Mercury Contamination in Prey Meas Goldmine, Cambodia
2007 Sampling
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Report to the Blacksmith Institute

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Abstract

In the last four years, the numbers of artisanal miners working around Prey Meas,
Ratanakirri, Cambodia has increased tenfold. All miners appeared to use mercury for the
first extraction of gold. Some miners used a second extraction with cyanide. Other
miners used a high concentration of mercury for a second extraction that extracts more
than 97% the gold. In spite of an efficient method, the high concentration of mercury
used (275,000 ng/g) resulted in considerable amounts of mercury in the tailings (8200
ng/g. Although the miners were concerned about the health risks of mercury, they had
not continued to use the retorts supplied to them a year ago. Washing with a detergent
and electron microscopic analysis indicated the mercury was not localized on the surface
of hair. A water filter that was provided prevented diarrhea effectively. No acid was
observed in the tailings. The acid volatile sulphide content of the ores was very low (4.4
ppm) so acid formation in the tailings was not a concern. Clams are good indicator of the
spatial distribution of mercury contamination. The higher concentration of total mercury
in clams nearest the goldmine reflects the mercury discharge from the mine and likely the
lower relative proportion of methylmercury in clams near the mine reflects suppression of
methylation by the mine effluents.

Introduction

Artisanal gold mining results in 12-23% of anthropogenic mercury emissions (Eisler
2003). The concern over the extraction of gold is a global problem but the local impact is
more serious. Once mine sites are contaminated, restoration is difficult. Moreover in
Cambodia, the rivers receiving mercury wastes are targeted to have hydroelectric dams
(Figure 1). Typically reservoir construction results in enhanced methylation of mercury,
increased bioaccumulation of mercury by as much as 10 fold. At the mines using
mercury, human health is impaired but not at the extremes associated with Minamata
disease. Because the general health of most rural people in the provinces of Cambodia is
poor, the anticipated low level of mercury toxicity is difficult to discriminate.

Gold mining in Cambodia is expanding both with increased artisanal mining and an
increased number of approvals for mine concessions from international mining firms.
The high price of gold and the successful development of mines in neighbouring
countries with similar geology are driving this development.   The first major review of
the gold mines in Cambodia outlined the problems but contained limited data (Sotham
2004). The deficiency of data reflects the lack of analytical services, isolation of the
mines in Cambodia and at times concerns about safety. Earlier we reported analyses of

goldmines in Cambodia and the intent of this report to resolve questions generated in the first two reports.

Methods

Site

The goldmine is in a dry forest about 48 km SE from Banlung (Figure 1). Miners use motorcycles to access the mine. Even when dry, the road access is extremely bad requiring a four-wheel drive vehicle with high clearance and a skilled driver.
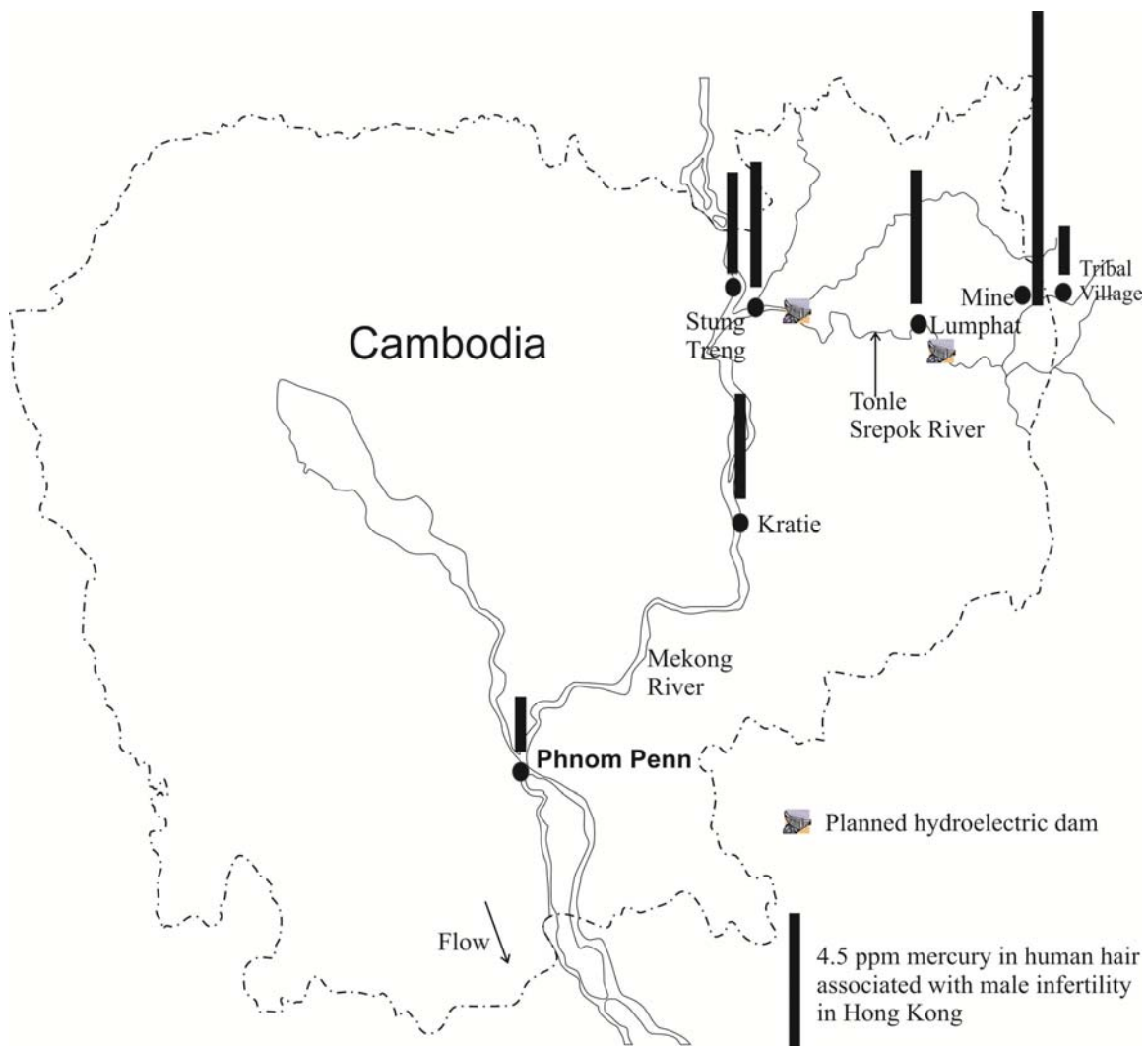

Figure 1 Location of Prey Meas Mine and Mean Mercury in Human Hair

Prey Meas Mine Complex
There are several discrepancies from our observations of the Prey Meas mines and those reported by the Oxfam team but some of the differences might reflect rapid expansion of the mine and the passage of time (Oxfam project sampled in Oct. 16-21, 2003). The number of miners is about 10 times higher than estimated in 2003. For example, in the earlier reports, the first mine that we sampled in the Prey Meas group of mines was called Prey Thmei mine. Now this mine is now commonly called Bay Ba. Prey Thmei means "new mine". We found a new mine called Prey Thmei that opened around March 2006. There are other differences in nomenclature and number of mines sampled in the Oxfam report varies from this report too. The Oxfam report said there were six active mines and one abandoned mine in the Prey Meas complex but it seems there are at least 10 active mines. The abandoned mine is now active again. The first essential aspect of the mines is their GPS location.

Prey Ba and Prey Thmei are the only mine sites that were sampled in this study.

Mine Names, April 2007
1) Bay Ba - 25 families, first site (13°31'22.9" N, 107°22'46.6" E)
2) Bay Bong
3) Bay Mok
4) Bay Hy
5) Phar Chas
6) Prey Marouse
7) Prey Thmei, opened around March 2006, (13° 32' 7.26" N, 107° 22' 15.36" W) 300 miners)
8) Prey Somroung
9) Phnom Pang, big, largest mine with 1000 to 2000 miners
10) Oh Kagna

The functional units of the mine complex are families who own the equipment. Families hire transient workers. The transient miners primarily work in the dry season but many of the families stay at the mines year round. There are about 400-500 families in the mine complex. Most families seem to have their own gold processing equipment so for first estimations the number of families could be used for the number of flumes used. The families have many children who have no access to school or medical services. Karaoke bars are common. The larger mines have shops selling various produce that is brought in by motorcycles.

The mines use shafts 30-35 m deep and about 1.2 m wide (Figure 2). At the bottom of the shafts are tunnels. The shafts are supported with minimal lumber. The shafts are partly dug by hand tools but explosives are used in the bedrock. Explosions were heard several times a day. Each family unit works independently of other mines and there is no coordination with other miners when an explosion takes place.

Figure 2 Mine shaft

Sampling

In 2007, Field trips to Ratanakirri were made Feb 19-21 and April 8-10th. GPS readings were done using a Garmin GPSMAP 60CSx.   We hired a four-wheel drive vehicle to drive to the sites.

Snakehead fish (Channa sp.) were collected from the Srepok River by fisherman and bought Feb. 21 at the market in Banlung. Fish were also collected April 9th at the junction of the Prey Meas Creek and Ou Tran River (13° 30' 57.3" N, 107° 26' .06" W). Subsamples of fish (5 g) were placed in plastic scintillation vials, frozen and shipped to Canada for analysis. Fish identification was done using the FAO Fishes of the Cambodian Mekong (Rainboth 1996).

Clams were collected at Lumphat in February 21, 2007 and April 9th, 2007 at Dol on the O Tran River (13° 31' 56.16" N, 107° 26' 40.80" W) and where the creek draining the Prey Meas mines enters the Ou Tran River. To get to the Dol River required a one-hour ride (6 km) on a motorcycle. The confluence of the Prey Meas Creek and Ou Tran River is 1.88 km downstream of the tribal village of Dol. We first used a small boat but rapids forced us to walk much of the way.   Samples were taken to Canada for analysis.

Clams and snails were held in fresh water for 24 hours to let their stomachs empty. Clams were weighed in Phnom Penh both with and without the shell. The complete body was removed from the shells, excess water was removed with adsorbent paper, frozen for shipment to Canada, and freeze dried in Canada to facilitate mixing prior to subsampling for mercury analysis. Samples that were processed for total and methylmercury were picked so that the weights of the clams between sites were similar. The clams at both sites appeared to be the same species. Snails (Pomacea) were much more difficult to process and their analysis was cancelled. Snails are also an alternative host for liver flukes (Schistosoma mekongi) so handing snails for an indicator species for mercury contamination has a greater health risk. Note that in Figure 3, there is a third type of shell that was too common – old munitions are a concern. Anyone working in this area should obtain the recent maps of the war records published by CIDA or look at the munitions maps in the following website: http://www.cambodiaatlas.com/map.

Figure 3 Shells collected at Lumphat

Mercury Analysis

For most mercury analysis, a DMA80 Direct Mercury Analyzer from Milestone was used in Environment Canada. Reference materials were processed with each batch of samples and the results were always within the range of expected values. The process is detailed in EPA Method 7473: Mercury in Solids and Solution by Thermal Decomposition Amalgamation and Atomic Absorption Spectrophotometry. This process is designated for the determination of total mercury in solids, aqueous samples and digested solutions. Solid and aqueous samples are dried and then thermally and chemically decomposed by controlled heating in an oxygenated decomposition furnace to liberate mercury. The decomposition products are carried by flowing oxygen to the catalytic section of the furnace where oxidation is completed and halogens and nitrogen/sulphur oxides are trapped. The remaining decomposition products are then

carried to an amalgamator that selectively traps mercury. After the system is purged with oxygen to remove any remaining residual by-products, the amalgamator is rapidly heated to release mercury vapour. The vapour flows through an atomic absorption spectrophotometer set at 253.7 nm to measure the concentration of mercury. Certified reference materials (CRM) are used for each set of analysis. Results are always within the standard deviation of the CRM. Relative standard deviations are typically around 3%. Blanks are run for each set of analyses and blank values are always much less than 0.1% of samples.

Samples for total and methylmercury in clams were analyzed by Flett Analytical Services in Winnipeg Manitoba, Canada.

Gold Extraction
0.2 g of crushed ore into glass scintillation vial
4 ml concentrated hydrochloric acid
1.3 ml concentrated nitric acid
Shake overnight.
Let settle 15 minutes
Decant and filter with GFC filter
Wash sediment with 5 ml 2N HCl
Make up to 50 ml in volumetric flasks with distilled water
Extracts were measured in a Varian Atomic Absorption Spectrometry using a graphite furnace (Elliot and Stever 1973 in Rowland 1982).

Sulphide analysis of ore
Ore samples that were first ground in the mines were further ground by hand in a laboratory in Canada with a mortar and pestle. Samples (0.5 g) were processed by the following procedure. More details are available in Brouwer and Murphy (1994).

Acid Volatile Sulphide
Under acidic conditions, the more reactive sulphides in sediment will be converted into H_2S, a gas at room temperature. The gas diffuses from the sediment suspension in acid into a sulphide antioxidant buffer trapping solution (SAOB) within the same container. H_2S is normally only slightly soluble in water, under acidic conditions its solubility decreases ever further, thus very little H_2S will remain in the aqueous phase. The SAOB solution contains sodium hydroxide (to react with the weakly acidic H_2S), ascorbic acid (to act as an antioxidant), and EDTA (to complex metals which may catalyze the oxidation of sulphides). Since the SAOB is a strong base, it will remove the H_2S from the headspace in the vial and convert the H_2S to S^{2-}. In addition, CO_2 produced from the sediments containing carbonates will also be removed by the SAOB solution; this does not interfere with the sulphide measurements. Nothing else in the sediment is likely to be transferred to the SAOB solution. The concentration of sulphide ion in the SAOB solution is measured using a sulphide ion selective electrode, which is standardized against known concentrations of sulphide in SAOB. Measurements are most conveniently and quickly made using a flow-through cell.

Hair washing experiment
Two large samples were collected from mine workers who work directly with mercury. The hair was cut into pieces 0.3 cm and mixed.

1-Control – no treatment
2- Wash with Triton-x (1%, non-ionic detergent, 10 ml, shake by hand for 1 minute), rinse with acetone
3- Wash with Triton-x (1%, non-ionic detergent, 10 ml, sonicate for 1 minute), rinse with acetone
4- Wash with Triton-x (1%, non-ionic detergent, 10 ml, let mix overnight on a shaker), rinse with acetone
5- Wash with Triton-x (1%, non-ionic detergent, 10 ml, let mix overnight on a shaker), rinse with acetone, repeat triton-x wash with sonication, rinse with acetone

Hair was also collected from goldsmiths in the Central Market of Phnom Penh.

Electron Microscopic Analysis
The intent of this analysis was to determine if we could detect surface contamination of mercury on hair and hopefully to discriminate from bioaccumulation via the body and blood.

All microscopic analysis was done at McMaster University, Canada. The thickness and nanoscale morphology of the layers were determined using transmission electron microscopy (TEM). All biological layers were stabilized and prepared for TEM analyses according to a fixation protocol designed to detect and help characterize nanoparticles deposited in or on membranes during experimental filtrations (Liss et al. 1996; Liao et al. 2002). Portions of each loaded membrane were prepared for ultrathin sectioning using a protocol which involved a primary fixation in glutaraldehyde plus ruthenium red, followed by subsequent processing and then embedding in a low-viscosity epoxy resin (see Liss et al. 1996 for details and the rationale for the overall approach). The ultrathin sections (80 nm) were cut with a diamond knife mounted in a Leica Ultracut UCT ultramicrotome (Leica Mikrosysteme, Wien, Austria). A comprehensive treatment of the use of TEM preparatory protocols and instrumentation, and the rationale for selecting a specific protocol for a specific research need is found in Mavrocordatos et al. (2007), accompanied by guidelines on how to detect, assess and minimize artefacts.

Ultrathin sections were observed and documented by a JEOL JEM 1200 EX TEMSCAN scanning/transmission electron microscope (JEOL, Peabody, MA, USA) operated at an accelerating voltage of 80 kV. On a "per particle" basis, an energy-dispersive x-ray analysis was used to identify individual elements (Chandler 1977) and to assess the relative abundances (Jackson & Leppard 2002) of pertinent elements ($Z>10$) detected in both virgin and experimental membranes. EDS spectra were obtained using a Tracor Northern X-ray detector (Noran, Madison, WI, USA) and EDS 2004 microanalysis software (IXRF Systems Inc., Houston, TX, USA).

With scanning/transmission electron microscopy, coupled to energy-dispersive spectroscopy (STEM-EDS), all morphological and spectromicroscopical analyses were based on a systematic search for representative images. Initially, searches of TEM images in many fields of view were made to document particle morphology for orientation purposes and to determine what might be candidate particles for STEM–EDS analyses. Then, for each of many ultrathin sections (taken from multiple blocks of embedded filters, sectioned at several levels within each block), a search was conducted, using both low (4,000x) and medium (15,000x) primary magnifications, to get an overview of the relative abundances of the most common particles present. This process was continued until all common morphotypes of conventional-sized particles became evident, with additional documentation done at 30 000× (for individual large colloids) and at 50 000× (for individual nanoscale colloids, or nanoparticles). Representative kinds of particles were thus defined after much observation, based on the relative abundances of specific individual morphotypes present. Individual particles showing complete or partial electron opacity were then selected at random from representative fields of view for spectromicroscopical analysis.

After initial EM analysis further spectromicroscopical analysis of miner's hair was done by STXM (scanning transmission **X-ray** microscopy) at a synchrotron. Instead of using an electron beam to eject X-rays for spectra of individual elements present, on a per particle basis (as is done with EDS at McMaster U.), a synchrotron uses an X-ray laser to cause atomic level changes in a visually selected part of the sample (providing spectra for individual chemical constituents, including organics).

Retort
Another retort was built with the intention of making the separation of mercury from gold faster. We continued to use a kitchen ware design.

Results

Mine Safety
The most chilling observation on the second field trip was a collapsed mineshaft at Prey Thmei (Figure 4). Fortunately, it happened at night when no one was down the shaft. The collapse was not triggered by an explosion or heavy rains. The shafts have too little support. The miners say that mineshaft collapses have happened when workers were down the shafts. A similar story was reported in a local newspaper Dec. 28, 2005 when four miners died in a mine collapse [http://thestar.com.my/news/story.asp?file=/2005/12/28/world/20051228203337&sec=world]. Because of the isolation of the mines, likely most such tragedies are not reported. These sudden deaths at the mines places any concerns about safety associated with mercury at a low priority to miners.

Figure 4 A collapsed mine shaft

The Prey Thmei mine had about 300 miners and was at least ten times the size of the Bay Ba mine. The miners at Prey Thmei use first mercury extraction with flumes followed by cyanide extraction. The maze of flumes and shafts at Prey Thmei is made more alarming by a variety of cyanide tanks with children and their pet dogs. Some of the cyanide is discharged to unlined pits in the ground.

In 2007, no attempts were made to sample groundwater. Earlier, we measured high levels of bacterial contamination but we could not detect any cyanide or mercury in the well water used at Prey Ba (Murphy et al. 2006b). The miners at Prey Ba had been provided with a ceramic filter from Resource Development International to remove bacteria. They have used the filter for a year; the diarrhea problem has gone. In 2007, miners at Prey Thmei complained of diarrhea being common and believed that mercury was the cause of their diarrhea.

Mine Processing
The miners said the new mine was the site of a new concession for an international firm and many people had visited the site.

Because of concern about the potential for waste tailings to produce acid, several samples of ore were evaluated for sulphide content. The acid volatile sulphide content of the ores

was low.   Miners talk about these ores having oxidized sulphide but such jargon is confusing. There is very little acid volatile sulphide (AVS, 4.5 ppm and 4.3 ppm in Prey Ba and Prey Thmei, respectively, table 1 and the concentration of other more acid resistant sulphides would be low. The AVS content of the two mines is not significantly different. In mines in many other countries, ores often have a high concentration of sulphide that oxidizes in the tailings forming sulphuric acid. The acid and associated dissolution of metals produces high toxicity. As expected from the AVS analysis, the pH of two mine tailings samples was 7.0.

With the intent of making a mercury mass balance, we measured mercury and gold at the top and bottom of flumes in mines in Prey Ba and Prey Thmei.   At Prey Thmei, samples were collected immediately after rocks were ground and applied to top of the flume and bottom of the flume. At Prey Thmei, the flume recovered about 72.7% of the gold. The concentration of mercury in the ore exiting the flume was about 992 ng/g. This is about 9% higher than in the ore applied to the ore. Some mercury must be lost from the flume but the difference is not statistically significant. At Prey Ba, were told that the sampled ore had already been processed once through the flume. This explanation is at least incomplete. The concentration of mercury in the ore at the top of the flume was 275,000 ng/g and 8212 ng/g as it exited the flume. The miners worked and lived near a pile of ore at least 1 m^{3}with 275,000 ng/g of mercury.

The flume was very efficient in adsorbing mercury and gold from this secondary ore (99.4 and 97.6%, respectively). The concentration of mercury in the processed ore (8212 ng/g) remained much higher than at Prey Thmei and is the same as detected in the spent tailings in this mine sampled in 2006 (7715 ng/g, Table 2). Note that the concentration of gold in the ore applied to the Prey Ba flume (35,900 ng/g) is about 10 times higher than found Prey Thmei or mines in similar geology in Laos (Wu 2004). It seems that the miners are concentrating the ore with a devise they hid from us. Likely they had a table shaker or other devise for density separation. The miners know we are concerned about mercury and deceit would reflect that worry. Between the two sampling trips in 2006, the mercury of hair increased more than 10 fold (Murphy et al. 2006) and one explanation for this change would be the introduction of another amalgamation process using more mercury.

We also tested the idea that perhaps mercury enhanced the extraction of gold in our laboratory extractions with aqua regia. We spiked the raw ore from Prey Thmei and repeated the analyses. There was more variability than with the first experiments but relative to the results obtained at Prey Ba, there is no enhancement of mercury in the aqua regia extraction (data not shown).

Table 1 Ore Acid Volatile Sulphide			
Sample	Triplicates (ppm, dry)		
Prey Meas Out 1	5.7	1.5	7.9
Prey Meas Out 2	1.7	8.2	1.0
Prey Meas Out 3	2.2	4.5	7.5
Prey Meas Out 4	6.1	1.7	2.3
Prey Meas Out 5	1.2	7.7	9.1
Prey Meas In 1	1.3	5.4	2.2
Prey Meas In 2	5.8	8.3	1.1
Prey Meas In 3	1.0	8.5	3.8
Prey Meas In 4	6.2	2.3	7.7
Prey Meas In 5	9.9	1.5	2.1
Prey Thmei Top 1	1.6	7.2	0.4
Prey Thmei Top 2	4.4	8.1	1.1
Prey Thmei Top 3	7.5	2.7	1.7
Prey Thmei Bottom 1	8.1	3.3	7.4
Prey Thmei Bottom 2	9.3	1.2	1.9
Prey Thmei Bottom 3	3.8	1.1	5.9

Table 2		
Gold and Mercury on Prey Ba and Prey Thmei Flumes		
Sample	Gold (ng/g)	Mercury (ng/g)
Prey Meas In 1	20500	230900
Prey Meas In 2	31900	229000
Prey Meas In 3	40400	339500
Prey Meas In 4	37600	265000
Prey Meas In 5	49000	308000
Prey Meas Out 1	590	4614
Prey Meas Out 2	2528	10315
Prey Meas Out 3	362	7426
Prey Meas Out 4	324	9860
Prey Meas Out 5	552	7285
Prey Thmei In 1	874	1118
Prey Thmei In 2	1860	827
Prey Thmei In 3	893	778
Prey Thmei Out 1	475	771
Prey Thmei Out 2	77	1027
Prey Thmei Out 3	437	1178

Each mercury value is a mean of triplicates.

Retort

The miners were not using the retorts we built for them. They say it takes too long. It took between 5 and 10 minutes to separate and recover the mercury. The added recovery of mercury was not financially motivating. In 2007, we built a smaller retort that continued to use the kitchenware design (Figure 5). The smaller size of the lower

mercury "pit" reduced the heat required to volatilize mercury but the smaller retort was not faster than the larger unit. Nothing is as quick as an open flame. Now that the miners know their diarrhea is caused by bad water and not by mercury maybe they may feel bolder with mercury? For sure they are prepared to go down mine shafts that clearly are unsafe (Figure 4) so to get them concerned about an unseen toxicity is a challenge.

Figure 5 Small Retort

Hair Analysis
Four types of analysis were done to further understand hair contamination with mercury: methylmercury analysis, more detailed hair washing experiments than were done in 2006, analysis of hair in goldsmiths and electron microscopic analysis of hair with mercury.

To evaluate the effect of residual mercury in gold in urban areas, hair samples were also collected in 2007 from goldsmiths in Phnom Penh (Table 4). For most of the workers, reworking of gold appears to be a moderate health hazard (Hg mean 3930 ng/g, n =9) but one individual had quite high levels of mercury (12000 ng/g). The goldsmiths worked in relatively open areas and if similar work were conducted in closed air conditioned shops, the goldsmiths could be accumulating more mercury. It appears that the reworking of gold cannot be the source of mercury contamination observed by Agusa et al (2005). They found high values in about 10% of hair in Phnom Penh and at times higher values than we did. But 10% of the population does not process gold and this occupation it is

not a major source of mercury contamination in Phnom Penh. In 2007 there was still an unknown source of mercury in Phnom Penh that results in high concentrations of mercury in hair.

Table 5				
Goldsmiths at Central Market, Phnom Penh				
Sample	Sex	Age	Hg (ng/g)	Hg (ng/g)
2005-1	M		1602	1592
2005-2	M		4207	4258
2005-3	M		2055	2122
2005-4	M		12552	11530
Lat Kosol	M	36	1924	2096
Sia	M	33	4841	5189
Son Lin	M	47	1730	1722
Sonousa	M	20	1425	1572
Sha Hon	F	49	1592	1892
Mean SD			4990	±1597

First 4 samples were collected in 2005; bottom set in 2007.

In two hair samples of miners in 2007 from Prey Meas, the mean total mercury content was 7675 ng/g, and 19% was present as methylmercury. The results for the two laboratories processing this sample for total mercury were identical. This degree of methylation is similar to what Akagi et al. (1995a) found in Brazil. The Brazilian fishers removed from goldmines had 90% of mercury in hair as methylmercury whereas in goldminers about 40% of hair was present as methylmercury and 13% of hair in goldsmiths was as methylmercury. Similarly Fréry (2001) found that more than 80% of the mercury in human hair was methylmercury and in general it is perceived that mercury in hair is from fish. Rodrigues et al. (2004) have shown that 60% of the mercury in the hair of gold workers can be inorganic. Contamination is always a concern and is addressed later. Akagi et al. (1995a) believed that much of mercury in hair was initially inhaled and later excreted in sweat. Less than 1% of dietary inorganic mercury is assimilated in the gastrointestinal tract but 50-100% of inorganic mercury is assimilated via the lungs (Gochfeld 2003). In general it is believed that most inorganic mercury is not methylated in the human body but this is not absolutely clear. The only common reports of inorganic mercury being methylated in the body are associated with dentistry (Guzzi et al. 2006) but the magnitude and significance of this pathway is still disputed (Björnberg et al. 2006). Studies with bacterial mediated methylation in the body, primarily dental, could be extrapolated from published laboratory studies with microbes (Clark 1997) to other diseases but the idea is very speculative. Conversion of inorganic mercury to methylmercury in Chinese industrial workers has been reported (Pang and Liang 1997).

Because of the concern associated with potential contamination of hair more complicated washing experiments were conducted in 2007. Two samples that were collected in February and April 2007 for a washing experiment (mean 6990 ng/g) were still rich in mercury but not as extreme as the last samples from 2006. We found that extensive washing with Triton X with sonication and acetone wash had no effect on the mercury content (Table 5).

Table 5			
Hair washing experiment			
Sample	Replicates		Mean
S1	7536	7409	7651	7532
S2	7644	7465	7556	7555
S3	7653	7499	7655	7602
S4	7493	7386	7589	7489
S5	7490	7467	7412	7456
Y1	6434	6490	6368	6431
Y2	6439	6459	6412	6437
Y3	6604	6323	6431	6452
Y4	6346	6355	6433	6378
Y5	6278	6436	6486	6400

Legend
S = miner, Sovannrita
Y = miner, Yutvolak
1= control (unwashed)
2=Triton-x1% 10 ml, hand-shake 1 min, 10ml acetone rinse (in 2 equal portions)
3=Triton-x1% 10 ml, sonicate 1 min, 10ml acetone rinse (in 2 equal portions)
4=Triton-x1% 10 ml, shaker overnight (200 rpm), 10ml acetone rinse (in 2 equal portions)
5=Triton-x1% 10 ml, shaker overnight (200 rpm), 10ml acetone rinse (in 2 equal portions); repeat Triton wash with sonication, rinse with 10 ml acetone in 2 equal portions.

Any notion of saying that the mercury in hair at the mine reflected surface contamination would have to invoke an unknown reaction whereby mercury in air bonded quickly and tightly with hair. For our purposes it is enough to say that the mercury in the miner's hair reflects exposure to high levels of mercury. The concentration of mercury in human hair downstream of the goldmines was still quite high and cannot represent surface contamination. The hair data shown in figure 1 includes the two samples from the goldmine collected in 2007 but does not include the goldsmiths which are not representative of the city. It would take a much larger sample than we collected (n=28) to represent the typical mercury in the city.

Electron Microscopic Analysis of Hair

(1) The cross-sections and longisections, for each hair sample type, showed no localized concentration of mercury with regard to morphologically identifiable components of internal hair structure.
(2) Particles were detected on the surface of individual hairs; these were colloids (e.g. submicrometre-size in least dimension) which is likely why they were not removed by washing.
(3) All morphologically distinctive colloidal particles on the hair surface appeared to be clay minerals; this identification was confirmed by EDS spot analysis showing Al and Si as major constituents.
(4) Mercury was not among the trace elements associated with the colloidal clay minerals.

The conclusion about there being no localized mercury concentration within the internal core structure of the hair was based on inference; conventional mercury analysis of hair mercury was close enough to the spectromicroscopical detection limit that a localized concentration should have shown up as a peak (or peaks). In fact, there were no peaks for mercury. This is disturbing since our major conclusion currently has to be based on inference rather than on actual numbers (namely, the counts under a mercury peak in the spectrum). The initial results were confirmed with STXM (scanning transmission **X-ray** microscopy) at a synchrotron at McMaster University.

There was a technical problem with instrumentation in the Medical School caused by the fact that the morphologically-distinctive components within the core of the hair are rich in sulphur, which has a tall wide peak at almost exactly the same place on the spectrum where we look for the tallest of the mercury peaks. We had to use STXM analysis to avoid this complication. Typical values of sulphur in hair are 2 to 6%; it is unfortunate that sulphur interferes with mercury analysis. We had good fortune to use equipment costing in excess of 500 million dollars. Perhaps this is why this approach has not been reported before. Note, that the observed surface contamination of clay did not contain mercury and clay is not rich in sulphur. It seems that the surface of hair is not contaminated with mercury rich particles. Either mercury is assimilated via the lungs or is adsorbed more readily to hair than the background clay dust. The analysis cannot absolutely be resolved with this method. To differentiate adsorption is not possible by "careful" washing. A more complicated biomedical analysis of blood might help but such an approach is not possible in Cambodia.

Fish Sampling
Two more sets of fish were processed. This time the mercury content of snakehead (Channa species) fish in the Srepok River was significantly less than we reported earlier (Table 6). Fish move and their residence time at any one location is not known. By chance we came across tribal people using explosives to collect fish in the Ou Tran River. They collected at least 100 fish but there were only two species. These small fish had a low concentration of mercury 67 ± 73 ng/g. These concentrations are not high, the risk to human health is insignificant but it is not appropriate to comment on limited data. To

compare piscivores to omnivores or herbivores is not wise. In 2006 we failed completely on one trip to net fish near the mine and on another trip in 2006 only got a few small fish. It would a major effort to collect fish of particular habits, i.e. piscivores like snakeheads (Channa species) and it might be that piscivores are preferentially poisoned by the mine effluent. In part because these concerns clams were collected to evaluate their utility for biomonitoring.

Table 6		
Snakehead fish collected Feb. 21, 2007		
Sample	Total Hg wet weight ng/g	Fish weight g
1a	116.4	1400
1b	115.2	
1c	122.3	
2a	30.3	800
2b	32.1	
2c	30.6	
3a	37.7	700
3b	35.4	
3c	38.1	
4a	41.8	700
4b	40.8	
4c	44.4	
5a	17.8	650
5b	17.1	
5c	19.6	
6a	9.6	500
6b	9.0	
6c	9.4	

Each fish was processed in triplicate

Clams
Clams were sampled to assess their bioaccumulation of mercury. It is quite difficult to collect fish at all sites and compare similar fish. If sites with different fish species are compared, then the comparison is dominated by the fish species and size of fish not the geographic distribution of mercury. Predator fish bioaccumulate mercury much more than omnivories or herbivores. Clams are widespread and do not move so their collection is a better representation of the site than fish, especially migratory fish. Also clams are eaten and knowing their mercury content has its own value.

The mercury content in the clams 1.8 km upstream of the confluence of the Prey Meas Creek and the Ou Tran River was not significantly different then at the mouth of the Prey Meas Creek (student T test, Table 7). However the total mercury of the same species of clams was significantly lower 45 km downstream at Lumphat (Mann-Whitney U test, Table 7). The concentration of methymercury was only slightly lower at Lumphat. Thus the proportion of methylmercury in clams (dry weight) relative to total mercury near the mine site was 26 and 17% whereas at Lumphat it was 42%. The higher concentration of total mercury nearest the goldmine reflects the mercury discharge from the mine likely

the lower relative proportion of methylmercury near the mine reflects suppression of methylation by the mine effluents.

The water content of the clams near the mines (88.6%) was also higher than observed at Lumphat (80.3%). Belanger et al. (1986) reported that stress from metals (zinc in their study) to Asiatic clams resulted in impaired osmotic regulation and a higher water content (control 85.8%-87.3% vs. treated 81.28%-85.59%). The water content in clams from the Srepok River seems to reflect a major change in the mercury content. There was not a strong relationship between the size of the clams and mercury content. Three variables are not consistent between the sites: mercury toxicity, clam harvesting, and nutrient supply. Clams were much easier to find and were much larger at Prey Meas Creek. The Prey Meas Creek site closest to the mine was quite remote, even from the tribal village. Clams were definitely collected by villagers at all sites. Nutrient loading (sewage) from the miners and tribal village might also be important for clam growth.

Relative to predator fish, clams have very little mercury and with respect to mercury are completely safe to eat. The fact that the relative content of methymercury varied between sites might indicate mercury toxicity and should be validated at other sites or in a controlled laboratory experiment. For accurate assessment of mercury toxicity of clams methylmercury and total mercury should be both processed but if the total concentration remains low, then total mercury is enough to ensure safety. It is much easier to process tissues for total mercury and for clams the total mercury concentration would have to increase 10 fold to justify the effort and cost of measuring methylmercury.

Discussion

Safety
The biggest concern for the transient miners is the potential for a shaft collapsing on them. There is no obvious simple solution. The miners cut some trees for lumber but they use too little lumber to work safely. Logging is illegal but the occasional removal of trees has little obvious change to the landscape. The roads near the mine are too bad for many logs to be removed; they are processed on site. By comparison, over the last three years, the road five to 10 kilometres from the goldmine has change greatly. As the section of good quality road improves, the forest is completely removed and replaced by agriculture, especially cashew plantations. The condition of the roads makes it obvious that most of the logs collected in this area are shipped by truck to Vietnam.

Further Health Analysis
Hair analysis indicates that levels of mercury are in the range where some health impairment is anticipated. Moreover, the extremes measured in 2006 are definitely in the range of health concerns. The data are all strong in that replication was excellent, blanks were low, and reference materials validated the response. Moreover the extreme values seen in 2006 were fairly consistent with all people handling mercury. Something changed with their handling of mercury and they were exposed to extreme levels of mercury. The ore handling seems to have changed with them using much more mercury

in open systems. Validation of the health risk is highly recommended with another procedure such as blood analysis. Hair analysis is useful but not absolute. The absolute discrimination between mercury excreted into hair from the body vs. a surface layer of contamination is not easy. Washing did not remove mercury. Washing procedures that were used were similar to those recommended by Akagi et al. 1995b and Veiga and Baker 2004

Table 7 Total and Methylmercury in clams (ng/g)

Sample	Total wet weight	Shell wet Weight	Meat wet weight	Meat dry weight	Meat % water	Meat dry methyl Hg	Meat dry total Hg	Meat wet methyl hg	Meat wet tot Hg
Dol Village on Ou Tran River									
1	7.5	3.7	2.6	0.256	90.15		235		23.14
#2	10.9	5.4	4.7	0.559	88.1	43	123	5.11	14.63
#3	13	5.7	6.2	0.572	90.8	38	148	3.51	13.65
4	22.5	13.1	8.7	0.993	88.6		164		18.72
#5	9.4	5.7	3.3	0.428	87.0	38	143	4.93	18.55
#6	19	10.1	8.1	1.08	86.7	37	180	4.93	24.00
mean						39	149		
Ou Tran River below Prey Meas Stream									
#1	15.9	8.8	6	0.692	88.5	31	134	3.58	15.45
#2	16.6	9.7	5.6	0.506	91.0	34	141	3.07	12.74
3	9.3	5.7	3	0.21	93.0		262		18.34
4	10.4	6.8	3.2	0.377	88.0		201		23.68
#5	13.7	8.4	5.1	0.583	89.0	24	301	2.74	34.41
#6	11.9	6.3	4.8	0.578	88.0	33	129	3.97	15.53
7	23.5	14.8	6.5	0.766	88.0		162		19.09
8	23	14.5	6.7	0.848	87.0		160		20.25
9	47.2	28.5	12.8	1.589	88.0		148		18.37
10	82.3	56.8	18.8	1.845	90.0		360		35.33
11	93.5	67.6	21.9	2.909	87.0				
12	41.1	25.6	10.5	1.404	87.0		175		23.40
mean						31	176		
Lumphat									
#1	15	10.4	4.6	1.03	77.7	27	68.3	6.02	15.23
#2	13	7.7	5.3	1.10	79.3	27	62.0	5.60	12.86
#3	16.9	10.6	6.3	1.35	78.6	25	60.6	5.34	12.94
#4	13.6	7.2	6.4	1.08	83.1	23	66.3	3.88	11.20
5	8.7	5.3	3.4	0.67	80.3	32	69.7	6.29	13.71
6	6.5	3.2	3.3	0.58	82.5	28	60.9	4.90	10.66
mean						27	65		

These samples were sorted and picked prior to analysis to have matched sets of similar size for analysis. Means of total only used the samples where methylmercury was also processed.

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The impact of mercury on the tribal people downstream of the goldmines needs more analysis. April 20, 2006, nine tribal villagers 6.1 km from the Prey Meas goldmine had a mean of 1.58 ppm (±0.34) of mercury in their hair. This low level of mercury relative to all other samples collected in Cambodia is not consistent with the high levels of mercury found further downstream at Lumphat. The clam data indicates a much lower proportion of methylmercury in clams near the mine. Likely either the high turbidity, toxicity of the mine tailings, lack of nutrients or oxic conditions of the site impedes methylation and bioaccumulation of mercury near the mine.

Health impairment from mercury should be considered. Of the published medical concerns at these levels of mercury, the enhancement of malaria by impairment of the immune system is the most relevant (Crompton et al. 2002, Silbergeld et al. 2005). There is some dispute over even this aspect (Alves et al. 2006). It is difficult to demonstrate cause and effect from one issue when the entire region suffers from malnutrition, anaemia and parasites (National Institute of Statistics 2001, SCW. 2006.). Downstream of the mines, liver flukes had infected >60% of Khmers (Urbani 1997) but there is no similar data near the mines. There is no analysis to determine if the anaemia is dietary or genetic (thalassemia). Another relevant disease to consider is tuberculosis (TB). Most Cambodians have been exposed to TB. In 2005 there were 35,535 case of TB with >10% mortality (Cambodian Weekly, June 29, 2007).

In the near future, the most cost-effective actions that could be taken to protect miners and people downstream would be to provide improved water sources. Water filters can effectively remove bacteria and pathogens like Giardia that cause diarrhea. For the rainy season, alternative water supplies such as rainwater collection should be considered too. Downstream from the mines, streams may no longer be reliable water supplies.

Future Engineering

Improved mercury recycling with a better retort should be evaluated again. It might be possible to build a retort that was faster. There are many types of retorts (Veiga at al. 2006) and perhaps the idea of using kitchenware for a retort is too simple. Our retort cost about $10 to make locally. The miners could likely pay more. But it is not clear that the miners would use it.   They only have to process their gold amalgam once a day and 10-20 minutes for the current design does not seem too long.

The engineering of a hidden mercury extraction should be resolved. The miners are using a large quantity of mercury in a concentration step prior to the flume. The major concern is the health of the miners and downstream export of mercury. It's not clear that they will cooperate. Their recovery of gold and mercury on the flume is quite good so the only cost benefit of an improved process would be mercury recovery in the retort. For 10-20 minutes, they would not use the retort so they act like the lost mercury is affordable.

Many other options exist for extraction of gold. Rather than improve the existing process, mercury could be substituted with a chemical such as cyanide. Extraction

procedures are somewhat site specific and it would be essential to have a laboratory in Cambodia for analysis of gold to guide pilot-scale evaluations

There are at least two factors that force further changes in the gold mining. Artisanal miners may be displaced by international mining firms. Concessions in Cambodia have been awarded recently to Southern Gold, Oxiana and others. There is considerable expectation that modern goldmines will be established. The artisanal miners expect to move to be forced to move but intend to continue mining. For a few reasons, enforcement to block the artisanal miners is unlikely. 1) Sotham (2004) report that the miners pay a fee to the military to manage their activities. Such fees are likely true in that most Khmers make little money and collect addition income by extra unofficial fees. 2) Finding their mines is difficult. It is currently expensive to buy satellite images for this purpose. The development of hydroelectric dams and potential for enhanced methylation and more mercury toxicity looms. Hopefully the EIA process of mine development would at least require documentation of the current degree of contamination before further development by international firms.

These firms might also want to establish a baseline of contamination before accepting responsibility. The precedent set by Newmont Mining Corp. has relevance. They were charged in Indonesia for mercury contamination but it is very possible that the mercury contamination was from artisanal miners. Senior company men had their passports withheld so that they could not leave Indonesia. An out-of-court settlement of $30,000,000 was paid (www.minesandcommunities.org/action). On April 24, 2007, Newmont was cleared in court but there were threats of appeals. As well as the significant cash settlement the disruption to business must have been major.

Oxfam America has a campaign called "No dirty gold" that is encouraging major jewellery chains to only buy gold from mines using clean technologies. There is yet another important link between international agencies and governments that should be discussed. The UNDP has attempted to restrict the sales of mercury to such poorly operated mines but the process moves slowly. Currently, Canadian and American governments encourage recycling of mercury that is then sold by recyclers as a commodity. The price of mercury increases three fold when it is sold in Cambodia.

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