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

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Removal of Barriers to Introduction of Cleaner Artisanal Gold Mining and Extraction Technologies

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Summary

Environmental Assessment (EA) is an effective decision-making tool to support mitigation actions. Depending on the stage and degree of the action, the EA can address different topics, but in general terms it must be designed to identify and predict adverse effects related to anthropogenic activities. A definition of EA is provided by Health Canada (1999):

*EA is a comprehensive and systematic process, designed to identify, analyze and evaluate the environmental effects of a project in a public and participatory manner; environmental assessment involves the use of technical experts, research and analysis, issue identification, specification of information requirements, data gathering and interpretation, impact prediction, development of mitigative proposals, design of any required follow-up monitoring, external consultations, and report preparation and review.*

The methodologies used in EA for mining projects involving heavy metals are not ideally suited to assess the effects caused by mercury (Hg) released by artisanal and small-scale gold miners (ASM) in developing countries. Therefore, conducting environmental assessments of ASM activities must be innovative and adapted to particular situations in the different countries in which the activities are taking place. This is especially true when working with artisanal miners in impoverished and developing countries because of the many challenges posed by working in remote and often difficult environments with little infrastructure and logistic support.

Metallic mercury, which is the main form of Hg released by ASM, is capricious and difficult to work with. It is liquid and volatile and there are other natural and anthropogenic sources of mercury emissions that bring confounding elements to data interpretation. Perhaps more importantly, the transformation of metallic mercury into its most toxic form, methylmercury (MeHg), is not clearly understood and there are no general rules governing the transformation of inorganic Hg into MeHg. 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 clearly defined 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 mercury 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:

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

The monitoring component of the Global Mercury Project (GMP) has specific goals described in the 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.”

It is important to establish protocols for monitoring steps in order to transfer sound techniques for sampling, sample preservation, transportation, analysis, etc. that are simple, reproducible and sustainable to environmental agencies, researchers, and artisanal gold miners. Local institutions must use the suggested methodologies in the future to assess the evolution of the mercury pollution at each mining site.

The monitoring work must be designed to establish procedures to observe how mercury levels in different environmental and biological samples change with time. Very few methodologies have attempted to do this. As bioavailability and bioaccumulation of mercury are the key components to be investigated, it is important to standardize procedures that can be used to assess the evolution of the mercury pollution in a mining area. This document highlights the relevance of sampling aquatic biota and diminishes the importance of sampling water due to the low Hg levels in solution. Biota are the ultimate indicators providing direct evidence that mercury in soil, sediments, water, or air has become bioavailable and is being bioaccumulated by the organism. 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 is more appropriate than other aggressive remediation measures. This, of course, is based on the acceptability to regulators.

An important objective of the monitoring steps of the GMP is the identification of mining hotspots, which are sites with high concentration of metallic mercury dumped by artisanal miners, usually into or near water streams. These are the sites with potential to be transformed into environmental hotspot, i.e. sites where metallic mercury can be transformed into methylmercury. Hotspots can have dimensions of few square meters to hundreds of square meters and are the main sources of Hg dispersion for the aquatic system impacting the lives of thousands of people both involved and uninvolved with the mining activities. Location and assessment of the risk posed by those hotspots must be a main objective of an E&HA.
These Protocols, designed for the GMP, do not provide many details of the sampling procedures as it is clear that this is a site-specific issue which must take into consideration the characteristics of the mining activity, the biodiversity of the region, accessibility, availability of resources, risks, logistics, etc. However, the scientific rationale behind the decisions on what must be preferentially sampled and the basic methodologies are presented herein. The researcher conducting E&HA must have a great deal of flexibility to adapt these concepts to the field conditions, and in many cases, to the available budget.

In Environmental and Health Assessments, researchers must be careful not to create false expectations among local stakeholders related to solutions regarding mercury contamination. 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 nor 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 vapor 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 homemade retorts to recover Hg, reducing the amount of mercury used, removing women and children from the amalgamation area, and strongly advising against burning of 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 the community.

To limit exposure of MeHg to individuals or families that consume large amounts of carnivorous fish, they should be encouraged to diversify their diet and consume fish with lower MeHg concentrations or dilute their meals with vegetables, when these are available.

Whether or not therapies should be discussed with Hg intoxicated people during a monitoring campaign is very controversial. For ethical reasons, 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.

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 learned through practical experience and from trial and error while conducting projects for a number of international institutions which are not usually reported in scientific journals. This information is offered to facilitate the implementation of Environmental and Health Assessment Protocols and to assist researchers operating in difficult field situations.
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PART 1 – Environmental Assessment

Small-scale or artisanal gold mining is an essential activity in many developing countries as it provides an important source of livelihood, particularly in rural regions where economic alternatives are critically limited. Artisanal mining encompasses small, medium, informal, legal, and illegal miners who use rudimentary processes to extract more than 30 different mineral substances worldwide. Artisanal mining activities are not necessarily limited to small-scale mining activities. 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 ASM site in the Brazilian Amazon where, during the 1980’s, more than 80,000 miners gathered to manually extract about 90 tonnes of gold from the same open pit. The International Labour Organization (ILO, 1999) estimates that the number of artisanal miners is currently around 13 million in 55 countries and rising, which suggests that 80 to 100 million people worldwide depend on this activity for their livelihood. Gold is easy to transport across borders and easily sold, and is by far the main metal being extracted. Worldwide it is estimated that more than 2.5 million women and 250,000 children are directly employed in artisanal mining (Hinton et al, 2003).

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

1. Occupational exposure to mercury vapor from amalgam burning or gold melting, and
2. Methylmercury from dietary sources, especially fish.

Inhalation of mercury vapor is the primary exposure pathway of miners, gold shop workers, and people living near areas where mercury is handled. High methylmercury concentrations in fish in waterways contaminated by mercury released from mining sites is the main means of exposure to local residents in rural communities. Because they are plentiful and inexpensive, fish are the main protein source for community residents. However, fish consumption can also result in the intake of greater amounts of methylmercury than health authorities advise.

Before conducting Environmental and Health Assessments in gold mining regions it is essential to understand the methods employed by miners to process gold ores and how mercury is being used. Mercury emissions depend fundamentally on the mining and processing methods. It is difficult to obtain reliable quantitative data about mercury emissions from active ASM, as miners do not provide information about the amount of mercury being used and their gold production is very unstable. 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 emissions and uncertainties associated with sampling processes prevents accurate determinations about of the amount of mercury emitted.
1.1. Amalgamation and Mercury Emissions

Mercury, because of its normal liquid state, has a unique capacity to form an amalgam with most metals except iron and platinum. Amalgam is a series of solid intermetallic compounds. Gold, in particular, can combine with mercury forming a wide range of compounds from $\text{AuHg}_2$ to $\text{Au}_8\text{Hg}$. The three main amalgams are: $\text{AuHg}_2$, $\text{Au}_2\text{Hg}$, and $\text{Au}_3\text{Hg}$. 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 known for more than 4000 years, the gold amalgamation process only became popular during the XIV century in Central Europe and was brought to Americas in the XVI century by Portuguese and Spanish miners. As recently as 1889, the gold amalgamation started to decline as an industrial process, to be replaced with a cyanidation process (Ciminelli and Gomes, 2002). Today, nearly all mining companies have halted the practice of amalgamation. Unfortunately, this is still the main process used by ASM and the probability of having an alternative, environmentally sound process that remains simple and popular is not encouraging (Veiga, 1997).

Although the use of mercury in mineral processing is illegal in most countries, mercury amalgamation is the preferred method employed by ASM. When used correctly, mercury is an effective, simple and very inexpensive reagent to extract gold (e.g. 1kg of Hg costs 1g of Au). A variety of mining and amalgamation methods are used in artisanal mining operations and they must be primarily surveyed to establish a reliable environmental assessment.

The extent of mercury losses from a specific site is defined by Au-Hg separation procedures; mercury is often discharged with contaminated tailings and/or volatilized into the atmosphere. Typical amalgamation methods used by ASM are listed below (Veiga et al., 1995):

- Whole ore is amalgamated: mercury is mixed with the whole ore in pump boxes or introduced in sluices during gravity concentration or amalgamated when copper plates are used.

- Only gravity concentrates are amalgamated: mercury is mixed with concentrates in blenders or barrels and separation of amalgam from heavy minerals is accomplished by panning in waterboxes, in pools or at creek margins.

A common practice in many countries is to amalgamate the whole ore, by either spreading mercury on the riffled concentration boxes or by using the old copper plate amalgamation method. When mercury loses its coalescence it “flours” i.e. forms a large number of small droplets that are carried with tailings. Mercury contaminated tailings from sluices are usually discharged directly into the river. In a few places where hydraulic monitors are used, miners spread large amounts of mercury on the ground with the belief that the “quicksilver” will “magically” move on the dirt to collect all available gold. Amalgamation actually occurs later, namely when the riffled sluices retain mercury 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 mercury to 1 part of gold produced and the chance to trap gold is remote. Although material derived from this processes is suitable for other mercury-free concentration methods, mercury is still frequently added during crushing and grinding,
gravity concentration or afterwards. In China, for instance, amalgamation using miller mills\textsuperscript{1} leads to losses of 40 parts of mercury for one part of gold produced (Gunson \textit{et al.}, 2001). In Peru, the use of the “quimbaliti” crusher/grinder, a round piece of rock revolved by the miner’s feet, is a clever technique to crush and grind the ore in order to liberate gold (Hrushka and Medina, 2001). Peruvian miners mix mercury with ore during the crushing step leading to very high mercury losses. Some bad practices have been abandoned or are used less frequently, such as the use of “Jack pots”, large mercury 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 emissions. Using this method approximately 14 grams of mercury is required to amalgamate 1 kg of concentrate (ratio Hg:concentrate $\approx 1:70$). Amalgamation is efficient for particles coarser than 200 mesh (0.074 mm) and for liberated or partially liberated gold (Wenqian & 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” (loss of mercury through formation of “inactive” droplets), as is the case in when amalgamation is performed in ball or rod mills. Some possible solutions are the use of shorter amalgamation periods, the use of large rubber balls to promote contact between mercury and gold particles, and application of oxidizing (e.g. KMnO$_4$) or complexing (chlorides) reagents to clean gold surfaces and avoid mercury flouring. Although chemical reagents can improve gold and silver recovery, they may promote metallic mercury dissolution and loss. Adding one gram of sodium hydroxide (NaOH) to every kilogram of concentrate to be amalgamated can easily 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 mercury to 100 parts of concentrate (Veiga, 1997).

When gravity concentrates are amalgamated, the mineral portion is separated from the amalgam by panning either in water boxes or in pools excavated in the ground or at creek margins. The heavy, mineral-rich amalgamation tailings frequently contain 200 to 500 ppm of residual mercury, which creates \textit{hotspots} when dumped into adjacent water bodies (Veiga, 1997).

In dredging operations in the Madeira River, Amazon region, Brazil, amalgamation is done on board using a blender and amalgamation tailings are steadily dumped into the rivers (Pfeiffer and Lacerda, 1988). In the Caroni River, Venezuela, miners used copper plates on board to amalgamate the entire dredged ore. Tonnes of mercury were dragged with the tailings to the river sediments (Veiga, 1996). In the Kahaya River in Central Kalimatan, Indonesia, more than 3000 dredges are currently dredging sediments over 200 km of river. The ore is concentrated 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 are widely dispersed, making them extremely abundant and very difficult to identify.

\textsuperscript{1}A heavy steel wheel that promotes the contact between mercury and gold particles while crushing the ore.
The most common method applied to remove excess mercury from amalgams is by manually squeezing it through a piece of fabric. The remaining amalgam usually consists of about 60% gold. When the amalgam is centrifuged, the mercury content in the amalgam drops to 20-30%. Only a few miners in Venezuela are using this centrifuging method (Veiga, 1996). Once the amalgam is obtained it is retorted or simply burnt openly in pans. Retorts can be used to capture volatilized mercury and condense it, allowing the mercury to be recycled. Condensed mercury contains residual gold (0.1%) in solution, which is claimed to improve amalgamation (Taggart, 1947, Sevryukov et al, 1950). This leads to Hg recovery above 95% and significant reductions in air pollution and occupational exposure. There are many types of retorts. Some are made with stainless steel while others use inexpensive cast iron. Mercury losses during retorting depend on the type of connections or clamps used. Unfortunately, the usual practice to separate Hg from gold is to burn the amalgam in a pan or shovel with a blowtorch that results in very high losses of Hg directly to the atmosphere. This oxidized Hg is easily transported and condensed elsewhere, and can be transformed into methylmercury in aquatic environments.

Metallic mercury is not strongly absorbed by the skin. A few cases of hypersensitivity or allergic dermatitis among dentists have been reported (WHO, 1991) but the main pathway of contamination by miners is inhalation of vapors. Natural mercury levels in air in rural areas usually range 0.001 to 0.004 µg/m³ and in urban areas from 0.01 to 0.17 µg/m³. Typically mercury is found in air as elemental mercury but 1 to 25% of Hg (II) can also be present depending on the type of emission source (US EPA, 1993). The limit for public exposure is 1.0 µg/m³ (Malm et al, 1990) and the recommended health-based exposure limit for metallic mercury is 25 µg/m³ for long-term exposure and 500 µg/m³ for short-term exposure (WHO, 1991). Table 1 compares the levels of Hg analyzed in air in different working places.

<table>
<thead>
<tr>
<th>Hg (µg/m³)</th>
<th>Workplace</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60,000</td>
<td>amalgam burning in a mine site</td>
<td>Malm, 1991</td>
</tr>
<tr>
<td>12,000</td>
<td>Dentist office (amalgam restorations)</td>
<td>Stopford, 1979</td>
</tr>
<tr>
<td>6,000</td>
<td>underground cinnabar mining</td>
<td>Stopford, 1979</td>
</tr>
<tr>
<td>3,000</td>
<td>police office - finger printing powder</td>
<td>Stopford, 1979</td>
</tr>
<tr>
<td>1,000</td>
<td>filling operation of fluorescent lamps</td>
<td>Stopford, 1979</td>
</tr>
<tr>
<td>300</td>
<td>gold dealer shop in Rondonia, Brazil</td>
<td>Malm et al, 1990</td>
</tr>
<tr>
<td>100</td>
<td>chloroalkali plant &amp; thermometer factory</td>
<td>Stopford, 1979</td>
</tr>
<tr>
<td>30</td>
<td>lighthouse in British Columbia</td>
<td>van Netten and Teschke, 1988</td>
</tr>
</tbody>
</table>

Malm (1991) measured up to 60,000 µg/m³ of Hg in air when amalgam was burnt in pans. When retorts are used, this concentration drops to as low as 10 µg Hg/m³. This is still high, but is lower than the 50 µg Hg/m³ limit for industrial exposure - TWA² (BC-MEMPR, 1992). It is obvious that miners are exposed to unacceptably high levels of

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.
mercury when they burn amalgam in an open pan or shovel. The WHO (1991) posed that an individual exposed to Hg levels in air above 80 µg/m³ has a high probability to develop symptoms of mercury vapor intoxication (tremor, erethism).

An example of an effective and creative amalgam process has been applied in Venezuela, where Amalgamation Centers were constructed to increase gold recovery and reduce mercury emissions. 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. Schulz-Garban (1995) found very high levels of Hg in air inside Amalgamation Centers in Venezuela. Under pressure from miners to rush the amalgamation process, employees of Amalgamation Centers have opened retorts while they were still hot. Wearing inappropriate dust masks, they were exposed to residual mercury vapor in the warm retorts. At that moment, Hg in ambient air was as high as 250,000 µg/m³, but reaching background in 20 to 40 seconds. Dust masks can retain a small part of mercury vapor, however contaminated masks must be discarded after use to avoid inhalation of Hg condensed on the mask. Appropriate respirators for mercury vapor must be used. The accumulation of Hg on mask cartridges is fast 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).

Mercury recovered by retorting often does not have the same amalgamating properties as new mercury. A thin layer of oxidation is formed, probably by absorbed oxygen on the mercury drop surface. In this condition, mercury forms thousands of droplets and loses its amalgamation capacity (i.e. flouring). Facing this problem, many miners simply discharge the retorted mercury. The most efficient way to reactivitate the surface of mercury is by using an ultrasonic bath, such those used by dentists, causing mercury droplets to coalesce in seconds (~US$200-400) (Hinton et al, 2003). A much less expensive method involves electrolytic activation using table salt and a simple flashlight battery to dissolve mercury oxides from the mercury 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) should accompany all activation methods. Despite the small amount of effluent, some soluble mercury could be transformed into methylmercury after being discharged to the aquatic environment.

In some countries, miners recover mercury 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 has some mercury and must be treated before disposal. Unfortunately this never happens. In addition mercuric nitrate fumes are highly toxic. Human beings have a tolerance of only 0.05 mg per cubic meter of air for the prevailing compound in the process, mercury pernitrate - Hg(NO₂)₂·H₂O. A very serious risk is also present 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 the aquatic systems. This form of mercury is readily available to be biotically or abiotically methylated (Veiga, 1997).
When the amalgam is retorted, a gold *doré* is obtained. This is sold in villages to gold shops that melt the gold to rid the *doré* of some impurities, before paying the miners. In fact, the *doré* still contains about 20 g of mercury per kg of gold, which is later released when gold is melted (CETEM, 1989). This operation is usually carried out by gold buyers under the miner’s supervision. Mercury levels in the interior of these shops are extremely elevated. Malm (1991) measured a mean concentration of 83 µg Hg/m³ over 2 hours when gold was not being melted. Fume hoods used for this are usually very rudimentary, consisting only of a fan, which blows the mercury vapors out into the urban atmosphere. Exposure of innocent people living near gold shops to mercury vapor 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.

In Indonesian goldfields on the Kahayan River, Central Kalimatan, where about 3000 dredges are operating, amalgam is burned in open pans at approximately 100 sites along the river, including in a floating restaurant. About 20 to 30 g of gold is produced per week by 3-5 miners working on each raft. In another mine site, Kereng Pangi, also in Central Kalimatan, a local gold dealer buys about 3 kg of gold daily from about 10,000 ASM extracting gold from dry alluvial ore. Melting the gold in the gold shop, mercury contaminated vapors drift straight by a nearby elementary school. Assuming that the gold bullion contains 5% residual mercury, approximately 55 kg of Hg is released annually by this one facility. This is the same amount of mercury released annually by waste treatment plants all over Austria (Beinhoff, 2003).

<table>
<thead>
<tr>
<th>Amalgamation Method</th>
<th>Hg lost : Au produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole ore</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Concentrates, no retort</td>
<td>1</td>
</tr>
<tr>
<td>Concentrates, with retort</td>
<td>0.001</td>
</tr>
</tbody>
</table>

In general, the amalgamation method defines the amount of mercury lost. When gravity concentrates are amalgamated properly and retorts are used, mercury emissions are very low (Table 2).

The ratio of $\frac{Hg_{lost}}{Au_{produced}}$ has been used as a parameter to quantify mercury emissions. One of the common and confusing points in reporting this ratio is that some authors report just $\frac{Hg_{used}}{Au_{produced}}$ ratio, which does not necessarily reflect the amount of Hg lost. In many cases the amount of Hg recycled is not reported. Maponga and Ngorima (2003) estimated that ASM in Zimbabwe used 6 tonnes of Hg in which about 3 tonnes was lost to the environment. The gold production from ASM in Zimbabwe is not known but it is estimated around 10 tonnes (UNIDO, 2002). Babut *et al* (2003) reported that the official mineral agency in Ghana has estimated that Hg:Au ratio is around 4. This seems a very high ratio to represent the proportion of mercury lost when only gravity concentrates are amalgamated. This ratio suggests that the miners are not recycling mercury in Ghana. Maurice-Bourgoin *et al* (2000), assuming a Hg:Au of 5, estimated that 330 tonnes of mercury have been released into the Bolivian environment by ASM. Farid *et al.* (1991) quantified the use of mercury in eight artisanal gold proceeding plants in Poconé, South of
the Amazon basin. Miners used either manual amalgamation or barrels to amalgamate gravity concentrates obtained from centrifuges, adding 1 kg of Hg per 60 to 100 kg of concentrates. Figure 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 mercury 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. It is clear that the main loss of Hg from the process occurs when excess mercury is squeezed off through a cloth. This mercury is recycled. The more gold produced, the less excess Hg is recovered. The ratio of $\text{Hg}_{\text{lost}}:\text{Au}_{\text{produced}}$ varied from one operation to another and, when very little gold is produced, the ratio provided a false idea that a high amount of mercury was lost.

It is very difficult to establish the gold production from artisanal miners and thus the amount of Hg emitted. Based on the official gold production and a ratio of $\text{Hg}_{\text{lost}}:\text{Au}_{\text{produced}} = 1$, 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 mercury emission from mining activities in Brazil alone was around 78 tonnes. Using published estimates, Lacerda (1997) estimated that the total amount of mercury emitted by ASM worldwide was around 460 tonnes in 1996. MMSD (2002) estimates that 20% of world gold production comes from ASM. In a UNEP report, there is a note that Gold Fields Mineral Service Ltd. estimated that between 500 and 800 tonnes of gold were annually produced by ASM. If an equal amount of mercury is being emitted when gold is produced, then at least 500 tonnes and possibly as much as 1000 tonnes of mercury is lost to the environment annually, representing almost 30% of all anthropogenic sources of global mercury emissions (UNEP, 2002).

![Fig. 1. Balance of mercury in the amalgamation steps](adapted from Farid et al, 1991)
When gathering information about ASM operations, it is very important to understand the labor relationship and the financial system. In order to introduce changes in the mining and processing procedures, the way in which miners think must also be understood. In this case, the economic aspects such as capital and operating costs must be evaluated. In many parts of the world the figure of the operation “owner” is well-known. A specific mining activity needs investment on sluice boxes, pumps, camp, transport, etc. After deduction of the 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. 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 costs just US$ 240, which represents less than 1% of the miner’s operating cost. Diesel for the pumps represents more than 90%. It is clear that mercury is not the main economic concern of miners and creative approaches must be developed to convince miners to introduce cleaner procedures to reduce Hg losses, environmental contamination, and impacts on human and ecological health.

Due to the scarcity of easily exploitable ores, low gold prices, and high operating costs, artisanal activities in many remote areas, in particular the Amazon, have declined since the early 1990s. However, the gradual increase of gold price in 2003 saw an immediate response from the ASM sector. Ore deposits abandoned by ASM or hitherto considered uneconomic are being re-mined. 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 and this signifies the beginning of the end of the artisanal operation. In Africa, miners excavate deep wells (20-30 m) to reach the ore body, which is usually a highly weathered material or a saprolite that is still easy to be ground using manual means. In places such as Brazil, Peru, China, and Venezuela, where sulfide-rich quartz veins are mined, the primitive underground work by ASM represents the main cause of fatal accidents in the mining sector. Primary ores must be ground to promote gold particle liberation from gangue minerals. Free or partially free gold particles can be concentrated or amalgamated. This is a fundamental principle of mineral processing, but unfortunately ASM do not know how fine they should grind. By examining tailings from ASM operations in quartz veins in Poconé, Brazil, it was possible to notice a great portion of gold still locked to quartz particles in the coarse fractions and another portion lost in the very fine fractions. As a result, rudimentary gravity concentration methods are not able to recover fine gold particles (CETEM, 1989). In the Philippines, it is also known that up to 30 ppm of gold is left in the tailings by artisanal miners (Murao et al., 2002).

Crushing and grinding (comminution processes), while relatively simple mechanical processes, are 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 lowers as a result of heterogeneity of the ore body, difficulty on the excavation and
hauling processes, and lack of gold liberation due poor comminution processes. When this occurs, miners use greater quantities of mercury. This does not work as gold is locked either in silicates or, in more difficult situations, in sulfides. Facing extraction problems, ASM seek technical assistance. Normally this support is not available. Engineering companies usually refuse to help artisanal miners and hiring consultants is too costly. Local governments are not prepared to provide specialized personnel or appropriate technology, and research institutions primarily offer 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).

More ASM are employing cyanidation for primary ores, as amalgamation usually does not extract the expected amount of gold. Amalgamation followed by cyanidation of the tailings creates an additional problem. Cyanidation in aerated vats promotes formation of mercuric compounds, which are more available to methylating organisms than metallic mercury. In high-grade (10 to 40 g/t Au) primary gold ore operations in China, miners add high amounts of mercury into the grinding circuit and the amalgamation tailings. After cyanidation, the tailings have 100 ppm Hg. Part of the residual mercury in the final tailings is mercury cyanide and part is non-reacted metallic mercury. All this material, together with cyanide solutions after gold precipitation with zinc, is discarded into the drainages (Gunson and Veiga, 2003).

---

Fig. 2. Main steps in bold amalgamation by ASM (Veiga and Hinton, 2002).
In summary, mercury emitted 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. 2). When no retort is used as much as 80% of the mercury initially introduced during amalgamation is lost to the atmosphere (CETEM, 1989). Additionally, the amount of mercury dumped with tailings is more significant when the whole ore is amalgamated. Amalgamation of the whole ore represents the main source of mercury 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 mercury emissions in ASM operations.

**Sampling ASM Operations**

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

It is important to carefully use the $\text{Hg}_{\text{lost}} : \text{Au}_{\text{produced}}$ ratio as an approximate and regional estimate of mercury emission from various operations in an ASM region. In order to obtain reliable figures about the amount of mercury lost and gold produced, a trustworthy relationship with miners is absolutely necessary to allow the researcher to have access to 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 mercury entering and leaving each unit operation must be carefully obtained weighing and analyzing mercury in 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 hints are suggested to obtain the $\text{Hg}_{\text{lost}} : \text{Au}_{\text{produced}}$ ratio:

- Interview operation **owners**, who are in charge of supplying mercury 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 mercury 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 day or 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 mercury being introduced and recovered.
- Sample amalgamation tailing and analyze Hg; knowing the weight of amalgamation tailings being produced per day 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.
- 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 in a mining region.

Once the source of mercury emission is characterized and the amount of mercury lost is quantified based on historical gold production data, the focus of the field campaign should be on:

• Identifying “hotspots”.
• Establishing the mercury pollution level of a region impacted by artisanal gold mining.
• Assessing risk of workers and the surrounding population to mercury exposure.
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, 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. 3). A risk assessment can predict if mercury in any geochemical compartment is or can become bioavailable. In this case, whatever the 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 bioaccumulated by the organism. 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 is 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.

![Risk Assessment Diagram](image)

**Fig. 3. Risk Assessment**

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

There are many methodologies for sampling geochemical and biological materials for Environmental and Health Assessment and, depending on the purpose of the monitoring, one procedure can be more practical than another. Table 3 outlines the main purposes of Environmental and Health Assessments in ASM areas and the relevance of those objectives for the UNIDO/GEF/UNDP Global Mercury Project (GMP).
### Table 3. Purpose of sampling geochemical and biological material in ASM areas

<table>
<thead>
<tr>
<th>Subject</th>
<th>Purpose</th>
<th>Material to be sampled</th>
<th>Technique</th>
<th>Relevance for the GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate Hg emissions from ASM</td>
<td>estimate the relevance of Hg emissions from ASM in a region.</td>
<td>Hg introduced and Hg recovered; Hg lost in amalgam, tailings</td>
<td>estimate $H_{\text{Hg introduced}}/H_{\text{Hg produced}}$ ratio through Hg balance in ASM operations.</td>
<td>high: location and management of hotspots are crucial</td>
</tr>
<tr>
<td>Identification of mining and environmental hotspots</td>
<td>apply remedial procedures depending on bioavailability</td>
<td>superficial soil and bottom sediment</td>
<td>total Hg analysis (semi or quantitative) or panning</td>
<td>high: important for remedial measures</td>
</tr>
<tr>
<td>Hg methylation potential</td>
<td>knowledge about how kinetics of MeHg generation</td>
<td>soil or bottom sediment</td>
<td>radiometric methylation rate or microbiological studies</td>
<td>low: complex and expensive</td>
</tr>
<tr>
<td>Hg bearing minerals</td>
<td>stability of Hg in sediments; bioavailability</td>
<td>soil or bottom sediment</td>
<td>sequential or selective extraction or mineralogical studies (SEM/XRD)</td>
<td>low/medium: can explain why Hg is or not bioavailable</td>
</tr>
<tr>
<td>Hg leaching potential</td>
<td>how easy Hg can be leached out from soil/sediment components</td>
<td>soil or bottom sediment</td>
<td>leaching tests; humidity cells</td>
<td>low/medium: indirect information about bioavailability</td>
</tr>
<tr>
<td>Hg size distribution</td>
<td>fines with Hg can be easily transported</td>
<td>soil or sediment</td>
<td>screening and centrifuging for $\leq 37\mu m$ fractions</td>
<td>high: predict transportation of Hg associated to fines</td>
</tr>
<tr>
<td>Atmospheric Hg deposition over time</td>
<td>Hg accumulation on soils and sediments</td>
<td>lake core sediments</td>
<td>analysis of core slices and dating</td>
<td>low: indirect information about Hg origin; lots of confounding factors</td>
</tr>
<tr>
<td>Atmospheric Hg dispersion in a region</td>
<td>extent of contamination</td>
<td>superficial soil analysis</td>
<td>total Hg analysis (semi or quantitative)</td>
<td>low/medium: hard to provide reliable quantitative data</td>
</tr>
<tr>
<td>Atmospheric Hg in workplace or public environment</td>
<td>public or workers exposure to Hg vapor; extent of contamination</td>
<td>ambient air, ash, soot, house dust and tar in smelting huts</td>
<td>air analysis over 8 hours or with sniffers; Hg analysis of dust using PIXE or other method.</td>
<td>medium: dust and soot provides Hg exposure over time</td>
</tr>
<tr>
<td>Hg mobility in aquatic system</td>
<td>extent of contamination</td>
<td>particulate matter</td>
<td>collect water and filter $\leq 0.45\mu m$</td>
<td>high: main Hg mobility mechanism in drainages and water courses.</td>
</tr>
<tr>
<td>Drinking water</td>
<td>to meet guidelines; health issues</td>
<td>filtered and unfiltered water</td>
<td>total Hg analysis</td>
<td>low: rarely Hg is detected in solution. Particulate has more Hg than water</td>
</tr>
<tr>
<td>Protection of aquatic life</td>
<td>to meet guidelines</td>
<td>filtered and unfiltered water</td>
<td>total Hg analysis</td>
<td>low: rarely Hg is detected in solution. Particulate has more Hg than water</td>
</tr>
<tr>
<td>Taxonomic Richness &amp; Abundance</td>
<td>check toxic effect on type and number of organisms</td>
<td>invertebrates (or other aquatic organisms)</td>
<td>number and type of organisms</td>
<td>low: laborious and complex in tropical countries</td>
</tr>
<tr>
<td>Hg in aquatic biota and/or toxicity</td>
<td>evidence of bioavailability</td>
<td>fish (preferentially carnivorous)</td>
<td>total Hg and/or MeHg; bioassays standardized fish can be analyzed over time</td>
<td>high: bioavailability control</td>
</tr>
<tr>
<td>Hg in invertebrates and/or toxicity</td>
<td>evidence of bioavailability</td>
<td>Invertebrates</td>
<td>total Hg and/or MeHg; bioassays</td>
<td>high: simple method to compare bioavailability from site to site</td>
</tr>
<tr>
<td>Hg in edible biota</td>
<td>health issues</td>
<td>edible biota (fish, etc.)</td>
<td>total Hg and/or MeHg</td>
<td>high: part of Health Assessment</td>
</tr>
<tr>
<td>Hg in edible vegetables and fruits</td>
<td>health issues</td>
<td>edible vegetables and fruits</td>
<td>total Hg and/or MeHg</td>
<td>low: usually low MeHg</td>
</tr>
<tr>
<td>Hg in hair</td>
<td>assess MeHg bioavailability; also health issue</td>
<td>human hair</td>
<td>total Hg or Hg in creatinine</td>
<td>high: to determine MeHg exposure through fish ingestion</td>
</tr>
<tr>
<td>Hg in urine</td>
<td>establish undue short-term exposure to Hg vapors</td>
<td>human first urine</td>
<td>total Hg or Hg in creatinine</td>
<td>high: to determine undue Hg vapor exposure</td>
</tr>
<tr>
<td>Hg in blood</td>
<td>establish long term exposure to vapor or MeHg intake</td>
<td>human blood</td>
<td>total Hg</td>
<td>medium/high: accumulates data on MeHg plus Hg vapor exposure</td>
</tr>
</tbody>
</table>
1.3. Soils and Sediments

Depending on the mercury 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 mercury used by miners has been conducted, geochemical and biological should be sampled either to verify the regional extent of the contamination or to locate hotspots.

Not all sites with high mercury concentrations necessarily have high methylation potential, but these sites are of high risk. Metallic mercury must first be oxidized and form soluble mercuric complexes to be available to methylating bacteria. 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 Hg for gold extraction. An environmental hotspot may be situated away from mining hotspots, in several locations, and is characterized by high organic (e.g. methylmercury) concentrations in sediments and/or aquatic biota in areas where inorganic mercury has been methylated and is bioavailable.

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 3, the use of soils and sediments in the environmental assessment may have distinct objectives, such as:

- Identify mining and environmental hotspots
- Predict and obtain evidence whether Hg associated with fine particles can be transported to other areas.
- Know how easy Hg can be leached out from soil and sediment components; these also are indirect ways to determine bioavailability
- Know 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 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 for the project. 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. Whether 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, it is recommended to analyze screened fractions. 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

Whatever is the objective of the soil and sediment monitoring program, 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 accomplished by locating areas with similar geological or physical characteristics in soil and sediment (e.g. grain size, lithology, organic content) uncontaminated by Hg in areas upstream or upwind of mining areas. 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 (HFO).

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):

\[
I_{geo} = \frac{\log_2 C}{l.5 \cdot B}
\]

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 it is recommended to “pre-concentrate” the sample by eliminating the coarse grain size fraction (Laird and Dowdy, 1994). 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.
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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 sample procedure for soils and sediments involves drying, disaggregation 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) prior to analysis of metals content (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 any other indication of the grain size of the samples. When comparing mercury levels in sediments from different locations it is very common that authors omit this information. With different mineralogy, composition and grain size, it is pointless to compare Hg from “non-contaminated” sediments from Africa with those from Amazon or Philippines. It is not uncommon to find authors that compare “contaminated and non-contaminated” sediment from completely different geological environments to make statements about mercury contamination.

Another interesting aspect when preparing geological samples is the drying procedure. Koksoy et al (1967), cited in Fletcher (1981), mentioned lithogenic mercury 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 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 that soil or sediment 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 Hg contaminated locations sampled (PSEP, 1997).

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. 25m² plots) and composited into a large container, mixed, sieved and subsampled for grain size and Hg analysis. Properly used, composite samples can provide a means of quickly assessing 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.

In the field whether sampling soils or stream sediments, the following basic information
about the site must be gathered:

- Date and time of sampling and location (GPS and map sheet 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 (m³/s) if
  a stream is sampled).
- 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\(^3\), 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 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.

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 prior to 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.

\(^3\) 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: \(Eh = E_{Ag/AgCl} + 0.199\) (in
volts)
• 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: did not contain large foreign objects (e.g. roots, branches, rocks); had adequate penetration depth (i.e. >10 cm); was not overfilled (sediment surface not touching the top of sampler); did not leak (there was overlying water present and no visible leaks); and was 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, either in the field or in the laboratory first to –2mm (remove debris) and then 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, cross-check 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 possibilities of a mining hotspot to become an environmental hotspot.

The following information must be collected from sediment samples:

• Total mercury 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 the same 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 procedures used to collect, prepare and analyze samples to establish background levels must be applied to samples from contaminated sites as well.

**1.3.2. Dispersed Contamination**

The intensity of atmospheric mercury 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 and consequently spreading of the 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 mercury 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 carrying natural mercury bound to old-intensively weathered soils to Amazonian aquatic systems. The authors stressed that this is a much more important source of mercury for the entire Amazonian environment than any other source, including ASM activities which are more relevant locally, near the mining sites.
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 and it is also used to control agricultural pests. Forest fires mobilize Hg contained in biomass and redistribute it into the atmosphere, either as vapor or attached to particulates. Today, with the high rate of deforestation by fire in developing countries, Hg emissions derived from wood combustion is significant. The amount of Hg annually emitted 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 emitted in the Amazon region is associated with biomass burning and 63% from gold mining.

![Diagram of Hg distribution in soils around gold shops of Alta Floresta (CETEM, 1992).](image)

Whether or not mercury emitted to the atmosphere by ASM travels long distances or is deposited near the emission source is another controversial point. According to Marins et al. (1991), the majority of Hg emitted from 32 gold smelting shops is deposited near the emission source (i.e. within 1 km). In Alta Floresta, a town in the South of the Amazon Basin, neither air analyses nor soil samples up to 600 m from gold shops show significant Hg concentrations in samples analyzed (CETEM, 1992) (Fig. 4). A simulation model of mercury emissions from gold shops in the same town concluded that Hg concentrations in air decrease quickly with distance (< 2km) from the source (Artaxo et al., 2000). Borochoff (2001), using mathematical simulation, estimated that mercury vapor 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 vapor. The authors monitored Hg vapor emissions from gold shops analyzing house dust from 30 residences located within 400 m

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4 Deforestation by fire in the Amazon in 1995 = 29,059 km² of jungle.
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 are those with higher levels of Hg in urine (Câmara et al, 1998). This procedure also revealed residents who were burning amalgam inside their houses. The use of mercury ‘sniffers’ (such as LUMEX, Jerome, Nippon, Genesis, etc.), i.e. portable instruments that analyze Hg in air, can detect high levels of Hg in houses of those who have been handling mercury. Murao et al. (2001) reported high mercury content, up to 15.7%, in dust, ash and soot in gold smelting and amalgamation huts.

Even when deposition is near the source, mercury from miners and gold shops can be re-emitted when a fire is ignited (Fig. 5). In his review on mercury in the Brazilian Amazon, Villas Bôas (2001) mentioned a simulation model which indicated that mercury from ASM can travel thousands of km when associated with aerosols.

Deforestation, as a result of human occupation of the jungle environment, also exposes soils to rainwater, increasing the leaching and erosion processes that mobilizes 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.*

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**Fig. 5.** Forest fires emit Hg from wood and redistribute Hg emitted by miners.
Global Mercury Project—Protocols for Environmental and Health Assessment

Atmospheric Deposition Rates

On a global scale, the atmospheric mercury cycle is dominated by elemental mercury vapor (usually >95% of total airborne Hg). However, the emission speciation of mercury is determined by the source characteristics (Ebinghaus et al., 1999). In a comprehensive review, Porcella (1995) describes mercury emission and deposition rates in the northern and southern hemispheres. He believes that much of the background emission is in the form of elemental Hg (Hg°) that 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 mercury from all sources is between 5000 and 6000 tonnes. Porcella also estimated that the mercury 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, mercury 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), making reference to a modeling study conducted by M. Roulet, indicated that the Hg deposition in the area between latitude 10°N and 10°S was around 13 μg/m².

An effective technique to estimate atmospheric Hg deposition rates is analysis of Hg in lake sediment cores. This controversial technique, known as “paleogeochemistry,” has been applied together with a dating procedure that can be derived from Carbon-14 analysis or from evaluation of lake sedimentation rates. Atmospheric mercury 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. A potential confounding factor is the vertical mixing of sediments by benthic worms, obscuring chemical signatures between sediment layers. 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 that diagenetic processes may bring Hg up to the sediment surface.

This method is not useful for soils because soil formation is different from lake sedimentation and the erosion and weathering processes substantially alter the mineralogical profile. A hypothetical example of this method is given in Fig. 6. Researchers must be aware that other factors can skew the results. Lakes can be partially dried during some months of the year. Interstitial waters (pore waters) are very reactive and have strong seasonal effect, i.e. migrate vertically. This is the environment where 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. Also, 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 and 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).
Hypothetical example of evaluation of atmospheric Hg deposition rates using lake sediment.

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

Rodrigues-Filho et al (2001) examined a sediment core sampled 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 mercury 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.

In recent work using lake sediment cores, Lacerda et al (1999) estimate that the current Hg deposition in the Brazilian Amazon basin ranges from 10 to 12 µg/m²/a. Fosberg et al (1999) analyzed Hg in rain water 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 very little mining influence. Assuming the average deposition rate from all sources of mercury emission the Brazilian Amazon is between 10 and 16 µg/m²/a, in an area of 5 million km², the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from different sources (Veiga et al, 1999).

**Atmospheric Deposition Forms**

Most mercury 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, from November to March, monthly precipitation ranges from 200 to 400 mm and only 25 to 50 mm from June to August.

The recent discovery of water-soluble species of mercury 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 HgCl₂. In Tennessee, the RGM form of mercury represents 3 to 5 % of the total gaseous mercury in the atmosphere (Lindberg and Stratton, 1998). In Florida, this species of mercury represents the dominant form of total mercury in the atmosphere associated with dry deposition (S. Lindberg - personal communication). Lindberg and Stratton (1998) indicated seasonal trends might exist in RGM concentrations; this variability was primarily associated with temperature, solar radiation, O₃, SO₂, 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 other 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 the carried to aquatic systems or bioaccumulated or methylated. Roulet and Lucotte (1999) studied the importance of soil erosion as a mode of transport of Hg from lithogenic and anthropogenic sources associated to particulate matter into the aquatic systems in Amazon. When organic acids from the soils contact metallic mercury (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 easily transport these contaminants 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 soil mercury can be considered largely Hg(II) complexes, although a small fraction of mercury in typical soils is elemental mercury (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 intestinal bacteria. They estimated that the total methylmercury synthesized from ingested inorganic mercury in humans is approximately 0.4 mg/day.

Hinton & Veiga (2002) used earthworms to study the mercury 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 mercury 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** apply sampling theory and involve random selection of sampling units. An essential feature of a probability-based sample is that each member of the population from which the sample was selected has a known probability of selection. When a probability-based design is used, statistical inferences may be made about the sampled population from the data obtained from the sampling units. Probability-based sampling designs provide the ability to calculate uncertainty associated with estimates, reproducible results within uncertainty limits, the ability to make statistical inferences, and can handle decision error criteria. As main disadvantages of this methodology one can list cost, the difficulty to randomly locate 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 (US EPA, 2002).

Sampling soils and sediments to establish the dispersion of Hg contamination is very 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 in which Hg is or was dispersed rarely can be used to calculate the amount of mercury (vapor) 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 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.

One of the most important aspects is to determine the appropriate sample size in order to quantitatively distinguish 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 $+\ 2 \ SD$ at a given level of power. The power analysis equation (Green, 1989) is as follows:

$$ N = 2(t_a + t_b)^2 \ (SD/ES)^2 $$
Where $n$ = sample size; $t_a$ = t value for a significance level; $t_\beta = t$ value for $\beta$ level significance; SD = standard deviation and ES = effect size (approximately 2 SD). Pre-calculated tables of $n$ are available for a variety of $a$, $\beta$ and SD values. If we assume that $a = \beta = 0.1$ and ES = 2 SD, five (5) replicate stations are required for each area sampled. To increase confidence, if we assume that $a = \beta = 0.5$, 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; Metal Mining Effluent Regulations).

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 mercury is dispersed. The best way to determine if mercury is becoming bioavailable within discrete areas is by analyzing 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 used for amalgamation of gravity concentrates. An environmental hotspot is characterized by the high risk (or evidence) of mercury bioavailability, which is usually associated with high methylation potential. For example, Baker and Allard (2002) observed a good relationship between MeHg and total mercury concentrations in mercury mining contaminated sediments from Pinchi Lake, BC, although there were several orders of magnitude difference in concentration. However, not all sites with high inorganic mercury concentrations have high methylation potential or high MeHg concentrations.

Hotspots can have dimensions of 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 that 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 4) and 50 ppm for industrial use. Freshwater sediments have more restricted guidelines in Canada (0.49 ppm aquatic life protection).

It is clear that the simple soil or sediment analysis does not provide enough evidence to support remediation actions. A disposal site for tailing 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.

Table 4. Some guidelines for total mercury in soil, sediment, water and air.

<table>
<thead>
<tr>
<th></th>
<th>Agri/Res</th>
<th>Com</th>
<th>Ind</th>
<th>ISQG</th>
<th>PEL</th>
<th>GV</th>
<th>CWQG</th>
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<tr>
<td>Soil (dw µg/g)</td>
<td>6.6</td>
<td>24</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Freshwater Sediment (dw µg/g)</td>
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<td></td>
<td></td>
<td>0.17</td>
<td>0.49</td>
<td></td>
<td></td>
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<tr>
<td>Drinking Water (µg/L)</td>
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<td></td>
<td></td>
<td>1</td>
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<td></td>
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<tr>
<td>Water for Protection of Aquatic Life (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


GV = Guideline Value (World Health Organization, 1996):


dw = dry weight

Knowing the strong influence of organic matter in dissolving metallic mercury in oxic environments, the resulting compounds, soluble mercuric-organic complexes, can be methylated by bacterial action (Veiga et al., 1999). So, when a mining hotspot exists in shallow creeks with considerable dissolved oxygen available and in contact with organic matter, the possibility of formation Hg-rich soluble complexes is high and represents a high-risk situation able 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.

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 is 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. Despite his suggestions as to the possible location of the mining hotspots, panning was the most effective and fast way to identify and demarcate sites with high Hg concentrations in a 1 ha flooded area that in the past had many amalgamation pools (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 were visible in the concentrate after panning, the sediment contained at least 3 µg/g 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 constantly moving from one site to another, the hotspots created by dumping Hg-contaminated amalgamation tailings into the river are very dispersed along the riverbed. In these cases, mercury contamination is very dispersed and it is advisable to focus on biota to evaluate bioavailability and to avoid sediment sampling.

The recent metallic Hg spill in Peru\(^5\) illustrated the importance of considering the form of Hg present when conducting sampling programs. During the clean-up following this accident, an analytical 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 sample analyzed (1 gram) was too small to sufficiently represent the content of Hg in soils from a given location. Later the company in charge of cleaning up the spill employed an effective procedure. Using a portable cold vapor atomic absorption analyzer, LUMEX RA915, able to detect 2 ng/m\(^3\) 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\(^\prime\) and length of 30 cm (distance from the soil). Mercury vapors 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 1000 ng/m\(^3\), 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 1850 soil samples were collected and submitted for analysis. Once a correlation between Hg in the vapor 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.

An 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 was heated in a Bunsen burner to temperature higher than 500 °C and the vapor was 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.

\(^5\) 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.
solution. Shaking the organic phase with soda, an orange color is developed indicating presence of mercury in solution. Comparing with other colorimetric standards, the procedure could analyze Hg concentrations up to a concentration of 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.

Another semi-quantitative process to detect presence of metallic mercury in soils and sediments is by using special silver-based amalgamation plates, which is a new technology recently developed in Brazil by two manufacturers to remove mercury 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 quickly pass 30 to 50 kg of sediment through its surface activated with drops of vinegar. Qualitatively, the process is very 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 5. As gold and mercury 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 mercury. In this case, to sample soil with 4 ppm Hg 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 mercury 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 (screened) grain sizes (e.g. 200 mesh or 0.074 mm).

<table>
<thead>
<tr>
<th>Size of Gold Particle (mm)</th>
<th>Kg of sample required</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 ppm Au</td>
</tr>
<tr>
<td>2.0</td>
<td>400</td>
</tr>
<tr>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>0.125</td>
<td>0.1</td>
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<tr>
<td>0.062</td>
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<tr>
<td>0.031</td>
<td>0.002</td>
</tr>
<tr>
<td>0.015</td>
<td>0.0002</td>
</tr>
<tr>
<td>0.008</td>
<td>0.00002</td>
</tr>
</tbody>
</table>

When dealing with metallic mercury, 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 it is required to handle samples with high concentrations of metallic mercury, 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.

CETEM (1989) observed that dry sieving of weathered geological materials is very inadequate as it does not disaggregate the clay and retain droplets of metallic mercury. Rodrigues-Filho and Maddock (1997) working with samples from mining hotspot noticed that that wet screening is not 100% efficient to send all metallic mercury to the fine fractions but definitely is more efficient than dry screening.

As mercury amalgamates very easily with copper and not with iron it is not advisable to use copper screens for sieving. Stainless steel sieves are more appropriate. Nylon screens are ideal but usually not quite available.

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 mercury and has created many political issues in the region because of the proximity to an ecological park in Brazil, the Pantanal. 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, 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 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 metallic mercury released by the ASM. Even knowing that amalgamation of the whole ore was practiced in this region, either the random sampling process was not efficient to detect the hotspots or mercury is present as very fine droplets diluted with the tailing material.

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

As an outline, the main steps are suggested to locate mining hotspots:

- **ASK FIRST**: Find out about the history of mercury 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 mercury content.
- **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 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.

- **CHECK Hg 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 (0.74 mm). 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 of the possibility of Hg being dispersed with fine particles.

- 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.

**Locating Environmental Hotspots**

It is not trivial nor easy to locate sites where mercury released from ASM has high potential to be transformed into methylmercury, thereby creating an environmental hotspot. Processes to evaluate methylation potential of different natural environments can be costly and complex. Methylmercury concentration in soil and sediment occurs as a result of the balance between methylmercury production and degradation of methylmercury into metallic Hg (i.e. demethylation). It has been recognized that methylmercury is mainly produced in sediments by methylating bacteria and either released into the water column where it is rapidly accumulated by biota (Jensen and Jenerlov, 1969; D'Itri, 1972), or incorporated by benthic invertebrates at the base of the food web.

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 (Provari and Verta, 1995).

Despite the uncertainties related to the transformation of metallic mercury into methylmercury in ASM regions, a simplified sequence of reactions is suggested:
Carmouze et al. (2001) observed that in the Amazon region, flooded forests, floating macrophytes, lakes with meanders, and anoxic places, etc. favor 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 also are acidic and thus promote desorption of mercury 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. In terms of risk assessment, the methodologies to identify environmental hotspots are those used to identify sites where mercury is or can be bioavailable (see Section 1.5).

Environmental hotspots can be identified using direct or indirect methods. Direct detection methods involve sampling resident biota (invertebrates and/or fish) for total and methylmercury concentration in areas suspected to be high methylation environments. Comparisons of Hg in similar biota species between reference areas and suspected environmental hotspots will identify the locations and relative importance of hotspots. Indirect methods involve using *in-situ* methods to determine methylation capacity or the methylmercury 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. Mercury can travel great distances between ASM activities and areas where inorganic mercury becomes deposited in sediments and encounters favorable methylating conditions, 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. It is absolutely necessary to identify and quantify the extent and magnitude of methylmercury production because these are the entry points of Hg into the aquatic food chain as MeHg 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 capture 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 methylmercury 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 primarily depend on (a) mercury complexing characteristics, (b) microbial metabolic activity of the sediment, and (c) total inorganic mercury concentration in the sediment.
Global Mercury Project— Protocols for Environmental and Health Assessment

(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 (1994), studying Canadian reservoirs, concluded that concentrations of total Hg in sediments was not a good predictor of MeHg production and that certain environments enhanced 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 favor 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 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 micro-organisms have the ability of methylating mercury, the procedures to determine the methylation potential in a laboratory is 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), was methylated in sediment or in other substrates, such as aquatic plant roots (Guimarães et al, 1998). Spiking sediments with radioactive traceable $^{203}$Hg, scientists observed that in the Amazon, higher methylation rates ($10^{-2}$ %,g$^{-1}$.h$^{-1}$) were found in rich, organic sediments in dark water 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 no clear correlation with organic matter content in sediment was observed. 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 methylmercury 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 methylmercury production in a contaminated soil or sediment over time. For screening purpose, simpler methods to evaluate the methylation potential of a soil or sediment should be developed.

Other studies have been dedicated to identifying bacteria capable of methylating Hg(II) and 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 tightly linked 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 from Univ. Central de Venezuela (personal communication, 2001) 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). As a large number of micro-organisms are capable of methylating Hg(II) species, it seems very laborious work to identify all types of methylating and demethylating bacteria in a sediment in order to predict potential risks.

**Sampling Environmental Hotspots**

Sampling materials 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. One can use soils and sediments or biota for this purpose. Methylmercury concentration of sediment determines the magnitude of methylation and provides an indirect measure of bioaccumulation potential. The same procedures must be applied to sampling suspected environmental hotspots as for reference areas. To directly determine if an area represents an environmental hotspot, resident biota (small fish or invertebrates) can be measured for total mercury. Analysis for total mercury 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 biota tissue, especially fish, analyzing for total Hg is less costly than for MeHg and therefore more samples can be analysed for less cost. 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.

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

If material from mining hotspots is visibly being transported 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 methylmercury than the mining hotspots. To select sites to measure as potential environmental hotspots the following criteria should be used:

- Seek depositional areas in streams – areas with fine sediments (silt/clay) or fine sand; avoid erosional 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 rapidly identify 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 very 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 areas 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 it 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 for fish.

1.3.4. Mercury Mobility

Metallic mercury dumped by ASM is heavy and not easily transported in drainages. Natural or anthropogenic mercury from various sources, including ASM, when deposited on ground is transported to water streams in solution or suspension through run-off waters. Either concentrated in “hotspots” or dispersed in sediments, mercury becomes mobile in terrestrial environments when mercury complexes (organic and inorganic compounds) adsorb on soil and sediment fine particles (main mechanism). Metallic mercury 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 mercury content of coarse fractions as a result of the interaction of Hg-oxidized complexes with soils and sediment components in particular clayminerals 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 (an important tributary of the Amazon River) system.

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 oxidation 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 has Hg concentration 7000 times higher than the one in non-impacted watercourses. However, within 100 meters from the tailing pond, the concentration of dissolved Hg dropped by 300 times as mercury was adsorbed by the suspended solids. These authors suggested that Hg entering the Tapajós River is not just the one emitted by ASM. Natural (lithogenic) mercury is the main source of mercury, 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.

One relevant environmental impact caused by ASM is river siltation. Once hydraulic monitors are extensively used in the weathered and placer ore extraction, miners do not use to impound tailings in ponds. Tailings are usually 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, Brazil. Physical impacts on biota are evident. Mercury and other heavy metals are also attached to the suspended particles to be transported to remote areas (Villas Boas, 2001), creating environmental hotspots.

In streams, particulate transport of Hg is much more evident than in lakes. CETEM (1989) investigated the transport of Hg from a mining hotspot, an abandoned tailing pond formed by irresponsible artisanal miners. Where tailings were deposited into a drainage near an important Ecological Park in Brazil, “Pantanal Matogrossense,” the project assessed the possibility of mercury transport and consequent bioavailability. The study concluded that particles released from the tailing pond did not travel long distances in small creeks and 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 impacted 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 mercury contacts organic acids (Meech et al., 1998). Fadini and Jardim (2001) concluded that mercury leached from soil is the major pathway to contaminate 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 mercury in solution or associated to 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 opposed to around 50 mm/month in the dry season (INPE, 2003).

It is a very time-consuming, complex, and uncertain process trying to determine how Hg moves in aquatic systems by sampling superficial water, especially run-off water. Water sampling provides a brief snapshot of the current conditions. In addition, there are many confounding factors that can influence 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 very difficult to determine the magnitude and range of Hg transport in aquatic systems.

The most appropriate method to evaluate Hg mobility is by sampling 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 introduction (mining hotspot) and determine where 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, and not by sampling the water column.

**Predicting Hg Mobility**

Variables controlling mercury toxicity can be divided into two major categories: 1) parameters affecting the release of mercury into the aquatic environment (mobility) and 2) parameters affecting mercury accessibility to biota (bioavailability). Most laboratory and field techniques concentrate in assessing bioavailability by chemical or biological methods. However, very few methods have been developed to predict the mobility of mercury 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 
the information about the hydrodynamics of the aquatic system can predict if the material 
from the hotspots can be transported to other sites.

Baker and Allard (2002) applied a method to predict mercury mobility from erosion of 
“mining hotspots” in Pinchi Lake, 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 
ocasionally 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 that evaluate the solubility of trace 
elements and the neutralization-adsorption capacity of the rock-forming minerals and the 
Kinetic Tests that use humidity cells to speed up the weathering procedure. 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 mercury release form the tailings. Test organisms (Daphnia magna) were 
exposed to the leachate. This supposes to simulate the reaction of natural organic acids 
with metallic mercury 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 material from a mining or 
environmental hotspot (when these are already characterized) 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 investigation, 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 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 also 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. One important point when seeking for depositional areas in streams is 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 mercury 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 sample 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 promoting 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 very difficult to obtain reliable relationship between magnitude of Hg on suspended solids and amount of Hg transport, especially without discharge information, which is usually seasonal and difficult to measure.

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 mercury 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 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. Also, when Hg concentrations are low, the risk of contamination of sampling vessels is high. In addition, organisms accumulate methylmercury very quickly, therefore the concentration of this compound analyzed in water is very low (D'Itri, 1990), often even in contaminated environments, undetectable by analytic methods.

In seawater the normal mercury concentration is around 0.05 µg/L Hg and 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 on the order of 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 (US EPA, 1997).

Mercuric ion (Hg$^{2+}$) is not stable as a free ion in natural aquatic environments. Mercuric species are combined forming 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 rapidly accumulated 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 mercury concentration in sediment (D'Itri, 1990).

Analyses of pH and redox conditions (Eh) of freshwater sediments impacted by ASM activities can provide useful information about mercury 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 very slow (Baeyens et al., 1979). The most toxic form of mercury, methylmercury is an example. It is thermodynamically less stable than inorganic species.

Assuming that metallic mercury is in equilibrium in a simple aquatic system, the predominant Hg species in solution would be undissociated mercury, Hg$^o$ (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), then Hg(OH)$_2^-$ and HgCl$^-$ are the predominant inorganic species depending on the pH. The full lines of Fig. 6 represent the equilibrium of Hg$^o$ (aq) and HgCl$^-$ (aq) and Hg(OH)$_2^-$ (aq) respectively. The dotted lines represent conditions in which concentration of uncharged species (HgCl$^-$ or Hg(OH)$_2^-$) are 1000 times lower than the Hg$^o$ (aq) concentration, i.e. in this case the importance of these complexes in the formation of MeHg is considered insignificant as metallic mercury has to be oxidized to...
become more soluble, i.e. to form Hg(II) species or complexes which are far more reactive. Mechanisms of methylmercury 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. 7) suggests that metallic mercury 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 mercury. 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) experimentally demonstrated that humic acid solutions increase the solubility of metallic mercury but the presence of calcium ions inhibits Hg solubilization.

Tromans et al. (1996) designed a Eh-pH diagram capable of predicting the stability of metallic Hg in contact with organic solutions (Fig. 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 mercury species. In the diagram, the organic ligand is represented as a diprotic acid H₃L. Two complexes are formed, HgL and Hg(H⁻L)⁻. The upper line of the diagram represents equilibrium between complexes and Hg⁰(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. 8. Equilibrium boundaries of Hg° (aq) and Hg-organic complexes

As the chemical composition of the organic acid which provides the complexing ligand is unknown, a molecular weight of 1000 g has been assumed and a concentration of 100 g/L (or 0.1 M) in the contaminated sediment. This condition is represented in Fig. 7 by the lower full line. The dotted lines represent a situation in which the Hg-complex concentration is 1000 times lower than that of Hg°(aq). If we consider a Hg° (aq) concentration of 63 µg/L, 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 are 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. 7 and 8. Since the early 1980s, miners have dumped Hg-contaminated tailings into these rivers and amalgams are burned usually without retorts on the barges or near river margins. As observed in Fig. 7, 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 impacted by mining activities, have shown higher Hg levels than “white water” rivers (i.e. those with high amount of particulate matter (clays) 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), which stated that organic acids may facilitate Hg(II) reduction to volatile Hg° while complexed to or dissociated from humic substances, determined that the amount of elemental Hg formed increased 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 reduction by humic substances is decreased by factors such as the presence of competing ions (particularly Cl\textsuperscript{-}), 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 1800 to 300 (Allard \textit{et al}, 1994). In the process of photodegradation, adsorbed mercury 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 (CEQQ, 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 \textit{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 \( \mu \)m), 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 favor of analyzing unfiltered water argue that many people drink unfiltered water, however mercury in the particulate matter (above 0.45 \( \mu \)m) 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 thoroughly flush the tubing 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 0.45\( \mu \)m 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 downcurrent direction to avoid disturbing and introducing sediment into the water column. Filtered and unfiltered water samples should be placed in ultraclean 250mL 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.
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. This transformation and bioaccumulation depends on several environmental factors and bacterial activity that controls chemical speciation of the metal (Carmouze et al., 2001). Sites with high Hg concentrations (mining hotspots) are not necessarily those with the greatest capacity of producing methylmercury (environmental hotspots) but they represent a risk situation. To assess environmental hotspots, analysis for MeHg would provide the most accurate information. However, MeHg analysis is costly, has a higher risk of 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 mineral surface. The stronger the bond, the lower the bioavailability.

Adsorption is the main mechanism to control availability of soluble mercury species to biota but it is also responsible for transporting mercury from mining hotspots to other locations where methylation is more favorable. For many lakes, adsorption and consequent sedimentation of Hg(II) and MeHg bound to particulate matter is expected to be the dominant process for removal of mercury from the water column (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 of mercury in the water column and to reduce concentration of mercury 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 very 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 was observed on HgCl\textsubscript{2} adsorption by clay minerals (Reimers and Krenkel, 1974). Clays may show an indirect effect in heavy metal adsorption due to the 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 clayminerals 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 mercury 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 mercury 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. Actually, mercury 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 6). In terms of risk assessment, it is very 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 mercury 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.

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

CaCl$_2$ or MgCl have frequently been applied 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 and therefore it is difficult to compare results (Quevauviller et al., 1997).

Ferreira and Veiga (1995) developed a sequential extraction to study the Hg distribution in a contaminated ferruginous sediment (17% Fe$_2$O$_3$) 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 mercury than the −0.074mm (200 mesh) fraction. As observed in Table 7, the procedure revealed that most mercury is associated with the hydrous ferric oxides (77.5%). The procedure used ammonium acetate (1M) to
determine the labile fraction associated with clayminerals, followed by attack with oxygenated water (30% vol.) + 0.02 M nitric acid in the proportion of 5 H₂O₂ : 3 HNO₃ for five hours to evaluate the mercury 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 mercury, 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+HClO₄+HNO₃ (5ml+5ml+5ml) at 60 °C. One of the main drawbacks of this procedure is the lack of selectivity between metallic mercury and organic matter. However, the method was useful to indicate that Hg adhered to very fine particles, most likely to be transported in streams, and was not easily extracted and therefore less bioavailable.

Table 7. Sequential extraction of Hg from different components of the clay fraction (-0.002 mm) of ferruginous sediment from Poconé.

<table>
<thead>
<tr>
<th>Hg-associated phase</th>
<th>% Hg extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exchangeable</td>
<td>3.0</td>
</tr>
<tr>
<td>Organic matter + metallic Hg</td>
<td>2.0</td>
</tr>
<tr>
<td>Hydrous ferric oxides</td>
<td>77.5</td>
</tr>
<tr>
<td>Residual Hg</td>
<td>17.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
</tr>
</tbody>
</table>

The adsorption capacity ferruginous sediments from Poconé revealed by the selective extraction procedure (Table 7) can also be responsible for almost no incorporation of mercury in test organisms 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 and 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 mercury 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. 9, could not discriminate between Hg associated with organic matter and metallic mercury. Using 1M ammonium hydroxide, the Hg weakly associated with humic substances was liberated. Exposing the sediments to concentrated nitric acid for 16 hours solubilized the fraction of Hg likely in the form of metallic mercury, mercury oxide, and/or as mercury 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. This preliminary investigation revealed that Hg in contaminated Pinchi Lake sediments exists primarily as a non-exchangeable, non-labile form (Fig. 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 that might explain greater bioavailability of Hg at some stations than others (Baker and Allard, 2002).

![Diagram of sequential extraction of Hg from Pinchi Lake, BC, Canada, sediments](image)

**Fig. 9.** Sequential extraction of Hg from Pinchi Lake, BC, Canada, sediments

![Bar chart showing % Hg Extracted](image)

**Fig. 10.** Mercury partitioning in sediment samples from Pinchi Lake
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 sulphhydryl groups. The combination of the lipophilic properties and affinity for the sulphhydryl groups of amino acid compounds results in rapid accumulation in the muscles and fat tissues until MeHg 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).

Because MeHg is assimilated rapidly and is eliminated slowly, its synthesis in sediments does not have to be rapid to promote bioaccumulation. The mechanisms and rates of accumulation and elimination are unclear, but appear to depend on the specific biological characteristics of each species of fish as well as the properties of the aquatic systems. A comparison of animals differing in species, size, and feeding habits confirms that the food intake of Hg is far more important than direct uptake from water. Thus, the Hg levels in the top predators are always higher than in their food (D'Itri, 1972; 1990; Lindqvist et al., 1991).

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 piranha (Serrasalmus rhombeus) are an ideal bioindicator (Veiga, 1994) because 80% of their diet is fish-based, they do not make long migrations, and they mainly live in quiet waters (Goulding, 1980). Akagi and Naganuma (2000) have shown that nearly all mercury analyzed in piranha as well as “peixe cachorro” (Rhaphidodon vulpinus) is MeHg. Unfortunately, black piranha is not found in all areas of the Amazon. 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). Therefore the mean mercury concentration of a sample very much depends 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 are 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 lakes or years, comparisons of standardized mean mercury 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 size range of fish 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 mercury analysis required that the fish be sacrificed. With technological advances made in analytical techniques, reliable estimates of fish mercury concentrations can now be made with very small sample sizes (<100 mg) that do not require sacrificing fish.

Baker et al (2003) have developed a non-lethal method of fish tissue sample extraction for Hg analysis. Using a Tru-cut™ tissue biopsy needle (14 gauge x 7.6 cm cannula with a 20 mm notch), ~50 mg of tissue is extracted from fish anaesthetized with clove oil. As Hg concentration increases with size and age, a wide size range of fish is needed to characterize Hg pollution in a given system. Using linear regression and analysis of covariance, quantitative comparisons of Hg concentrations can be made among populations and over time based on a standardized fish size.

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. An example of the standard protocol to describe the optimal sample size per size interval in British Columbia for mountain whitefish, rainbow trout, and bull trout from which to derive standardized mercury concentrations is shown in Table 8. These standardized sizes fall near the middle of the size range of fish collected and have been used as standardized sizes elsewhere in British Columbia and Canada, which facilitates comparisons between lakes and years for the same species (Fig. 11 and 12).

Table 8. Example of collection protocol to collect size-stratified samples for Hg analysis (Baker 2002)

<table>
<thead>
<tr>
<th>LENGTH INTERVAL (mm)</th>
<th>WHITEFISH</th>
<th>RAINBOW TROUT</th>
<th>BULL TROUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-199</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>200-299</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>300-399</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>400-499</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>500-599</td>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>600-699</td>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>&gt;700</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Standardized Length (mm)</td>
<td>300</td>
<td>350</td>
<td>550</td>
</tr>
</tbody>
</table>
Very few methodologies have been adopted in regions impacted by artisanal mining in order 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. Because of the lack of a standardized procedure it is not possible 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, fish migration, and movement between lakes, although Hg concentration in most individuals exceeded 0.5 ppm.

In an extensive study in the Tapajós River, Brazil, dos Santos et al (2000) noticed 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.

The correlation between fish length and mercury 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 the fish is caught, the Hg-weight relationship can be significantly different. Feeding also has similar a effect. For example, a large fish can consume up to 20 - 30% of its body weight, so depending on the time of capture and whether the fish has eaten, this can also dramatically affect the Hg-weight relationship. Differences in length also 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 exists 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.

An interesting approach to compare mercury bioavailability from one site to another was adopted by Castilhos et al (2001). Using the tucunaré (Cichla ocellaris) as a bioindicator, the authors analyzed the concentration of Hg from samples. They then calculated its daily uptake of MeHg in order to reach the analyzed concentration. The advantage of using the tucunaré was due to some peculiarities of these fish such as low change in weight during the seasons of the year, sedentary habits, carnivorous, and sedentary lifestyle. Additionally, its meat is very 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 the time was reduced to 1 and 2 years respectively. However, the highest Hg bioavailability was noticed in the tucunaré from the Tucuruí hydroelectric reservoir in the Brazilian Amazon (Castilhos and Lima, 2001). Povari (1995) also observed high levels of mercury in the tucunaré from this Brazilian 2.430 km² hydroelectric reservoir. He noticed that 92% of carnivorous fish samples have mercury levels above 0.5 ppm and the average of 1.2 ppm Hg (n = 33) was found in the standardized 700g tucunaré. Some authors (Aula et al, 1995) attribute Hg in Tucuruí 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.
The reservoir impoundment effect is well known in increasing Hg concentration in fish after flooding (Kelly et al., 1999; Bodaly and Fudge, 1999). Airborne mercury of natural or anthropogenic origin is captured by vegetation, deposited, and ultimately retained in forest soils. Following impoundment, 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 mercury sources were not identified but the influence of the submerged vegetation, type of organic matter, and bacteria in flooded sediments were recognized as important factors in the magnitude of Hg response. Mercury bioavailability is related to quality and amount of flooded vegetation and humus.

The impoundment effect is exacerbated in darkwater systems, such as in the Guri reservoir, a 4,000 km² 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 colour, 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 mercury in carnivorous fish as high as 8 ppm. It is interesting to note that in the lower part of the river, a 35 km sector intensively impacted by ASM, fish show lower Hg levels than in the Guri reservoir (Veiga, 1997) despite the 5 tonnes of mercury dumped by miners over the years. This result suggests that mercury bioavailability in the Guri reservoir is strongly affected by flooding (Fig. 13), perhaps even more so than by ASM.

**Sampling Fish**

The objective of the fish Hg assessment must be very 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 by
communities as well as by other nearby villages. Fish markets can also provide useful samples as well (see Chapter 2).

Regarding the locating 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 and 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 they are relatively easy to capture, are well known 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).

A strategic, quantitative approach to fish mercury sampling must be adopted for determining Hg concentrations for 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 (see Table 8 above). For the reasons discussed above, it is advocated developing a 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. Using fish length eliminates or reduces variability, as described above. The protocol for describing the relationship between mercury (ppm) and length (mm) is well known (Johnson, 1987; Bodaly et al, 1988; McMurtry et al, 1989) and is of the form:

\[ \log_{10}[\text{Hg}] = a + b(\log_{10}[\text{Length}]) \]

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 protocol to collect fish achieve an appropriate sample size and size range of fish to derive 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 8). 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 (e.g. 40 cm), 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.
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 to be used as “standard” is as follows.

1. A carnivorous species with greatest Hg concentrations.
2. A species with sedentary habit (limited migratory habits).
3. Easy 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. 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™ or Whirlpac™ 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.
- As soon after sample collection as possible tissue samples should be placed on ice and/or frozen. 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 vapor 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 mercury (ppm wet weight) data and plot the log (length) and log [mercury] 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 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 very unlikely for carnivorous fish, especially in Hg contaminated environments. But 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 mercury 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 mercury 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 [mercury] and log [length] for a particular species between years or sites. If the slopes (i.e. the rate of mercury 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 mercury concentrations are significantly different between groups of fish being tested. An example of this procedure is illustrated in Figure 12, above and in Figure 14, below.
Fig. 14. Example of annual variation of Hg in fish of a standardized size.

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

If slopes are significantly different \( (p<0.05) \) from each other, then the relationship between the rate of mercury 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 mercury 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 multiple years, can be compared is illustrated in Fig. 14.

By employing this simple data collection protocol and analytical procedure, differences in fish mercury 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 very 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 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 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, then 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.

**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. Also by using toxicity tests the relative toxicity of a mixture of chemicals can be assessed by taking into account synergistic or antagonistic interactions among chemicals (SCOPE, 1995). Mortality is the most commonly used endpoint. The classical approach to 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, very 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*) (US EPA, 1994-June) and inland silversides (*Menidia beryllina*) (US EPA, 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 variegatus*) (US EPA, 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, very 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 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. 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 very controversial issue. For neurotoxins such as mercury
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 adds difficulty in correlating results from one study to another and how to translate
results to 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, attached either 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 in turn make the data more difficult
to interpret. The potential for escape of test organisms or for a predator somehow to enter
the test enclosure 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, low or almost no mercury was
incorporated into the organisms. The presence of iron oxides in these sediments likely
explained the low bioavailability. The main criticism to caging fish studies is that
normally these fish 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. But
certainly in situ bioassays 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 fiber-glass-water tanks of 200 L
each, 20 kg of sediment spiked with 2 grams of 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 and after 60
days they were sacrificed and Hg in tissues analyzed. Algae, grown on the sediments, have
been used as food source by the test animals. Except for the shallow depth most natural
conditions were reproduced. This can represent the worst-case scenario, since aeration of
the contaminated sediments enhances the reaction between soluble organic acids of the
dark waters and metallic mercury from the sediments. This ingenious experiment was also
conducted adding organic matter and iron oxides to observe variations in Hg
bioavailability (Luis Perez, 2003 – personal communication).
When conducting a **health risk assessment**, the best indicator of MeHg exposure are fish acquired directly from local fishermen or in fish markets. In this case the edible parts are analyzed followed by a detailed questionnaire about population diet, gender, age, weight, habits, type of fish consumed, and quantities. 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 (e.g. organism physiology, internal solubilization capabilities, food quality and feeding behavior, etc). 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 methyl mercury 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. Invertebrates sieved from sediment from areas that had received Hg rich roasted ore (calcines) and areas away from the calcine sediments that were suspected of being in methylating environments were analyzed for Hg and MeHg. 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 this environmental hotspot.

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, effect 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 HgCl₂. Accumulation occurred rapidly. After one week of exposure to contaminated sediments, Hg accumulated has already 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, either for inorganic and organic forms. For the inorganic compound, 80% of Hg was localized in the intestines (Saouter *et al.*, 1989). Crayfish has been used as an efficient bioindicator. Results indicate that half-life⁶ 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;

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⁶ Half life is the time required to eliminate 50% of the pollutant from an organism.
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 easily bred, have been extensively studied, and are approved for use in toxicity testing by the US EPA, 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 the most appropriate indicators of mercury bioavailability in sediment. It can however 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. The results of bioavailability are based on tissue concentration determined by exposing earthworms in laboratory to contaminated soils, sediments or 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 80mL of distilled water (in solids evaluation) or solution (effluent solution) and manually homogenize in a 900ml 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 fetida) 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 to prepare 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”. Prior to analysis these worms must starve to void gut contents for 24 hours. 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 ASM 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. 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 environmental hotspots. In spite of requiring total Hg analysis, the method is easy to operate, reproducible, and appropriate in developing countries.

![Fig.15. Mercury in worm tissues versus Hg in contaminated sediments (substrate) (Hinton, 2002).](image)

**Sampling Aquatic Invertebrates**

In addition to the above, aquatic invertebrates can be directly sampled 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 eventually is transferred 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 and can be easily sampled, provided that 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. Clams should either be depurated in the bucket with clean water for at least 24 hours, or alternatively, the stomach containing sediment (possibly contaminated with Hg) should be removed from the clam tissue.

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 analysed for total mercury and MeHg. The ratio of inorganic Hg : MeHg 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 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 and then freeze as soon as possible after collection. Transport frozen. The entire snail from each composite should be homogenized and analyzed for Hg in the laboratory.

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.

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 and 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. Using Taxonomic Richness and Abundance to Assess Bioavailability

There is no simple or straightforward 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 methylmercury concentrations than these same groups in sediments uncontaminated with mercury. 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 methylmercury concentrations in resident biota.

In theory, Hg contaminated sediments might have a more depauperate 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 mercury 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, in spite of the effect of each separate variable on Hg bioaccumulation being 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 (1995) 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 colour), sediment colour, 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; Miele et al, 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 coloured 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 colour (brown) is well correlated with organic matter content in water. Fish caught in dark waters of the Amazon region are almost always higher in Hg than fish from white water 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 characteristic of blackwater rivers as evidenced in the Caroni River, Venezuela (Bermudez, 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 are controversies 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 bioindicator for mercury bioavailability is human beings. 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. 16). The Amazon region has been used as a living laboratory by academic researchers. In many monitoring programmes, human beings are seen merely as donors of hair, blood, or urine samples. In most cases, affected people never learn the results of the monitoring programme.

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 vapor 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 (GEDEBAM, 1992; Wilhelm, 1996), blood analysis gives a combined picture of both metallic and MeHg contamination.

![Fig. 16. Distribution of samples collected in the Amazon region for Hg monitoring (Silva, 2001).](image)

**Urine**

Tsuji et al (2003) evaluated ten studies reporting paired air and urine mercury 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 (1991) described a
relationship between Hg in air (A) ion µg/m³ and in urine of exposed workers (U) expressed as µg/g creatinine: \( U = 10.2 + 1.01 \times A \). 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 (1991).

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).

![Fig. 17. Hg in blood and urine of workers burning amalgam daily (adapted from GEDEBAM, 1992).](image)

Urine samples from miners who frequently burn amalgams in open pans show Hg levels >20 µg/L (Fig. 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. One of the highest mercury concentrations in urine in ASM regions was analyzed by Malm et al (1995a) in workers of gold shops in Amazonian villages. With values as high as 1,168 µg/L, the gold shop workers, working in confined environments, had higher concentrations of Hg in urine than miners burning amalgam outdoors. 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 has between 1 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).
A large number of studies have been dedicated to analyze urine of miners burning or handling mercury. It seems very obvious that somebody burning mercury in an open pan will accumulate mercury and exhibit large concentration of mercury in urine. Studies dedicated to prove this fact are pointless unless they are combined with medical exams and solutions for miners to reduce the Hg exposure and/or to remove Hg from their bodies. It seems that urine analysis is more useful to evaluate undue Hg vapor 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 time to sample urine is first thing in the morning. This analysis reflects mercury excreted by the body during the night. However collection during early morning 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 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 agent) is present in urine, the mercury analysis, especially reduction by stannous chloride, can be difficult (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) prior to analysis by CVAAS (cold vapor atomic absorption spectrometry) or CVAAS (cold vapor atomic fluorescence spectrometry). The authors described a method to analyse urine without pre-treatment. In this case sodium-borohydride is used to reduce mercury 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 mercury.

Mercury concentrations in urine should be corrected to the creatinine excretion. 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 (Wilhelm et al, 1996).

**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 comprised of Brazilian and British scientists who analyzed blood and urine of residents of Jacareacanga (Fig. 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 are considered to be normal (6 to 12 μg/L; Krenkel, 1971), and theoretically pose a great health risk to indigenous people of this region. WHO (1991) 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.

![Graph of Hg in blood and urine of fish-eating people from Jacareacanga](image)

As blood is difficult to sample, preserve, and transport, most researchers prefer to analyze hair in communities not directly exposed to Hg vapors (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 vapors and MeHg from fish ingestion, blood analysis provides useful information (Drasch et al., 2001).

**Hair**

Mercury in hair from the scalp is a good indicator of MeHg exposure (Malm et al., 1995b). Hair grows about 1 cm per month and 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 vapor 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), and Hg concentration in fish in ppm (F) is approximately: \( H = 0.2375 \times W \times F \). A relationship derived by a Japanese study of 765 people obtained somewhat different results: \( H = 0.167 \times W \times F \) (Kojima & Araki, 1972). A 70-kg person consuming 200 g of "non-contaminated" fish on a daily basis containing an average of 0.3 ppm Hg, as commonly observed in riverine populations of the Amazon (Barbosa et al., 1995; Castilhos and Bidone, 1999), would be expected to have a daily intake of 0.86 µg of
Hg per kilogram of body weight and may show around 11 ppm of Hg in hair samples. This is almost 9 times the recent American Reference Dose\(^7\) 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 vapor 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 vapor 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 to the amount of mercury in the environment which ultimately is 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, 1991). These data illustrate that people consuming fish once or more per day will have Hg levels in hair exceeding 10 ppm (WHO, 1990).

Fréry et al (2001) also confirmed the high levels of Hg in hair from Amerindians in French Guyana not directly impacted by ASM activities. Results showed that 57% of the Amerindians had Hg levels above 10 µg/g (ppm) as a result 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 where fish is extensively consumed but not impacted by gold mining. 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 Caxiuana.

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 must be developed to indicate to the public which species have the lowest Hg concentration.

Methylmercury usually comprises at least 70% of the total mercury analyzed in hair (Vanconcellos et al, 1999). Hair from the scalp of people with no direct contact with

\(^7\) 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.
"garimpos" was collected in different sites along Tapajós River (Akagi et al., 1995). The study concluded that riverine 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 analysed from fishing villagers in the Amazon with no exposure to mercury vapor. In this case, nearly all (>90%) mercury 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 where 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 very 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 mercury 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 mercury 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 more herbivorous fish at the end of the rainy season and more piscivorous fish at the end of the dry season.

**Sampling Hair and Blood**

Urine, hair, blood, and any other biological samples (e.g., nails) can be used for two purposes: 1) monitoring mercury 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 fewer cultural issues involved. This is not a universal rule as there are lots of 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 which contain Hg poses additional problems for the evaluation of MeHg exposure. Chemical speciation can differentiate the inorganic (mercury chloride) and methylated Hg compounds. No study of washing procedures to remove inorganic mercury from hair contaminated with Hg-soaps was found.
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 and kept the samples in an air-tight plastic bag. This seems an adequate procedure when working in hot and humid environments.

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 analytical lab.

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 can 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 vapor exposure as urine. It is clear that when chemical speciation is available the difference between MeHg and (inorganic) Hg⁰, 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 type of exposure (i.e. Hg vapor 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 vapor can help estimate the contribution of MeHg.

As indicated by the study of Dolbec et al (2001), mercury concentration in hair has seasonal variations. Thus, long hair can be used to observe these variations. Assuming that hair grows at a rate of 1 cm per month, 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.

Drasch *et al* (2001) suggested that 10 mL of blood was 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.

### 1.6. Analytical Procedures for Mercury

This section briefly describes recommended analytical procedures for analysis of total mercury and methylmercury in sediment, water, biota tissue (invertebrates and fish), and human health samples (urine, blood, hair). This document does not intend to describe the analytical procedures as they are fraught with details that concern the analytical laboratories. A very 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. US EPA) 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

A method of determining total mercury in water is suggested by Bloom and Crecelius (1993). It has since been adopted (1996) by the US EPA as Method 1631 – *Mercury in water by oxidation, purge and trap and CVAFS*. 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, followed by addition of a reducing agent, stannous chloride. 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 methylmercury 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 Hg⁰ before entering a cold vapour atomic fluorescence analyser 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 1000 g) 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. Half of the pulverized sample (125 to 250 g) is sent to the analytical laboratory. Store the other half.

Akagi et al (2000) described an analytical method to analyze total Hg in soils and sediments. 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), 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 and an aliquot of the solution is introduced into the analyzer (cold vapor or atomic fluorescence) where a solution of 10% stannous chloride 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 subsamples into acid cleaned test tubes. About 10 mL of a 1:2.5 nitric/sulphuric acid mixture is added and heated at 180°C for 6 hr in an aluminium hot block. After 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 analysed in duplicate. Detection limit is on the order of 1 ng Hg per gram (0.001 μg/g) wet sediment.

The 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 (see above, Section 1.6.1). Matrix spikes/matrix spike duplicates should be performed to determine recovery and to correct reported values. The detection limit is about 0.05 ng methylmercury per gram dry weight of sediment (0.00005 μg/g).

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 US EPA 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 Vapor Atomic Fluorescence Spectrometry.

Prior to 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 HNO₃/H₂SO₄. For tissue, weigh (nearest mg) approximately 0.2 – 0.4 gm of homogenized sample and add 10 mL of HNO₃/H₂SO₄. 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 to sit 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 (SnCl₂) containing water. Purge the solution onto a gold trap for 20 minutes. Change the SnCl₂ 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 vapor atomic absorption spectrometry (CVAAS) is 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 and 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, 0.5 g of finely chopped fish muscle or 10 mg of finely cut hair or 5 mL of blood is placed in a 50 mL volumetric flask to which 2 mL of nitric-perchloric acid (1+1), 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 and an aliquot of the solution is introduced into the analyzer (cold vapor 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 very 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 conduct Hg in urine and blood (30 µL of samples) in about 5 minutes.
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 that samples are collected using specified standardized procedures, analyzed by laboratories that have been certified for all applicable methods, and by 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 material (CRMs) samples. 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, whether it be sediment, soil or fish tissue.

The general DQOs for a typical field project are:

- **Analytical Precision** = ± 25% Relative Percent Difference (%RPD).
- **Analytical Accuracy** = 80 to 120% recovery of matrix spikes and CRMs.

**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) or replicate (i.e. more than 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:

\[
\text{RPD} = \frac{(A - B)}{((A + B)/2)} \times 100
\]

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 sometimes lead to a belief that the level of precision is less than it actually is.

Analytical precision is measured in the laboratory by:

- The analysis of laboratory duplicates, in which the same sample is analyzed at least twice; and
• By the analysis of matrix spike duplicate samples, in which a matrix spike sample is analyzed twice.

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

**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 known target analytes that are spiked into a field sample (i.e., a surrogate or matrix spike) or reagent water (i.e., Laboratory Control Sample [LCS] or blank spike) before extraction at known concentrations.

The analysis of CRM samples also provides an indication of analytical accuracy. A variety of CRMs are 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: \(A = \text{obtained value}\); \(B = \text{true value}\).

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 and CRM samples 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. That is, if average recovery from a reference material 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.
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 primarily be attributed 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 vapor 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 mercury in contact with organic-rich soils becomes soluble and eventually converts into its most toxic species, methylmercury, which is rapidly bioaccumulated. Communities reliant on fish, especially carnivorous species, as a primary food source may be particularly susceptible to ingestion of dangerous levels of methylmercury. To assess human health it is useful to assess the environmental 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 is designed to complement the Environmental Health Assessment, providing indications of the level of mercury poisoning and their health effects on ASM communities either by exposure to mercury 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 vapor 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 in which mercury is bioaccumulated in humans. The main ways are through metallic mercury vapor from amalgam burning (and gold melting) and ingestion of fish with high methylmercury concentrations. Other pathways may include Hg evaporation from amalgamation tailings and dirt ingestion by children.
2.1. Health Effects Caused by Mercury Vapor

Inhalation of Hg vapor is more significant for mining and gold shop workers directly involved in handling metallic mercury, but can also indirectly affect surrounding communities. 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 mercury can easily diffuse across the blood-brain barrier, which is a series of multiple systems which regulate 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).

Hg vapor is completely absorbed through the alveolar membrane and complexes in the blood and tissues before reacting with biologically important sites (Mitra, 1986). The biological half-life of Hg in blood absorbed as vapor 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, 1991). 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). 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 (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. 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 (Stopford, 1979).

Symptoms typically associated with high, short-term exposure to Hg vapor (1000 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. The common manifestation of chronic exposure to excessive levels of Hg vapor is metallic taste and gum diseases such as gingivitis, ulcers and formation of a blue line at gum margins (Stopford, 1979). Long-term, low-level Hg vapor exposure has been characterized by less pronounced symptoms of fatigue, irritability, loss of memory, vivid dreams, and depression (WHO, 1991). 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 (WHO, 1991). 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).

Experiments with animals indicate continuous exposure to Hg above 0.3 µg/m³ of air may present a health hazard. 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).

Since inorganic Hg poisoning affects liver and kidneys, high Hg levels in the urine can indicate undue exposure to Hg vapor. WHO (1991) collected a large amount of evidence
to conclude that a person with a urine Hg level of 100 µg/g creatinine has a high probability of developing symptoms such as tremors and erethism (abnormal irritability). For Hg levels 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 30-50 µg/g creatinine, mild effects can occur in sensitive individuals but it seems more difficult to observe symptoms.

A recent metallic mercury spill in the road from the Yanacocha mine, Peru resulted in health problems for the local people who collected, handled, and eventually burned mercury in their homes, hoping to find gold associated with the Hg. Pulmonary problems and dermatitis due to high levels of mercury vapor exposure were the main symptoms reported by the local health post (CAO, 2000).

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 and were treated with chelating agents (Malm et al., 1995a). Schulz-Garban (1995) in a study of 20 amalgamation workers in Venezuela detected that 8 individuals with high mercury levels in urine, exceeding 50 µg/L and 4 of them with 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 creatinine9;
2. HBM II comparable to LOAEL (lowest observed adverse effect level): blood: 15µg/L and urine: 20 µg/g creatinine10.

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 high Hg levels in urine (median 4.1, max 76.4 µ/L) relative to an outside control group (median 1.7, max. 7.6 µg/L). Miners with median levels of 11 and max 294.2 µ/L showed classical symptoms of mercury intoxication such as tremor, 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 vapors because the houses in which they live were also sites where amalgamation and amalgam burning were being carried out. The authors diagnosed a chronic mercury intoxication based on high blood/urine and/or hair mercury levels 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

9 this is around 7 µg/L of Hg in urine
10 this is around 25 µg/L of Hg in urine
intoxication was 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 distributed in other body tissues was concentrated in the kidneys to be eliminated via urine (up to 86 times than before treatment). After a short time treatment significant improvements in some symptoms were detected by medical examination. Through chemical speciation of Hg in hair, chronic inorganic mercury intoxication was found in the mining area whereas downstream from the mine site higher percentage of MeHg intoxication was found (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 or the treatment must be performed in more than one cycle to guarantee the excretion of Hg from other tissues. The necessary reduction of exposure was not possible in the area.

Mercury contamination in Tanzania due to ASM was recently reviewed by Mutakyahwa (2002). The author estimates that from 1991 to 1995 more than 20 tonnes of mercury was emitted into the environment, in particular around Lake Victoria and Lake Tanganyika. This is reflected in the high concentration of mercury in urine of miners burning amalgam in open crucibles (130-410 µg/L). Harada et al (1999) clinically examined 118 gold miners working 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 objective symptom, the authors found that 13.6% of the examined miners had gingivitis. Other objectives symptoms found in this study were: sensory disturbances and tremors in 8.5% of the miners, decrease in tendon reflex (in 5.1%), neurasthenia (in 3.4%), night blindness (in 3.4%), and hyperreflexia (in 2.5%).
2.2. Health Effects Caused by Methylmercury

In many rural communities fish is the primary, inexpensive, and sometimes the only source of animal protein. When contaminated fish is consumed, methylmercury is the main form to be transferred to human beings. Methylmercury is easily bioaccumulated and biomagnified and becomes concentrated in fish, particularly in carnivorous fish. These species are usually the preferred species of consumption by most people and represent the main exposure pathway of MeHg to humans.

Methylmercury (CH$_3$Hg) poisoning was first identified in the early 50s by an infamous incident at Minamata Bay, Japan, in which a plastic factory discharged about 82 tonnes of mercury (mostly already methylated) into the river and bay. Up to March 1997, the number of patients officially recognized as Minamata disease victims was 10,353. Of these, almost 3,000 victims have been compensated (Osame and Takizawa, 2001; SSSGMD, 2001).

Organomercurials such as methylmercury are more available for intestinal absorption (> 90% in mice). These pass into the blood stream and are distributed throughout the tissues. Kidney accumulation is lower than with inorganic Hg compounds, but the brain is affected significantly. According to Dr. H. Akagi from the National Institute of Minamata Disease, Japan (personal communication), in order to characterize "Minamata disease", i.e. poisoning by high levels of methylmercury, an individual must exhibit five typical symptoms:

2. Numbness of the extremities.
3. Impairment of hearing.
4. Impairment of speech.
5. Impairment of gait.

The first two symptoms are strongly indicative of the beginning of the illness. Muscular atrophy and mental disturbance are prominent in acute intoxication. Some cases of long-term effects of mercury 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 not confirmed neurological symptoms at the first examination (Suzuki, 1979).

As typical symptoms of Minamata Disease, Tokuomi (1960), studying patients from Minamata, reported that sensory disturbance and constriction of the visual field were observed among 100% of individuals, coordination disturbance among 93.5%, dysarthria among 88.2%, hearing disturbance among 85.3% and tremor among 75.8%. The symptoms of methylmercury poisoning are very variable as a combination of various symptoms with various degrees can occur depending on the individual and the level of poisoning.

Thousands of cases of MeHg poisoning were documented in Minamata as well as Niigata in Japan. Adults who depended on these 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. Neurological disorders and death
observed in these tragic incidents were results of consumption of high levels of MeHg.
At lower levels of exposure, continued interference with biochemical and cellular processes potentially cause neurophysiological and psychological functions to undergo slow alterations, which in the early stages may go undetected 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 groups of exposed persons, with respect to control groups or to internal or external exposure parameters (Mergler, 2002).

The effect of MeHg on the human body in terms of the degree of contamination is thought to be as follows: When large doses\(^\text{11}\) 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).

MeHg can penetrate into the placental barrier transferring mercury 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 \textit{et al}, 1973).

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 on excretion of MeHg compiled by Nelson \textit{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 mercury is poorly absorbed by the gastrointestinal tract, i.e. the majority is flushed out of the organism (WHO, 1991).

The normal Hg level in hair is less than 1-2 ppm (WHO, 1991). 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). 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. 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. Recent evaluation considers 5 ppm Hg in hair a safety guideline for pregnant women (Yagev, 2002).

\(^{11}\) 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 \textit{et al}, 1971).
Akagi et al (1995) reported that after clinical examination, no typical Minamata Disease symptoms (all five described above) have been identified in the ASM impacted areas in the Brazilian Amazon. However, many authors described neurological effects.

Studying fish-eating inhabitants in the Tapajós River, Brazil, Grandjean et al (1999) noticed neuropsychological dysfunctions in children whose mothers had less than 10 ppm mercury in hair. The authors also found that more than 80% of 246 children studied had mercury levels in hair above 10 ppm which was believed to cause adverse effects on brain development. Also in the Tapajós region, Harada et al (2001) examined clinically 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 as well as disturbance in balance and coordination, tremors, hyperreflexia, and dysarthria (difficulty in articulating words) in about 10% of the examined individuals. The authors have described three individuals out of fifty with symptoms of “mild Minamata disease.” With Hg in hair ranging from 16 to 71.5 ppm those individuals used to consume large amounts of fish (as much as 1,000 g per day). They failed in performing some neuro-physiological tests such as two-point discrimination, finger-to-nose, knee-to-knee and exhibited glove-and-stocking type sensory disturbance, tremors, 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 mercury 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 mercury levels in hair. They confirmed previous remarks by WHO (1990) that 50 ppm of total mercury 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.
2.3. Guidelines and Reference Doses

The guideline\textsuperscript{12} 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 \textit{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 guideline is enforced by the Canadian Food Inspection Agency (CFIA) and is intended to regulate commercial sales of fish. Canned tuna is frequently consumed and is subject to inspection and enforcement of the 0.5 ppm guideline. The use of smaller, younger tuna in the canning process makes it possible for mercury levels in canned tuna to fall within this guideline concentration (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 does 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, 1991) 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 TDI level established in Canada in 1998 is 0.47 µg/kg body weight for adults and 0.2 µg/kg bw for children and women of reproductive age. In 1989, the US EPA had a daily methylmercury reference dose of 0.3 µg/kg bw and in 1995 this was revised to 0.1 µg/kg bw (UNEP, 2002).

\textsuperscript{12} 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 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, 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 market shopkeepers 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 and 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, however, either due to high prices or the difficulty in capturing them. In the Amazon region, the frequency and type of fish consumed also varies 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 to follow 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 facility is not available or for screening purposes (Yallouz, 2001). 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 mercury 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, insomina, and disturbance in taste (metallic taste). As more objective symptoms the authors listed those investigated in specific tests. Some of them are: sensory disturbance (glove-and-stocking type), disturbance in balance, disturbance in coordination, tremor, 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, 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 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.

Before starting the field assessment, questionnaires must be translated into the regional/national language. 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 mercury exposure by vapor and a simplified diet advisory. Some formalities must be observed:

1. The volunteers must be informed that all personal information (especially identity) will be kept confidential.
2. They must be instructed about the goals of the assessment.
3. They must be informed that the Health Assessment will follow ethical procedures recommended by the Code of Conduct of the World Health Organization.
4. They will receive all results of the analyses of biomonitoring materials and will be clearly informed about their health situation and any possibility of being intoxicated by mercury and how (this is a job to be conducted together with local health authorities).
5. When high certainty about mercury intoxication exists, they will be informed about available ways to reduce exposure.
6. In areas of high incidence of tropical diseases, malaria or tuberculosis incidence, it is suggested to test and recommend therapy for these treatable diseases.
7. 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.

The importance of a well-designed Health Assessment is stressed by Wyzga and Yager (1999). The authors compared two studies, one in Seychelles Islands and the other in the Faroe Islands, undertaken to understand the health risks associated with fish consumption. Beside the fact that both studies were well conducted, the Seychelles study could not find any statistically significant association between MeHg ingestion and several developmental tests. The study in the Faroe Islands found a clear relationship and reported decrements in neuropsychological test performance relate to those children exposed in utero to methylmercury. The authors explained that the Seychelles study examined children at 6, 19, 29, and 66 months with intention to evaluate development effects with age caused by MeHg ingested by their mothers. The authors commented that it is possible that effects may not be easily observed at younger ages since children develop more rapidly when they are young.

The 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 child-bearing age are the group most sensitive to mercury poisoning, the Health Assessment can be designed to evaluate this specific group (Boischio and Cernichiari, 1998; Boischio and Henshel, 1996). Lebel et al (1998) evaluating effects of MeHg in a riparian community in the Amazon, selected a group for medical examination based on hair measurements, age (range of 10 years), gender and education level. It was observed that mercury in hair of men and women decreases with age, being higher in the group with ages from 15-24.

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
mercury level in maternal milk was around 6 ppb with values as high as 24.8 ppb Hg. Correlation analysis revealed that mercury in hair was significantly affected by maternal MeHg ingestion during pregnancy, but not during the post-natal breast feeding period and the Hg levels in milk does not correlate with mother’s or infant’s hair (Barbosa and Dorea, 1998).

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).

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 previously applied to a large number of people in a community to select just the individuals at higher risk of being exposed to mercury vapor 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, and then those clusters are compared to each other. This is useful method when large variation on habits and living conditions occur within a community (IPCS, 2000).

In the Global Mercury Project the random sampling approach is preferred. 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 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.

In the Appendix, there is an example of a questionnaire and the content of a medical exam developed by Dr. Stephan Boese O’Reilly of the Institute for Forensic Medicine, Ludwig-Maximilians University in Munich, Germany. The questionnaire is divided into the following parts:

1. Personal data.
2. General questions related to:
   - occupational exposure to mercury (routes of exposure)
   - diet issues (frequency and type of food)
   - health problems (subjective, based on symptoms described by the patient)
   - alcohol ingestion habits (frequency and type; confounder)
   - other confounding factors
3. Specific health questions related to mercurialism (metallic taste, salivation, etc.).
4. Physical examination (blood pressure, signs of gingivitis, tremors, reflexes, etc.).
5. Specific neuropsychological tests (memory, coordination, etc.).

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

**In the Physical 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 dysarthria.
- 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 to test 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 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 and 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, first of all because regions with high concentration of artisanal miners also have high alcohol consumption, and secondly, due to cultural reasons, it is hard to obtain reliable information about the amount of alcohol ingested by an individual. Alcohol can influence or bias results of medical and neurophysiological tests. There are possibilities for toxico-kinetic as well as toxico-dynamic interactions between alcohol and mercury. Ethanol as an inhibitor of the enzyme catalase, reduces oxidation of mercury vapor into ionic mercury 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-mercury 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 mercury 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 low MeHg:total Hg ratio as most gold miners and residents are subjected to high burden of Hg vapor 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.

2.5.3. Data Management

Data management consists of two basic processes: 1) to represent the data quantitatively; and 2) to accumulate knowledge on health effects caused by mercury poisoning.
Once the information is obtained from questionnaires and chemical analyses, statistical methods must be employed to validate the findings. IPCS (2000) published a very comprehensive book on Human Exposure Assessment where statistical considerations for data management are extensively discussed. 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, characteristic 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 monitor 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 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). The 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.

When considering ranking or scoring methods, many procedures do not discriminate the importance of an observation over another. The question is: is discoloration of the gums more significant as a symptom of undue exposure to Hg vapor than a chronic headache or 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 is only considered in specific rules are 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 mercury 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 to employ human analysis to provide an approximate and yet effective means to describe behaviour of 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 one adopted by Rosenblatt in 1957 (Minsky and Papert, 1969) in his famous Perceptron system, an expert system 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 (1995) 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\text{conclusion}) in a conclusion (e.g. Hg poisoning is occurring) combining the importance of each factor (W\text{f}) with the Degree of Belief (DoB\text{i}), given by the interviewer (e.g. nurse or doctor), on the intensity or frequency that the factor (e.g. symptom) is occurring.:

\[
\text{DoB}_{\text{conclusion}} = \text{MIN} (100, \sum_{i=1}^{n} \text{DoB}_i \times W_i)
\]

This procedure is very easily adapted and transparent. The method was also designed to accommodate cultural, and socio-economic and political differences from one region to another (Veiga and Meech, 1994).

Table 9 shows a hypothetical example of results of a medical evaluation and the Degree of Belief in the conclusion (in this case “mercury intoxication is likely and biomonitoring materials must be collected from this individual”). In this hypothetical example, the experts, in consensus before starting the Health Assessment, have agreed with the importance (W\text{f}) of all factors 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. 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% 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, the conclusion on Hg intoxication has 80% of certainty and this can be checked by analysis of biomonitoring materials from this individual. Naturally, this conclusion should be compared with other pieces of information from the socio-economic-demographic questionnaire and neuropsychological tests. A similar process can be used to accumulated knowledge when pieces of information from other questionnaires or tests are combined.
Table 9. Hypothetical example of knowledge accumulation using weighted inference method.

<table>
<thead>
<tr>
<th>Factor/evidence</th>
<th>Intensity and/or Frequency of the evidence (DoB&lt;sub&gt;i&lt;/sub&gt; (%))</th>
<th>Importance of the evidence (W&lt;sub&gt;i&lt;/sub&gt;) from -1 to 1</th>
<th>DoB&lt;sub&gt;i&lt;/sub&gt; x W&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>discoloration of gums</td>
<td>50</td>
<td>0.5</td>
<td>25</td>
</tr>
<tr>
<td>salivation</td>
<td>80</td>
<td>0.2</td>
<td>16</td>
</tr>
<tr>
<td>hearing problems</td>
<td>40</td>
<td>0.3</td>
<td>12</td>
</tr>
<tr>
<td>visual constriction</td>
<td>20</td>
<td>0.6</td>
<td>12</td>
</tr>
<tr>
<td>tongue tremor</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>recent malaria</td>
<td>70</td>
<td>-0.5</td>
<td>-35</td>
</tr>
<tr>
<td><strong>Total (DoB&lt;sub&gt;conclusion&lt;/sub&gt;)</strong></td>
<td></td>
<td></td>
<td><strong>80</strong></td>
</tr>
</tbody>
</table>
2.6. 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 following the cohorts obtained in the socio-economic-demographic study.
4. Inform volunteers about the purpose of the assessment and instruct them.
5. Apply criteria to form groups or clusters (age, gender, education, occupation, fish consumption, proximity of the Hg vapor source, etc.).
6. Apply general (work, diet, health history and possible confounders) questionnaire.
7. Apply specific health questionnaires related to Hg poisoning.
8. Apply physical neurological (medical) exam.
9. Select volunteers with suspicion of MeHg or Hg vapor exposure.
10. Apply simple neuropsychological tests (Wechsler Memory test, Match Box test, Finger Tapping, Frostig test, Visual Field test, etc.).
11. Collect biomonitoring samples: hair, urine and/or blood.
12. Apply knowledge accumulation process (scoring) using clinical + biomonitoring sample results.
13. Re-examine those individuals with high scoring.
14. 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.).
15. 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 to avoid control groups with history of high ingestion rates of fish.
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http://www.motherisk.org/updates/oct02.php3


Appendix 1

**Example of Health Assessment Questionnaire**

by Dr. Stephan Boese O’Reilly, Prof. Dr. Gustav Drasch, Stefan Maydl, Dr. Milan Vosko  
Institute for Forensic Medicine, Ludwig-Maximilians University, Munich, Germany.  
and  
Dr. Claude Casellas and Dr. André Rambaud,  
Dept. Sciences de l’Environment et Sante Publique, Faculté de Pharmacie  
Université de Montpellier, France

**Removal of Barriers to the Introduction of Cleaner Artisanal Gold Mining and Extraction Technologies**

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

**Health Assessment**

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. Personal Data

Participant ID Number: ______________________

Family Name: ....................................................................................................

First Name: ....................................................................................................

Date of Birth: ........................................Age:............(years)

Gender: ____ Female              ____ Male

Address:  .................................................................................................................

..............................................................................................................................

Any telephone for contact: ..........................................................................................

2. General Questionnaire

Date of interview:.............................

Name of the interviewer:............................Code of the interviewer ___________

2.1. Work Exposure

How long have you been living in this area?      ______ year(s)

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
___ School child (not working)
___ Other job..................................................................................................

Have you ever worked in the ___________________________  area?

0 ____  No
1 ____  Yes

If yes, for how many _______ year(s)?

Have you ever worked as a miner with direct contact with mercury?

0 ____  No
1 ____  Yes

If from when to when: ___________________________________________

Have you ever worked burning amalgam in open pans or melting gold in inadequate fume

hoods?

0 ____  No
1 ____  Yes

If yes, from when to when: __________________________________________

Have you ever used a retort?

0 ____  Yes, when_________________ and which type _____________________
1 ____  No
Have you stored mercury containers or flasks?
0  ____ Never
1  ____ At work
2  ____ At home

Have you kept your dirty working clothes at your home?
0  ____ No
1  ____ Yes

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

### 2.2. 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 in the meal the interviewer estimate the approximate mass of fish consumed:

_______ grams (___ 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)

<table>
<thead>
<tr>
<th>Fish Name</th>
<th>Species</th>
<th>% (dry season)</th>
<th>% (wet season)</th>
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</table>

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 impacted by mining

Can you name the river and local where you catch most fish you have consumed?
____ No
____ Yes, the river (or lake or pool) is ______________________________
Has this river (or water body) dark water (Coca-cola color)?
0 _____ don’t know the origin of the fish (buy in the market)
1 _____ Yes, mild
2 _____ Yes, very dark

Name the place where you obtain drinking water:
______________________________________________

Do you consume from local production 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 from local production meat (> 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 from local production vegetables, fruits?
0 ____ Never
1 ____ At least once a month
2 ____ At least once a week
3 ____ At least once a day

2.3. Confounders

Have you ever had any neurological disorders (epilepsy, stroke, Parkinson, 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 many time ago you had your last malaria? _______ (days or months or weeks)

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

Have you been constantly handling gasoline and kerosene? (this can develop tremors)
0 ____ No
1 ____ Yes
If yes, how many years you have been doing this? ______ (years)

Have you been constantly handling insecticides or pesticides?
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 ___ Medium (10-20 cigarettes per day)
3 ___ Lots (more than 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

Do you or did you suffer from Leprosy?
0 ___ No
1 ___ Yes

Have you ever used whitening soap (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 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)? ____________________________

Did you have any serious accidents (did you have to go to hospital)?
0 ___ No
1 ___ Yes, but not severe
2 ___ Yes, and it was severe (more than 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)
Exclusion criteria from statistical evaluation
Severe neurological disease such as Parkinson, stroke, severe accident (brain injury), birth trauma, tetanus, polio, hyperthyroidism, epilepsy, malaria or any acute severe disease, etc. may introduce too many factors that confound with Hg intoxication symptoms.

Based on the confounders, should this individual be excluded from the Health Assessment?

To be filled in by project doctor.

____ No
____ Yes

Why this individual should be excluded from the assessment:
______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

3. Health Questionnaire

Date of interview:.............................................

Name of the interviewer:....................................…Code of the interviewer ___________

Do you feel a kind of a metallic taste?
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)

Did you loose weight within the last year?
0 ____ No
1 ____ Yes

Did you loose hair within the last year?
0 ____ No or only rarely
1 ____ Yes, slight to moderate
2 ____ Yes, marked to sever

Have you been coughing within the last year for more then for 3 month?
0 ____ No
1 ____ Yes
Have you ever had kidney disease except 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 actual or former health problem worsened since exposure to mercury occurred?
0 ____ No mercury exposure
1 ____ Mercury exposure, but no worsening effects
2 ____ Yes, mercury exposure and worsening

TREMORS
Have you had any problems with tremor (shaking)?
(Clinical Tremor Rating Scale)
0 ____ I have no tremor or tremor does not interfere with my job
1 ____ I am able to 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 tremor
3 ____ I am unable to do a regular job, I may have changed to a different job due to tremor; 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 the state of fatigue (Wessely S, Powell R: Fatigue syndrome)

Have you got 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

Can you no longer start anything?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you always 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: __________ score sum**

**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 to find correct words when you speak?
0 ____ Same as usual
1 ____ Worse then usual
2 ____ Much worse than usual

Do you have problems with eyestrain?
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: __________ score sum**

**WELL BEING**

Do you feel nervous?
0 ____ Never
1 ____ at least once a **month**
2 ____ at least once a **week**
3. How is your current sexual life? (for men)
   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 have a headache?
   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, aching at any location of 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

4. Clinical-Neurological Examination
   Date of neurological examination:.................................................................

   Name of the neurological examiner:...................................................Code ____________

   Weight and Height
   Weight: ______ Kg
   Height:______ cm
   Blood pressure:_________/__________ mmHg

   **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  
(n) ___ 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 p 435)  
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 and rates  
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, 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.

**Mentalabial 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

**PSR (quadrizeps reflex)**
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 heel the knee of the other leg. Then to move with 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 heel-to-shin 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 there you observed an hypo mimic expression of the face during the examination) -t

0 ____ Absent  
1 ____ Present
5. Specific Tests

Date of the test: .................................................................................................

Name of the tester: ............................................................................................ Code ___________

Memory Disturbances: (different memory tests can be used)

**Forward** digit span test (part of *Wechsler Memory Scale*)
Please repeat each column of numbers. Score longest series correctly repeated forward

<table>
<thead>
<tr>
<th>Score</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6-4-3-9</td>
</tr>
<tr>
<td>4</td>
<td>7-2-8-6</td>
</tr>
<tr>
<td>3</td>
<td>4-2-7-3-1</td>
</tr>
<tr>
<td>3</td>
<td>7-5-8-3-6</td>
</tr>
<tr>
<td>2</td>
<td>6-1-9-4-7-3</td>
</tr>
<tr>
<td>2</td>
<td>3-9-2-4-8-7</td>
</tr>
<tr>
<td>1</td>
<td>5-9-1-7-4-2-3</td>
</tr>
<tr>
<td>1</td>
<td>4-1-7-9-3-8-6</td>
</tr>
<tr>
<td>0</td>
<td>5-8-1-9-2-6-4-7</td>
</tr>
<tr>
<td>0</td>
<td>3-8-2-9-5-1-7-4</td>
</tr>
</tbody>
</table>

**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

**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**.

______ points
**Frostig Score**

Draw a line from one symbol to the other. Do not interrupt while drawing. Do not touch the lines.

Score: _____

Please connect with a pencil the symbols. Please try to stay within the lines. ??

---

**F1**

---

0-2

---

**F2**

---

0-2

---

**F3**

---

0-2

---

**F4**

---

0-1
Please connect the symbols with a straight line.
MEMORY DISTURBANCES (new battery of tests):

**Orientation to time** - season:
0 ___ correct response
1 ___ incorrect response

**Orientation to time** - part of the day:
0 ___ correct response
1 ___ incorrect response

**Orientation to place** - name of the village
0 ___ correct response
1 ___ incorrect response

**Orientation to place** - name of the country:
0 ___ correct response
1 ___ incorrect response

**Episodic memory (registration of 3 words):** example: Fish, Ball, Tree
0 ___ Registered all 3
1 ___ Registered just 2
2 ___ Registered just 1
3 ___ Registered none

**Dexterity and Coordination**
Copy figures
Copy two five-sides figures
6. Specimens

Date of the specimen: .................................................................

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: ____
7. Laboratory Analysis Results

<table>
<thead>
<tr>
<th>Material/test</th>
<th>Result</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mercury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylmercury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mercury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl mercury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mercury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl mercury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others (saliva, nails, breast milk, feces...)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments:
8. Medical Score Sum

<table>
<thead>
<tr>
<th>Test</th>
<th>Score Points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anamnestic data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metallic taste</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Excessive salivation</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Tremor at work</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Sleeping problems at night</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Health problems worsened since Hg exposed</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bluish coloration of gingiva</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Ataxia of gait</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Finger to nose tremor</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Dysdiadochokinesis</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Heel to knee ataxia</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Heel to knee tremor</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Mento labial reflex</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neuropsychological tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory test</td>
<td>0/1/2</td>
<td></td>
</tr>
<tr>
<td>Matchbox test</td>
<td>0/1/2</td>
<td></td>
</tr>
<tr>
<td>Frostig test</td>
<td>0/1/2</td>
<td></td>
</tr>
<tr>
<td>Tapping test</td>
<td>0/1/2</td>
<td></td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Medical score sum ____________

13 Proteinuria 1 = more then trace, 0 = 0 or trace (correctness of this borderline needs to be checked, same test material should be used, eg.)

14 Memory test: 2 = score 0, 1 = score 1-2, 0 = score 3-4

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

16 Frostig test: 2 = 0-9 correct answers, 1 = 10-12 correct answers, 0 = 13-16 correct answers

17 Tapping test: 2 = 0-53 dots, 1= 54-64 dots, 0 = 65 or more dots
9. Decision for the diagnosis of “chronic mercury intoxication”

Threshold limits for mercury

<table>
<thead>
<tr>
<th></th>
<th>Hg-blood (µg/L)</th>
<th>Hg-urine (µg/L)</th>
<th>Hg-urine (µg/g creatinine)</th>
<th>Hg-hair (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBM I</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>HBM II</td>
<td>15</td>
<td>25</td>
<td>20</td>
<td>5 (in analogy)</td>
</tr>
<tr>
<td>WHO</td>
<td></td>
<td>50</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>BAT for metallic and inorganic Hg</td>
<td>25</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAT for organic Hg</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEI (Biological exposure index)</td>
<td>15 (after working)</td>
<td>35 (before working)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table: 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 a “chronic mercury intoxication”

<table>
<thead>
<tr>
<th></th>
<th>Medical Score Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 – 4</td>
</tr>
<tr>
<td>Hg in all biomonitors</td>
<td>&lt; HBM I</td>
</tr>
<tr>
<td></td>
<td>&gt; HBM I</td>
</tr>
<tr>
<td>Hg at least in one biomonitor</td>
<td>&gt; HBM II</td>
</tr>
<tr>
<td></td>
<td>&gt; BAT</td>
</tr>
</tbody>
</table>

Table: Decision for the diagnosis “chronic mercury intoxication”

Intoxication

________ no

________ yes
Appendix 2

**Example of a Socio-Economic-Demographic Questionnaire**

by Mrs. Susan Wagner, Dar Es Salaam, Tanzania

STUCTURED 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) Separate

Q4. Number of children:

<table>
<thead>
<tr>
<th>Sex</th>
<th>F</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Q5. What is the highest level of education achieved of:

<table>
<thead>
<tr>
<th>Father</th>
<th>Mother</th>
<th>Respondent</th>
<th>Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.</td>
<td>3.</td>
<td>4.</td>
</tr>
<tr>
<td>5.</td>
<td>6.</td>
<td>7.</td>
<td>8.</td>
</tr>
</tbody>
</table>

01. Illiterate, 02. Primary education, 03. Secondary Level, 04. University 06 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 economic activities are you doing?
   ..............................................................
   ..............................................................

Q12. On average, where does your monthly income stand?
   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, what is your expenditure on the following per month?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Food</td>
<td></td>
</tr>
<tr>
<td>b) Water</td>
<td></td>
</tr>
<tr>
<td>c) Rent</td>
<td></td>
</tr>
</tbody>
</table>
d) Health

e) School fees

f) Clothing

g) Transport

h) Energy

i) Servants

j) Others (specify)

Total expenditure

Q14. What is the source of 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:

<table>
<thead>
<tr>
<th>Activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Domestic use</td>
<td></td>
</tr>
<tr>
<td>b) Washing/bathing</td>
<td></td>
</tr>
<tr>
<td>c) Mining/sluicing</td>
<td></td>
</tr>
<tr>
<td>d) Irrigation</td>
<td></td>
</tr>
<tr>
<td>e) Livestock</td>
<td></td>
</tr>
<tr>
<td>f) Others, specify</td>
<td></td>
</tr>
</tbody>
</table>

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)

Distance to the water source:  
a) <0.5 hrs  
b) 0.5- 1 hr  
c) 1-2 hrs  
d) 3-4 hrs  
e) > 4 hrs

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
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? 

Q21. When did you start? .................

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? .............................................................................

Q24. What kind of support you are getting from the association?

......................................................................................

Q25. Who are employed/working in the enterprise?

<table>
<thead>
<tr>
<th>Self</th>
<th>Spouse</th>
<th>Children</th>
<th>Other dependants</th>
<th>Casual Employment</th>
<th>Permanent Employment</th>
<th>Seasonal Employment</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
</tbody>
</table>

Indicate in the boxes above how much they have been paid are paid in cash? Kind? Both.
E. EQUIPMENT AND INPUTS (MERCURY, TOOLS AND 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 encounter any problems in 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. Who informed you?
      ........................................................................................................................................
      ........................................................................................................................................
      ........................................................................................................................................
      ........................................................................................................................................

F. PROPERTIES OWNERSHIP

Q31. Indicate kinds of properties you have access to and control that means those you own outright by your name, specify amount or type where relevant.

<table>
<thead>
<tr>
<th>Properties with specifications</th>
<th>Access</th>
<th>Value</th>
<th>Control</th>
<th>Self</th>
<th>Spouse</th>
<th>Both</th>
<th>Others (specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold pit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livestock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm Equipment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milling Equipment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
G. DECISION MAKING ON INCOME AND EXPENDITURE

Q32. Who decide on 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 business? a)Yes b) No  
Explain:
Q37. How do you compare you present situation financially since 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. Any recommendations you have for improving training provision? …………
………………………………………………………………………………………….
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 are your comments incase of the introduction of proposed 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 do you expect if you might encounter in the change over?

Thanks for your cooperation, do you have any questions?
Appendix 3

**Example of a Socio-Economic-Demographic Questionnaire**

by Earth System Lao, Vientiane, Lao PDR

---

**Introduction:**

The purpose of this Study is to conduct a survey of mining practices along the Nam Khong and Nam Ou rivers. This will involve a village and household level survey to gather baseline socio-economic data and to describe the mining methods being used.

Request to speak to the person who knows best about the livelihood activities of the household. In most cases this is likely to be the head of the household. Where possible request that the interview is conducted with both the male and female head of the household.

Request the consent of the household to be interviewed

---

**Questionnaire ID No.:**

**Household ID No.:**

**Village Name:**

**District Name:**

**Date of survey:**

**Name of Principal Surveyor:**

**Name of Enumerator 1:**

**Name of Enumerator 2:**

**Responder (male):**  
First Name: ______________
Family Name: ______________

**Responder (female):**  
First Name: ______________
Family Name: ______________
## A1 PERSONAL INFORMATION

**For all persons**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Who is a member of this household?</strong></td>
<td>1</td>
<td>Head</td>
<td>2</td>
<td>Spouse</td>
<td>3</td>
<td>Son/Daughter</td>
<td>4</td>
<td>Parent</td>
<td>5</td>
<td>Other relative</td>
</tr>
<tr>
<td><strong>What is relationship to head of household?</strong></td>
<td>1</td>
<td>Male</td>
<td>2</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Is male or female?</strong></td>
<td>1</td>
<td>Male</td>
<td>2</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>What is age?</strong></td>
<td>1</td>
<td>Head</td>
<td>2</td>
<td>Spouse</td>
<td>3</td>
<td>Son/Daughter</td>
<td>4</td>
<td>Parent</td>
<td>5</td>
<td>Other relative</td>
</tr>
<tr>
<td><strong>How old?</strong></td>
<td>1</td>
<td>Number of years living in this village?</td>
<td>2</td>
<td>What is citizenship?</td>
<td>3</td>
<td>What is ethnic origin?</td>
<td>4</td>
<td>What is marital status?</td>
<td>5</td>
<td>What is religion?</td>
</tr>
<tr>
<td><strong>What is major sickness in the last 2 years?</strong></td>
<td>1</td>
<td>Never married</td>
<td>2</td>
<td>Married</td>
<td>3</td>
<td>Divorced/ separated</td>
<td>4</td>
<td>Widowed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### For persons aged 6 years and above

<table>
<thead>
<tr>
<th></th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Can read and write?</strong></td>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>Has ever attended school?</strong></td>
<td>1</td>
<td>Never</td>
<td>2</td>
<td>At school</td>
<td>3</td>
</tr>
<tr>
<td><strong>What is highest level of education completed?</strong></td>
<td>1</td>
<td>Number of years living in this village?</td>
<td>2</td>
<td>What is citizenship?</td>
<td>3</td>
</tr>
<tr>
<td><strong>What was main activity the last 12 months?</strong></td>
<td>1</td>
<td>Head</td>
<td>2</td>
<td>Spouse of head</td>
<td>3</td>
</tr>
<tr>
<td><strong>What was main occupation during the last 12 months?</strong></td>
<td>1</td>
<td>Head</td>
<td>2</td>
<td>Spouse of head</td>
<td>3</td>
</tr>
</tbody>
</table>

### For persons aged 10 years and above

<table>
<thead>
<tr>
<th></th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Can read and write?</strong></td>
<td>1</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td><strong>Has ever attended school?</strong></td>
<td>1</td>
<td>Never</td>
<td>2</td>
</tr>
<tr>
<td><strong>What is highest level of education completed?</strong></td>
<td>1</td>
<td>Number of years living in this village?</td>
<td>2</td>
</tr>
<tr>
<td><strong>What was main activity the last 12 months?</strong></td>
<td>1</td>
<td>Head</td>
<td>2</td>
</tr>
<tr>
<td><strong>What was main occupation during the last 12 months?</strong></td>
<td>1</td>
<td>Head</td>
<td>2</td>
</tr>
</tbody>
</table>

## A2 SOCIO-ECONOMIC INFORMATION

### 16 What is the approximate average annual income of your household?

- [ ] 0 - 2M Kip
- [ ] 2M Kip to 5M Kip
- [ ] 5M - 10M Kip
- [ ] > 10M Kip

### 17 Who in your household manages the income?

- [ ] Head
- [ ] Spouse of head
- [ ] Son/Daughter of head
- [ ] Other

### 18 Who in your family manages the expenditure?

- [ ] Head
- [ ] Spouse of head
- [ ] Son/Daughter of head
- [ ] Other

## A3 HOUSING CHARACTERISTICS

### 19 What is the tenure status of the household?

- [ ] Owner / purchaser
- [ ] Tenant
- [ ] Lodger
- [ ] Other

### 20 Type of dwelling unit?

- [ ] Concrete
- [ ] Timber
- [ ] Bamboo
- [ ] Other (specify):
21 Is the dwelling unit electrified?  
- No  
- Yes (own meter)  
- Yes (own generator)  
- Yes (share meter)  
- Yes (car battery)

22 What is the household's main source of energy for cooking?  
- Electricity  
- Paraffin  
- Charcoal  
- Gas  
- Wood  
- Coal  
- Sawdust  
- Other

23 What is the living area of the dwelling unit?  

m²

<Mark the location of the dwelling on the village map - include Household ID No.>

### A4 WATER FOR DRINKING AND COOKING

24 What is the household's main source of water for drinking and cooking?  
- Piped water in/outside  
- Well/borehole  
- River/stream/dam  
- Rainwater from tank/jar  
- Other (specify):

25 Distance from house to the main source of water for drinking and cooking?  

m

<Mark the location of the water source on the village map>

26 Is drinking water treated before use?  
- Yes  
- No

If so how?  
- Boiled  
- Filtered  
- Other (specify)

27 Are you satisfied with the quality of your drinking water?  
- Yes  
- No

If no, why not?

28 Who most commonly collects the drinking / cooking water in your household?  
- Head  
- Spouse of head  
- Son/Daughter of head  
- Other
### A5 SOURCES OF FOOD

For each of the following food groups identify:

(i) The number of meals over the past 7 days when this food group has been eaten;

(ii) The source of the food.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>No. Times</th>
<th>Source (tick the appropriate boxes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red meat</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family livestock</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Chicken / duck</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family livestock</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family livestock</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Garden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swidden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Garden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paddy field</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swidden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td>Other aquatic food</td>
<td></td>
<td>Fishpond</td>
</tr>
<tr>
<td></td>
<td></td>
<td>River</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>Market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family livestock</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest</td>
</tr>
</tbody>
</table>

### A6 DEATHS IN THE HOUSEHOLD AND HYGIENE

Did any death occur in the household in the last 12 months? (also children at birth)

- Yes
- No

If Yes:

- Was the deceased male or female?
  - 1 Male
  - 2 Female
- How old was the deceased?
  - Age in years
- For woman aged 15 to 49 years: Did she die while pregnant, while giving birth or within 42 days after giving birth?
  - 1 Yes
  - 2 No

What type of toilet facility is mainly used by the household?

- Flush toilet
- Dry toilet
- Other
- None

Has anyone in your family been engaged in mining activities? (Either currently or previously)

- If yes, continue to PART B of the questionnaire
- If no, thank the respondent for their cooperation, and ask the respondent whether they would be prepared to participate in a follow-up health survey at a later date?
  - Yes
  - No

Additional observations of the Surveyor:
B.1 ARTISANAL MINING INFORMATION

1. How many years ago did you start mining? □ years

2. Do you continue to engage in mining activities each year? □ Yes □ No

2.1 If not, why did you stop mining?

3. Over what period of the year do you engage in mining activities?

3.1 On average how many hours per day do you spend mining? □ hours

4. Who inspired you to start mining? □ Yourself □ Partner □ Parent □ Other

5. When you are mining, do you work by yourself? □ Yes □ No

5.1 If not, how many people do you work with? □ Family □ Friends □ Labour

6. Where exactly do you conduct your mining activities?

B.2 EQUIPMENT AND INPUTS

7. Briefly outline each step in the gold extraction process, including: the technology/equipment; quantity of materials used; and time taken.

Collection of the ore:

Preparation of the ore:

Amalgamation:
Gold recovery:

8. Do you have any plans to change this process in the future? ☐ Yes ☐ No
   If so, how?

9. Have you ever used mercury for amalgamation of the gold? ☐ Past ☐ Present ☐ Never

10. Where do you buy your mercury?

10.1 From whom do you buy your mercury?

11. What is the average cost of the mercury per unit weight? ☐ per ml or; ☐ per kg

12. On average, how much mercury do you use per week? ☐ ml or; ☐ kg

13. On average, how much gold can be amalgamated with this quantity of mercury? ☐ grams

14. How do you store the mercury?

15. How frequently do you burn amalgam? ☐ Several times a day ☐ Once a day ☐ Once a week
   ☐ Several times a week ☐ Other

16. Do you bring your work clothes / equipment into the house at the end of the day? ☐ Yes ☐ No

17. What are the major problems you encounter when producing gold?

18. Are you aware of any health hazards associated with the use and handling of mercury? ☐ Yes ☐ No
   If yes, what are the hazards?
   Who informed you about these hazards?
B.3 MARKET

19. Where specifically do you sell your gold?

20. Do you encounter any problems when selling your gold?

21. What is the average market value of the gold you sell? __________ per gram

B.4 TRAINING

22. Have you received any training regarding your mining activities?  
   □ Yes    □ No
   If so, who provided the training?
   □ Yes    □ No
   Where was the training provided?

B.5 IMPROVED MINING TECHNOLOGIES

<Provide a short description of the improved mining technology>

23. Would you be interested to apply these methods to your mining activities?  
   □ Yes    □ No
   Explain why: ___________________________________________________________________

24. Would the introduction of these methods adversely affect your mining activities?  
   □ Yes    □ No
   Explain why: ___________________________________________________________________

Thank the respondent for their cooperation.

Would the respondent be prepared to participate in a follow up health survey at a later date?  
   □ Yes    □ No

Additional observations of the Surveyor: _____________________________________________  
_________________________________________________________________________________  
_________________________________________________________________________________  
_________________________________________________________________________________