Hg vapour exposure.
11. Apply neuropsychological tests (memory test, Match Box test, Finger Tapping, Frostig test, Visual Field test, etc.).
12. Collect biomonitoring samples: hair, urine and/or blood.
13. Apply knowledge accumulation process (scoring) using clinical + biomonitoring sample results.
14. Re-examine those individuals with high scoring.
15. Suggest simple and easy to implement remedial measures (technical improvements such as use of retorts, filters, amalgamation of concentrates, safe disposal of amalgamation tailings, removal from source, diet advisory, etc.).
16. Use a control group (non-exposed group, distant from the site) to collect biomonitoring materials and to apply the same clinical examination and neuropsychological tests; try to avoid control groups with history of high ingestion rates of fish.

What immediate advice can be provided to miners and exposed people?

A: It is important to inform the public about the results obtained in the E&HA. "Immediate advice" to local people, especially related to fish consumption, heightens the likelihood of mistakes. Fish consumption advice should be based on reliable facts about routes of exposure. Such advice should also include recommendations on what types of local fish may be consumed because they are not contaminated or contain mercury levels that do not adversely affect human health. Local people should not discouraged from eating fish and they need to know that MeHg is not unique to their fish but rather a phenomenon occurring in fish around the planet. Fish consumption advisories must not be holistically developed only based on mercury concentrations, without considerations of dietary options or health benefits of eating fish.

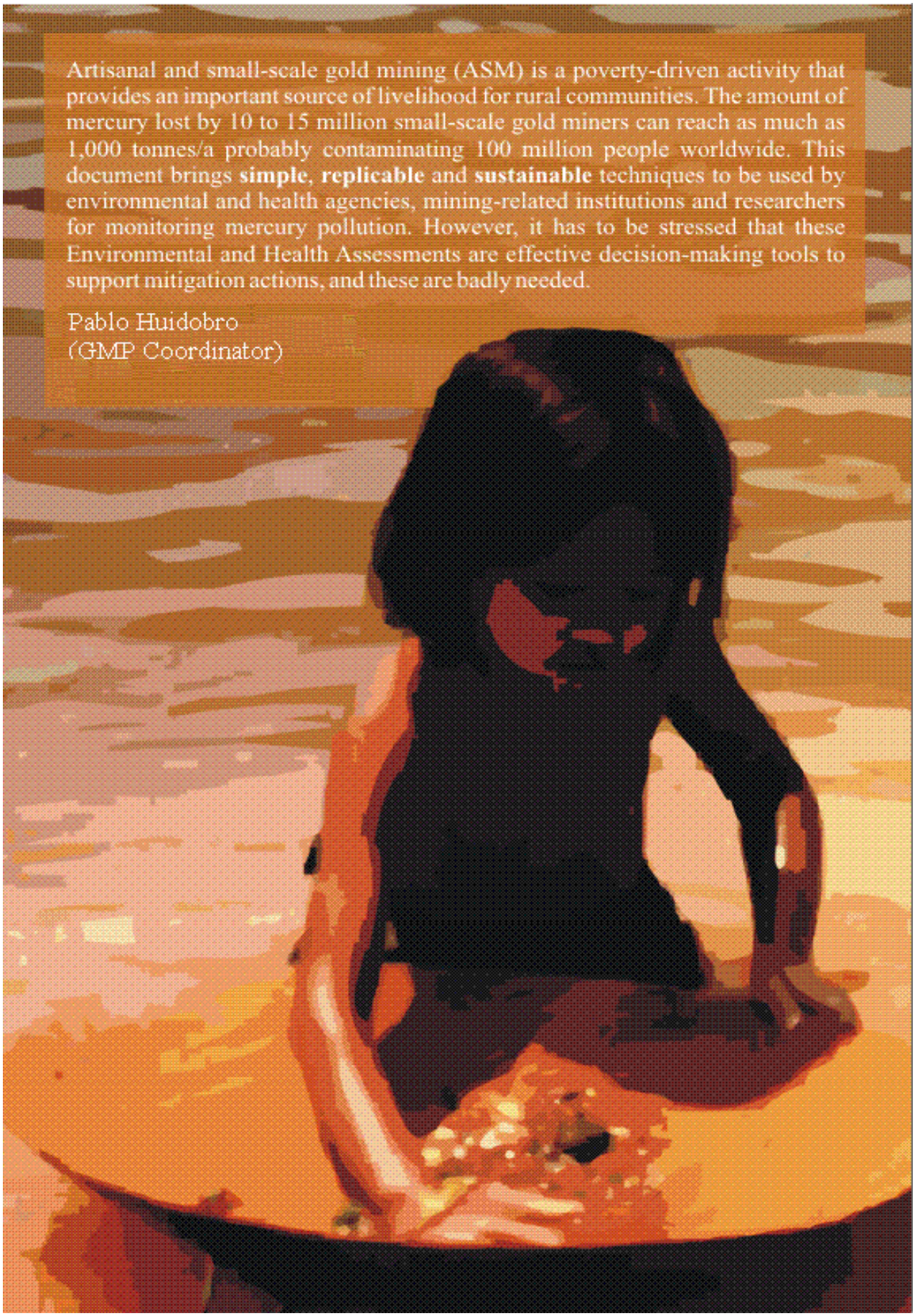
In many regions, especially in the Amazon, there are other sources of mercury that can release even more mercury to the aquatic system than ASM activities (e.g. erosion) Most people tend to blame miners for all mercury pollution of the ecosystem. In many cases, communities living hundreds of kilometers distant from mining activities have pointed to mining as the sole polluting source. When the mining activity diminishes, they believe that fish will be adequate for consumption again. It is important to show that there are other sources of mercury and likely carnivorous fish will not be clean of mercury because mining stopped.

Health effects of Hg vapour must be brought to the public attention to show the benefits of using retorts. It is important that any initiative related to public awareness or distribution of retorts should be accompanied by a strong educational campaign. Information packages must consider issues such as illiteracy rates, which can be high in many regions, and other social and cultural aspects.

In Environmental and Health Assessments, researchers must be careful of creating false expectations related to solutions regarding mercury contamination among the local stakeholders. Environmental assessment work is merely an initial step in addressing the issue by identifying problems and introducing solutions. This is frequently not understood by local stakeholders and government regulators who want to see procedures implemented and problems solved as soon as possible.

In terms of technical solutions, when a situation where Hg vapour exposure is identified, such as when miners are burning amalgam in open pans, there are a number of quick and simple solutions that can be immediately implemented to reduce mercury exposure. These include the use of home-made retorts to recover Hg, reducing the amount of mercury used, removing women and children from the amalgamation area, and advising against burning amalgam in closed areas such as kitchens. These simple measures can easily be brought to the attention of miners and other individuals exposed to Hg and significantly reduce Hg exposure to these people.

It is controversial whether therapies should be discussed with Hg intoxicated people during a monitoring campaign. For ethical reasons, UNIDO has adopted the approach to simply inform the local and regional health care authorities when a mercury intoxication problem is detected and not to undertake active intervention. As UNIDO's mandate is to provide assistance to eradicate pollution sources, the organization understands that the health conditions of affected communities must be considered. However, medical intervention can only be responsibly applied by competent organizations and qualified physicians.



Artisanal and small-scale gold mining (ASM) is a poverty-driven activity that provides an important source of livelihood for rural communities. The amount of mercury lost by 10 to 15 million small-scale gold miners can reach as much as 1,000 tonnes/a probably contaminating 100 million people worldwide. This document brings **simple**, **replicable** and **sustainable** techniques to be used by environmental and health agencies, mining-related institutions and researchers for monitoring mercury pollution. However, it has to be stressed that these Environmental and Health Assessments are effective decision-making tools to support mitigation actions, and these are badly needed.

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