# AN ASSESSMENT OF THE EXTENT OF ENVIRONMENTAL MERCURY CONTAMINATION IN THE VICINITY OF THOR CHEMICALS, CATO RIDGE, KWA ZULU-NATAL AND THE SUBSEQUENT HEALTH RISK COMMUNITIES CONSUMING FISH IN THE AREA ARE EXPOSED TO

by

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# ABSTRACT

An assessment of the extent of environmental mercury contamination in the vicinity of Thor Chemicals, Cato Ridge, Kwa Zulu-Natal, South Africa and the subsequent health risk communities consuming fish in the area are exposed to.

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Environmental mercury pollution of the Valley of a Thousand Hills area of Kwa Zulu-Natal, South Africa, in particular the river system below the Thor Chemicals mercury recycling plant, has been a topic of heated debate for a number of years. Thor Chemicals was established as a mercury recycling plant in the mid -1980's and it processed mercury waste imported from various countries. A number of factory workers were subsequently exposed to high levels of mercury vapour causing the death of a worker. Upon investigation it was found that in addition to the occupational exposures of workers, mercury waste had been discharged into the river systems of the Valley of a Thousand Hills. During the 1998 South African Parliamentary session, questions were raised regarding the lack of adequate monitoring and research directed at quantifying human health risks in the region. A number of Government departments were accused of apathy and incompetence in adequately addressing the issue.

Fish forms an important part of the diet of the local community living in the Valley of a Thousand Hills. Children, in particular, are frequently observed fishing in the rivers, thus placing these individuals at risk should the fish be contaminated with mercury. The aims of this study were: to determine the extent of environmental mercury pollution of the river system downstream from the Thor Chemicals plant, and to quantify the human health risk associated with fish consumption in the region. Samples of streambed sediment, algae, cattle hair, fish and human hair, were obtained from the study area as well as from a control area upstream from the Thor Chemicals plant. These were analysed to determine the concentration of mercury in each sample. Mercury levels in the study group were compared to mercury levels in the control areas.

Sediment samples indicate that the area directly below Thor Chemicals, within 500 metres of the boundary, is heavily polluted. A composite sample in this area measured the mercury concentration to be 54 µg/g, this being 491 times higher than the mean baseline mercury level measured in the control area (0.11 µg/g).

At the confluence of the Mngcweni and u'Mgeni Rivers, approximately 2 – 3 km downstream from Thor Chemicals, mean mercury levels were found to have increased over a seven year period from 0.017 µg /g in 1991 to 0.74 µg/g in 1998 ($p = 0.04$). This trend suggests that mercury is becoming mobilised in the ecosystem and is polluting areas further downstream from the Thor Chemicals plant.

Cattle throughout the study area were observed wading into rivers and streams, stirring up sediment and drinking muddy water. Since cattle meet all the criteria determined by Burger (1994) for the selection of suitable animal species for monitoring, it was decided to include them in the study. All cattle hair samples taken from both the study area as well as the control area were found to be below the level of detection of the laboratory procedure used (<0.5 µg/g). There was therefore no measurable difference between the two groups.

The median mercury level in young fish (0.36 µg/g) caught in the u'Mgeni River was significantly higher than the median mercury level (0.19 µg/g) measured in older, larger fish taken from the Inanda Dam ($p = 0.01$). This finding indicates that fish populations in the river, which is closer to the Thor Chemicals plant, have higher mercury levels than fish further downstream in the Inanda Dam.

The median mercury level in fish taken from the Nagle Dam, located in the control area (0.07 µg/g) was lower than the median mercury level measured in fish taken from the Inanda Dam (0.19 µg/g) which is located in the study area ($p = 0.47$).

All fish sampled in the study area (u'Mgeni River and Inanda Dam) as well as the control area (Nagle Dam) had mercury concentrations well below the United States Food and Drug Administration mercury action level of 1 µg/g. However, 20 % of fish taken from the control area, exceeded the 0.5 µg/g limit set by the South African Foodstuffs Cosmetics and Disinfectants Act (U. S. Environmental Protection Agency 1997 and South African Foodstuffs Cosmetics and Disinfectants Act 1972). The appropriateness of the South African limit is debatable, as it is not revised regularly. Furthermore the FDA level may not be appropriate in this study area due to the quantity of fish consumed.

For risk assessment purposes an assumption was made that all mercury in fish would be in the form of methyl-mercury. The daily fish consumption rate of the exposed population was estimated to be 200g per day. Total daily methyl-mercury consumption per kilogram body weight per day was calculated for each person included in the study. These data were compared to the tolerable daily intake of 0.48µg methyl-mercury per kg body weight published by the United States Food and Drug Administration and the World Health Organisation. In addition the data were compared to the United States Environmental Protection Agency reference dose of 0.1 µg methyl-mercury per kg body weight per day. The results of the risk estimation indicated that the exposed population was deemed to be at risk with a median hazard quotient of 4.6 when using the FDA criteria and 22 using the U.S. EPA criteria.

In order to obtain an objective measure of human exposure, scalp hair samples were collected from people deemed to be at risk. All hair samples were found to contain mercury levels lower than the level of detection of the laboratory test used (<0.5µg/g). The hair mercury standard accepted by the World Health Organisation is 11 µg/g. The U.S. Environmental Protection Agency has no established "standard" for mercury levels in hair. Based on data from Iraq, The U.S. Environmental Protection Agency calculated that a mercury hair concentration of 11 ppm approximately corresponds to the $95^{th}$ percentile lower statistical bound on the bench mark dose. This is considered to be approximately equal to an empirically derived NOAEL. This value was used in the derivation of their reference dose (U.S. Environmental Protection Agency 1997).

Although there is evidence of serious mercury contamination in the immediate vicinity of the Thor Chemicals plant, it appears as if mobilization of mercury in the area is slow, in spite of frequent heavy rains. The area is flat and marshy and is covered with dense vegetation (grasses and reeds). Further research needs to be done in order to determine the most suitable remediation option. Care must be taken not to accidentally mobilize mercury, should physical removal of the sediment be decided upon. In-situ methods must be investigated as an alternative option. The contaminated zone around Thor Chemicals must be fenced off, in order to restrict access. Sediment and fish mercury levels in the Mngceweni and u'Mgeni Rivers as well as the Inanda Dam must be routinely monitored.

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# PREFACE

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this dissertation was carried out under the auspices of the Department of Public Health, University of Cape Town and was supervised by Professor Rodney Ehrlich (UCT), and Professor Jerome Nriagu (Michigan University), as external advisor.

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## DEFINITIONS

The following risk assessment definitions were formulated by the World Health Organisation (1994).

Adverse effect: Change in morphology, physiology, growth, development or life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress, or increase in susceptibility to the harmful effects of other environmental influences. Decision on whether or not any effect is adverse requires expert judgement.

Critical effect: The adverse effect judged to be the most appropriate for determining the tolerable intake.

No-observed-adverse-effect-level (NOAEL):

Greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure. Alterations of morphology, functional capacity, growth, development or life span of the target may be detected which are judged not to be adverse.

No-observed-effect-level (NOEL):

Greatest concentration or amount of a substance, found by experiment or observation, which causes no alteration of morphology, functional capacity, growth, development or life span of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Lowest-observed-adverse-effect-level (LOAEL):

Lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development or life span of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Benchmark dose: The lower confidence limit of the dose calculated to be associated with a given incidence (e.g., 5 or 10% incidence) of effect estimated from all toxicity data on that effect within that study.

Uncertainty factor (UF):

A product of several single factors by which the NOAEL or the LOAEL of the critical effect is divided to derive a tolerable intake. These factors account for adequacy of the studies, interspecies extrapolation, inter-individual variability in humans, adequacy of the overall database, and nature of toxicity. The term uncertainty factor was considered to be a more appropriate expression than safety factor since it avoids the notion of absolute safety and because the size of this factor is proportional to the magnitude of uncertainty rather than safety. The choice of UF should be based on the available scientific evidence.

Toxicodynamics: The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects.

Toxicokinetics: The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body.Both the amounts and the concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics,but the latter term should be restricted to the study of pharmaceutical substances.

Tolerable intake (TI): An estimate of the intake of a substance, which can occur over a lifetime without appreciable health risk. It may have different units depending upon the route of administration. Though not strictly an "intake".

Default value: Pragmatic, fixed or standard value used in the absence of relevant data.

Guidance values (GV's):

Values, such as concentrations in air or water, which are derived after appropriate allocation of the TI among the different possible media of exposure. Combined exposures from all media at the guidance values over a lifetime would be expected to be without appreciable health risk. The aim of the guidance value is to provide quantitative information from risk assessment for risk managers to enable them to make decisions concerning the protection of human health (WHO, 1994).

# CHAPTER 1

## INTRODUCTION

The most widely documented and severe incident of environmental mercury pollution occurred at Minamata in Japan where an acetaldehyde manufacturing plant used inorganic mercury salts as catalysts. Some of the mercury was chemically converted to form methyl-mercury compounds which were released as wastewater into a large ocean bay (Minamata Bay) with devastating consequences for fishermen, their families and fish consumers in the area. The outbreak, which occurred in the 1950's, illustrated the unique property of methyl-mercury to accumulate in such high concentrations in fish tissue that it caused widespread fatalities among fish consumers. The bio-accumulation factor of methyl-mercury from water to edible fish tissue exceeds 10 million for certain species of fish (Clarkson, 1992).

Human exposure to methyl-mercury may occur through various routes including the ingestion of contaminated drinking water and food sources (other than fish) as well as dermal uptake (through soil and water). Fish consumption is however the most important pathway of exposure particularly among regular fish consumers (U.S. Environmental Protection Agency 1997).

A worldwide survey of mercury discharges into the environment indicated that most of the increases in environmental mercury pollution occur in less developed regions of the world (Kannan et al. 1998). This could be attributed to a combination of factors such as: a lack of knowledge about the harmful effects of mercury pollution among officials in the developing countries, a lack of enforced environmental protection regulations and the economic benefits gained from accepting the waste of other countries. The highest concentrations of mercury in the environment are found in the vicinity of chlor-alkali plants used to process mercury waste (Kannan et al. 1998).

## 1.1 BACKGROUND TO THE PROBLEM

The genesis of local concern around the Thor Chemicals plant and mercury pollution was brought to the fore during the mid 1980's and early 1990's through television news coverage, newspaper reports and a scientific paper published by Green Peace, an environmental advocacy organisation (Johnston et al. 1991).

In order to fully appreciate the concerns regarding mercury pollution from Thor Chemicals it must be noted that the plant was built in very close proximity to the source of a stream that feeds into the Mngceweni River, a tributary of the u'Mgeni River. Ultimately the u'Mgeni River flows into the Inanda Dam, which provides the City of Durban with drinking water. Furthermore the area which is part of the former Kwa Zulu homeland, is densely populated and people in this area (Valley of a Thousand Hills) rely upon the affected rivers and the Inanda Dam for drinking water, watering of their livestock and fishing. Figure 1 (a and b) are sections of two topographical maps, namely 2930 DA Cato Ridge and 2930 DB CatoRidge and these maps indicate the location of Thor Chemicals and the affected rivers, as well as the population density in the region. Sampling positions are marked in red (study area) and green (control area).

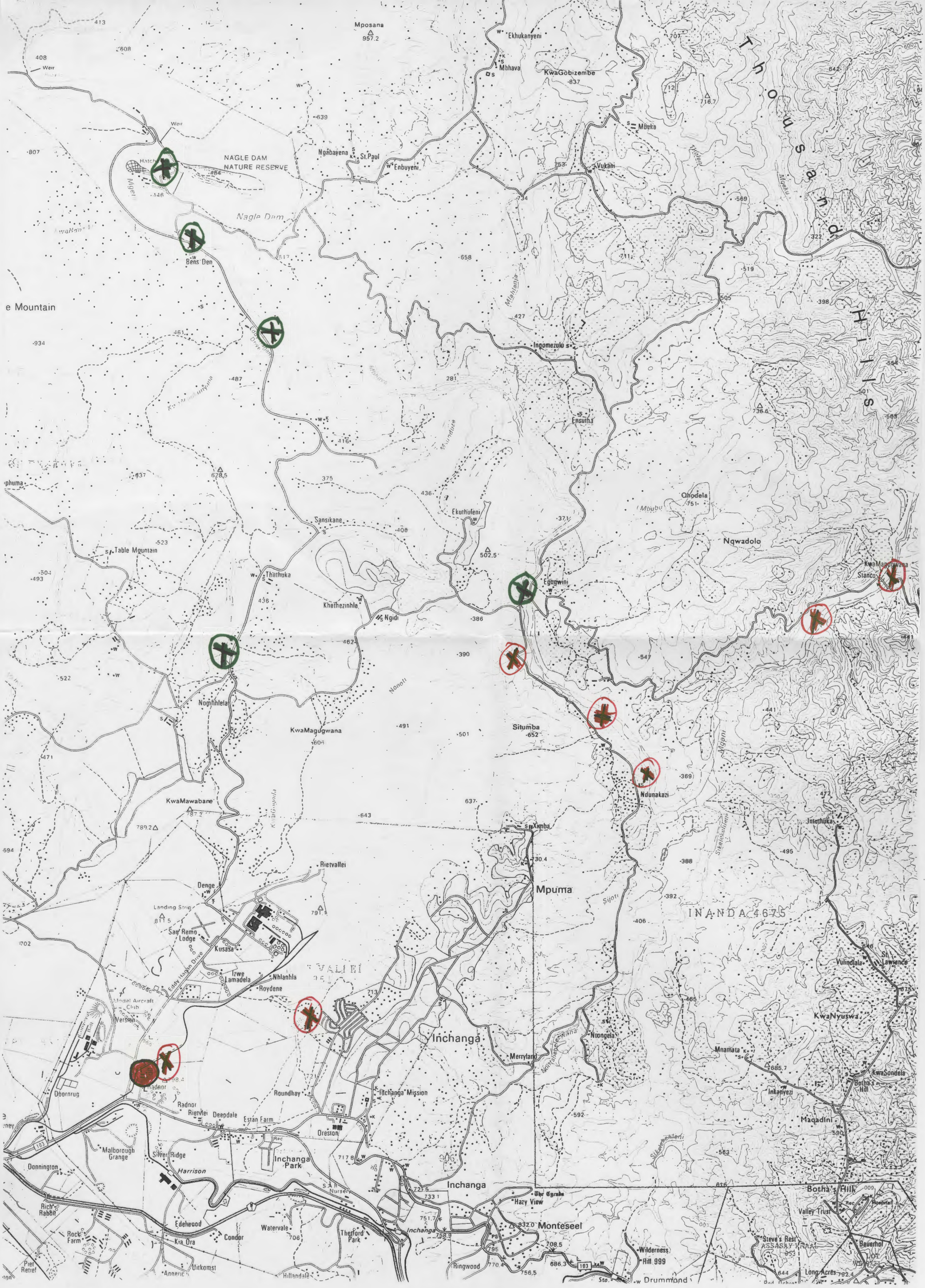


Figure 1 (a) Sampling positions (red = study area samples, green = control area samples) Thor chemicals circled in red




Figure 1 (b) Sampling positions up to the Inanda Dam indicated in red

### 1.1.1 Initial newspaper reports

The world's largest mercury incinerator plant (Thor Chemicals) is located in the Cato Ridge area of Kwa-Zulu Natal, between Pietermaritzburg and Durban, South Africa (Thor Chemicals under scrutiny 1994). Thor Chemicals received and processed waste at Cato Ridge for a number of years (since 1986) from a variety of local sources, such as African Explosives and Chemical Industries (AECI) as well as from overseas companies, including Lederle Laboratories (USA) a division of the company Cyanamid.

These companies shipped spent mercury chemicals, mercury contaminated water and mercury sludge as well as contaminated pipes and rings, in drums to Thor Chemicals for processing. Toxic organic mercury waste was incinerated at the plant in order to reclaim mercury (Hotbed of toxic waste 1990).

As early as 1989 it was reported that unknown quantities of effluent-containing mercury from holding ponds had over flown into the Mngcweni River, the source of which is near the Eastern boundary of Thor Chemicals. In early April 1990 environmental pollution was blamed on workers who had stolen contaminated drums and emptied the contents into a stream near Thor Chemicals. It was claimed that seven drums had been stolen from the site (Hotbed of toxic waste 1990).

An Assistant Director of the Department of Water Affairs stated that at the time the majority of the mercury was inorganic and not in the more hazardous form of methyl-mercury. Green Peace argued that the ecological system, including the fish life, was perfectly capable of converting organic to inorganic mercury (methyl-mercury) in a short time period.

By April 1990, Green Peace had conducted an investigation, their findings were that the mercury levels a few hundred meters from the boundary of Thor Chemicals were 8600 times higher than the United States' limit for toxic waste. It was also reported that families living downstream from the Thor Chemicals Plant were using the contaminated water for both domestic and agricultural purposes (Hotbed of toxic waste 1990).

A Green Peace spokesperson stated at the time that the mercury pollution did not pose a serious problem at the lower reaches of the river system. However, fears were expressed that the mercury could be biologically converted to methyl-mercury and ultimately threaten the drinking water supplies of the greater Durban area, in particular the Inanda Dam, a major reservoir in the Durban water supply system. Furthermore it was reported that mercury was probably leaving the area and was being carried along with sediments into the major river basin, and as such could compromise the integrity of the ecosystem. This situation was very dangerous for both the natural environment as well as human health. It was also reported that gross environmental contamination took place over a number of years. However, this claim had not been adequately investigated and the potential impact of such pollution on local communities was largely unknown (Hotbed of toxic waste 1990).

The exposure of a number of workers from Thor Chemicals, with serious side effects and a subsequent death, made headlines all over the world in the early 1990's. Senior management of the plant was subsequently prosecuted for negligence in not enforcing proper occupational hygiene control measures and the recycling facility was closed down, however stockpiles of mercury remain upon the premises (Thor fined after pleading guilty 1995).

### 1.1.2 Green Peace study

Sediment samples were obtained from the vicinity of the Thor Chemicals plant, situated at the head of the Mngceweni River, a marshland area at the permanent settlement of Fredville, which is situated within the Mngceweni River valley and the confluence of the Mngceweni and u'Mgeni Rivers (Figure 2). The principle objective of the Johnston study was to evaluate the extent of mercury pollution and to pinpoint the source of contamination. Access to the Thor Chemicals site itself was not granted, while access to the lower Mngceweni River was made difficult because of the nature of the terrain.

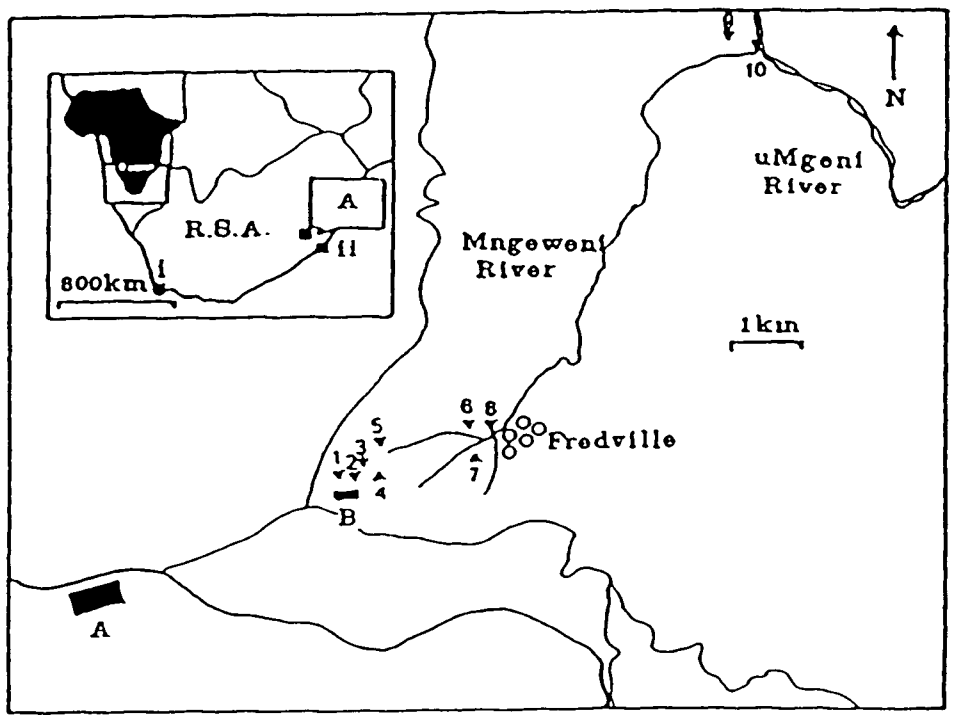


**Figure 2. Study area and sample sites in the vicinity of Thor Chemicals (Johnston et al. 1991).**

Sample 1 was taken 50m downhill of the evaporation ponds, an area clearly receiving site run-off from Thor Chemicals, which was bare of vegetation. Sample 2 was obtained adjacent to the site landfill area, close to the perimeter fence. Sample 3 was taken from a dry riverbed below the plant and sample 4 from the riverbank on the same side of the valley as the Thor Chemicals plant. Four samples were taken at sample site 5, which marks the point at which a ground water spring beneath the plant establishes permanent flow into the Mngceweni River. Further sediment samples were taken (sample 6) approximately 50m upstream of the confluence of the tributaries. Sample 7, for comparative purposes, was taken from the bed of an un-named tributary to the Mngceweni River. Sample 8 consisted of river sediment taken from the settlement of Fredville; and sample 9 was obtained directly above the confluence of the Mngceweni and u'Mgeni rivers.

Sample 10 consisted of alluvial material, apparently carried down the Mngceweni River during periods of heavy flow, which was deposited at the confluence with the u'Mgeni River. Samples 1,2 and 4 were taken in areas which are subjected to storm water runoff from the Thor Chemicals plant and these contained mercury levels in excess of 10 μg/g. Soil polluted to this extent would require remediation under Dutch law. Sample 3 contained 49.6 μg/g which, in the Netherlands, is close to the 50 μg/g limit used to classify material as chemical waste (Table I). This level of environmental pollution is extremely high and potentially dangerous should it become mobilized in the ecosystem and bio-accumulate in the form of methyl-mercury in fish (Johnson et al. 1991).

**Table I Results of mercury analyses for environmental samples taken in the vicinity of the Thor Chemicals recovery plant.**

| SITE | SAMPLE | TYPE | Hg (µg/g) |
|---|---|---|---|
| 1 | 1 | Soil | 21.4 |
| 2 | 2 | Soil | 12.5 |
| 3 | 3 | Sediment (dry river bed) | 49.6 |
| 4 | 4 | Soil (dry riverbank) | 11.4 |
| 5 | 5a | Sediment (dry riverbank) | 6.4 |
| 5 | 5b | Soil (dry riverbank) | 0.85 |
| 5 | 5c | Sediment (Mngeweni source) | 1764.0 |
| 6 | 6 | Sediment (river) | 0.91 |
| 7 | 7 | Sediment (un-named tributary) | 0.03 |
| 8 | 8 | Sediment (upstream, Fredville) | 0.33 |
| 9 | 9 | Sediment (uMgeni/Mngeweni) | 0.03 |
| 10 | 10 | Sediment (uMgeni/Mngeweni) | 0.004 |
| 5 | 12 | River water | nd |
| 8 | 13 | River water | nd |

nd = none detected
(Johnston et al. 1991).

The Thor Chemicals plant was identified as the point source of contamination due to the fact that there was a progressive reduction in mercury levels with distance from the site. Moreover, mercury levels in soil taken from the river bank opposite the plant (sample 5b) were an order of magnitude lower than those found in soil from the same side as the plant (sample 5a). An order of magnitude difference was found in the sediment content of mercury between the unnamed tributary, which does not receive direct run-off from the plant, and the Mgceweni River. The extraordinarily high mercury content of sample 5c was due to the fact that there is an earth dam at this point which allowed for the accumulation of mercury from upstream areas. Samples taken near the Mgceweni and u'Mgeni confluence show much lower mercury levels, which could be regarded as normal for many soils. In this lower part of the Mgceweni river (sample 9) mercury levels are similar to those measured in the sediment of the un-named tributary (sample 7). Significant mercury contamination is likely to be associated with the fine fraction of sediments that tends to be removed to the u'Mgeni River during vigorous wet-season flows.

Increased rainfall contributed to the mobilization of mercury from the contaminated soils and the riverbed areas found in the vicinity of the plant. Under dry conditions, the major mobilization pathway from soils would most likely be by volatilization. Furthermore, Johnston et al. (1991) suggested that sediment transport from the Mgceweni River may have important implications for the new Inanda Dam complex on the u'Mgeni as a result of siltation. Johnston et al. (1991) advised that any fishery activities taking place in the impounded waters should be monitored carefully to ensure adequate protection of public health. Furthermore the authors suggested that a hydro-geological survey should be carried out to assess ground water impact. In addition it was recommended that a general remediation programme should be implemented urgently in the contaminated area and the exposure of both the local and wider communities be exhaustively evaluated. It was also suggested that extensive contamination would persist for many years. Although ground water may have been contaminated, mercury was not detected in river water samples.

Johnston et al. (1991) clearly highlighted the need for additional research in the area. To date no action has been taken to implement environmental remediation in the contaminated zone. A limitation of the study, was the fact that it was restricted to the sampling of soil and sediment and other sampling media such as aquatic plants, fish, animal and human hair were not included. It could however be argued that the study took place shortly after the spill and bio-accumulation and transformation would not have occurred at this early stage.

Chester et al. (1996) * measured total mercury levels in water, soil and vegetation at three points along the river system below Thor Chemicals. Sediment samples indicated high levels of pollution (1150 $\mu g/cm^3$ - max). Vegetation, which was collected from the river within 50 metres of the flood plain, did not contain elevated mercury levels.

* Chester S, Euripidou E, Maharaj R, Oosthuizen M, Randolph R. 1996. Mercury Poisoning: Thor Chemicals, Unpublished Thesis, Technikon Natal Library: 1-10.

**Table II Mercury levels measured by Chester et al. (1996)**

| SAMPLE NUMBER | SAMPLE TYPE | Hg (ug/cm$^3$) |
|---|---|---|
| A - 0m | Water | 0.42 |
| A - 0m | Water | 0.002 |
| B - 0m | Water | 47.3 |
| B - 0m | Water | 0.0001 |
| B - 0m | Water | 0.001 |
| C | Water | 0.001 |
| C | Water | 0.001 |
| C - 0m | Water | 0.001 |
| A1 - 0m | Sediment | 174.0 |
| A1 - 5m | Soil | 33.6 |
| A2 - 0m | Sediment | 326.0 |
| A2 – 0m | Sediment | 1150.0 |
| A2 – 5m | Soil | 140.0 |
| B1 – 0m | Sediment | 1.9 |
| B1 – 50m | Soil | 155.0 |
| B2 – 50m | Soil | 14.0 |
| B2 – 50cm | Soil | 173.0 |
| C – 0m | Soil | 45.7 |
| A1 – 0m | Vegetation | 4.88 |
| A1 – 5m | Vegetation | 0.22 |
| A2 – om | Vegetation | 0.56 |
| A2 – 5m | Vegetation | 0.03 |
| B1 – 50m | Vegetation | 0.002 |
| B2 – 50m | Vegetation | 0.02 |
| C – 50m | Vegetation | 1 |
| B | Cows milk | 0.004 |

A = Marsh area directly below Thor Chemicals, B = River below Thor, Chemicals C = Inanda Dam, upper confluence, m = distance from river bank
Chester et al (1996).

After attracting considerable negative media attention the Thor Chemicals saga culminated in a call for action during 1999 by the South African Parliament. Previously most of the attention had centred on the occupational exposure of workers. The environmental aspects were never satisfactorily dealt with. In recent years "Nkosi" – (Chief) Malaba and the residents of the Valley of a Thousand Hills expressed their concern about the possible effects that mercury pollution might have on people living in the area. Verbal interviews with the community indicated that locally caught fish forms a major part of their diet.

Although several years have passed since the last known discharge of mercury waste by Thor Chemicals, it was necessary to conduct a study in order to ascertain to what extent mercury in the environment may have impacted upon the health of people living in the area.

## 1.2 RATIONALE FOR THE STUDY

i) Fish forms an important part of the diet of the community residing in the study area, in particular young children, who swim and fish in the rivers daily, thus placing these individuals at a potentially elevated risk should the fish be contaminated with mercury. It was therefore important to determine whether or not people residing in the vicinity of Thor Chemicals are exposed to a mercury health hazard or not and if so, to quantify the risk.

ii) Community leaders and people residing in the area, Parliament, affected Government Departments (National and Provincial), as well as Thor Chemicals need to know what the pollution and risk levels are, in order to plan interventions in the region and to take the steps necessary to eliminate or minimise health impacts.

## 1.3 PURPOSE OF THE STUDY

i) To determine the extent of environmental mercury contamination of the river system downstream from the Thor Chemicals plant, up to and including the Inanda Dam, when compared to a control area.

ii) To quantify the extent to which mercury has become mobilized in the ecosystem.

iii) To identify pathways of exposure of people living in the area and to quantify the health risks communities may be subjected to.

iv) To ascertain the levels of mercury in hair samples obtained from the exposed population.

v) To recommend appropriate interventions if deemed necessary.

## 1.4 THESIS FORMAT

i) Chapter 2 will discuss relevant and current literature on methyl-mercury exposure and monitoring.

ii) Chapter 3 will expand on the methods employed in sample collection, as well as information pertaining to the study design and sample selection.

iii) Chapter 4 will present the findings (results) of the study as well as a discussion of statistical methods used to analyse the data.

iv) Chapter 5 will discuss the results and findings of the study and the relevance thereof in terms of the original study aims. Reference will be made to literature in support of arguments, assumptions and conclusions.

v) Chapter 6 will summarize the main findings of the study in the form of pertinent concluding remarks.

vi) Chapter 7 will propose specific recommendations for intervention, future monitoring in the area and additional research.

# CHAPTER 2

# LITERATURE REVIEW

Mercury cycles in the environment as a result of natural and human (anthropogenic) activities. The amount of mercury mobilized and released into the biosphere has increased since the beginning of the industrial age. Most of the mercury in the atmosphere is in the form of elemental mercury vapour, which circulates for extended periods of time (up to a year), and hence can be widely dispersed and can be transported thousands of miles from likely sources of emission. Most of the mercury in water, soil, sediment, or plants and animals is in the inorganic form. Inorganic mercury, when bound to airborne particles, or in a gaseous form, is readily removed from the atmosphere by precipitation and dry deposition. Wet deposition is the primary mechanism for transporting mercury from the atmosphere to surface waters and land. Even after it deposits, mercury is commonly emitted back to the atmosphere, either as a gas or associated with particles, to be re-deposited elsewhere. As it cycles between the atmosphere, land and water, mercury undergoes a series of complex chemical and physical transformations, many of which are not completely understood (World Health Organisation 1991).

## 2.1 CHEMICAL TRANSFORMATION OF MERCURY IN THE ENVIRONMENT

The inorganic forms of mercury undergo transformations in the environment, mainly by oxidation-reduction reactions. Mercury vapor is oxidized to ionic divalent mercury (Hg++) in water, in the presence of oxygen. The oxidation of metallic mercury to inorganic divalent mercury is greatly favored when organic substances are present in the aquatic environment.

Ionic mercury, once present in water, is capable of forming a wide variety of complexes and chelates with organic materials. Ionic divalent mercury (Hg++) in nature is converted to methyl-mercury and dimethyl-mercury compounds. Methyl-mercury is the predominant form of mercury in fish, regardless of the nature of the mercury pollutant; therefore it is clear that transformations of mercury compounds occur in the environment Two biochemical pathways of methylation of mercury have been identified, one anaerobic and the other aerobic. Methylation significantly increases the ability of mercury to cross biological membranes this is why aquatic organisms contain mainly methyl-mercury. If conditions of pH are favourable, dimethylmercury will be formed (World Health Organisation, 1991).

Mercury compounds are converted by bacteria to form methyl-mercury, which is soluble, mobile, and rapidly incorporated into aquatic food chains. Mercury concentrates as it moves up the food chain, accumulating in carnivorous fish to levels of between 10 000 and 100 000 times the concentrations in surrounding water. Between 70% and 90% of the mercury detected in fish muscle is in the bio-available form of methyl-mercury and hence is readily absorbed (World Health Organisation, 1991).

Mercury present in the bottom sediment of rivers and lakes is subject to methylation by micro - organisms. Methyl-mercury enters aquatic food chains, starting with uptake by small organisms such as plankton and eventually attaining its highest concentration in large predatory fish. Methyl-mercury is poorly eliminated and it accumulates throughout the lifetime of the fish. Thus the highest concentrations are found in the longest-lived, top predatory species. Several factors such as the acidification of bodies of fresh water by acid rain and the impoundment (damming) of rivers and lakes raises methyl-mercury levels in fish. It has been suggested that the flooding of vegetation will enhance the substrate supply to micro - organisms, including those species that methylate mercury (World Health Organisation 1991).

## 2.2 MERCURY IN SEDIMENT

Since mercury settles in the sediment of contaminated river systems, sediment sampling is a fundamental component of most study designs. It was therefore important to review literature on the topic.

Once mercury enters a body of water, it can:

i) Remain in the water.

ii) Be lost from the water body through drainage.

iii) Be re-volatized into the atmosphere, or

iv) Settle into sediment and be taken up by aquatic biota, (U.S. Environmental Protection Agency 1997).

The movement of mercury through any water body is dependent on many variables. Of particular importance and concern is the fact that, once in aquatic systems, mercury can exist in dissolved or particulate form and can undergo a number of chemical transformations. Contaminated sediments at the bottom of surface waters tend to serve as an important mercury reservoir, with sediment-bound mercury recycling back into the aquatic ecosystem for decades or longer. Mercury has a long retention time in sediments, as a result mercury that has accumulated in sediments may continue to be released to surface waters and other media for long periods of time, possibly hundreds of years, (U.S. Environmental Protection Agency 1997).

The lengthy period of mercury retention and release into the environment emphasise the need to evaluate the extent of pollution resulting from mercury discharges by Thor Chemicals. A study by Johnston et al. (1991), on the levels of environmental mercury pollution in the vicinity of Thor Chemicals argued that mercury contaminated sediments could have a direct toxic effect on aquatic life in the region. It was hypothesized that the potential bio-accumulation of mercury in the food chain could pose a future risk to humans, wildlife and aquatic organisms in the area.

The transfer of mercury into aquatic systems is dependent on the methylation thereof. The World Health Organisation (WHO), in a mercury review, referred to several studies which demonstrate that the methylation of inorganic mercury in the bottom sediment of lakes and rivers, is a key step in the transportation of mercury into the aquatic food chain. Some of the studies reviewed showed that the degree of methylation correlated well with the overall level of microbial activity in sediment. The following were considered to be key factors in the methylation of mercury:

i) The rate of methylation under oxidising conditions is greater than under anaerobic conditions.

ii) The output of methyl-mercury doubles for a ten-fold increase in inorganic mercury.

iii) Temperature affects methylation as a direct result of its effects on the rate of microbial activity. Higher microbial growth rates increase methylation (World Health Organisation 1989).

The above findings have been confirmed by several authors (Sittig 1976, Jackson 1988, Grosheva 1993, Jackson 1993, Aula et al. 1995, U.S. Environmental Protection Agency 1997, Bidone 1997, Kannan et al. 1998 and Fabbri et al. 1998).

Aula et al. (1995) found that coarse particles absorb less mercury than finer particles and that there is a positive correlation between mercury content and the percentage of organic material in the sediment. It is therefore important to consider, in addition to the percentage of humic matter, the particle size of sediment samples collected for laboratory analysis. Barghigiani et al. (1996) confirmed that the highest concentration of mercury in sediment is found in the <20 μg grain size fraction. The mercury concentration in the >20μg fraction is negligible.

Park and Curtis (1997) concluded that the exchange of sediment bound mercury back to the water column is generally low. Water is therefore not considered to be a good sampling medium to quantify mercury pollution as mercury binds to humic matter within sediment and from there is transferred to other tropic levels. Mercury concentrations in free flowing water are much lower than those measured in sediment. Guimaraes et al. (1998), in South America, found that mercury levels in sediment penetrated down to 4 cm below the surface. Methyl-mercury was detected only in the upper layers (0-2 cm) of the sediment.

These findings, confirmed by other authors (Rasmussen 1994, Kannan 1998 and U.S. Environmental Protection Agency 1997) dictated the methodology adopted in this study. Samples of finely graded sediment with high humic content were thus collected from the top 10 cm of the surface. Samples containing sand or grit were discarded. Furthermore this methodology is consistent with the sampling procedure prescribed by the US National Water Quality Program for Sediment Sampling of Trace Elements and Organic Contaminants (Shelton et al. 1994).

## 2.3 THE ROLE OF pH AND TEMPERATURE IN MERCURY MOBILIZATION

Several studies reviewed by WHO describe the influence of temperature and pH on microbial activity and the resultant methylation of mercury (World Health Organisation 1989). Subsequently these findings were confirmed in separate studies. Hintelmann and Wilken (1995) demonstrated that seasonal variation showed larger amounts of methyl-mercury compounds under warmer summer conditions than in cold winter and spring weather. Park and Curtis (1997) identified temperature as an important factor in the seasonality of mercury methylation and availability. Methylation increases from spring to late summer and decreases in the fall in the Oregon reservoirs of the USA.

Lenka et al. (1992) in a study conducted at a chlor-alkali plant in India, demonstrated that pH has a significant effect on the leaching of mercury from sediment. The pH of the sediment at the sites being studied varied between 6.4 and 7.4, with an average value of 7. The selective leaching of mercury from the sediment samples increased with decreasing pH, irrespective of the site. Experiments carried out by selective leaching from sediment samples within a pH range (2 to 6) indicated that mercury removal from sediment increases exponentially or linearly with decreasing pH. It was concluded that these findings provided an explanation for the elevated concentrations of mercury in fish from low pH lakes.

Table III demonstrates the importance of pH in the transfer of mercury from sediment to other trophic levels within an aquatic system. Sediments may provide a reservoir for mercury, where it is bound to humic matter and may be released over a lengthy period of time. High temperature and low pH could however accelerate mercury mobilization from the sediment (Lenka et al. 1992).

**Table III: The role of pH in the transfer of mercury to higher trophic levels.**

| Site | pH | Concentration of total Hg in sediment (µg/g) |
|---|---|---|
| Control | 6.6 | 0.38 |
| 1 | 6.7 | 1.6 |
| 2 | 6.4 | 4.72 |
| 3 | 7.3 | 54.53 |
| 4 | 7.0 | 42.46 |
| 5 | 7.3 | 192.00 |
| 6 | 7.4 | 107.89 |
| 7 | 7.3 | 73.66 |
| 8 | 7.2 | 44.2 |

Lenka et al. (1992)

The Valley of a Thousand Hills, where Thor Chemicals is located, is a sub tropical region with hot, wet summers. Daytime temperatures frequently reach the mid to high 30's (degrees centigrade) and remain fairly high throughout the year, thereby creating conditions favoring the methylation of mercury from the sediment bed. It was decided to conduct this study during the Southern Hemisphere summer of December 1998 - February 1999 when methylation due to high ambient temperatures and bacterial activity was optimal.

## 2.4 ALGAE AS INDICATORS OF MERCURY POLLUTION

Bailey and Stokes (1985), demonstrated that the concentration of metals in algae is generally much higher than in the surrounding water (1 000 - 10 000) times greater. The World Health Organisation (1989) reported on a study where cultures of algae were grown for 48 hours in the presence of mercuric chloride. In that time approximately half the mercury was retained in the algae. However when the algae were fed to copepods for 5 days, neither the copepods, nor their eggs or faeces, retained mercury in detectable amounts, suggesting algae may not be an effective route to bio-magnify mercury into higher trophic levels within the ecosystem.

A more recent study by Guimaraes et al. (1998) measuring mercury methylation in the Pantanal flood plains of Brazil suggested otherwise. Algal mats were clearly demonstrated as being more important mercury methylation sites than sediment and this held true in different phases of the hydrological cycle and in river basins with different geo-chemical features. Aquatic vegetation was found to be a potentially important site for the production of highly bio-available methyl-mercury, because it is in close contact with the water and has a high relative surface area.

Shrivastava and Rao (1989) determined that certain aquatic weeds absorb and incorporate mercury into their tissue rapidly and effectively and can be used for the removal of mercury from contaminated environments. Gonzales (1991), in a study conducted in Sagua la Grande, Cuba, demonstrated the capacity of aquatic plants to accumulate mercury acting as good indicators of mercury bio-accumulation.

Lenka et al. (1992), in a study, conducted in Ganjam, India, using a wide range of aquatic plants, indicated that most plants bio-concentrated mercury to different degrees. Rasmussen (1994) in a study of the Great Lakes Region of Canada suggested that sampling both sediment and aquatic plants is more informative than sampling sediment alone, due to problems with sediment heterogeneity and because some plants are sensitive indicators of mercury pollution. Furthermore the sampling of a selection of sediments and aquatic plants provides valuable information about mercury bio - availability. The U.S. Environmental Protection Agency (1997) states that mercury and methyl-mercury complexes in soil are available theoretically for plant uptake and translocation, potentially resulting in the transfer of mercury through the terrestrial food chain.

In view of these findings, it was decided that algae samples would be included in this study.

## 2.5 METHYLATION AND BIO-ACCUMULATION OF MERCURY IN FISH

Jackson (1991) identified several complex factors involved in the movement of mercury throughout aquatic systems. These differences may be due to regional and temporal variations in mercury content as well as differences in fish habitat preference, metabolic rate, age, growth rate, size, bio - mass, diet and excretory pathways.

A study by Kim (1995) on the North Island of New Zealand indicated that there were distinct variations in methyl-mercury concentration attributable to the length and age of fish. Older, larger, predatory fish having higher methyl-mercury levels. It was also found that virtually 100% of the mercury in fish tissue is in the form of methyl-mercury. These findings were confirmed by a number of other authors (Holsbeek et al. 1996, Bidone et al. 1997, Park and Curtis 1997 and Wagemann et al.1997).

The bacterial conversion of inorganic mercury to methyl-mercury is important for three reasons:

i) Methyl-mercury is much more toxic than inorganic mercury.

ii) Organisms require considerably longer periods of time to eliminate methyl-mercury.

iii) Methyl-mercury containing bacteria may be consumed by the next higher level in the food chain, or the bacteria may release the methyl-mercury to the water, where it can quickly adsorb to plankton, which are also consumed by the next level in the food chain, (U.S. Environmental Protection Agency 1997).

Numerous factors such as the acidity of the water, length of the aquatic food chain, temperature and the concentration of dissolved organic matter influence the bio-accumulation of mercury in aquatic biota. In addition physical and chemical characteristics of a watershed, such as soil type and rate of erosion affect the amount of mercury that is transported from soils to water bodies. The exact mechanisms by which mercury enters the food chain remain largely unknown and probably vary among ecosystems. Certain bacteria play an important role in this process. Bacteria which process sulphates take up mercury in its inorganic form and through metabolic processes convert it into methyl-mercury (U.S. Environmental Protection Agency 1997).

The elimination of methyl-mercury from fish is such a slow process that long-term reductions of methyl-mercury levels in fish are often due to growth of the fish (increased mass) and not true elimination from tissue. By comparison other mercury compounds are eliminated relatively quickly. Humans may become exposed to methyl-mercury as a result of their ingestion of contaminated drinking water and food sources (other than fish), as well as dermal uptake through soil and water. However, the fish consumption pathway dominates all others (U.S. Environmental Protection Agency 1997).

Kannan et al. (1998) in a study conducted in the South Florida Estuaries (US), indicated that whereas the methyl-mercury content of the mercury in sediment was 0.77%, the content of methyl-mercury in fish muscle from the same area accounted for 83% of total mercury. The highest methyl-mercury concentrations were found in two different species of catfish. Mercury accumulation in fish was found to be dependent upon the availability of inorganic mercury in sediment and the water column, trophic interactions and the rate at which micro flora transformed mercury to methyl-mercury. This again confirms the complexities of mercury as a pollutant in its mode of transfer and bio-accumulation within a given ecosystem.

In addition Kannan et al. (1998) examined the relationship between mercury concentrations in fish and sediment collected from corresponding locations within the South Florida estuaries. The concentrations of total mercury in sediment were positively correlated with those measured in fish ($r=0.52$; $p < 0.05$). Similarly, total sediment mercury concentrations were related to fish methyl-mercury concentrations ($r=0.42$; $p<0.05$). Sediment methyl-mercury concentrations were also correlated with those in fish ($r=0.33$; $p< 0.05$). Therefore, irrespective of its state, mercury was shown to accumulate in higher trophic levels of fish.

In a study of mercury levels in fish conducted over a period of 16 years in 18 reservoirs, a model was designed which was based on pH, concentration of organic matter in water, the water level and reservoir age. This model predicts that mercury levels in fish will normally exceed 1 μg/g for the first twelve years after flooding. The mercury load introduced into these reservoirs originates from newly inundated soils and vegetation. Moreover the addition of organic material to the reservoir increased the methylation of mercury leading to the bio - accumulation of methyl-mercury in fish (Porvari 1998). These findings were confirmed by Jackson (1988) who reported that the creation of reservoirs by the impoundment of river water and flooding of adjacent land commonly caused an appreciable increase in the mercury content of fish inhabiting the water.

The Inanda dam, which was commissioned in 1989 (www.umgeni.co.za 1999) is a relatively recent impoundment with a high organic load due to poor sanitation in the catchment area and access by livestock. It is therefore expected that the dam could have elevated background methyl-mercury levels. This is a confounding exposure that needs to be considered when attempting to ascribe mercury pollution levels in the dam to a point source discharge from Thor Chemicals.

## 2.6 ANIMAL SAMPLES AS INDICATORS OF MERCURY POLLUTION

This section will discuss the use of animal samples in mercury assessment of environmental contamination.

A number of studies have assessed mercury levels in the flesh and internal organs of animals (Shaw et al. 1986 and Halbrook et al. 1994). Burger et al. (1994) clearly indicated that bio-accumulation of heavy metals (including mercury) takes place in the tissue of animals. Once in an animal, the heavy metal can be stored in tissue or it can be eliminated by excretion or by deposition in feathers or hair. Both mammalian hair and avian feathers can be used as alternative indicators of heavy metal levels in other tissues.

Hair samples are reflective of the exposure of an animal and can be obtained without sacrificing the animal, particularly in the case of mercury where there is little opportunity for external contamination. Mercury levels in hair correspond with internal tissue levels and can thus be used as bio-indicators. The hair to organ mercury ratio is in the order of 200:1, (Burger et al.1994) and (Palherta et al.1995). In addition Palherta et al. (1995) emphasized the appropriateness of animal hair as an indicator of long term exposures, as opposed to the use of blood and organ analysis which are more appropriate for recent exposures.

In order to determine the appropriateness of the animal group to be used in a study, Burger et al. (1994) suggest that the following criteria should be considered.

i) The species should be relatively common and available.

ii) The animal should be sufficiently large to provide an adequate sample for analysis.

iii) Age and gender should be determinable.

iv) Life span should be appropriate (use short lived or new-born animals to measure recent exposure and long-lived animals to integrate cumulative exposure).

v) The animal should accumulate the chemical (or have a biological response) to an appropriate trophic level.

vi) Home range must be appropriate (sessile animals to monitor point source; mobile animals to integrate exposure over space).

vii) Small or abundant animals will be particularly useful as bio-indicators because they are relatively sedentary and will thus reflect local contamination.

Cattle kept by the local community in the study area are of great social and cultural importance. The animals are not only a source of meat or milk but also an indicator of wealth and status. In this relatively poor community, cattle are only slaughtered for traditional feasts and usually old (less productive) animals are used for this purpose. Cows are milked regularly. Interviews conducted with cattle owners suggested that the animals were not moved around much and most animals have been in the area since birth. The only sources of water for cattle in the study area are the rivers contaminated by the Thor Chemicals spill. Cattle in the area were observed wading into shallow pools or along riverbanks where they stirred up fine sediment when drinking. It is therefore assumed that they could be at risk of drinking mercury contaminated sediment during watering and as such cattle are potentially a prime source for the transformation of mercury from the sediment beds to higher trophic levels within the ecosystem.

The local community consumes chickens more regularly than cattle, therefore consideration was given to the use of feathers of domestic chickens as a sample medium in this study. Feathers were however not deemed to be an appropriate sampling medium, since a feather grows rapidly over a period of a few weeks after which the blood supply shrivels up.

The feather remains on the bird for a month as a dead structure, and therefore represents an archive of exposure during the weeks prior to feather formation. Hair, on the other hand, grows continuously and maintains a blood supply connection with the body. Growing from the base, each segment of hair contains a record of elements circulating in the blood at the time that segment of hair was formed. From base to tip a hair represents a continuous archive of blood levels (Burger et al.1994). These findings were confirmed by World Health Organisation (1989) and Becker et al.(1994).

Due to their watering habits and age, local cattle hair was selected as the animal sampling medium of choice. An additional benefit being the fact that the need to sacrifice animals was eliminated.

## 2.7 HEALTH EFFECTS ASSOCIATED WITH MERCURY EXPOSURE

This section will review literature on animal and human effects associated with mercury exposure, as well as epidemiological studies and risk assessments conducted in other parts of the world. The main thrust of the review will be related to methyl-mercury exposure, although exposures to inorganic forms of mercury will be discussed in brief.

### 2.7.1 Kinetics and metabolism

The absorption, distribution, metabolism and excretion of mercury are highly dependent on the form of mercury to which a receptor has been exposed. The absorption of elemental mercury vapour occurs rapidly through the lungs, but it is poorly absorbed from the gastrointestinal tract. Once absorbed, elemental mercury is readily distributed throughout the body; it crosses both placental and blood-brain barriers.

Once elemental mercury crosses these barriers and is oxidized to the mercuric-ion, return to the general circulation is impeded and mercury can be retained in brain tissue. Elemental mercury is eliminated from the body via urine, faeces, exhaled air, sweat and saliva (U.S. Environmental Protection Agency 1997).

#### 2.7.1.1 Inhaled mercury vapour

The body retains 80% of inhaled metallic mercury. Inhaled inorganic mercury aerosols are deposited in the respiratory tract where they are absorbed, the rate of absorption being dependent upon particle size. The faecal and urinary routes are the main pathways for the elimination of inorganic mercury in humans, although some elemental mercury is exhaled. One form of depletion is the transfer of maternal mercury to the foetus (Clarkson 1992).

#### 2.7.1.2 Ingested methyl-mercury

Less than 1 % of ingested liquid metallic mercury is absorbed via the gastrointestinal tract. The absorption of inorganic mercury varies with the particular mercuric salt involved, absorption decreases with decreasing solubility (U.S. Environmental Protection Agency 1997).

Studies in both animals and humans have demonstrated that more than 90% of ingested methyl-mercury is absorbed. Methyl-mercury is distributed to all regions of the body and within 30 hours, 7% thereof is found in the blood. Distribution to the brain takes approximately 3 days. On the other hand elimination from the body is a slow process and thus tissue concentrations stay relatively constant and do not fluctuate with excretion. In primates and humans the brain to blood ratio is in the range of 5 to 1. Blood levels are therefore predictive of levels in the target organ, which makes blood a valuable indicator medium.

Scalp hair however is the indicator medium of choice as it can reveal both past and present blood concentrations and can be collected non-invasively. It is also easily stored and transported. The hair to blood ratio is approximately 250 to 1 (Clarkson 1992).

### 2.7.2 Review of human toxicity

This section will focus on human exposure to organic (methyl – mercury) as opposed to mercury vapours, which are associated with occupational exposures. No discussion on human methyl-mercury exposure will be complete without reference to the Minamata Bay incident that occurred in the 1950's in Japan. This incident is in fact of such significance that Methyl-mercury poisoning is commonly referred to a Minamata disease (M.d.)

#### 2.7.2.1 Methyl-mercury poisoning at Minamata Bay

Minamata disease is one of the first and most serious documented cases of disease resulting from environmental contamination. The disease was caused by the discharge of wastewater containing methyl-mercury from an industrial plant. Early investigations revealed the following main findings:

i) The disease affected adults as well as children.
ii) The first case was reported in 1953.

The Minamata disease research group consisted mainly of members of the Kumamoto University School of Medicine, who established that the disease was a form of poisoning that occurred with the ingestion of marine life taken from the area, and that it was not in itself contagious (World Health Organisation 1991).

Minamata city was the only industrialized city in the area where the Chisso Co. Ltd operated a chemical plant. In view of the fact that the cause of the disease was undoubtedly water contamination, it was strongly suspected that the source of pollution could only have been the Chisso chemical plant. The plant however accepted no responsibility and took no measures whatsoever to prevent further contamination, claiming that the cause of the disease was unknown. Chisso also refused to co-operate with investigations on the grounds that doing so would reveal confidential corporate information (World Health Organisation 1991).

During the 1950's people began to witness strange phenomena in and around Minamata Bay. For no apparent reason fish rotated continuously and floated belly up to the surface, shellfish opened and decomposed and birds fell while in flight. The most shocking observation however, was the frenzied death of cats. Cats suffered from excessive salivation and general convulsions or violent rotational movements, were unable to walk straight and often collapsed, dead. Many cats jumped into the sea to drown and eventually cats were no longer seen in the area. Cats in fact played an important role in helping to establish the aetiology of the disease. In February 1957 cats were brought to Minamata from Kumamoto City which was located 100 km away. All immigrant cats developed similar symptoms within 32 to 65 days of arrival. Mercury was detected in high concentrations in the brains of cats (18.6 µg/g - max), livers (145.5 µg/g - max), kidneys (36.1 µg/g - max) and hair (134.0 µg/g - max). (World Health Organisation 1991).

2.7.2.1.1 Early patients

The first patients diagnosed with Minamata disease were considered to be cases of acute and sub-acute poisoning. Of the 34 patients diagnosed, 16 died within 3 months of the onset of disease. Within 6 months of the onset, an additional 4 died, which demonstrated that the disease advanced rapidly.

All patients had common symptoms, including constriction of their visual field, sensory disturbances, ataxia, dysarthria, auditory disturbances and tremor. However, some bias in early diagnosis may have occurred, due to the fact that only serious cases manifesting all these symptoms were diagnosed as having the disease. Pathological findings demonstrated a number of common features such as damage to the central nervous system (CNS) particularly the cerebellar cortex. In addition, the disease was characterized by notable damage to the calcarine region of the occipital lobe (visual centre), the pre and post-central cortex (motor and sensory centres), and temporal cortex (auditory centre). In the cerebellar cortex, deciduation of granular cells was remarkable while Purkinje cells were relatively well retained, showing a granular cell-type cerebellar atrophy. Destruction and demyelination of the dorsal roots or sensory nerve fibres of the peripheral nerve system were also noted (World Health Organisation 1991).

2.7.2.1.2 Hair samples

Due to developments that were taking place in analytical methods, hair samples were only analysed 4 to 5 years after the onset of the disease. In spite of this delay, mercury levels in hair ranged from 2.46 μg/g to 705 μg/g (World Health Organisation 1991).

2.7.2.1.3 Congenital Minamata disease

Soon after the official discovery of Minamata disease, it became clear that a considerable number of children were born with congenital cerebral palsy. These patients had a number of common symptoms, including; mental retardation (100%), primitive reflex (100%), cerebellar ataxia (100%), disturbances in physical development and nutrition (100%), dysarthria (100%), deformity of limbs (100%), hyperkinesia (95%), and hyper salivation (95%).

Paroxysmal symptoms (82%), strabismus (77%), and pyramidal symptoms (pathological reflex) (75%). Such extensive damage to the brain is, of course, not limited to the symptoms of Minamata disease. Without data on mercury contamination, individual diagnosis is impossible, but group diagnosis or diagnosis in terms of epidemiological data is possible (World Health Organisation 1991).

#### 2.7.2.1.4 Mothers

While mothers were at first thought to be asymptomatic, subsequent, detailed examinations revealed a high incidence of mild symptoms of Minamata disease among them. Mothers suffered from sensory disturbances (100%), focal cramps (100%), mild ataxia (79%), auditory disturbances (75%), pain in the limbs (64%), constriction of the visual field (57%), dysarthria (43%), and tremor (39%). However, the degrees of severity of their symptoms were much milder than those displayed by the congenital patients.

#### 2.7.2.1.5 Infantile diagnosis

Generally, infantile cerebral palsy is only noted or diagnosed 3 to 4 years after birth. This is also true of congenital Minamata disease, with diagnosis first taking place in Minamata in 1962. No data on the mercury content in mothers or children at the time of birth existed. Fortunately, people in this region of Japan, have a custom of preserving the umbilical cords of new-born babies, which provided researchers with an opportunity to analyse mercury levels of infants at the time of birth. These analyses revealed elevated methyl-mercury levels at the time when the incidence of acute and fulminant cases was highest. A positive temporal correlation also existed between the production of acetaldehyde from the Chisso plant and the methyl-mercury levels in umbilical cords (Harada 1995).

#### 2.7.2.2 Methyl-mercury poisoning in Iraq

In Iraq, three epidemic mercury poisonings were reported: one in 1955/56, another in 1959/60 and the third and largest outbreak in 1971/72. All three outbreaks were caused by the distribution of seed grain treated with mercury compounds. Rural people consumed the grain instead of planting it. The total number of official victims was 6530 including 459 deaths. In the investigation of the tragedy, dose-effect and dose-response relationships were established. In addition, a relationship between mercury concentrations in hair and blood was established. Since mercury concentration in hair strands recapitulates the history of methyl-mercury exposure, the analysis of hair mercury levels provided abundant information about the course of the exposure (World Health Organisation 1991).

##### 2.7.2.2.1 Foetal exposure to methyl-mercury in Iraq

In the Iraqi outbreaks, babies with in utero exposure to methyl-mercury were investigated for their physical and mental development and mothers were interviewed. Methyl-mercury exposure was estimated by the peak mercury concentration in a single hair strand, from each mother. A scoring system of examination results was adopted in the investigation. Although individual scores exhibited variability, a dose-response relationship was found. Statistical analysis suggests a greater effect in boys than in girls. The data were statistically analysed in detail to establish a dose-response relationship between the effect and the hair mercury concentration. Both logit and hockey-stick models were fitted to the data. From these analyses, an estimated lowest effect level (ELEL) of 10 μg/g mercury / g in hair was proposed as a threshold for human populations (World Health Organisation 1991). This being a level providing protection to foetuses.

#### 2.7.2.3 Additional human toxicity studies

Due to the fact that the earliest effect of methyl-mercury poisoning is the non-specific symptom of paraesthesia, the diagnosis of incipient methyl-mercury poisoning is very difficult (Clarkson 1992). Except at very high doses, all signs and symptoms of methyl-mercury exposure are due to selective damage to the nervous system. In humans the brain is the primary target and even within this organ, damage is selective or focal. Certain anatomical areas of the brain are more susceptible to damage. These include the visual cortex and the granule layer of the cerebellum. Severe damage manifests itself as a loss of neuronal cells in these areas. In adults the most conspicuous visual impairment, associated with methyl-mercury exposure, is constriction in visual fields and deficits in spatial and temporal visual function. Developmental exposure at high levels may result in oculo-motor manifestations and blindness, whereas less severe poisoning may result in changes in acuity and constriction of visual fields. The primary visual cortex is a major site of damage (Rice 1995).

##### 2.7.2.3.1 Prenatal toxicity

It was first discovered during the Minamata outbreak that the most hazardous form of mercury exposure is prenatal. Pregnant women exposed to methyl-mercury gave birth to infants suffering from severe brain damage. The mothers only experienced asymptomatic or mild effects such as transient paraesthesia during pregnancy. In later follow up studies, a milder form of prenatal damage characterised by psychomotor retardation was also noted. The nature of prenatal damage appears to differ fundamentally from that of adult damage to the central nervous system (CNS). Unlike focal damage in adults, damage to the developing brain is diffuse and widespread. In severe cases, ectopic neurons are seen, suggesting that methyl-mercury interferes with neuronal migration.

The foetus may be 5-10 times more sensitive than the adult brain to damage by methyl-mercury thus prenatal dose-response relationships are the ones most relevant to human risk assessment (Clarkson 1992).

The human brain forms over an unusually long time period, compared to other organs. While most of the basic structure is laid down before birth, neuron proliferation and migration continue in the postnatal period. The blood-brain barrier is not fully developed until the middle of the first year of life. The number of synaptic connections between neurons reaches a peak around age two and is then trimmed back by about half. Similarly, there is great postnatal activity in the development of receptors and transmitter systems as well as in the production of myelin. Many of the toxic agents known to damage the developing brain interfere with one or more of these developmental processes. Agents with antimitotic action, such as X-rays and methyl-mercury, have distinctly different effects on structure depending on which neurons are forming at the time of exposure. Guidelines designed to protect human populations from developmental neurotoxicity need to take into account the changing sensitivity of the brain as it passes through different developmental stages (Rodier 1995).

Data from human mother-infant pairs suggest that infants are born with higher blood mercury levels than their mothers. Neonatal and in utero exposure to methyl-mercury often results in more severe signs of intoxication in the offspring than in the mother. These include cerebral palsy, mental retardation and delayed walking and speech, as well as deficits in motor, language, psychological, scholastic and behavioural tests. In postnatal exposure, damage is more diffuse with neuronal loss in all areas of the visual cortex, as well as in many other brain areas. Retina and optic nerves are presumed not to be involved. Hearing deficits in adults range from 42 - 85 %. Severe hearing impairment, deafness and delayed speech development have resulted from in utero exposure to methyl-mercury (Rice 1995).

#### 2.7.2.3.2 Chronic exposure effects

In Japan, people on the coast of the Shiranui Sea had consumed fish containing low-dose methyl-mercury for decades, until 1968. The effects of long term consumption of methyl-mercury on those people were studied 10 years after the methyl-mercury dispersion had ended. An epidemiological study clarified that people in a fishing village (Ooura) on the coast of the Shiranui Sea showed a significantly higher frequency of neurological signs characteristic of methyl-mercury poisoning (hypothesia, ataxia, impairment of hearing, visual change and dysarthria), in comparison to people in a non-polluted fishing village (Ichiburi). These results suggest that people on the coast of the Shiranui Sea were affected by long-term dietary exposure to methyl-mercury (Harada et al. 1995).

#### 2.7.2.3.3 Treatment

In its severe form methyl-mercury poisoning is essentially irreversible, due to the destruction of neuronal cells. Treatment is therefore directed toward early removal of methyl-mercury from the body before irreversible damage occurs. Only complexing or chelating agents that contain SH- ligands such as D-penicillamine and N-acetyl-D-penicillamine are effective in reducing blood methyl-mercury levels. A method involving haemodialysis together with a diffusable thiol compound such as amino acid cysteine introduced into the arterial blood has also been used with success (Clarkson 1992).

It is of utmost importance that the health risk to people in the study area of this thesis should be properly quantified and monitored in order to establish if they should be treated to prevent further long term neurological damaged.

## 2.8 METHYL-MERCURY MUTAGENICITY AND CARCINOGENICITY

There are no human data available regarding the effects of inorganic mercury on human germ cell mutagenicity or from studies on the induction of mutations in animals. Results of tests for mutagenicity have been variable. Generally test results in prokaryotes may be positive for DNA damage, and results in eukaryotes are positive for clastogenicity. Table IV contains a summary of results from genetic toxicity testing in vitro, and table V a summary of genotoxicity data in cats (Schoeny 1996).

**Table IV Summary of in vitro genotoxicity studies of methyl-mercury.**

| System | Effect |
|---|---|
| Primary human lymphocytes | Chromosome aberrations, aneuploid |
| Primary human lymphocytes | Sister Chromatid Exchanges |
| Muntjac fibroblasts | Sister Chromatid Exchanges |
| V79, rat glioblastoma cells | DNA strand breaks |
| E.coli B/rWP2, WP2, Salmonella TA11535,TA1537,TA1538,TA98,Ta100 | Negative spot test |
| V79 cells | Weak mutagenicity at cytotoxic dose. Chromosome nondisjunction, negative for gene mutations and recombination. |
| Saccharomyces Cerevisiae | Decreased DNA synthesis, single strand breaks |
| L5178Y cells | Negative |
| Micronucleus, Salmonella Mutagenicity Bacillus rec assay | DNA damage |

(Schoeny 1996).

**Table V Genotoxicity of methyl-mercury in cats**

| Exposure duration | Dose (µg/g/day) | Effects, limitations, BML |
|---|---|---|
| 39 months, 7 days per week | 0.008<br>0.020<br>0.046 | No dose-related changes in unscheduled DNA synthesis in cultured lymphocytes or frequency of chromosomal aberrations in bone marrow of cats fed Hg contaminated fish or a fish diet supplemented with MeHg chloride. Limitations: no positive control, no assessment of cytotoxicity. BML range: 500 – 13 500 µg/L, Hg in blood . |

BML = Blood Hg level, Hg = mercury, MeHg = methyl-mercury
(Schoeny,1996).

Data from animal studies provide limited evidence to suggest that methyl-mercury is carcinogenic, see Table VI.

**Table VI Carcinogenic effects of methyl-mercury in animals, oral exposure**

| Species sex + number | Exposure duration | Dose mg/kg/day | Effects, limitations BML |
|---|---|---|---|
| Rat (ns) 25M,25F | 2yr,ad libitum in feed | 0, 0.004, 0.02, 0.1 | Tumours at comparable incidence (all groups). Small sample size, failure to achieve MTD. BML average 850ug/L in blood at 0.004, 6500 ug/L at 0.02 and 29 000 – 36 000 ug/L at 0.1 |
| Rat (sd) 56M,56F | 130 weeks ad libitum in feed | M (0.011, 0.05, 0.28) F (0.014, 0.064, 0.34) | No increase in tumour incidence |
| Mice (swiss) 54M,54F | from weaning until death | 0, 0.19, 0.19-0.95 | No increase in gross tumour incidence; limitation: histological examination not performed. |
| Mouse (ICR) 60M,60F | 78 weeks ad libitum in feed | 0, 1.6, 3.1 | Increased incidence of renal adenomas and adenocarcinomas in low dose M, limitations: very poor survival in both M dose groups |
| Mouse (ICR) 60M, 60F | 104 weeks ad libitum in feed | 0, 0.02, 0.03, 0.11, 0.15, 0.6 0.73 | Carcinoma significantly increased in M at 0.73, not invasive. Limitations: MTD exceeded (including severe renal damage in high dose M) |
| Mouse ($B6C3F^1$) 60M,60F | 2 yr ad libitum in feed | M(0.03, 0.14, 0.69) F(0.03, 0.13, 0.6) | Renal epithelial carconomas and adenomas in M at 0.69. Limitations: MTD exceeded in high dose M |
| Mice Swiss ns | 15 week ad libitum in water | 0, 0.03, 0.07, 0.27 | Number of lung adenomas, tumour size increased with dose |
| Cat (domestic) 4-5M, 4-5F | 2 years ad libitum in feed | 0, 0.0084, 0.02, 0.046, 0.074, 0.176 | No increase in tumour incidence. Limitations: small group size, short exposure duration, no pathological data for the lowest 3 doses |

Abbreviations: NS, not specified, M male, F female, BML blood mercury level, MTD maximum tolerable dose.
(Schoeny 1996)

### 2.8.1 Evaluation of methyl-mercury for human germ cell mutagenicity

Methyl-mercury is widely distributed in the body, breaching both blood-brain and placental barriers in humans. There are data indicating that methyl-mercury administered by intra-peritoneal injection reaches germ cells and may produce adverse effects. Because there are data for mammalian germ cell chromosome aberrations and limited data from a heritable mutation study, methyl-mercury is placed in a group of high concern for potential human germ cell mutagenicity.

The only reason why methyl-mercury is not classified as highest level of concern is the lack of positive results in a heritable mutation assay (Schoeny 1996). Recently published study results on the genotoxicity of methyl-mercury in humans are presented in Table VII.

**Table VII Genotoxicity of methyl-mercury in humans**

| Number / sex | Exposure duration | Dose mg/kg/day | Effects, limitations and BML |
|---|---|---|---|
| 24-63 both sexes | NS | NS | Incidence of SCE's in culture's peripheral lymphocytes correlated with intake of seal meat in an Eskimo population (as a surrogate for Hg intake), p=0.001. Other factors also correlated with SCE's, but multiple regression analysis found that some of the effect was attributable to Hg. Limitation: limited exposure data. BML not reported. |
| 51M | Measured as seafood meals/week; range 2- 14 | NS | Incidence of micronuclei positively correlated with blood Hg levels and age. No correlation with smoking or number of seafood meals /week. Limitation: no control group. BML range: 10.08 - 403.11 µg/g blood. |
| 18M exposed 10M contrrol | 10.5 years (occupa-tional) | 0.15-0.44 ($HgCl_2$) | Increased frequency of chromosomal breaks. Limitations: workers also exposed to mercuric chloride and one worker had a history of benzene poisoning: control group was not matched for sex, smoking habits or sample size. BML = 890 µg/L in urine (average). |
| 6M,3F exposed 3M,1F control | > 5 years > 3 x per week | NS | Correlation between blood Hg concentration and chromosome breaks in lymphocytes cultured from people who ate Hg contaminated fish. Limitations: small sample size, limited exposure data. BML range 4-650 µg/L in blood. |

Abbreviations: M-male; F-female; NS-not specified; BML-blood mercury level; Hg-mercury, SCE – Sister Chromatid Exchanges.
(Schoeny 1996).

## 2.9 EPIDEMIOLOGICAL STUDIES

The evaluation of health risks attributable to environmental agents relies heavily on evidence gleaned from epidemiological studies. It is important to emphasise procedures that should be adopted in assessing the value of such investigations by identifying shortcomings inherent in the epidemiological method. Initially evaluations require value judgements regarding the quality of the design and execution of the study. Thereafter an assessment is needed of groups of studies to estimate the likelihood or otherwise that the relationship between the exposure and the disease is causal (World Health Organisation 1989: 150).

### 2.9.1 Correlation between fish consumption data and hair methyl-mercury levels

Several studies have been conducted in areas where people consume large quantities of fish and a positive correlation between hair mercury levels and fish consumption rates has been demonstrated. The levels of hair mercury are dependent on the fish species and quantity consumed as well as the environmental levels of mercury pollution, (Buzina et al. 1995, Holsbeek 1996 et al. and Batista et al. 1996).

Furthermore, Holsbeek et al. (1996), demonstrated a positive correlation between the mean total mercury concentration in hair and the calculated daily methyl-mercury intake by the following formula ($Y = 183X + 0.155$) Y = µg/g hair, X = mg methyl-mercury intake/day ($r = 0.99$, $p = 0.01$).

In an attempt to determine the influence of a number of variables on mercury concentrations in scalp hair, 233 school children aged 6 - 16 years in the Tarragona Province (Southern Catalonia, Spain) were studied. The influence of place of residence ($r = 0.2143$), sex ($r = 0.2656$), fish and seafood consumption ($r = 0.2509$) were significantly ($P<0.001$) correlated with the concentrations of mercury in hair.

By contrast age, number of dental amalgam fillings, hair colour, occupation of the parents, as well as smoking habits of the household members did not show a significant correlation with hair mercury levels (see tables VIII and IX) (Batista et al. 1996).

**Table VIII Correlation between mercury concentrations in the hair of children from Tarragona Province (Spain) and various independent variables.**

| Variable | r | P |
|---|---|---|
| Place of residence | 0.2143 | <0.001 |
| Age | -0.0701 | ns |
| Sex | 0.2656 | <0.001 |
| Fish consumption | 0.2509 | <0.001 |
| Number of dental amalgam fillings | 0.0694 | ns |
| Hair colour | -0.0496 | ns |
| Occupation of parents | -0.0250 | ns |
| Smoking habits of members of the household | -0.0213 | ns |

NS, not statistically significant (P >0.05) (Batista et al. 1996)

**Table IX The effect of various variables on hair mercury concentrations of school children living in Tarragona Province**

| | Number of subjects | total Hg[a] | interval[b] |
|---|---|---|---|
| Sex | | | |
| Male | 79 | 0.54 | 0.203 - 1.460 |
| Female | 154 | 0.92 | 0.386 - 2.207 |
| **P*** | | <0.001 | |
| Consumption of fish (number of times per week) | | | |
| 0 | 5 | 0.45 | 0.26 - 0.79[c] |
| 1 | 113 | 0.66 | 0.26 - 1.72[c] |
| 2 | 85 | 0.80 | 0.32 – 202[c] |
| 3 | 25 | 1.25 | 0.54 - 2.88[d] |
| 4 | 5 | 1.93 | 1.18 - 3.15[d] |
| **P*** | | <0.01 | |
| Place of residence | | | |
| Tortosa | 63 | 0.97 | 0.472 – 1.977[c] |
| Tarragona city | 100 | 0.83 | 0.296 – 2.320[c] |
| Flix | 70 | 0.57 | 0.224 – 1.446[d] |
| P* | | <0.05 | |

*ANOVA P values, [a] total mercury concentrations are presented as geometric means (G) and are expressed in µg/g, [b] intervals are given as geometric mean (G) +/- typical deviation, [c, d] indicate statistical significance differences (Batista et al. 1996).

### 2.9.2 Human hair as a sampling medium for the determination of mercury exposure

A number of studies have demonstrated that human scalp hair is an ideal medium for the monitoring of methyl-mercury exposures in humans. This form of biological monitoring has a number of advantages over the sampling of other specimens (such as blood and urine). Of particular importance is the fact that it is a non-invasive method and allows for easy collection, storage and transport of samples. A number of studies have revealed distinct features of the accumulation of methyl-mercury in human hair. Hair methyl-mercury levels are generally 250-300 times greater than methyl-mercury levels measured in blood. The concentration of methyl-mercury in newly formed hair has been shown to be directly proportional to its simultaneous concentration in blood; thus it is possible to recapitulate previous blood levels of methyl-mercury through longitudinal analysis of hair strands. In most epidemiological studies, hair analysis has served as the sole basis for the estimation of methyl-mercury exposure in humans (Shi et al. 1990, Soria et al. 1992, Suzuki et al. 1993, Holsbeek et al. 1996 and Barbosa et al. 1998).

Human scalp hair was selected as the sampling medium of choice in this study. Cultural taboos regarding the possible use of hair for witchcraft purposes was anticipated to be a potential problem in this rural study area (Valley of a Thousand Hills). It was therefore necessary to have preliminary meetings with leaders in the community in order to gain support for the study.

### 2.9.3 Pregnant women

The observation of children affected by prenatal methyl-mercury exposures from Japan and Iraq suggests that the most critical periods for methyl-mercury exposures during pregnancy appear to be the late embryonic and foetal periods after the seventh week of gestation (Choi 1989).

Pregnant women are at a particularly high risk when exposed to methyl-mercury because the foetus is most susceptible to mercury and its compounds (Soria et al. 1992).

In a study by Holsbeek et al. (1996) of 251 subjects, the correlation between maternal hair mercury and mercury in the hair of infants (less than 2 years of age) still breast feeding was found to be statistically significant ($r = 0.55$ $p < 0.001$). The correlation between length of the breast feeding time period and mercury concentration in infant's hair was found to be significant for Indian children ($r = 0.51$, $p = 0.03$) but not for non-Indian children ($r = 0.03$; $p = 0.83$). Segmented hair analysis was done on a sub-sample of 30 mothers. The results of this analysis showed a mean decrease of 20% in methyl-mercury body burden during pregnancy, thus indicating the extent of placental transference of mercury to foetuses. One could however argue that there could be increased excretion of methyl-mercury via other routes due to metabolic changes during pregnancy.

The threshold of maternal hair mercury concentration indicative of adverse effects in the foetus is in the order of 10-20 µg mercury / g hair (Holsbeek et al.1996). There was a positive correlation between the mean total mercury concentration in hair and the calculated daily methyl-mercury intake ($Y = 183X + 0.155$), Y = µg/g hair, X = µg methyl-mercury intake/day; $r = 0.99$; $p = 0.01$, (Holsbeek et al.1996).

Prenatal methyl-mercury exposure is generally determined by measuring total mercury in a segment of the mother's hair that was growing during pregnancy. Methyl-mercury enters hair follicles in direct proportion to its level in the blood and is incorporated into the hair shaft. Once in the hair shaft the mercury content does not change. Furthermore, inorganic mercury such as the vapour released by dental amalgams does not appear to be taken up by the hair follicle. Human hair grows at approximately 1.1 cm per month and the segment that was growing during pregnancy can be determined with some accuracy.

By measuring the mercury concentration in short segments of hair the exposure history for the entire pregnancy can be recapitulated. Foetal exposure can also be determined by measuring total mercury in cord blood samples at birth. Blood provides an excellent measure of recent exposure with an average half time of 52 days (Myers and Davidson, 1998).

### 2.9.4 Developmental problems in exposed children

Since the dose-response analysis from Iraq clearly raised concers that lower prenatal exposures might be associated with adverse neuro-developmental effects, studies were undertaken to determine if adverse effects could be confirmed in such populations. Initial studies from Canada and New Zealand and more recently the Faroe Islands have supported the Iraqi conclusions. In contrast, studies from Peru and the Republic of the Seychelles have not found adverse associations. The studies have varied in multiple ways including the end points evaluated. Table X lists the general categories of end points evaluated (Myers et al. 1995).

**Table X: Categories of tests used to detect an association between prenatal methylmercury exposure and neuro-development in reported studies.**

| Testing category | Iraq | Canada | New Zealand | Peru | Faroe Islands |
|---|---|---|---|---|---|
| Neurologic | + | + | - | - | - |
| Developmental milestones | + | | | - | - |
| Developmental screening | - | + | | | - |
| Psychological | | | + | | + |
| Educational | | | - | | |
| Neuro-psychological | | | | | + |
| Neurophysiological | | | | | + |

+ studies in this category were done and a positive association with prenatal methyl-mercury was found.
- studies in this category were done and no association with prenatal methyl-mercury exposure was reported.
(Myers et al. 1995)

Myers et al. (1995) identified a cohort of 804 children, who had foetal methyl-mercury exposure from a maternal diet high in oceanic fish, in the Republic of Seychelles. Individual mercury exposure was determined by measuring maternal hair mercury levels during pregnancy. The median foetal mercury exposure was 6.6 μg/g. Children were evaluated once between 5 -109 weeks of age, using the revised Denver Development Screening Test (DDST-R) and a neurological examination. The association between maternal hair mercury levels and developmental outcome was evaluated by multiple logistic regression analysis. Covariates for the child included gender, birth weight, one and five minute Apgar score, age at testing and medical problems, and, for the mother, age, tobacco and alcohol consumption and medical problems during pregnancy.

An association between foetal methyl-mercury exposure and development was found when DDST-R scores of "questionable" and "abnormal" were combined, a procedure used by previous investigators. However, the association no longer existed when DDST-R scores of "questionable" were treated in the standard manner as passes. The importance of traditional lifestyles, such as fishing, to the social, cultural and economic wellbeing of indigenous people should not be ignored. In addition, there is growing evidence that fish consumption has cardiovascular protective benefits for adults. Concern about foetal exposure to methyl-mercury from fish consumption, should be tempered by its importance in brain development and other benefits (Myers et al. 1995).

A study during 1986 – 1987 on new born infants in the Faeroe Islands was followed up after seven years (n= 917). Clinical examination and neurophysiological testing did not reveal any clear-cut mercury related abnormalities, however, mercury related neurophysiological dysfunction was most pronounced in the domains of language, attention and memory and to a lesser extent in visiospatial and motor functions.

It was concluded that in this study population effects on brain function was associated with prenatal methyl-mercury exposure and that early dysfunction is detectable at exposure levels currently considered safe (Grandjean et al. 1997)

More recent studies among pregnant women and their offspring have also been conducted in the Faeroe Islands, in order to determine whether neonatal neurological function is adversely affected by seafood contaminants (such as methyl-mercury) from the maternal diet during pregnancy (n = 182). Each child's neurologic optimality score was determined at 2 weeks of age and adjusted for gestational age. Predictors were assessed by regression analysis. After adjustment for confounders, a 10-fold increase of the cord-blood mercury concentration was associated with a decreased neurologic optimality score of 2 (p=0.03). This effect corresponds to a decrease in gestational age of about 3 weeks. It was therefore confirmed in this population that prenatal exposure to methyl-mercury from contaminated seafood is associated with an increased risk of neuro-developmental deficit (Steuerwald et al. 2000).

The Seychelles Child Development Study is testing the hypothesis that prenatal exposure to low doses of methyl-mercury from maternal consumption of fish is associated with the child's developmental outcomes. No deleterious relationships between exposure to methyl-mercury and cognitive functions were identified in the primary analysis of the main cohort (n=740). A secondary analysis was subsequently performed to determine if effect modification from social and environmental factors was affecting associations between methyl-mercury and outcomes. Interactions between methyl-mercury level and both caregiver intelligence and family income were analyzed. The median prenatal methyl-mercury exposure was 5.9 ppm (0.5 – 26.7).

In terms of the Bayley Scales of Infant Development No effect modification occurred for preferential looking or visual attention at 6.5 months, psychomotor development at 19 or 29 months, or activity level at 29 months. Interactions between between methyl-mercury level and both caregiver intelligence and family income were statistically significant at 19 but not at 29 months.
It was therefore concluded that no major effect modification due to social or environmental factors were identified (Davidson et al. 1999).

In the study area of this thesis (Valley of a Thousand Hills) people are generally poor and do not have many opportunities for employment in the region. Most families survive as subsistence farmers and frequently somebody who receives an old age pension supplements this income. Locally caught fish is an important source of protein in the community, particularly among young boys, and as such the fish supply needs to be monitored and protected from contamination. It was decided to focus the hair sampling strategy on children found fishing in the river.

## 2.10 RISK ASSESSMENT

In order to appreciate the risk assessment process it was necessary to review World Health Organisation (WHO) and U.S. Environmental Protection Agency (EPA) policies and procedures employed to quantify risk. Although the most frequently measured health outcome in risk assessments is cancer, the same procedures are applied to non-carcinogenic agents such as mercury, where neurological damage is the health effect of concern.

Chemicals are classified into two broad groups, namely those that have a threshold of effect (such as mercury) and those that present a risk at any level (no threshold), which include genotoxic carcinogens and germ cell mutagens. The data available for risk assessments, include studies in humans and animals, structure-activity relationships and in vitro investigations.

Risk assessments should be based on all available data at the time of review, but it is appreciated that recognition of additional hazards or risk may emerge which will require subsequent re-evaluation (World Health Organisation 1994).

Wherever possible, appropriate human data should be used as the basis for risk assessments. As a result of a number of tragic incidents of methyl-mercury poisoning that have occurred in the past, there is a great deal of data available on human exposure effects for this chemical. For threshold effects, where data in humans are used as the basis for the development of tolerable intakes (TI's), uncertainty factors should be applied to observed effect levels, (OEL's). This will allow for the magnitude of any effect seen in the exposed group and their sensitivity to be extrapolated to the general population or target group. For compounds with critical effects, such as mercury, a primary objective of a literature review is to consider the comparability of experimental animal and human data, and to determine the highest doses that humans can be exposed to, without producing a critical effect.

In studies on experimental animals, the value of the No Observed Adverse Effect Level (NOAEL) is an observed value that is dependent on the protocol and design of the study from which it was derived. There are several study-dependent factors that influence the magnitude of the value observed. These include, the species, sex, age, strain and developmental status of the animals studied. The size of the study group. The sensitivity of the methods used to measure the response. The duration of exposure and the selection of dose levels, which are frequently widely spaced, so that the observed value of the NOAEL can in some cases be considerably less than the true no-adverse-effect-level (World Health Organisation 1994).

### 2.10.1 Toxicokinetic and toxicodynamic data.

Toxicokinetics includes data on the rate and extent of absorption (bio-availability), pattern of distribution, rate and pathway of bio-activation, and rate, route and extent of elimination.

Factors such as peak plasma concentration (Cmax), and area under the plasma concentration-time curve (AUC) of the toxic entity are particularly important since they are usually indicative of the extent and duration of exposure of the target organ. Dosimetric adjustments of administered animal dose to equivalent human dose are also possible. However, it is important to define which parameter is relevant to the toxicity since some are dependent on the Cmax and not AUC, while for long-term bioassays, the AUC may be of greater importance. Appropriate toxicodynamic factors include the identification of the toxic entity, the nature of the molecular target, the presence and activity of protective and repair mechanisms and the in vitro sensitivity of the target tissue.

These toxicokinetic and toxicodynamic parameters should be compared between the test species and humans for derivation of interspecies factors where this is possible. Modification of the 10-fold factor for inter-individual variability in humans would require data on toxicodynamics in a wide and fully representative sample of the general or exposed population, including an assessment of neonates, if appropriate. In the absence of reliable information on toxicokinetics and dynamics, the default values for these factors become the commonly used composite value of 100 (i.e., 10 for inter-individual variability and 10 for interspecies variation) (World Health Organisation, 1994).

### 2.10.2 Uncertainty factors

There is enormous variability between different data bases for risk assessment. In some cases a risk evaluation must be based on limited data obtained from studies on experimental animals. In other cases, detailed information on the mechanism of toxicity and/or toxicokinetics may be available, while in some other instances a risk evaluation can be based on data obtained from exposed human populations.

Consequently, for the general population the range of uncertainty factors applied in the derivation of TI's has been wide (1-10 000), although a value of 100 has most often been used. Historically a factor of 100 has applied where animal data are used. More recently, additional uncertainty factors have been incorporated to account for deficiencies in the data bases, such as the absence of chronic data or of a NOAEL. If data from well-conducted studies in human populations are the basis for the safety evaluation, a safety factor of 10 (for sensitive populations) has been considered appropriate. The value of 100 has thus been regarded as comprising two factors of 10 each to allow for interspecies and inter-individual (intraspecies) variations. A scheme has been proposed which retains the two 10-fold factors as the cornerstone for extrapolation from animals to man but which allows subdivision of each to incorporate appropriate data on toxicokinetics where these exist. This approach improves the extrapolation process, and where appropriate data can be introduced, it has the effect of replacing "uncertainty" factors with correction factors, (World Health Organisation, 1994).

There is a greater potential for differences in kinetics than in dynamics between humans and laboratory animals, so that an equal split of the 10-fold factor is inappropriate. The usual 10-fold factor (log 1) should be split into default values of 2.5 (100.4) for dynamics and 4 (100.8) for kinetics. A similar split has been proposed for inter-individual differences between humans in toxicokinetics and toxicodynamics (using pharmacokinetic - pharmacodynamic modelling).

However the variability for both aspects is similar and WHO concluded that the 10-fold factor should be split evenly between both aspects, i.e. 3.2 (100.5) for kinetics and 3.2 (100.5) for dynamics. The commonly applied 100-fold uncertainty factor should therefore be split as indicated in Figure 3 (World Health Organisation, 1994).

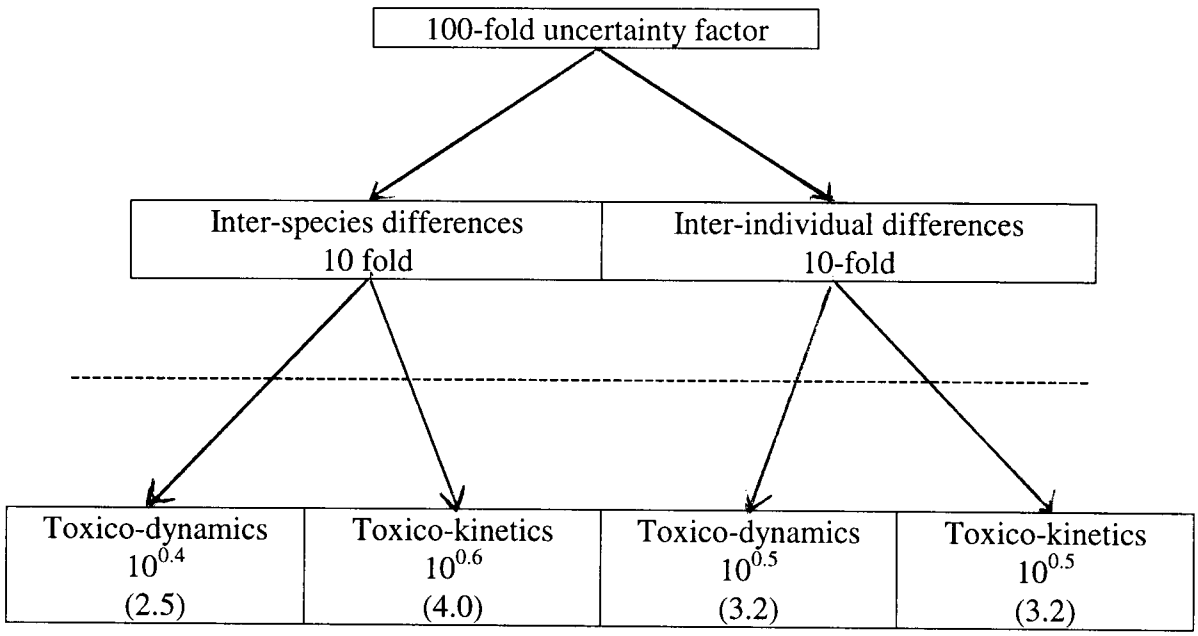


**Figure 3.** **Subdivision of the 100-fold uncertainty factors showing the relationship between the use of uncertainty factors (above the dashed line) and proposed subdivisions based on toxicokinetics and toxicodynamics (WHO, 1994).**

### 2.10.3 Steps in the risk management process

The following steps in the risk management process have been identified by Martin and Crane (1996).

i) Initial screening of potential areas of concern.

Screening assessments are performed to identify sites that may pose a potential threat to human health or ecological receptors based, in part, on sediment contamination (Martin and Crane, 1996).

ii) Risk assessment planning

Risk assessment planning provides an organisational framework for the subsequent steps that follow in the risk management process. Existing information is compiled to describe the physical features of the area of concern, the general distribution of sediment contaminants and their potential sources, and the human and ecological receptor populations likely to be present.

Contaminants of concern, biological species, endpoints (measured biological or ecological qualities), and primary exposure pathways for human and ecological receptors are identified. This information is used to develop preliminary remediation objectives, which are general descriptions of what remedial actions should accomplish, including the reduction of risks associated with exposure to contaminated sediments. Potential remedial actions may then be identified. Deficiencies in the available data that might preclude an adequate baseline risk assessment should be identified. Step iii (supplementary field sampling) may then be conducted if necessary (Martin and Crane, 1996).

iii) Supplementary field sampling

If data gaps were identified in the previous step, supplementary field sampling efforts may be required to collect the information necessary for a detailed site assessment. Additional information may have to be gathered on the physical, biological and chemical conditions of the system to further characterize the nature and extent of the sediment contamination problem. The data are also used to develop appropriate sediment remediation alternatives, to support mass modeling and to conduct the comparative risk assessment of the remediation alternatives (Martin and Crane, 1996).

iv) Baseline risk assessment

Baseline risk assessments estimate current risks to humans, wildlife and aquatic organisms resulting from direct and indirect exposure to contaminated sediments in the absence of any sediment remediation. Baseline risk estimates are developed using conservative or health protective assumptions to determine which contaminants and exposure pathways pose the greatest risk. This is done in order to determine whether remediation is likely to be required and to provide a baseline against which any future remedial action can be evaluated (Martin and Crane, 1996).

### 2.10.4 The U.S. Environmental Protection Agency reference dose

The U.S. EPA define the reference dose (Rfd) as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during an average lifetime (U.S. Environmental Protection Agency 1997).

EPA scientists review RfD's for accuracy, appropriate use of risk assessment methodology, appropriate use of data and other scientific issues. When consensus has been reached by the workgroup, information on the RfD is made available to the public through one of the U.S. EPA databases namely The Integrated Risk Information System (IRIS). The RfD is based on the best available data that indicate a critical effect. This is generally the first indicator or most subtle indicator of an adverse effect in the species under study. In calculating RfD's, U.S. EPA generally uses a no observed effect level (NOAEL). This is found from either inspection or modeling of dose-response data on the critical effect. It is a means of estimating the threshold for effect in the reported study.

The NOAEL is most useful when it is from a study in which a determination of the lowest observed adverse effect level (LOAEL) can also be done. The LOAEL is the lowest tested dose at which the critical effect was seen in the species under study. In calculating the RfD the U.S. EPA divides the NOAEL or LOAEL by a series of uncertainty and modifying factors in order to extrapolate to the general human population (U.S. Environmental Protection Agency 1997).

The uncertainty factors (which may be as much as 10 each) are for:

i) Extrapolation of data to sensitive human sub-populations;

ii) Extrapolation from animal data to humans;

iii) Lack of chronic data;

iv) Lack of certain other critical data; and

v) The use of a LOAEL in the absence of a NOAEL.

The RfD is used for risk assessment judgements dealing with evaluations of general systemic toxicity. It is intended to account for sensitive members of the human population: the rationale is that if exposure to the RfD is likely to be without appreciable risk for sensitive members of the population, then it is without appreciable risk for all members of the population.

The RfD is generally applicable to men and women and to adults, to children and to the aged, unless data support the calculation of separate RfD's for these groups. The RfD is a quantitative estimate of levels expected to be without effect even if exposure persists over a lifetime. It is not intended to be compared with isolated or "one off" exposures. Exceeding the RfD does not mean that risk will always be present. The acceptability of an uncertain risk is a risk management decision. Risk management decisions may consider the RfD but will also take into account other exposures, as well as other risk factors and non-risk factors. At the RfD or below, exposures are expected to be safe. The risk following exposures above the RfD are uncertain, but risk increases with increasing exposures (U.S. Environmental Protection Agency 1997).

### 2.10.5 Methyl - mercury exposure guidelines published by various agencies

#### 2.10.5.1 World Health Organisation, International Programme on Chemical Safety (WHO/IPCS)

Reference values for mercury concentrations (expressed as total mercury) in biological materials, commonly used to indicate human exposures to mercury have been published by the WHO International Programme on Chemical Safety (IPCS). A number of different estimates exist for hair mercury levels that are associated with low risks of neurological endpoints such as paraesthesia. These estimates are sensitive to variables such as half-life of mercury in the body (time to eliminate half the dose).

The half-life of methyl-mercury is usually estimated as an average of 70 days, with extremes of about 35 to just over 200 days, reported for different individuals. The methyl-mercury half - life has not been directly measured in pregnant women, however it has been found to be shorter during lactation, possibly due to the excretion of mercury into milk.

WHO recommendations regarding risk associated with hair mercury concentrations is facilitated by data reported by WHO on 559 samples of human head hair from 32 locations in 13 countries. The WHO report found that mercury concentrations in hair increased with an increase in the frequency of fish consumption (see table XI) (World Health Organisation 1991).

**Table XI Correlation between fish consumption and hair mercury levels.**

| Frequency of fish consumption | Average mercury concentration in hair (µg mercury / g of hair) |
|---|---|
| No unusual Hg exposure | 2 |
| Less than one fish meal /month | 1.4 (range 0.1 – 6.2) |
| Fish meals twice a month | 1.9 (range 0.2 – 9.2) |
| One fish meal a week | 2.5 (range 0.2 – 16.2) |
| One fish meal each day | 11.6 (range 3.6 – 24.0) |

World Health Organisation (1991)

WHO analysed the Iraqi data and established that the risk of infants developing abnormal neurological signs when maternal hair mercury concentrations were over 70 µg/g was increased by 30%. Furthermore it was estimated that there was a 5% increased risk of neurological disorders in infants when maternal hair concentrations were between 10 and 20 µg mercury / gram of hair. The recommendations of WHO/IPCS are based on clinically observable neurological changes as the indicator of effect.

In addition to their recommendations on hair mercury concentrations WHO/IPCS recommend that as a preventative measure, in any sub-population that consumes large amounts of fish, hair mercury levels of women of child bearing age should be monitored. It was estimated that a daily methyl-mercury intake of 0.48 µg mercury per Kg body weight will cause no adverse effect (World Health Organisation 1991).

#### 2.10.5.2 U.S. Food and Drug Administration

In 1969, in response to the poisonings in Minamata Bay and Niigata, Japan, the U.S. Food and Drug Administration (FDA) proposed an administrative guideline of 0.5 µg/g for mercury in fish and shellfish moving in interstate commerce. This limit was converted to an action level in 1974 and was increased to 1.0 µg/g in 1979, in recognition that exposure to mercury was less than originally considered. In 1984, the 1.0 µg/g action level was converted from a total mercury standard to one based on methyl-mercury. The FDA's action level is based on consideration of the tolerable daily intake (TDI) for methyl-mercury, as well as information on fish consumption patterns and associated exposure to methyl-mercury. The neurological endpoint evaluated being paraesthesia (Cordle and Tollefson 1994).

U.S. FDA and WHO established a TDI based on a weekly tolerance of 300 µg of total mercury per person, of which no more than 200 µg should be present as methyl-mercury. These amounts are equivalent to 5 µg and 3.3 µg, respectively, per kilogram of body weight. Using the values for methyl-mercury, this tolerable level corresponds to a methyl-mercury intake of approximately 230 µg/week for a 70 kg person or 33 µg/person/day, (Cordle and Tollefson 1994).

The resulting TDI of 0.48 μg/kg-day was calculated from data developed in part by Swedish studies of Japanese individuals poisoned in the episode of Niigata, which resulted from the consumption of contaminated fish and shellfish and the consideration of other studies of fish-eating populations. Based on observations from the poisoning event later in Iraq, U.S. FDA acknowledged that the foetus might be more sensitive than adults to the effects of mercury. In recognition of these concerns, the U.S. FDA provided advice to pregnant women and women of childbearing age to limit their consumption of fish known to have high levels of mercury (Cordle and Tollefson 1994).

Due to the uncertainties associated with the Iraqi study U.S. FDA has chosen not to use this study as a basis for revising its action level. Instead, it has chosen to wait for the findings of prospective studies of fish-eating populations in the Seychelles Islands and the Faroe Islands to be published (Cordle and Tollefson 1994).

#### 2.10.5.3 U.S. Environmental Protection Agency limits for methyl-mercury

The current U.S. EPA reference dose for methyl-mercury, was based on data concerning neurological changes in 81 Iraqi children who had been exposed to methyl-mercury in utero. Mothers of the children had eaten methyl-mercury-contaminated bread during pregnancy.

Data were collected by interviewing mothers and by clinical examination by a paediatric neurologist approximately 30 months after the poisoning episode. The incidence of several endpoints such as, late walking, late talking, seizures or delayed mental development and scores on clinical tests of nervous system function were mathematically modeled in order to determine a mercury level in hair which was associated with no adverse effects.

These effects were defined as delays in motor and language development, such as:

i) Inability to walk two steps without support by the age of 2 years.

ii) Inability to respond to simple verbal communication by the age of 2 years among children with good hearing.

iii) Scores on physical examination by a neurologist that assessed cranial nerve signs, speech, involuntary movements, limb tone, strength, sensation, posture, and ability to sit, stand, walk and run.

iv) Assessment of mental development or the presence of seizures based on interviews with the child's mother (U.S. Environmental Protection Agency 1997).

In calculating the mercury level in hair, which was associated with no adverse effects, the U.S. EPA chose a benchmark dose approach, based on modeling of all effects in children. The benchmark dose is the intake of methyl-mercury associated with the lower bound (that is the lower limit) of the 95% confidence interval around the dose producing a 10% prevalence of adverse effects. The symptoms used as end-points of adverse neurological effects included delayed walking, talking and abnormal neurological scores. This lower bound was determined to be 11 µg/g methyl-mercury in hair. A dose-conversion equation was used to estimate a daily intake (including the uncertainty factor) of 0.1 µg methyl-mercury /kg body weight/day. This dose, when ingested by a 60 kg individual, will maintain a blood methyl-mercury concentration of approximately 44 µg/L in blood or 11 µg Hg/g hair (11 ppm).

Due to variability in the way individuals process methyl-mercury in their bodies and the lack of data on observed adult male and female reproductive effects, an uncertainty factor of 10 was used to derive the U.S EPA, RfD from the benchmark dose. The RfD for methyl-mercury was determined to be 1 x $10^{-4}$ mg/kg-day; that means that a person could consume 0.1 µg MeHg for every kg of his/her body weight every day for a lifetime without anticipation of risk of adverse effect. This also applies to sensitive sub-populations, over a lifetime.

The RfD may be considered the midpoint in an estimated range of about an order of magnitude. This range reflects variability and uncertainty in the estimate. At the RfD or below, exposures are expected to be safe. The risk following exposures above the RfD is uncertain but risk increases with increasing exposure. It is important to note, however, that the RfD is a risk assessment tool, not a risk management decision. Judgements as to what constitutes a safe dose and exposure represent decisions that involve risk management components (U.S. Environmental Protection Agency 1997).

The current methyl-mercury RfD is supported by investigations of laboratory animals under controlled exposures to methyl-mercury. Data from experimental animals (including primates with long-term exposures) show methyl-mercury-induced nervous system damage, particularly on the visual system, although the animals appeared clinically normal. The endpoints described in the animal literature are important and these have been conducted by dosing protocols that are relevant to human exposures. In experiments using non-human primates, sensory (visual, somatosensory, auditory), cognitive (learning under concurrent schedules, recognition of faces), social play, and schedule-controlled operant behaviour are all identified as having been adversely affected by methyl-mercury. The sensory, cognitive and motor deficits appear reliably over a consistent range of doses in non-human primates exposed to methyl-mercury during development. Subtle but important deficits appear in several functional domains.

These are identifiable signs of methyl-mercury effects when appropriate testing conditions are applied (U.S. Environmental Protection Agency 1997).

Furthermore the RfD is supported by studies of children exposed in utero. These studies include investigations among Cree Indians in Canada and New Zealanders consuming large amounts of fish. Hair samples were used to monitor mercury exposure over time. Conclusions by the investigators in their official reports cite developmental delays among children born of mothers whose hair mercury concentrations during pregnancy were 6 to 18 μg/g, this being consistent with the benchmark dose of 11 μg/g (U.S. Environmental Protection Agency 1997).

Currently a number of prospective research studies are underway in the Seychelles and the Faeroe Islands and some papers on the results of the research are just being published. These studies further address the question of methyl-mercury exposures through fish consumption and associated neurological disease. The studies include more subjects than the Iraqi study and they utilize endpoints that are more sensitive than the clinical signs and symptoms of methyl-mercury poisoning observed in Iraq. Early findings seem to suggest that the benchmark dose for maternal hair should be approximately 1 μg/g (Budtz-Jorgensen 2000).

These data should be useful in decreasing the uncertainty surrounding both the benchmark dose and the RfD. However, statistical analyses for purposes of risk assessment have been recommended by the U.S. EPA's Science Advisory Board. In addition to these two major prospective investigations, additional studies evaluating the effect of methyl-mercury exposures from fish and shellfish in human subjects from other geographic areas are expected to be published in peer-reviewed literature in due course. The U.S. FDA has determined that revision of its action level for mercury concentrations of fish in interstate commerce should wait until the new studies have reduced the level of uncertainty.

The availability of results from the above studies will likewise enable U.S. EPA to re-examine and adjust its RfD as needed (U.S. Environmental Protection Agency 1997).

## 2.11 EXPOSURE MODELING

Boischio and Henshell (1996) evaluated the risks associated with methyl-mercury exposure of a fish eating population along the Madeira River in the Amazon. Their assessment was based on hair mercury concentrations. Given the vulnerability of the developing nervous system, a pre-natal LOEL of 0.7 µg/ kg bodyweight, and an adult and childhood LOEL of 3 µg mercury / kg body weight was used.

Based on hair mercury concentrations, it was observed that approximately 95% of infants were at risk as a result of mercury absorption through previous placental exposure and/or by ingesting mercury from mother's milk and/or fish consumption, at a level as great as the LOEL. The hazard quotient (see Table XII) derived from the LOEL for neuro-behavioural effects was 64, based on an estimated mean daily mercury intake of 4.5 µg/kg bw. Approximately 45% of the mothers of infants and other women of child-bearing age were at risk of ingesting mercury at a level equivalent to the LOEL. Mercury ingestion at this rate translates into a derived hazard quotient for neuro-behavioral effects of 17 for all potential mothers in the population.

The non-infant population at the highest risk was fish eating children under 5 years old. This sub-population had a mean estimated mercury daily intake of 6.4 µg/kg bw. This resulted in a probability that almost 60% of the sub-population ingested mercury at a level equivalent to the LOEL or higher. For this sub-population, there was a hazard quotient of 21. These data strongly indicate that the young children of the study population may be ingesting mercury doses that have been correlated with neurological damage from mercury poisoning.

A mean daily mercury intake of approximately 70 µg was calculated from the observed mean fish mercury concentration of 0.36 µg/g and the fish consumption estimate of 200 grams per day. The inter-conversion of the observed mean hair mercury level - into mercury intake, also resulted in an estimated intake of approximately 70 µgHg/day (see table XII). Hazard quotients were determined according to the equation: Hazard quotient=Hg intake / Reference dose (RfD) (Boischio and Henshel, 1996).

**Table XII** **Mean mercury intake and respective hazard quotient estimated for the population distributed by gender / age sub-groups, according to their mean hair mercury concentration, in a fish eating population along the Amazon River, Brazil.**

| Gender / age group | N | Hair Hg µg/g | Body weight (kg) | Hg intake* µg/kg bw | Hazard** quotient |
|---|---|---|---|---|---|
| <24 months old, not eating fish | 19 | 7.5 | 7.1 | 4.5 | 15/64 |
| those eating fish < 5yrs old | 31 | 18.3 | 12.1 | 6.4 | 21 |
| from 5 – 14 yrs old | 61 | 22.8 | 25.6 | 3.8 | 13 |
| from 15 – 48 yrs old, female | 57 | 14.7 | 53.5 | 1.2 | 4/17 |
| above 49 yrs old, female | 19 | 9.7 | 51.5 | 0.8 | 3 |
| above 15 yrs old, male | 50 | 19.2 | 58.8 | 1.4 | 5 |
| total population | 237 | 17.2 | 35.5 | 2.2 | - |

**The following RfD's were used in these calculations: A single hazard quotient refers to results obtained using the adult RfD (0.3 µg/kg bw). If there are two values the first (left) RfD = 0.3 µg/kg bw is for the adult and children population; and the second (right) RfD = 0.07 µg/kg bw for the prenatal and perinatal population.
(Boischio and Henshel, 1996).

The highest hazard quotients obtained were those for infants. Children (up to 5 years old) who ate fish were at the next highest risk, as the hazard quotients for this gender/age group were lower, but still relatively high. The correlation coefficients (Spearman rank correlations) between the estimates of mercury intake and hair mercury are strongly positively correlated (r = 0.83 to 0.97). Body weight is negatively correlated with mercury intake (r = - 0.52 to 0.19), according to the gender/age group.

A high percentage of the population under 15 years old was at risk of ingesting mercury at levels at least as great as the LOEL. This was estimated to be approximately 95% for breast-fed infants, 60% for children up to 5 years old who eat fish and 35% for children between 5 and 14 years old (Boischio and Henshel, 1996). The Boischio and Henshel study is of relevance to this thesis, as young children, boys in particular, play and fish in the u'Mgeni river and tributaries and they consume fish from the river daily.

The highest levels of methyl-mercury in fish, predicted with the aid of exposure models, are found in the 4th trophic level (predatory fish species at the top of the food web). In quantifying risk, one needs to establish fish consumption rates and the average body weight of populations. The FDA advises pregnant women, and women of childbearing age intending to become pregnant, to limit their consumption of fish species with methyl-mercury levels around 1 µg to 200 g per week (about 1 serving). For fish with levels averaging 0.5 µg/g, regular consumption should be limited to two servings per week. Both uncertainty and variability characterize estimates of the number of individuals who exceed various recommendations on exposures to mercury (U.S. Environmental Protection Agency 1997).

WHO recommends that a risk analysis be done for people who consume more than 100 g of fish per day. The 100 g/day recommendation of WHO can be used as a screening threshold to identify populations potentially at increased risk, particularly pregnant women. The significance of the risk, as mentioned above, is also a function of the methyl-mercury concentrations of the fish consumed. Children may be at a higher risk of methyl-mercury exposure than adults because they appear to have higher exposures per kilogram body weight and they may be inherently more sensitive than adults given the developmental state of the nervous system. Whether or not children differ from adults in sensitivity to methyl-mercury neuro-toxicity is, however, still unknown (U.S. Environmental Protection Agency 1997).

There are various ways available to assess the risk to populations from methyl-mercury exposure. These include the following approaches.

i) Quantification of risk by predicting increases in methyl-mercury concentrations in fish, due to anthropogenic emissions and subsequent exposure levels of human populations. This method is flawed by virtue of the fact that many assumptions about environmental concentrations and pathways of exposure have to be made.

ii) The use of dietary surveys to identify the amount and type of fish consumed by populations. This method provides data on pathways of exposure and the quantity of methyl-mercury consumed is estimated from hypothetical fish methyl-mercury levels. Data related to fish methyl-mercury levels may not be readily available.

iii) The use of hair mercury levels as a method of determining whether members of a population are at risk. Hair mercury levels reflect methyl-mercury exposures for general populations. This type of assessment is an appropriate measure of actual mercury exposure since biological samples are utilized (U.S. EPA 1997).

In view of the above criteria it was decided that a direct measure of exposure by means of dietary surveys and the analysis of fish and animal and human hair samples would be done in order to calculate human health risks associated with the consumption of fish in the Valley of a Thousand Hills study area.

## 2.12 CONCLUSIONS DRAWN FROM THE LITERATURE REVIEW: RELEVANCE FOR THIS STUDY

i) The initial levels of contamination measured in the vicinity of the Thor Chemicals Plant were very high and exceed acceptable environmental standards.

ii) Mercury waste discharged by Thor chemicals into the environment has probably been converted to methyl-mercury.

iii) Mercury has probably been mobilized in the ecosystem of the Valley of a Thousand Hills and this needs to be confirmed.

iv) Sediment and fish from the u'Mgeni River and Inanda Dam will reflect most accurately the extent of mercury pollution in the Valley ecosystem.

v) It can be accepted that 90 % - 100 % of mercury in fish tissue will be in the form of methyl-mercury.

vi) Human populations consuming fish in the region are at risk, and monitoring of hair mercury levels is the most appropriate method of quantifying their levels of exposure.

vii) Pregnant woman and children are considered to be a "high risk" group and sampling should be directed towards them.

# CHAPTER 3

# METHODS

## 3.1 ETHICAL CONSIDERATIONS

Since a whole community relies upon a river system, known to be polluted with mercury, for its supply of fish and domestic water, professional ethics demand that the health risk of the exposed group be investigated.

Data were obtained from people living in the study area, thus principles of ethical research were considered during the design and execution of the study.

The following ethical principles were observed:

i) The University of Cape Town - Higher Degrees and Ethics Committees approved the study proposal prior to the collection of data.

ii) The research conformed to generally accepted moral and scientific principles.

iii) Non - invasive techniques ensured that the study population was not subjected to any discomfort or risk.

iv) The relevant literature was reviewed in detail. The human sampling method selected (hair samples) was the least invasive method available and only a limited number of individuals were sampled. Environmental and animal samples were used to gather additional data.

v) Individual personalities, rights, wishes, beliefs, consent and freedom to withdraw from participation was respected.

vi) The researcher was deemed to be qualified and competent by the University Ethics Committee to collect the samples and data.

vii) This research involved no experimental or therapeutic methods.

viii) Prior to the collection of hair samples, free consent was obtained from individuals and the parents of children under the age of 18. A translator was used to ensure that all participants understood the purpose, methods, aims and objectives of the study. Participants were requested to sign an informed consent form (annexure 1). Feedback regarding the results of hair analysis was provided to participants.

ix) Subjects were informed that they could withdraw from the study at any stage.

x) All data was used in group data format and the names of individuals were not reported.

xi) Participants in the study were paid R10 for fish samples and R5 for both cattle and human hair samples.

xii) The community were informed of the findings and recommendations generated by the research.

## 3.2 STUDY DESIGN

An analytical risk assessment was conducted during the period November 1998 to April 1999. Data were collected to quantify levels of mercury pollution in various media and to evaluate the risk the community may be exposed to. Permission to conduct the study and to take samples was obtained from the Faculty of Health Sciences of the University of Cape Town, the local traditional leader, as well as the police commissioner in the area, and from all persons who participated in the study.

The study was designed to collect and analyse data to determine:

i. The existing levels of mercury contamination in the polluted river system from below Thor Chemicals, along the Mngceweni and u'Mgeni Rivers up to the Inanda Dam (the study area) compared to control area samples.

ii. Mercury levels in algae, cattle hair and fish samples from the study area and a control area.

iii. Human fish consumption rates and scalp hair mercury levels obtained from people living in the study area, as compared to controls and international standards.

## 3.3 STUDY AREA

Two 1:50 000 topographical maps, obtained from the Land Surveyors Office, Cape Town, were used to identify and select sampling positions, these are highlighted on the map (figure 1) which was introduced in Chapter 1 of the thesis. Sampling in the study area was conducted from directly below the Thor Chemicals plant, along the Mngceweni (tributary) and u'Mgeni Rivers, with the final sampling point being the Inanda Dam. Control samples were collected upstream from the Mngceweni / u'Mgeni confluence the u'Mzinduzi River and the Nagle Dam.

## 3.4 STUDY POPULATION

The study population consisted of all people residing in the study area. Amongst this population a "high risk" sub-group was identified. This group consists of young boys who swim, play, drink and fish in the u'Mgeni River daily. A control group which consisted of young boys from the u'Mzinduzi River, Nagal Dam area, located upstream from Thor Chemicals, was also selected.

## 3.5 SAMPLING METHODOLOGY

i) Sediment, animal hair, human hair and river fish samples were collected during the period 30 November 1998 to 4 December 1998. Fish samples were collected from the two dams during the period 7 December 1998 and 8 June 1999.

ii) Sampling personnel wore clean cotton clothes and gumboots and un-powdered vinyl gloves during sample collection and handling. Gumboots and gloves were thoroughly washed and rinsed at each sampling point.

iii) Each sample was identified numerically starting with the number 1 followed by the specific name (e.g. sediment). Furthermore, each sample area was identified alphabetically starting with the first sampling area being identified as - (A).

For example; 1 sediment A.

Where:

- 1= sample number;
- sediment = type of sample.
- A = sample area

Where more than one sample was taken at a particular point, the sample was further identified by the allocation of an additional number.

All samples, immediately after collection, were placed in Styrofoam insulation boxes, which were packed with ice in order to reduce the temperature as rapidly as possible. The samples were dispatched to the South African Bureau of Standards (SABS) approved laboratory of Umgeni Water in Pietermaritzburg for analysis, on the day of sampling. On occasions when samples could not be dispatched on the same day (fish), they were placed in cold storage at -15$^0$ C, until transfer of the sample to the laboratory could take place.

### 3.5.1 Sediment samples

#### i) Sampling Equipment

The following sampling equipment was used:

- sterile plastic bags.
- 1Metre Poly Vinyl Chloride pipe (30cm diameter), with an airtight plastic bung on each side.
- un-powdered vinyl gloves.
- heavy-duty plastic spoon/scoop.
- tie on labels.
- water proof black marking pen.
- heavy-duty plastic bags.
- note book and pen.

#### ii) Method

10 Sediment samples were obtained along the stream bed of the study area, at approximately three kilometer intervals, as detailed by the United States, Geological Survey (USGS) (Porter et al. 1993).

Depositional zones, representative of upstream influences and various flow regimes, were selected as sample points, in order to ensure that sediment samples were representative of the depositional patterns of the river. The sampling areas extended approximately 100 meters in length along the riverbed. The depth of the river and the strength of the flow of water determined the width of the sampling area. Finely grained sediments (less then 2-mm fraction) with high organic loads were selected for inclusion in the sample.

All the collected sub-samples were composited in the laboratory using the "coning" method, to ultimately obtain one single homogenous sample, which was analyzed. The number of samples obtained from each site was based on the size of each depositional zone. This technique was used in order to reduce local scale variability and to obtain a result representative of the average contaminant levels present at each site. In addition analytical costs were reduced considerably. The minimum number of sub-samples taken at each point was 5 and the maximum was 10 (Porter et al. 1993).

As prescribed by the USGS, one sample site was selected randomly (area C) and all sub-samples obtained from this area were analyzed individually, prior to the mixing (coning) of the sub-samples. The mean of the sub-samples was calculated and compared to the composite sample result, in order to verify the reliability of the methodology (Porter et al 1993).

- The date and time each sample was collected was recorded.
- Labels were prepared with a waterproof pen and the labels were firmly attached to the plastic sample bags immediately after sample collection.

- The sample area was approached from downstream in order to reduce disturbance of the sediment bed and to prevent contamination of the sample.

- The plastic bungs were removed from both ends of the corer. The corer was inserted into the sediment bed to a depth of approximately 20-cm and prior to removal from the sediment bed the plastic bung was inserted into the top opening of the corer, so as to retain the sample core within the corer.

- The corer was removed from the sediment bed and prior to removing it from the water the second bung was placed in the bottom end of the corer thereby retaining the sediment.

- The corer containing the sample was removed to the riverbank where the top bung was removed. Excess water was drained off by tilting the corer carefully. The contained sediment sample was emptied into a plastic, sampling bag.

- The sediment sample was inspected visually to determine if it contained an adequate fine fraction (<2mm) and sufficient organic material. If the sample was considered to be unsuitable, it was discarded. If suitable, the bag was sealed and placed in the cooler box for storage and transport to the laboratory (Porter et al. 1993).

### 3.5.2 pH and Temperature

In order to determine the reduction potential of the river area being sampled (ability of the water to reduce inorganic mercury to mercury) pH and temperature were measured. All readings were taken with the aid of a Beckman 200, pH and temperature metre. The instrument was calibrated prior to sampling with buffers of pH 4 and pH 10. Readings were taken simultaneously within a central region of the sampling area. The probes were placed in the river and once the readings stabilized they were recorded.

### 3.5.3 Algae

Depending on availability, algae samples were collected at the same sample sites used for sediments, in accordance with prescribed USGS methodology, as detailed by Porter et al. (1993).

The randomly selected site (C) was used for sample verification. All sub-samples taken from this site were analyzed individually, prior to the coning of the combined sub-samples. The mean of the individual sub-sample results was determined and compared to the composite sample result. Reliability was accepted to a 2 standard deviation (95% confidence level).

i) Sampling Equipment

- sterile plastic bags
- un-powdered vinyl gloves
- plastic scraper / spoon
- plastic, tie-on labels.
- water proof black marking pen.
- note book

ii) Method

The same sampling sites used for sediment sampling were used for the collection of algae. Where possible 5 to 8 sub-samples were collected from within the sampling area. The sub-samples were composited in order to obtain one single sample for analysis. Algae samples were taken at the same time as sediment samples, according to the prescribed USGS methodology (Porter et al. 1993).

- Algae samples were collected after sediment sampling had been completed, in order to minimise disturbance of the river sediment.

- Samples were collected by hand, using un-powdered vinyl gloves, in wade-able areas only.

- Where algae adhered to the substrate and as such could not be removed by hand, a plastic spoon was used to dislodge the algae, taking care not to contaminate the sample with substrate particles.
- The sampling area was approached from downstream to avoid contamination.
- Five to eight sub-samples of algae collected from each sampling area were placed into separate sterile plastic bags.
- Algae sub-samples were composited in order to obtain a representative sample of the total mercury value at each site.
- Tie labels containing all relevant information were prepared and attached to each sample bag. The bags were sealed and placed in cooler boxes containing ice for transport to the laboratory.

### 3.5.4 Cattle hair

Cattle in the study area are penned only at night. During the day they graze freely and drink water from the Mngceweni and u'Mgeni Rivers as well as the Inanda Dam. Cattle generally were observed wading into the water when drinking, thereby stirring up sediment, which increases the likelihood, that they could be consuming deposited mercury in the water they drink. Local communities were approached and samples of hair from the tails of their cattle were purchased. Sampling was restricted to the area below the confluence of the Mngceweni and the u'Mgeni Rivers up to the Ndunaizi Village. No distinction was made between breed or sex of cattle. Control samples were collected upstream of the Mngceweni and u'Mgeni confluence in the vicinity of the Nagle Dam.

i) Sampling equipment

- un-powdered vinyl gloves
- white metal free envelopes
- stainless steel scissors
- notebook and ballpoint pen
- questionnaire (as detailed below)

ii) Method

- Local inhabitants were interviewed and their permission was obtained for the collection of samples. Only cattle that had been living in the immediate area for three years or longer were sampled. The owners of the cattle were requested to complete a short questionnaire. All instructions were conducted through interpreters so as to prevent misunderstanding and owners were requested to collect the samples under supervision. Owners were required to wash their hands thoroughly prior to sample collection and were paid R5 for each sample.

- Hair was cut with stainless steel scissors from the tip of the tail and was placed into a clearly marked envelope (Porter et al. 1993).

- The sample area was identified as for sediment sampling.

Questionnaire

1. Sample Number.
2. Name of owner:
3. General description of location
4. Length of time residing (animal) in the area.
5. When was the sample animal obtained by the owner
6. Where was the sample animal obtained by the owner.
7. General health of the animal since obtained
8. Food source

### 3.5.5 Fish

Since the Inanda Dam is deemed to be the major receiver of sediment deposition from the study area, the sampling of large fish from this source was considered to be of importance. Sharp Toothed Catfish and Wide Mouthed Bass of between 3 to 5 kg in weight or between 40 to 50 cm in length were caught from Inanda Dam, (situated approximately 20 km downstream from Thor Chemicals). Control samples of fish were taken from the Nagle Dam, which is situated approximately 20 km upstream from Thor chemicals.

In the u'Mgeni River (study area) local fishermen, in particular young boys were observed catching and consuming fish ranging from 15 cm to 25 cm in length and approximately 600 g in weight. Due to the fact that these fish were consumed regularly by the children, it was considered relevant to include them in the study, in spite of their small size. Catfish and carp were selected for inclusion in the study. The main motivation for their selection being the fact that visual observation and interviews with the community indicated that these were the most popular and abundant species caught and consumed by locals. *Cyprinus Carpio Linnaeus*, the most common species of Carp in the region, is omnivorous, taking a wide range of plant and animal matter mainly by grubbing in sediment. The Sharp tooth Catfish (*Clarias gariepinus*) is also a common resident fish of the region and the most abundant of the Catfish in South Africa. The fish preys, scavenges or grubs on virtually any available organic food, including fish, birds, frogs, small mammals, reptiles, snails, crabs, shrimps, insects, other invertebrates and plant matter, and is even capable of straining fine plankton if necessary (Skelton 1993).

i) Sampling Equipment

- Un-powdered vinyl gloves.
- Sterile heavy-duty plastic bags
- SABS approved scale and tape measure.
- Tie on labels and marking pen

ii) Method

- Samples of fish were clearly identified by the area where they were caught and the areas were correlated with the numbering system used for sediment samples. Fish caught for control purposes were obtained from the Nagle Dam.

- Local fishermen and boys, using nets in the river and fishing rods in the dams caught all fish sampled. Fresh fish was purchased from the local community and was immediately packed in ice for transport to an overnight storage facility and subsequently to the laboratory. Due to distances that had to be traveled and the working hours of the laboratory in Pietermaritzburg, samples had to be stored overnight and were frozen at -15 $^{0}$ C, prior to their dispatch to the laboratory within 24 hours.

- Fishermen were requested to complete the following questionnaire.

QUESTIONNAIRE

1. Sample Number.
2. Name of fisherman.
3. General description of the location
4. Specific site where fish were caught.
5. Date and time of catching.
6. Method used to catch the fish.
7. Date and time fish was purchased from fisherman
8. Weight of fish:
9. Length of fish:

### 3.5.6 Human hair

Whilst collecting fish samples, the fishermen (mostly young boys) were requested to provide a sample of their scalp hair. Hair sampling was restricted to the area below the confluence of the Mngceweni River and the u'Mgeni River up to the Ndunaizi Village and the Inanda Dam. Control samples were obtained from fishermen in the Nagle Dam area. Problems were encountered, as some people objected to providing hair samples for fear of witchcraft practices.

i) Sampling equipment

- Un-powdered vinyl gloves
- White metal free envelopes
- Stainless steel scissors
- Ballpoint pen.
- Questionnaire (as detailed below)

ii) Methodology

- Local fishermen or their parents were interviewed and their permission was obtained prior to any samples being taken. Only people who consumed fish regularly and have been living in the immediate area for three years or longer were sampled. All persons sampled were requested to complete a short questionnaire.

- All instructions were conducted through interpreters so as to prevent misunderstanding.

- Hair was cut with stainless steel scissors and the sample was placed into a clearly marked envelope (Porter et al. 1993).

- The sample area was identified as for sediment sampling.

Questionnaire

1. Sample Number.
2. Name:
3. Frequency of fish consumption.
4. Length of time residing in the area.
5. When was the sample was obtained
6. Where was the sample was obtained.
7. General state of health as perceived by the individual.
8. Other sources of food (homegrown crops and meat).
9. Is river water used for domestic purposes, swimming and drinking?
10. Is river sediment (mud/sand) used for the building of houses?

### 3.5.7 Control (reference) samples

Control samples of sediment, pH and temperature, algae, fish, cattle hair and human hair were taken from the Nagle Dam and surrounds. The control area extended along the u'Mgeni River to the bridge at Egogwini, approximately one kilometre above the confluence of the Mngceweni River and the u'Mgeni River, which formed the "upper-end" boundary of the study area. The sampling protocols employed in the collection of control samples were identical to those followed in the collection of study area samples.

The following control samples were collected:

1. Sediment: 5 composite samples, consisting of between 5-8 sub-samples.
2. Algae: 4 composite samples consisting of between 5-8 sub-samples.
3. Cattle hair: 5 samples.
4. Fish: 5 samples from Nagle Dam.
5. Human hair 5 samples

## 3.6 LABORATORY ANALYSIS OF SAMPLES

The U.S. Environmental Protection Agency (1999) recommend that due to cost and other considerations fish samples should be analysed for total mercury content as one can assume that between 90 and 100% thereof will be in the form of methyl-mercury. All samples were transported to the SABS approved analytical laboratory of Umgeni Water, immediately after collection, or were frozen at $-15^{0}$C, (except hair) until they could be taken to the laboratory. Samples were analysed, in accordance with the methodology detailed in Appendix B.

## 3.7 STATISTICAL ANALYSIS OF DATA

Environmental samples were classified into groups according to sample type and compared to control samples. These data were statistically analysed non-parametrically with the aid of the Mann - Whitney test. The level of significance of the tests did not exceed 0.05. In addition, due to small sample size, a Monte Carlo analysis was done to verify the significance of tests.

Descriptive statistics were used to compare results to international standards and mercury results obtained in earlier studies. The mean fish mercury levels measured in the various sampling locations were used to estimate the daily methyl-mercury intake of individuals. Body weight and daily fish consumed rates were estimated. The individually calculated daily intakes, expressed as μg mercury per Kg body weight, were compared to international norms and hazard quotients were calculated.

The SPSS statistical computer software package was used for data entry and analysis.

# CHAPTER 4

# RESULTS

The following results were obtained:

## 4.1 DEMOGRAPHIC DATA

All subjects were male Zulu speaking residents of a rural area. The mean age of the control group (n = 5, 19.6 years) was slightly higher (p = 0.55) than that of the study group (n = 9, 17.3 years).

## 4.2 ENVIRONMENTAL MEASUREMENTS

### 4.2.1 Sediment samples

In an attempt to maximize the number of samples taken, composite sampling techniques were employed. Approximately 7 - 10 samples were taken at each point. The samples were mixed into a batch and through a process of coning a homogeneous sample was selected for analysis (see Table XIII).

**Table XIII Sediment samples obtained from directly below Thor Chemicals (A/1) and at regular intervals as far as the Inanda Dam. Control Samples were obtained above Thor Chemicals at regular intervals as far as the Nagle dam.**

| STUDY AREA Sample number | Hg levels (µg/g) | CONTROLS Sample number | Hg levels (µg/g) |
|---|---|---|---|
| **A/1 #** | 54.0 | | |
| B/2 | 0.19 | V/11 | 0.006 |
| C/3* | 0.86 | W/12 | 0.1 |
| D/4 | 0.12 | X/13 | 0.1 |
| E/5 | 0.48 | Y/14 | 0.03 |
| F/6 | 0.07 | Z/15 | 0.3 |
| G/7 | 0.1 | | |
| H/8 | 0.09 | | |
| I/9 | 0.2 | | |
| K/10 | 0.1 | | |
| **Mean excluding #** | **0.25 (sd 0.26)** | | **0.11 (sd 0.12)** |

sd Standard deviation
* (Verification of sediment sampling technique, composite sample)
\# Outlier analysed and discussed separately

The composite sample obtained directly below the Thor Chemicals plant contained a very high level of mercury (54 µg/g) and was excluded from the statistical analysis of the data. This result is to be discussed separately. A two tailed Mann-Whitney test for non – parametric statistics was performed (p=0.2). There was no significant difference between the mean mercury levels of the two groups. This was confirmed by a Monte Carlo analysis (p = 0.22).

In order to verify the composite sampling technique as specified by the USGS, one sample area was selected randomly (C) all individual sediment and algae samples collected in this area were analysed separately and the mean result was compared to the result of the composite sample (Porter et al. 1993). Table XIV demonstrates the reliability of the compositing technique employed.

The mean of the individual results (0.74 μg/g) was deemed to be comparable to the composite result of 0.86 μg/g as there was no significant difference between the results (p = 0.28) Sd = 0.19.

**Table XIV Verification of compositing (sample C/3).**

| Sample number | Hg (μg/g) |
|---|---|
| C/3a | 0.63 |
| C/3b | 0.83 |
| C/3c | 0.52 |
| C/3d | 0.57 |
| C/3e | 1.109 |
| C/3f | 0.72 |
| C/3g | 0.78 |
| **Composite** | **0.86** |
| **Mean** | **0.74** |
| **Standard deviation** | **0.19** |

4.2.2 Algae samples

The composite USGS sampling techniques employed for sediment analysis was also used in the algae sampling strategy (Porter et al. 1993). Approximately 7 - 10 samples were collected at each point and they were mixed into a homogenous batch, which was then analysed, as illustrated in Table XV.

**Table XV Algae samples obtained from both the study area and the control area.**

| Study area Sample number | Hg (µg/g) | Control area Sample number | Hg (µg/g) |
|---|---|---|---|
| B/1# | 2.1 | | |
| C/2* | <0.5 | V/1 | <0.5 |
| D/3 | <0.5 | Y/2 | <0.5 |
| E/4 | <0.5 | Z/4 | <0.5 |
| F/5 | <0.5 | | |
| G/6 | insufficient | | |
| I/7 | <0.5 | | |
| **Mean** | **<0.5** | **Mean** | **<0.5** |

* composite sample
\# outlier, analysed and discussed separately
threshold of detection = 0.5 µg/g.

Sample B/1 was collected from an area close to Thor Chemicals and is discussed separately, as with the sediment sample. Since the means of the study group and control group were below the threshold of detection, the two groups are deemed to be identical in terms of their mercury content. Table XVI demonstrates the reliability of the compositing technique employed.

**Table XVI Verification of composite sample (C/2).**

| Sample number | Hg (µg/g) |
|---|---|
| C/2a | No algae |
| C/2b | <0.5 |
| C/2c | <0.5 |
| C/2d | <0.5 |
| C/2e | <0.5 |
| C/2f | <0.5 |
| C/2g | <0.5 |
| C/2h | <0.5 |
| **Mean** | **<0.5** |
| **Composite** | **<0.5** |

Threshold of detection 0.5 µg/g

All sub-samples obtained from area C were analysed individually and all sub-samples, as well as the composite sample were found to be below the threshold of detection of the test (<0.5 µg/g) and therefore there is no measurable difference between the two sample areas.

## 4.3 BIOLOGICAL SAMPLES (ANIMAL)

### 4.3.1 Cattle hair

Hair samples were taken from the tails of cattle in both the study area and the control area and were analysed to determine the levels of mercury in each area.

**Table XVII Cattle hair samples obtained from the study area and control area.**

| Study area Sample number | Hg (µg/g) | Controls Sample number | Hg (µg/g) |
|---|---|---|---|
| D1a | <0.5 | 1 | <0.5 |
| D1b | <0.5 | 2 | <0.5 |
| D1c | <0.5 | 3 | <0.5 |
| D1d | <0.5 | 4 | <0.5 |
| D1e | <0.5 | 5 | 0.6 |
| D1f | <0.5 | 6 | <0.5 |
| D1g | <0.5 | 7 | <0.5 |
| E2a | <0.5 | | |
| E2b | <0.5 | | |
| E2c | <0.5 | | |
| E2d | <0.5 | | |
| **Mean** | **<0.5** | | **<0.51** |

Threshold of detection 0.5µg/g.

As can be seen from the results in table XVII, all study area samples were below the level of detection (<0.5 µg/g). Control samples were also below the level of detection, except for one which was 0.6 µg/g. There is no significant difference between the two groups ($p = 0.21$).

### 4.3.2 Fish samples

Fish caught in the u'Mgeni River and the Inanda Dam (located in the study area) and the Nagle Dam (control area), were purchased from local fisherman. The fish had been caught either by netting or with baited lines. The results of the laboratory analysis are listed in table XVIII.

The U.S FDA's revised action level of 1 µg/g, is deemed to be the fish tissue mercury concentration which is considered safe for consumption on a daily basis, by sensitive populations (U.S. Environmental Protection Agency 1997). All fish mercury levels from both the study and control areas were found to be well within this limit. The South African prescribed level is half that of the U.S. FDA (0.5 µg/g). All fish samples in the study area were within this limit, however, 2 control samples (20%) taken from the Nagle Dam exceeded the 0.5 µg/g limit (Foodstuffs Cosmetics and Disinfectants Act of 1977).

The median mercury level of fish taken from the u'Mgeni River, in the study area, was 0.36 µg/g.

The median fish mercury level in the Inanda Dam, which is located in the study area was 0.19 µg/g) and in the control area the median values was 0.07 µg/g. A two tailed Mann-Whitney test for non – parametric statistics was performed ($p=0.47$). There was no significant difference between the mean mercury levels of the two groups. This was confirmed by a Monte Carlo analysis ($p = 0.47$).

**Table XVIII Fish mercury levels, u'Mgeni River, Inanda and Nagle Dams, KwaZulu-Natal, South Africa**

| u'Mgeni River (study area | | | Inanda Dam (study area) | | | Nagle Dam (control area) | | |
|---|---|---|---|---|---|---|---|---|
| sample species | Fish size (cm) | Mercury concen-tration (µg/g) | Sample species | Fish size (cm) | mercury concen-tration (µg/g) | Sample species | Fish size (cm) | mercury concen-tration (µg/g) |
| Carp | 16 | 0.33 | Carp | 30 | 0.05 | Carp | 31 | 0.1 |
| Carp | 18 | 0.36 | Carp | 28 | 0.1 | Carp | 33 | 0.1 |
| Carp | 16 | 0.36 | Carp | 27 | 0.07 | Carp | 33 | 0.55 |
| Carp | 17 | 0.33 | Carp | 31 | 0.08 | Carp | 35 | 0.66 |
| Catfish | 18 | 0.36 | Carp | 30 | 0.25 | Carp | 30 | 0.44 |
| Catfish | 17 | 0.45 | Catfish | 35 | 0.19 | Catfish | 35 | 0.05 |
| Catfish | 18 | 0.4 | Catfish | 30 | 0.21 | Catfish | 34 | 0.24 |
| | | | Catfish | 34 | 0.20 | Catfish | 34 | 0.13 |
| | | | Catfish | 35 | 0.20 | Catfish | 31 | 0.08 |
| | | | Catfish | 36 | 0.20 | Catfish | 32 | 0.2 |
| Median | 17 | 0.36 | Median | 30.5 | 0.19 | Median | 33 | 0.07 |
| Range | 16-18 | 0.33-0.45 | Range | 27-36 | 0.05-0.25 | Range | 30-35 | 0.05-0.66 |

## 4.4 HUMAN RISK ASSESSMENT

The Hazard Quotient is frequently used to quantify non-cancer hazards such as exposure to methyl-mercury. Intake is determined over a specific exposure duration in µg of the chemical /kg body weight x day. The reference dose is a level of exposure below which no adverse health effects are expected. If the hazard quotient is less than 1, no harm is expected, if greater than 1 the threshold has been exceeded and toxicity is likely to occur (Fan and Chang 1996, p 245).

### 4.4.1 Risk calculations based on the World Health Organization's tolerable daily intake

The U.S. Food and Drug Administration and the World Health Organisation established a tolerable daily intake of 0.48 μg methyl-mercury per kg body weight per week (U.S. Environmental Protection Agency 1997). In order to perform a risk assessment for the most sensitive population, daily fish consumption was estimated to be 200g per day and the weight of exposed individuals determined by interview. Total daily mercury consumption per kilogram body weight per day was calculated, and compared to the World Health Organization's criteria.

An assumption was made that 100% of the mercury in fish is in the form of methyl-mercury (U.S. Environmental Protection Agency 1997). From the data presented in Table XIX there is clearly a potential risk to people consuming fish from the study area on a daily basis with a median hazard quotient of 4.6 (0.8-6.3).

**Table XIX Total mercury consumption per kg body weight of the study group, compared to the World Health Organisation Tolerable Daily Intake**

| Age/sex | Estimated weight | Mean fish Hg level in area (µg/g) | Estimated daily intake (µg)/kg body wt. | WHO TDI | Hazard Quotient |
|---|---|---|---|---|---|
| 14 | 30 | 0.33 | 2.2 | 0.48 | 4.6 |
| 10 | 25 | 0.36 | 2.9 | 0.48 | 6.0 |
| 17 | 65 | 0.36 | 1.1 | 0.48 | 2.3 |
| 34 | 70 | 0.33 | 0.9 | 0.48 | 1.9 |
| 13 | 25 | 0.36 | 2.9 | 0.48 | 6.0 |
| 11 | 20 | 0.3 | 3 | 0.48 | 6.3 |
| 21 | 65 | 0.45 | 1.4 | 0.48 | 2.9 |
| 14 | 30 | 0.4 | 2.7 | 0.48 | 5.6 |
| 22 | 70 | 0.15 | 0.4 | 0.48 | 0.8 |
| median 14 (10-34) | Median 30 (20 –70) | median 0.36 (0.15-0.45) | Median 2.2 (0.4-3) | | Median 4.6 (0.8-6.3) |

Note; a worst case scenario assumption was made that 100% of the mercury was in the form of methyl-mercury. Hazard Quotient below 1 indicates no risk.

4.4.2 Risk calculation based on the Environmental Protection Agencies reference dose

The U.S. Environmental Protection Agency has published human fish consumption limits for the American public, using a reference dose of 0.1 µg/g. Calculations were based on an adult population (weight 72 kg) consuming 227 g of fish per meal. A month was estimated as being 30.44 days, these limits are summarized in Table XX (U.S. Environmental Protection Agency 1999).

In terms this risk estimate, adults in the study area should not be eating more than 1 or 2 fish meals per month given the mercury levels measured in the locally caught fish. However, the subjects interviewed stated that they consumed at least one fish per day.

**Table XX U.S. Environmental Protection Agencies fish consumption limits for methyl-mercury.**

| Risk-based consumption limit Fish meals per month | Fish tissue concentrations (μg/g) |
|---|---|
| 16 | >0.03 – 0.06 |
| 12 | >0.06 – 0.08 |
| 8 | >0.08 – 0.12 |
| 4 | >0.12 - 0.24 |
| 3 | >0.24 – 0.32 |
| 2 | >0.32 – 0.48 |
| 1 | >0.48 – 0.97 |
| 0.5 | >0.97 – 1.9 |
| none | >1.9 |

(U.S. Environmental Protection Agency 1999)

Using the estimated daily intake of methyl-mercury obtained from Table XIX, and the current U.S. Environmental Protection Agencies reference dose of 0.1 μg methyl-mercury/kg/day (U.S. Environmental Protection Agency 1999) the hazard quotient for individuals in the exposed sample was calculated (see Table XXI). This showed that in terms of EPA criteria, the population should be regarded as being "at risk" since the median hazard quotient was calculated to be 22 (4-30).

**Table XXI U.S Environmental Protection Agency, mercury reference dose, Individual hazard quotient calculations**

| Age/sex | Estimated weight | Mean fish Hg level in area (μg/g) | Estimated daily intake (μg)/kg body wt. | Hazard Quotient |
|---|---|---|---|---|
| 14 m | 30 | 0.33 | 2.2 | 22 |
| 10 m | 25 | 0.36 | 2.9 | 29 |
| 17 m | 65 | 0.36 | 1.1 | 11 |
| 34 m | 70 | 0.33 | 0.9 | 9 |
| 13 m | 25 | 0.36 | 2.9 | 29 |
| 11 m | 20 | 0.3 | 3 | 30 |
| 21 f | 65 | 0.45 | 1.4 | 14 |
| 14 m | 30 | 0.4 | 2.7 | 27 |
| 22 m | 70 | 0.15 | 0.4 | 4 |
| median 14 (10-34) | Median 30 (20-70) | median 0.36 (0.15-0.45) | Median 2.2 (0.4-3) | median 22 (4-30) |

Note; a worst case scenario assumption was made that 100% of the Hg was in the form of MeHg. Hazard Quotient below 1 indicates (no risk)

## 4.5 HUMAN HAIR SAMPLES

Human hair mercury levels and fish consumption data in the study group (n = 9) and the control group (n = 5) are presented in Table XXII, together with the mean fish mercury levels found in the area where subjects reside. Human hair mercury levels in both exposed and control groups were all < 0.5 μg/g and well below the U.S. EPA reference dose of 11μg/g (U.S. Environmental Protection Agency 1997).

**Table XXII Personal data, fish consumption data and human hair mercury levels: study and control groups.**

| Age & sex | Years of Residence in the area | mercury amalgam fillings | Mean fish mercury level in the area (µg/g) | Human hair mercury (µg/g) Study group (n=9) | Human hair mercury (µg/g) Control group (n=5) | Minimum fish meals per week |
|---|---|---|---|---|---|---|
| 14 m | 1 | 0 | 0.33 | <0.5 | <0.5 | 7 |
| 10 m | 10 | 0 | 0.36 | <0.5 | - | 7 |
| 17 m | 17 | 0 | 0.36 | <0.5 | <0.5 | 7 |
| 34 m | 6 | 0 | 0.33 | <0.5 | <0.5 | 7 |
| 13 m | 13 | 0 | 0.36 | <0.5 | - | 7 |
| 11 m | 11 | 0 | 0.3 | <0.5 | <0.5 | 7 |
| 21 m | 21 | 0 | 0.45 | <0.5 | - | 7 |
| 14 m | 14 | 0 | 0.4 | <0.5 | - | 7 |
| 22 m | 3 | 0 | 0.15 | <0.5 | <0.5 | 7 |
| Median 14 (10-34) | Median 11 (1-21) | | Median 0.36 (0.15-0.45) | Median <0.5 | Median <0.5 | Median 7 |

**NOTE: Sensitivity level of the test (0.5 µg/g)**

# CHAPTER 5

# DISCUSSION OF RESULTS

## 5.1 LIMITATIONS OF THE STUDY

In any study there are limitations that need to be identified and overcome where possible. These limitations must be brought to the attention of the reader, as they may influence the validity of findings. The following limiting factors were identified in this study.

i. Due to the geographic nature and extent of the study area as well as budgetary constraints, a limited number of environmental samples could be taken. A sampling technique (compositing) was used to optimize the representativeness of each sample. Samples were collected at approximately 2 km intervals in areas where fine sediment deposition was evident, this being in accordance with the U.S. Geological Survey methodology (Porter et al. 1993).

ii. Obtaining permission to collect hair samples proved to be difficult due to superstitious beliefs regarding the use of hair or nail clippings to perform witchcraft. A limited number of samples were obtained (n = 9) and controls (n = 5). Care was therefore taken to ensure that people were selected that were deemed to be most at risk, owing to their fish consumption patterns and age in order to obtain a worst case scenario.

iii. At the time of sample collection (December 1998) it was hot. Most of the fishermen interviewed were boys swimming in the river while fishing with nets. The children may have overestimated their annual fish consumption rates, as fishing was good at the time and this may not necessarily be the case throughout the year particularly during the colder winter months.

iv. The Mngcweni river is a small shallow stream which is not used for fishing. All study area fish and hair samples were thus collected beyond the confluence of the Mngcweni and u'Mgeni rivers which is at least 2 – 3 km from the plant.

v. The small sample size, particularly for human hair was deemed to be a problem, this was addressed through statistical uncertainty analysis.

## 5.2 SIGNIFICANCE OF RESULTS

Mercury pollution from Thor Chemicals has been a subject of heated debate and argument within South Africa and abroad for a number of years. Occupational Health issues were thoroughly investigated and appropriate action was taken to limit exposures of this nature (Randolph 1998). The environmental impact, however, was never adequately researched and resolved. Ongoing debates about the extent and impact of the environmental pollution have often been based on non-validated hearsay evidence and not scientific data. Towards the latter quarter of 1998 Parliament took an interest in the environmental debate on Thor Chemicals and it was clear that a need existed for this study to be conducted.

## 5.3 ENVIRONMENTAL SAMPLES

### 5.3.1 Sediment

Table XXIII is a summary of sediment mercury levels collected from; below Thor Chemicals, Fredville and the confluence of the Mngceweni and the U'mgeni Rivers over a period of 7 years.

The area directly below the Thor Chemicals boundary (500 m) was found to be heavily polluted and the measured mercury concentration in sediment in this study was 54 µg/g, which is 491 times greater than the mean baseline level of 0.11 µg/g measured in the control area. This level of pollution accords well with results obtained 7 year earlier (49.6 µg/g) by Johnston et al. (1991).

The fact that mercury levels in this area are still excessively high supports the view that mercury can exist in stream sediment for decades, providing an important reservoir for mercury to cycle back into the ecosystem (U.S. Environmental Protection Agency 1997).

**TableXXIII Summary of mercury levels (µg/g) in sediment samples over a period of 7 years**

| Sampling area | Johnston et al (1991) µg/g | Chester et al (1996) µg/g *** | This study (1998) µg/g |
|---|---|---|---|
| Directly below Thor Chemicals | 49.6<br>1764* | 174<br>326<br>1150* | 54<br>composite sample mean |
| Fredville | 0.91<br>0.33 | 1.9 | 0.48<br>composite sample mean |
| Confluence of Mngweni and u"Mgeni<br><br>**Mean** | 0.03<br>0.004<br><br>**0.017 (sd 1.8)** | Not sampled | 0.6 3<br>0.83<br>0.52<br>0.57<br>1.109<br>0.72<br>0.78<br>**0.74 (sd 0.19)** |
| Control area<br><br>**Mean** | <br><br>**0.85** | Not sampled | 0.006<br>0.1<br>0.1<br>0.03<br>0.3<br>**0.11** |

* Samples taken in this area were very high as they were taken in an earth dam, where mercury accumulated in the bottom sediment.

*** The Chester et al. study was not conducted according to strict scientific protocols, sample containers did not conform to standards and contamination may have occurred. These results are therefore of limited use for comparison purposes.

sd standard deviation

The highly contaminated area below the Thor Chemicals plant is isolated and does not support livestock or human habitation. The stream is shallow and densely overgrown which makes access difficult and it does not support fish. The root systems of the vegetation in the streambed serves to bind the contaminated sediment and soil, thereby decreasing the amount of contamination further downstream.

No immediate intervention, except for access control, is deemed necessary in this area (directly below Thor Chemicals). Attempts to remove the contaminated sediment could create a greater problem downstream, due to the removal of the vegetation currently containing the pollution. This contaminated zone is an ideal area to conduct further research on the use of plants or bacteria for bio-remediation of mercury contaminated land, (U.S. Environmental Protection Agency 1997).

When comparing the results of Johnston et al 1991, to the results obtained in 1998 at the confluence of the Mngceweni and u'Mgeni Rivers (Figure 4) an interesting trend is noted. The mercury level in sediment in this area increased from a mean value of 0.017 µg/g in 1991 to 0.74 µg/g in 1998. A two tailed Mann-Whitney test for non – parametric statistics was performed (p=0.04). The increase in mercury levels is therefore significant. This finding was confirmed by a Monte Carlo analysis (p = 0.05).

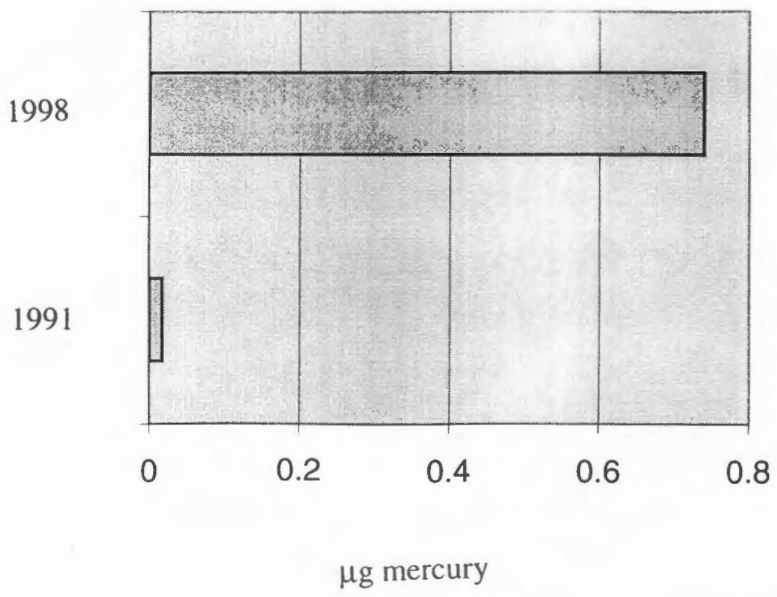


**Figure 4. Comparison of the mean mercury levels at the confluence of the Mngceweni and u'Mgeni Rivers in 1991 and 1998.**

If the samples collected from below Thor Chemicals up to the confluence of the Mngceweni and u'Mgeni Rivers are excluded from the statistical analysis, there is no significant difference ($p = 0.46$) between the mean mercury levels of the control (0.11 µg/g) and study areas (0.12 µg/g) see figure 5. Dilution of the sediment as it flows into the u'Mgeni River is possibly the main factor responsible for the lower mercury levels measured further downstream.

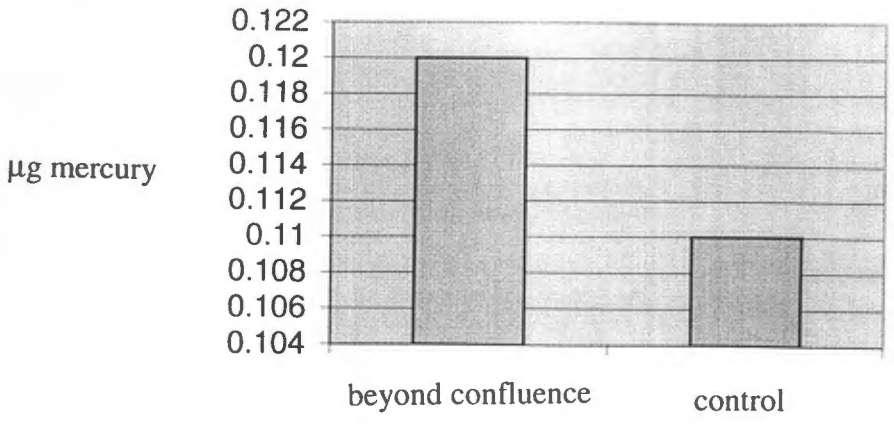


**Figure 5. Mean mercury levels beyond the confluence of the Mngceweni and u'Mgeni Rivers compared to the mean control sample concentration.**

### 5.3.2 Algae

Aquatic plants are deemed to be good indicators of mercury pollution and the bio-accumulation thereof in the environment (Bailey and Stokes 1985, Shrivastava and Rao 1989, WHO 1989, Lenka et al. 1992, Porter et al. 1993, Rasmussen 1994, U.S. EPA 1997, Gonzales 1997 and Guimaraes et al 1998).

Sample B, which was obtained within 2 km of the Thor Chemicals plant had a higher mercury concentration than all other samples. The mercury level at this point was 2.1µg/g, which is at least 4.2 times greater than control area levels. The remainder of the samples obtained from the study area, were found to be below the threshold of detection (<0.5 µg/g) of the laboratory test employed.

## 5.4 BIOLOGICAL MONITORING (ANIMALS)

Burger (1994) confirmed that animals are good environmental bio-monitoring indicators of mercury pollution. Local cattle were sampled in order to ascertain if animals in the study area have elevated levels of mercury in their tissue when compared to controls.

### 5.4.1 Cattle

Throughout the study area, cattle were observed wading into rivers and streams, stirring up sediment and drinking muddy water. Since cattle meet all the criteria determined by Burger (1994) for the selection of suitable animal species for monitoring, it was decided to include them in the study. All cattle hair samples were found to be within acceptable limits and in fact all samples were below the level of detection of the test. Therefore there was no statistical difference between mercury levels in cattle from the study area as compared to controls.

### 5.4.2 Fish

Mercury bio-accumulates readily in fish thus fish are frequently used as indicators of mercury pollution. Furthermore, human fish consumption data are used to calculate mercury poisoning risks for fish consumers. Between 80 and 100% of the mercury found in all fish species is in the form of methyl-mercury, which is of great public health concern.

Furthermore older and larger fish are expected to have higher levels of mercury due to their diet (higher up the food web) and the amount of time that they have been exposed to mercury (Kjellstrom et al. 1989, Kim 1995, Holsbrook et al 1996, Bidone et al. 1997, U.S. Environmental Protection Agency 1997, U.S. Environmental Protection Agency 1999).

This study initially proposed only to obtain older larger fish from Inanda Dam (study area) and to compare the mercury levels of these to fish obtained from the Nagle Dam (control area). While conducting the study, however, it was noted that smaller, younger fish caught in the rivers formed an important part of the diet of people living along the riverbanks. In particular it was found that young boys net and consume small fish from the rivers daily. In order to conduct an accurate assessment of risk to the whole population in the area it was decided that young "river" fish would be included in the study.

A disturbing finding was the fact that the median mercury level in young fish, caught in the u'Mgeni River 0.36 (0.33-0.45) μg/g, was significantly higher than the median mercury level of 0.19 (0.05-0.25) μg/g, measured in older adult fish taken from the Inanda Dam. The older fish were however obtained at a distance of approximately 20 km downstream from Thor Chemicals ($p = 0.01$). This level of significance was obtained for both the Mann – Whitney and Monte Carlo tests.

This finding is a cause for concern as it indicates that fish populations in the river, closer to Thor Chemicals have significantly raised mercury levels. Dilution of sediment being carried to the dam could be a factor in explaining the relatively lower mercury concentration in Dam fish.

It must be emphasized that all fish sampled in the u'Mgeni River were found to be well below the South African and U.S. FDA action levels for mercury, which are 0.5 µg/g and 1 µg/g respectively. The fish is therefore assumed to be safe to consume (S.A.FCD Act 1972 and U.S. EPA 1997). Possible future increases in mercury levels are of concern and fish should be sampled regularly in order to ensure that this important food source remains safe.

Although not statistically significant for either the Mann-Whitney, or the Monte Carlo test (p=0.47). The median mercury level in fish obtained from the control area of Nagle Dam, 0.07 (0.05-0.66) µg/g, was lower than the median mercury level obtained from fish in the Inanda Dam, 0.19 (0.05-0.25)µg/g, which is located the study area.

Fish taken from both dams were found to be well below the U.S. FDA limit of 1.0 µg/g, however 20% of the fish samples obtained from the control area (Nagle Dam) exceeded the South African limit of 0.5 µg/g (FCD Act 1972 and U.S. Environmental Protection Agency 1997).

## 5.5 HUMAN RISK ASSESSMENT: HAIR SAMPLES

In terms of both the World Health organization and US Environmental Protection Agency criteria, the exposed sample was deemed to be at risk with median hazard quotients of 4.6 and 22 respectively.

It was decided that human biological samples should be included in the study design, in order to verify the results of the risk assessment.

The use of human scalp hair as an indicator of methyl-mercury exposure has been well established. The main advantage of this sampling method being the fact that it is non-invasive and allows for easy collection, storage and transport. Blood and hair mercury concentrations are related, with the hair mercury levels being 250 times greater than blood mercury levels. Hair analysis has therefore served as the sole basis for the estimation of methyl-mercury exposure in a number of studies, (Shi et al. 1990, Soria et al. 1992, Suzuki et al. 1993, Holsbeek et al. 1996 and Barbosa et al. 1998).

All of the hair samples were obtained from a "high risk" group of frequent fish consumers in the study area as well as from the control area had concentrations below 0.5 μg/g, (see Table XXII). Using the U.S. Environmental Protection Agency (1997) reference dose of 11 μg/g in human hair, the hazard quotient was calculated to be $< 0.045$ for all persons sampled. The inference is therefore that there is no measurable risk to humans, given the low level of mercury in their hair.

It seems likely that the fish consumption rates of the study group may have been overestimated and possibly the uncertainty factors built into the risk assessment criteria may have caused the calculated risk to be exaggerated.

# CHAPTER 6

# CONCLUSIONS

For many years the "Thor" issue has stimulated heated debate, arguments, accusations and counter accusations amongst various groups. Unfortunately the voices and wishes of the most important stakeholders, the exposed community, were seldom heard. The fact that negligence and subsequent occupational and environmental pollution occurred can not be ignored. However, the whole debate thus far has been based on assumptions and emotionally charged outrage rather than fact. Environmental health issues in particular were poorly understood and investigated. This study was designed to shed some light on the main issues of concern, namely the extent of environmental pollution and the subsequent health risk to which the surrounding community may be exposed.

It is evident that extensive mercury pollution occurred in the area a number of years ago. High levels of mercury in sediment have been found in the direct vicinity of the Thor Chemicals plant since 1991. The area directly below Thor Chemicals is still heavily contaminated and will remain a source of mobilization and the potential bio-accumulation of mercury into the ecosystem for many years to come. The bulk of the mercury pollution, however, seems to be well contained in the immediate vicinity of the Thor Chemicals plant. It is hypothesised that this can be attributed to the unique topography and vegetation of the area.

The source of the Mngceweni River is a relatively small and slow flowing stream (even during the rainy season) and the area, including the streambed, is covered by extremely dense vegetation. This serves to minimise access to the stream by animals and humans and binds the sediment, even during periods of high flow. In addition the area is not inhabited and there is no evidence that livestock have access to the stream. The upper reaches of the stream do not support fish.

When comparing sediment mercury levels over a period of 7 years (between 1991 and 1998) as well as mercury levels in algae, it appears as if mercury is being transported further downstream from Thor chemicals. Raised mercury levels in both sampling media were found as far downstream from Thor Chemicals as the confluence of the Mngceweni and u'Mgeni Rivers a distance of approximately 3 kilometres.

Levels of mercury in algae and cattle hair were not found to differ significantly from control area mercury concentrations. Mercury levels in fish were found to be well below the current South African and WHO guidelines. River fish were found to have higher mercury levels than fish obtained from the dam but were deemed to be safe to consume in terms of legal standards. Human risk assessments based on estimated daily fish consumption rates showed that in terms of both (U.S FDA / WHO) and U.S. EPA guidelines, the study population was deemed to be at risk. The analysis of hair obtained from fish eating members of the community, indicate that people in the region have not yet consumed dangerous quantities of mercury. However, this situation could change in future as contaminated sediment will be released into the river system for many years to come, particularly if disturbed by the removal of vegetation and during periods of high flow. Furthermore it is likely that the methyl mercury levels in fish tissue will increase in future.

The contaminated area in the immediate vicinity of Thor Chemicals thus presents a potential source for the release of mercury into the ecosystem, placing human fish consumers at risk for many years to come, unless adequate steps are taken to eliminate the source of pollution.

# CHAPTER 7

## RECOMMENDATIONS

i) Although there is evidence of mercury contamination near the Thor Chemicals plant, it would appear as if mercury mobilization in the area is slow, in spite of frequent heavy rains. The area involved is flat and marshy and is covered with dense vegetation (grasses and reeds). Further research needs to be done in order to determine the most suitable remediation option. care must be taken not to accidentally mobilize mercury should physical removal of the sediment be decided upon. In-situ methods must be investigated as an alternative option.

ii) The contaminated zone around Thor Chemicals should be clearly identified and fenced off, in order to restrict access.

iii) The community, government agencies, Thor Chemicals, The Valley Trust and other interested parties will be informed of the outcome of the research.

iv) Fish consumers will be informed that fish taken from the u'Mgeni River and Inanda Dam are currently safe for human consumption in moderation.

iv) Umgeni Water is encouraged to monitor sediment and fish mercury levels in the u'Mgeni River and Inanda Dam in future, as a routine precaution. In the event of the contaminated sediment being mobilized, the logical endpoint of sediment transportation would be the u'Mgeni River and the Inanda Dam.

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**APPENDIX A**

**UNIVERSITY OF CAPE TOWN**

**FACULTY OF MEDICINE**

**DEPARTMENT OF COMMUNITY HEALTH**

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Informed consent form:

1. I, (name) _____

   hereby consent to the following procedure being conducted upon myself.

   **Collection of a sample of my scalp (head) hair by means of cutting it with a pair of sharp stainless steel scissors.**

2. I acknowledge that I have been informed by Mr. Jacques De Villiers Oosthuizen about the study and the reasons why it is being done.

3. I freely consent to the hair sample being collected from me.

4. I am aware that I may withdraw my consent at any time without prejudice.

signed _____ date: _____
subject

signed _____ date: _____
witness

signed _____ date: _____
informant (translator)

signed _____ date: _____
researcher

## INFORMATION GIVEN TO SUBJECTS

My name is Jacques Oosthuizen, we are conducting a survey to see if any pollution from the factory (Thor Chemicals) has come down the river. Mercury pollution builds up in fish and we are checking to see how much mercury is in the river and the fish. We would also like to check if there is any mercury in your body and we can find that out by taking some of your hair to the laboratory for tests.

We need to do this to make sure that the fish you eat is safe.

As soon as we have the results from the laboratory we will inform you of the outcome and advise you whether or not the fish in your area is safe to eat.

Jacques Oosthuizen

**APPENDIX B**

Laboratory methods

Umgeni water analytical services laboratory methods manual, method 34, rev (amendment no 5, April 1998).

1. Introduction

All forms of Hg, including organic derivatives, are oxidised to ionic Hg by acidic potassium permanganate. Inorganic Hg is rapidly reduced by sodium borohydride in an acidic solution to Hg vapour. The vapour is purged continuously with argon into an unheated quartz tube in the light path of an atomic spectrometer where absorbance is determined at 253.7 nm.

2. Scope

This method is applicable for the analysis of trace quantities of Hg in clean waters and wastewaters.

3. Interference

Water vapour can result in loss of sensitivity and baseline drift. Condensation of water can occur in the measuring cell and this can be prevented by using a drying tube on the inlet to the cell.

4. Hazards

Nitric acid and sulphuric acid
Potassium dichromate
Potassium permanganate
Mercury

See the manufacturers handbook for instrument related hazards.

Ensure that you are familiar with the dangers and treatment associated with each of the above substances.

5. Sample collection and preservation

Samples should be collected in 250ml glass bottles with 1 ml dichromate solution and 1ml nitric acid (refer 7.6) per 100 ml sample.

6. Apparatus

6.1 Atomic Absorption spectrometer
Varian Spectra AA 220

6.2 Hydride generator
Vapour generation accessory with pump system.

6.3 Sampler
Varian SPS-5 autosampler or manual sampling.

6.4 Glassware
Certified measuring cylinders as required.

6.5 Pump tube sizes

| | |
|---|---|
| sample line : | purple-white (3 pump tubes) |
| acid line : | orange-orange |
| borohydride line: | orange-orange |

7. Reagents

7.1 Nitric acid (minimum assay 65%m/m).

7.2 Sulphuric acid (minimum assay 98%m/m).

7.3 Sodium borohydride solution:
3.0g Sodium borohydride.
2.5g Sodium hydroxide.
Dissolve and make up in a 500ml volumetric flask, using ultrapure water.
Must be prepared daily.

7.4 Bleach solution
60g Sodium Chloride
60g Hydroxylammonium chloride.
Dissolve and make up in a 500ml volumetric flask, using ultrapure water.
Must prepare daily.

7.5 Potassium permanganate solution (5% m/v):
100g Potassium permanganate (mercury free)
Dissolve and make up in a 2 000 ml volumetric lask, using ultrapure water.
Discard after three months.

7.6 Nitric acid stock solution (50% v/v):
Dilute concentrated nitric acid (refer 7.1) 1:1 with ultrapure water.

7.7 Dichromate stock solution (5% m/v):
50 g Potassium dichromate.
Dissolve and make up in a 1 000ml volumetric flask, using ultrapure water.
Discard after three months

7.8 Sulphuric acid stock solution (50% v/v):
Dilute concentrated sulphuric acid (refer 7,.2) 1:1 with ultrapure water.

7.9 Dichromate/nitric acid bath for soaking glassware and bottles

Pour 10l reverse osmosis water into a 25l bath. Add 1 000 ml nitric acid (refer 7.1). Add 10g potassium dichromate and mix well in order to dissolve . Add another 10l reverse osmosis water to the bath, and mix thoroughly. Make up fresh every three months, or if required, more frequently.

All glassware used in the sampling, digestion and analysis of mercury samples must be soaked in a potassium dichromate/nitric acid bath, preferably overnight, and thoroughly rinsed with ultrapure water before use.

7.10 Hydrochloric acid
Minimum assay 32%

7.11 Hydrochloric acid stock: (5m)
Dilute concentrated hydrochloric acid (refer 7.10) 1:1 with ultrapure water.

8. Preparation of standards

8.1 Mercury standard stock:
Spectroscopic standard 1 000mg/l. This solution must be discarded 2 years after opening.

8.2 Mercury Standard intermediate Stock (1 000ug/l):
Pipette 0.5 ml of the mercury standard stock into a 500 ml volumetric flask, add 5 ml dichromate solution (refer 7.7) and 5ml nitric acid (refer 7.6) and make up to the mark with ultrapure water. This solution must be discarded after 3 months.

8.3 Mercury working Standards:
Std 1 2ug/l: 0.4 ml intermediate stock (make up to 200ml using ultrapure water).
Std 2 5ug/l: 1.0 ml intermediate stock (make up to 200ml using ultrapure water).
Std 3 10ug/l: 2.0 ml intermediate stock (make up to 200ml using ultrapure water).

Thereafter, add 2ml dichromate solution (refer 7.7) and 2 ml nitric acid (refer 7.6) to each 200ml volumetric flask. These solutions must be prepared fresh or every run.

9. Preparation for analytical quality control (AQC)

9.1 Mercury AQC Stock:
Spectroscopic standard 1 000mg/l. This solution must be discarded 2 years after opening.

9.2 Mercury AQC intermediate stock (1 000 ug/l):
Pipette 0.5ml of the mercury AQC into a 500ml volumetric flask, add 5ml dichromate solution (refer 7.7) and 5ml nitric acid (refer 7.6) and make up to the mark with ultrapure water. This solution must be discarded after 3 months.

9.3 Mercury working solution:

5ug/l: 1.0ml intermediate AQC stock (make up to 200ml using ultrapure water). Thereafter, add 2ml dichromate solution (refer 7.7) and 2ml nitric acid (refer 7.6). This solution must be prepared fresh for every run.

10. Analytical procedure

10.1 Sample pre-treatment

(i) Transfer 100ml of the sample into a beaker, using a certified measuring cylinder.
(ii) Add 5ml sulphuric acid (refer 7.8) and 5ml potassium permanganate (refer 7.5).
(iii) If the sample has a high turbidity or a high organic content, more potassium permanganate (refer 7.5) may be added e.g. 10ml potassium permanganate. Samples may also be diluted to compensate for high turbidities i.e. take 50ml of sample and add 50ml potassium permanganate. This sample is now diluted twice. It is important to maintain the purple colour throughout the digestion.
(iv) Cover the beaker with a petri dish and digest the sample in a preheated water bath (set at 80 +-5$^0$C) for at least 8 hours.
(v) Remove the sample from the water bath and allow it to cool to room temperature.
(vi) Add 2.5ml bleach (refer 7.4) and mix well in order to remove excess potassium permanganate. If more potassium permanganate is used for digesting, then add sufficient bleach to decolourise all the potassium permanganate e.g. for 10ml potassium permanganate add 5ml bleach and for 50 ml potassium permanganate add 25 ml bleach.
(vii) Make the sample up to 150ml with ultrapure water, using a certified measuring cylinder.

10.2 Standards and AQC pre-treatment

(i) Transfer 200ml of each standard and the AQC solution into separate beakers and add 10 ml sulphuric acid (refer 7.8) and 10ml potassium permanganate (refer 7.5) to each beaker.
(ii) Cover the beakers with petri dishes and digest the solutions in a preheated water bath (set at 80+-5$^0$C) or at least 8 hours.

(iii) Remove the solutions from the water bath and allow them to cool to room temperature.
(iv) Add 5ml bleach (refer 7.4) and mix well in order to remove excess potassium permanganate.
(v) Make the solutions up to 300ml with ultrapure water, using a certified measuring cylinder.

10.3 Blank preparation

(i) Add 10ml nitric acid (refer 7.6) and 10 ml dichromate solution (refer 7.7) to 1 000ml ultrapure water. Transfer 1 000ml of this solution into a glass bottle.
(ii) Add 50ml sulphuric acid (refer 7.8) and 50ml potassium permanganate (refer 7.5).
(iii) Cover the bottle with a petri dish and digest in a preheated water bath set at 80+-5$^0$C for at least 8 hours.

(iv) Remove the solution from the water bath and allow it to cool to room temperature.
(v) Add 25ml bleach (refer 7.4) and mix well in order to remove excess potassium permanganate.
(vi) Make the blank up to 1 500ml with ultrapure water, using a certified measuring cylinder.

10.4 Instrument set-up

The mercury lamp must be allowed to warm up for at least ten minutes before analysis is started.

11. Calculation of results

The instrument is calibrated by means of standards from which a calibration graph is established. Sample concentrations are read from the calibration graph. The instrument does not perform automatic dilutions. Calculations or manual dilutions need to be indicated on the run sheet.

12. Sources of error

Contaminated glassware
Water vapour
Incorrect pump tubes

13. References

13.1 SCA Method: Mercury in waters, effluents and sludges by Flameless Atomic Absorption Spectorphotometry (1978 version).

13.2 Refer to the instrument operating manuals for further details regarding the setting up and running of the AA.

14. Analysis of mercury in hair, sediment and algae samples

14.1 In the case of sediment samples the following procedure was adopted:
Duplicate masses of each sample were weighed out and then 40ml of potassium permanganate and 10ml sulphuric acid was added to each sample. The sample was made up to 100ml before digestion on the water bath. Due to the extra potassium permanganate added, 20ml bleach was added before analysis was done. The reported results reflect the average of the duplicate results obtained. Due to the difficulty in obtaining a completely homogenous sediment sample, this procedure was followed for all such samples.

14.2 In the case of hair and algae samples the following procedure was adopted:
Same as above, except that no duplicate analysis was performed due to the quantity of the sample received.

15. Analysis of mercury in fish samples

15.1 reagents:
5% Potassium persulphate
Sulphuric acid (minimum assay 98%m/m)
5% Potassium permanganate (see method 34)
Nitric acid (minimum assay 65% m/m)

15.2 preparation of standards:
As for method 34 (individual samples were spiked with mercury in order to check recoveries).

15.3 Preparation of AQC
As for method 34.

15.4 Analytical procedure

Sample pre-treatment

(i) Weigh out +-1g of sample into a beaker.
(ii) Add 10 ml potassium persulphate and 10 ml sulphuric acid.
(iii) Cover the beaker with a perti dish and digest the sample in a pre-heated water bath (set at $60^0$C) for at least 5 hours.
(iv) Leave samples overnight and the following morning add 40ml potassium permanganate.
(v) Leave overnight once again and then add 1ml nitric acid.
(vi) Add 20ml of bleach (refer to method 34) and analyse by cold vapour generation AAS.

## CONE AND QUARTER METHOD

1. Take a large sample and mix it well.
2. Form a cone.
3. Separate the cone into quarters.
4. Discard the bottom left and top right corners.
5. Mix the remaining 2 quarters and form another cone.
6. Separate into quarters.
7. Discard the bottom right and top left.
8. Mix the remaining quarters and form another cone.
9. Repeat steps 3 to 8 two more times.

The remaining sample is then analysed