Chapter 1

Introduction

Among all the heavy metals, mercury has become one of the most concerns due to its potential accumulation and toxic effect towards human (Ana Isabel et al, 2002). Mercury in nature present in silver color metallic and odorless liquid form in room temperature. Mercury has atomic number of 80 and weight of 200.59 respectively. The density of mercury is 13.6 g/ml and has low melting point of -39 C with high boiling point of 397 C.

1.1 Source of Mercury

The major natural sources of mercury are degassing of the earth crust, emission from volcanoes, and evaporation from natural bodies of water (National Academy of Science, 1978). The earth crust is also an important source of mercury bodies of natural water. Some of this mercury may be from natural sources but some may have been deposited from the atmosphere.

Levels of mercury in the environment are increasing due to discharge from hydroelectric, mining, pulp, and paper industries. Incineration of municipal and medical waste and emissions from coal-using power plants also contribute to high levels of mercury.

Mercury released from ongoing human activity in the can be separated into four broad categories. The first category is “area sources”. Landfills, dental preparations, and laboratory use are defined as area sources. The second category is combustion processes. These include coal-fired power generation, medical waste incinerators, and municipal waste combustors. The third category is the manufacture of metals, alkali, and cement. Other industrial processes fall into the fourth category.

In the past, mining was a substantial source of mercury in some areas. For example, the hydraulic placer-gold mines of the Sierra Nevadas released several thousand tons of mercury to the environment from the 1860s to the early 1900s. The U.S Geological Survey (USGS) believes that high levels of mercury in fish, amphibians, and invertebrates downstream of hydraulic mines are a result of historic mercury use.

1.2 The Mercury Cycle

Mercury from atmosphere to land or water at any one location is comprised of contributions from:

- The natural global cycle
- The global cycle perturbed by human activities
- Regional sources
- Local sources

Recent advances allow for general understanding of the global mercury cycle and impact of anthropogenic sources. It is more difficult to make accurate generalization of mercury cycle on a regional or local scale due to the site specific nature of emission and deposition process.

According to United state environmental and protection agency, several authors have used different numbers of techniques to estimate the pre-industrial mercury concentration in environmental media before anthropogenic emission become a part of the global mercury cycle. It is difficult to separate current mercury concentration by origin due to the continuous cycling of the element in the environment. For example, anthropogenic release of elemental mercury may be oxidized and deposited as divalent mercury far from the source, the deposited mercury may be reduced and re emitted as elemental mercury only to be deposited again continents away.

Comparison of contemporary measurements and historical records indicate that the global atmospheric mercury burden has increased since the beginning of the industrialized period by factor of two and five. For example, analysis of sediment from Swedish lakes shows mercury concentration in the upper layer that two to five time higher than those associates with pre-industrialized times.

1.3 Mercury Transformation and Transport

Elemental mercury has an average residence time in the atmosphere of about one year and will thus be distributed evenly in troposphere. Oxidized mercury which is inorganic mercury, Hg (II) may be deposited relatively by wet and dry deposition process, leading to a residence time of hours to month.

The transformation of elemental mercury to inorganic mercury in cloud water demonstrate a possible mechanism by which natural and anthropogenic sources of elemental mercury to air can result in mercury deposition to land and water. This deposition can occur far from the source due to slow rate of elemental mercury uptake in cloud water. It has been suggested that this mechanism is important in a global sense for mercury pollution.

Residence time between elemental mercury and other mercury species leads to very larger scale of transport and deposition for elemental mercury. Generally, air emission of elemental mercury from anthropogenic sources, fluxes of elemental mercury from contaminated soil and water body and natural fluxes of elemental mercury all contribute to a global atmospheric mercury reservoir with a holding time of half to two years. Global atmospheric circulation system can take mercury emission from their point of origin and carry them anywhere on the global before transformation and deposition occur. Emissions of all other forms of mercury are likely to be deposited to earth surface before they thoroughly dilute in to the global atmosphere.

1.4 Bioaccumulation and Mercury Toxicity

Mercury can chemically combine with other element to form organic mercury and inorganic mercury compounds (ASTDR, 1999). For people who not exposed to mercury in their work, the most probable source of this element is dietary intake of fish and fish product (Susan C. Hight et al, 2004).

There are several forms of mercury which are elemental mercury, ionic form of mercury and organic mercury (Falchuk et. al. 1997). However, the contribution towards toxicity is the organic mercury, particularly methyl mercury (Hwang et. al. 2004). Elemental mercury efficiently transported as gas around the globe. Besides elemental mercury, the major form of mercury in water is ionic mercury which is bound to chloride and sulphide. Majority of organic mercury is in form of methyl mercury.

Atmospheric deposition largely contribute to the large portion of mercury found in lake and soil. Mercury emitted into atmosphere by combustion, incineration or manufacturing process that may later be deposited in the lake. In the atmosphere, mercury transported by wind either as a vapour or particles. Mercury reaches water through direct deposition or through run off from soil after rain. Figure 1.1 shows the natural fate of mercury in the environment.

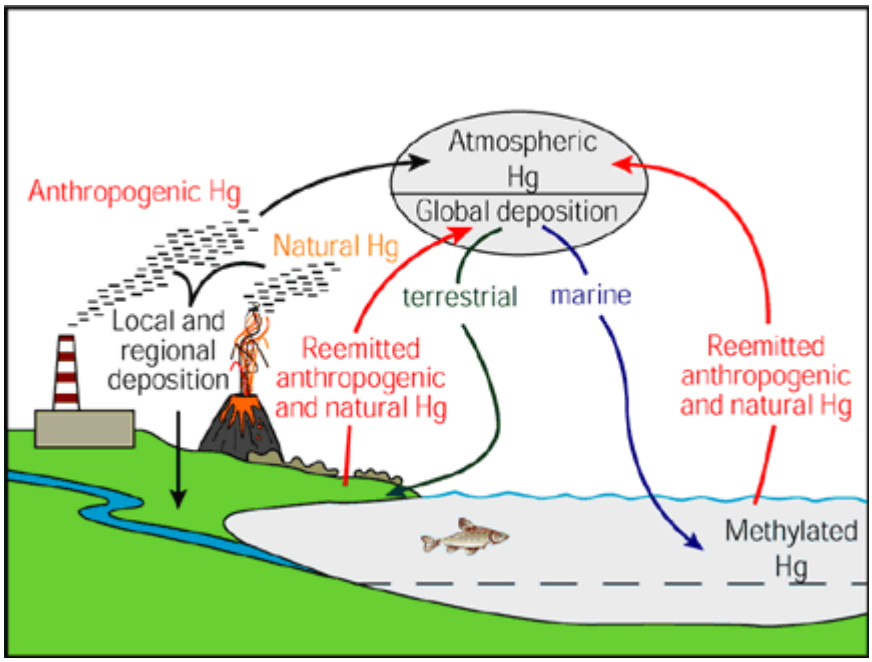

Figure 1.1: Natural fate of mercury in the environment

In water system, mercury is converted from inorganic mercury to methyl mercury by biotic reaction through methylation process. The toxic effect of methyl mercury is well established and extensive studies have been carried out for the past ten years, the methyl mercury toxicity lies in its ability to accumulate in fish tissues through the food chain and will lead to the biomagnifications of the toxic (Logar et. al. 2002). The tendency of bioaccumulation in aquatic food chain will build up the amount of methyl mercury deposited in the fish tissues although the atmospheric input of methyl mercury is low (Selvendiran et. al. 2008).

Figure 1.1 shows the bioaccumulation pyramid of mercury through the food chain. Methyl mercury biomagnifies through the food chain as predators eat other organisms and absorb the contaminants that their food sources contained. Over time, an individual who consumes plants or prey contaminated with methyl mercury will acquire levels greater than in either its habitat or its food. As a result, a top predator which is human acquires greater body burdens of mercury than the fish they consume.

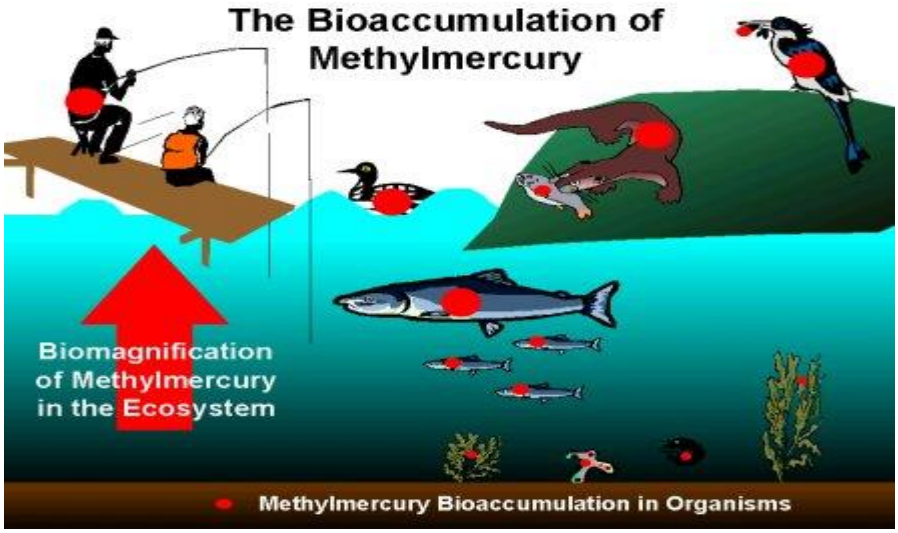

Figure 1.2: Methyl mercury bioaccumulation in organism.

Most people and wild life can tolerate with extremely low levels of this substance. When mercury enters the body it becomes concentrated in the tissue due to bioaccumulation process. Mercury vapor easily absorbed by lung and is a potential health threat to people who breathe it. On the other hand, methyl mercury which is the organic form of mercury can completely absorbed from the gut in to the blood and distributed throughout the body, passes into the brain and reaches a nerve system. In human adult, organic mercury damage nervous system and cerebellum (F. Ubillus et. al. 2000).

The developing fetus is the most sensitive to the effect of mercury, so maternal consumption during pregnancy of methyl mercury can cause variety of abnormalities to their offspring including delayed of walking and talking and reduce neurological development. Mothers consuming diet containing mercury will pass the toxicant to fetus and infants through breast milk (Farhana Zahir et. al. 2004).

1.5 Regulation

Due to human health concerns, the United State Food and Drug Administration (FDA) has set an action limit for methyl mercury content in fish for human consumption at 1ug/g (wet weight) (Hwang et. al., 2004). The food and Agriculture/World Health Organization (FAO/WHO), recommends a maximum intake without exceeding weekly consumption of 5 ug/kg of total Mercury and 1.5 ug/kg of methyl mercury in diet for adult with body mass of 60 kg. Malaysian Food regulation, 1985 adopt the similar limit as WHO of 0.5 mg/kg of total mercury in food for human consumption.

1.6 Fresh water and marine water fish

Fresh water fish lives in fresh water with salinity <0.05 %. There are 41.24 % of all known species of fish are found in fresh water. Fresh water fish is differing physiologically from salt water fish in several aspects. Their gills must be able to diffuse dissolve gasses while keeping the salt in the body fluids and the scale will reduce water diffusion through the skin.

Marine water fish lives in water with >0.05% salinity. As for salt water fish, they will take the salt water to replace lost fluid and then eliminate the excess salt. Their kidneys produce small volume of fluids containing high concentration of salt.

1.7 Malaysia Fisheries

Fisheries have been a long practiced since of food acquisition by mankind. It has maintained its importance as the top natural protein provider in diet of many nations in the world. Malaysia is one of the top fish consuming countries in Asia, almost double average of Thailand and China, but still below the level of Japan and South Korea.

Based on the studies done by Ministry of Agriculture Malaysia, in 2010 an average malaysian consumed more fish (54 mg/year) compared to 20 mg/year in 1970, a drastic increased in demand for fish over four decades due to the rapid population growth. This study raises serious concern of whether the available marine fish resources able to catch up with the demand.

Due to the demand for fish in Malaysia, ministry of Agriculture Malaysia suggests commercialized of fresh water fish as one of method to sustain fish stock in Malaysia.

1.8 Objective of the study

1. To determine total and methyl mercury in fresh and marine water fish muscle tissues purchased from a supermarket in Malaysia.
2. To compare the concentration of total and methyl mercury in fresh and marine water fish muscle tissue.
3. To assess the ratio of methyl mercury to total mercury in fish purchased from a supermarket in Malaysia.

1.9 Scope of study

In this study, five species of each fresh water and salt water fish will be digested by wet digestion followed analysis by Cold Vapor Atomic Absorption Spectroscopy for total mercury and alkaline digestion and analyzed by automated Gas Chromatography Cold Vapour Atomic Florescence Spectroscopy to determine and quantify the concentration of Total and Methyl mercury in the samples of fresh and salt water fish. In the same time, these two different samples will be compared in term of concentration and the ratio of methyl mercury to total mercury in fish will be determined.

Chapter 2

Literature Review

2.1 Sample pretreatment for mercury compounds in Fish

Among all the heavy metals, mercury has become one of the subjects of most concern due to its potential accumulation and toxic effect to aquatic organism and human health (Ana Isabel Cabanero et al. 2002). Therefore, it is necessary to develop rapid, sensitive and accurate method for extraction, separation, identication and quantification of Total and Methyl mercury. Before instrument detection, sample pretreatment is required to remove possible interference presence in fish samples.

Based on Ong et al. (2000) there is possible matrix interference during determination of total and methyl mercury in fish if organic matter in the fish are not completely decomposed or removed. Digestion of samples is necessary step before determination of mercury concentration using spectroscopic technique. It generally involves heating of samples with different combination of mineral acid such as hydrochloric acid, nitric acid and other oxidizing agent such as peroxide.

The main problem of sample digestion method is the possibility of losses through volatilization or incomplete digestion as well as contamination from different souces (Voegborlo et al,2008). Hajeb et al (2009) reported good matrix spike recovery for total mercury in fish by using nitric acid decomposition and heated at 40 °C. Combination of three types of acids (Nitric Acid, Perchloric acid and Sulphuric acid) are practically performed on certified reference material by Veogborlo (2007) with recovery between 94 to 116 %. Good recoveries of CRM demonstrate the accuracy of the method used.

The most recent sample pretreatment method for Total mercury in fish sample by using microwave oven with open or closed pressurized system provide an alternative that allowed reduction in total analysis time and risk of contamination as well as volatilization of analyte of interest.

The mercury speciation analysis by GC-CVAFS was developed by Liang et al (1994). Prior to analytical method, sample undergoes alkaline digestion rather than acid digestion. Acid digestion will decompose all mercury species in the sample in to total mercury. There are several method for methyl mercury sample preparation done by Ana Isabel Cabanero et al (2002) such as mercury extraction using copper sulphate and SDS Extraction.

2.2 Analytical method for determination of mercury

Various techniques have been used to determine total mercury in fish. The methods are Inductive Coupled Plasma Mass Spectrometer (Jiang and Chen, 1998), Inductive Coupled Plasma Atomic Emission Spectrometer and Cold Vapor Atomic Absorption Spectrometer (Veogborlo, 2007).

The analytical method most commonly used for determination of methyl mercury is gas chromatography using electron captured detector (GC-ECD). The disadvantage of ECD is unselective response, thus, extraction into the organic phase is required. Uria and Medel, 1998 reported that mercury halides in the sample will not able to provide reproducible chromatographic peak due to strong interact with the packing material of the column. Thus, the best method to evaluate organic mercury in fish samples are hyphenated method which required initial separation to separate the presence of methyl mercury compound in the samples.

The hyphenated methods are gas chromatography inductive coupled plasma mass spectrometer (Baxter et.al, 2007) and gas chromatography cold vapour atomic fluorescence spectrometer (Liang et al, 2004). The recent hyphenate instrument that able to detect mecury species in fish sample is liquid chromatography inductive couple plasma mass spectrometer (Hight and Cheng, 2006).

Chapter 3

Methodology

Introduction

In this study, 10 different species of fish that is largely consumed by Malaysian, five from seawater species and five from fresh water species was purchased and digested for determination of Total and Methyl mercury. Then, sample will be analyzed by Flow injection mercury system for total mercury and cold vapor gas chromatography atmic fluorescence for methyl mercury. Fish species that will be analyzed in this study as stated in table below.

Table 3.1 List of fish sample

Fresh water Fish	**Salt water Fish**
Striped snake head	Wolf Herring
Black Tilapia	Mackerel
Short Barbel Pangas	Snake Head
Red Tilapia	Red Snapper
Catfish	Barramundi

3.1 Chemical and Reagent

3.1.1 Total Mercury

All chemical used in the digestion and analysis of total mercury is analytical grade. Blood acid was prepared by mixed hydrochloric acid, sulphuric acid and perchloric acid with ratio 5:1:2. Stannous chloride (5% w/v) reducing agent was prepared freshly by dissolving 50 g stannous chloride (Systerm) in Hydrocholoric acid (5% v/v)

For calibration, Mercury nitrate standard of 1 mg/L was prepared by diluting mercury nitrate stock standard solution (1000 mg/L) in 100 ml nitric acid (2%v/v). Then, one set of calibration standard with range from 0.5 ppb, 2.0 ppb, 5.0 ppb and 10.0 ppb were prepared.

3.1.2 Methyl mercury

For alkaline digestion method, methanolic potassium hydroxide solution (25% w/v) was prepared by dissolve 125 g of potassium hydroxide (Fisher Scientific) in 500 ml methanol (Fisher Scientific)

Ethylating agent of sodium tetraethylborate (1%w/v) was prepared by dissolving 1 g of sodium tetraethyborate in chilled potassium hydroxide (2%w/v). This reagent stored in the freezer and is mostly thawed just prior to use. Sodium tetraethyborate solution should not be used if they show any discolorization. The frozen reagent has been provento be stable for several months if not thawed.

Acetate buffer was prepared by dissolving 136 g of sodium acetate pentahydride (Merck) in ultrapure glacial acid (Merck). It is recommended to check the PH of the samples periodically after buffer adition by withdrawing a small aliquot and pipetting it in to PH paper.

For calibration, 1 mg/L Methylmercury (II) htdroxide standard was prepared by diluting mercury stock standard of 10 mg/L in 100 ml ultrapure glacial acetic acid (0.5% v/v) and trace metal grade hydrocholoric acid (0.1% v/v) (Merck). Concentration range of methyl mercury was 2 pg, 5pg, 10pg, 50pg, 100pg, 250pg, 500pg, 1000 pg.

3.2 Instrumentation

3.2.1 Total Mercury by Flow Injection Mercury System

Analysis for total mercury was done by using flow injection mercury system (FIMS-400) from Perkin Elmer. This instrument consist of 2 peristaltic pump, manifold tubing, gas liquid separator and quartz cell. Detection based on atomic absorption technique and detector used was photomultiplier tube. Date processing and pump flow is controlled by WinLab32 software. The parameter of the instrument for this analysis is stated as table 3.2.

Table 3.2 Instrument parameter for determination of total Mercury

Sample Loop	500 uL
Reductant	Stannous Chloride (5% w/v HCL)
Carrier	HCL (3%v/v)
FIAS Injection Time	20 seconds
FIAS Fill Time	10 seconds
Measurement Time	Peak height in 20 seconds
Light Source	UV Lamp at 253.7 nm
Wavelength	253.7 nm

3.2.2 Methyl mercury by Automated Gas Chromatography Cold Vapor Atomic Fluorescence Spectroscopy

Methyl mercury concentration is determined by using Gas chromatography cold vapor atomic fluorescence spectroscopy by Brooksrand. This instrument consists of automated probe for purge and trap system, conventional gas chromatography packed coloumn for separation and pyrolitic column for breaking down all mercury species into atomic mercury. Detection done by photomultiplier tube with atomic fluorescence technique. Table 3.3 is list of instrument parameter for methyl mercury analysis.

Table 3.3 Instrument parameter for determination of methyl mercury

Gas Chromatography Column	15 % OV-3 GC Coloumn
GC Temperature	34 ºC
Wavelength	253.7 nm
Light Source	UV Lamp
Nitrogen Flow rate (Purge & Trap)	50 mm scale reading
Argon Flow rate (Carrier gas)	35 mm scale reading
Desorption Temperature	200 ºC
Pyrolitic coil temperature	850ºC

3.3 Analytical Procedure

3.3.1 Total Mercury

3.3.1.1 Sample Preparation for Total Mercury Analysis

1.5 g homogenized fish tissue sample were weight into 50 ml closed polypropylene tube. 10 ml of blood acid was added followed by 5 ml 65% Nitric acid. The sample mixtures were allowed to leach at room temperature overnight and digest at 90ºC for one and half hour. Digested sample allowed to cool at room temperature before marked up to 50 ml volume of Deionized water.

3.3.1.2 Analysis of Total Mercury

Digested sample transferred to 10 ml rest tube and analyzed by FIMS-400. Sample was introduced to 500 uL sample loop. Continuous flow of carrier (Hydrochloric Acid 3% v/v) pushed the sample solution to the mixing manifold where solution mixed with reductant. Reductant acts as reducing agent to reduce inorganic mercury to elemental mercury. The reaction mixture flows into gas liquid separator where the atomic mercury vapor was separated from liquid solution. Atomic mercury vapor enters the quartz absorption tube.

The mercury vapor absorbed UV Light at wavelength 253.7 nm, excited at higher energy level and relaxed at same wavelength by emitting light that detected by photomultiplier tube. The concentration of mercury present in the sample determined quantitatively by using calibration curve of absorption versus concentration.

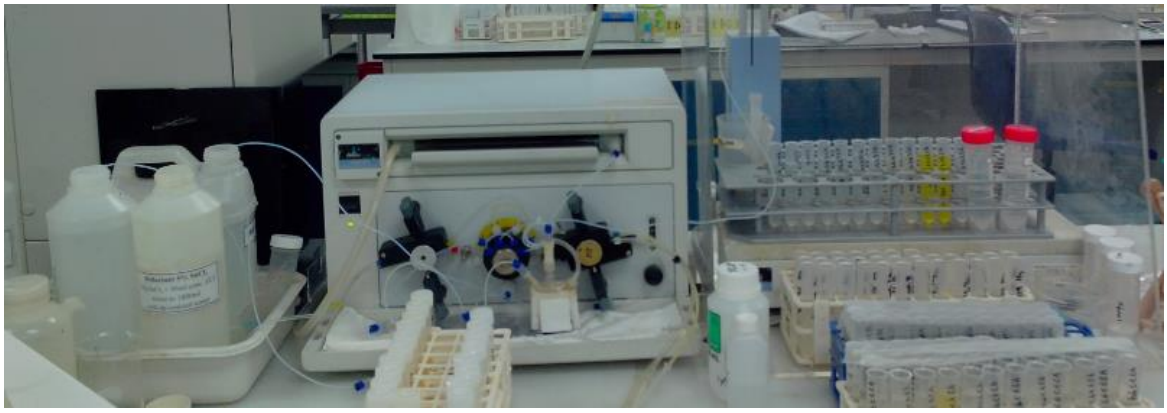

Figure 3.1: Cold Vapour Atomic Absorption Spectrometer

3.3.2 Methyl mercury

3.3.2.1 Sample preparation for methyl mercury analysis

0.1 g homogenized fish sample were weight in closed Teflon digestion vessel. 1 ml methanolic potassium hydroxide (25% w/v) was added and sample mixture were vortex to ensure it homogeneity. Sample mixture was digested at 75ºC for 3-4 hours. The mixture allowed to cool at room temperature and marked up to 2.5 final volumes by methanol. Digested sample were vortex once more to ensure it homogeneity before analyze by cold vapor gas chromatography atomic fluorescence spectroscopy.

3.3.2.2 Analysis of Methyl Mercury

30 uL of digested sample were pipette in 40 ml reaction vial containing 30 ml deionized water. 300 uL of acetate buffer was added to ensure the optimum PH of solution from 3.5 to 5.5. If 300 uL of buffer is not sufficient to bring the sample PH between 3.5 to 5.5, more buffer may be used without any impact on the results. This is because ethylating agent reaction optimum in pH 3.5 and 5.5.

Then, 100 uL ethylating agent of tetraethyl borate was added. Ethylation of the sample should be the last step before sealing the vial, and each vial was sealed quickly after ethylating agent was added. This is because the ethylating reagent makes the mercury species volatile, and they will be lost if the vial left uncapped for too long. Last but not least, the solution was marked up to final volume of 40 ml before the vial closed tightly and swirled to ensure it homogeneity. The mixture allowed reacting for 10 minutes before analysis was started.

Analysis using automated model of MERX GC-CVAFS. The principle is the same with the manual GC-CVAFS model except for the auto sampler and the design is safer to be used. Compared to manual model, analyst exposed to high temperature of nichrome wire for desorption and pyrolysis step.

After reaction step, sample was purged and volatile mercury compound was trapped in the tenex trap. Tenex trap will be heated with nichrome wire at 200 C and all mercury species in the tenex trap will vaporized and go to guard column before reach packed column for separation. Mercury species will go to packed column in gas chromatography and separated based on weight. Lastly, each of the mercury species (Elemental, Organic and Inorganic) will pass through pyrolitic column heated at 800 C to atomize the mercury species and detected by photomultiplier tube by Atomic Fluorescence Spectrometry technique.

Figure 3.2: Automated – Gas Chromatography Cold Vapor Atomic Fluorescence Spectrometer

Chapter 4

Result and Discussion

4.1 Analytical Performance

Cold vapor technique used for both total and methyl mercury. The different between both techniques were the detection method, where total mercury was absorption and methyl mercury was fluorescence.

Atomic absorption method use reducing agent, stannous chloride to convert all inorganic mercury into elemental mercury. The elemental mercury will absorb UV light source at 253.7 nm and excited to higher energy level at same wavelength and emit an energy which detected by photomultiplier tube. This process gives an absorption peak as shown in Figure 4.1.

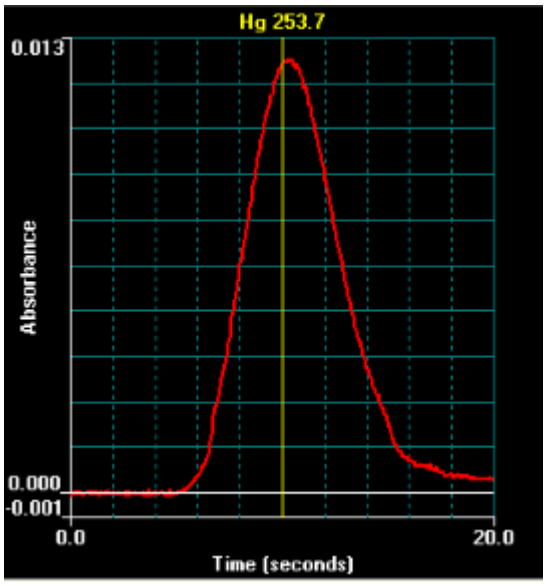

Figure 4.1: Absorbance versus time peak for CVAAS Technique.

In CVAFS technique, ethylating agent was used to convert all involatile mercury compounds to volatile mercury compound able to be separated by the conventional packed column gas chromatography in isothermal temperature. Detection by fluorescence technique produce peaks as in figure 4.2. Three peaks will observe. The first peak is elemental mercury followed by second peak which methyl mercury peak, peak of interest and the last peak is inorganic peak.

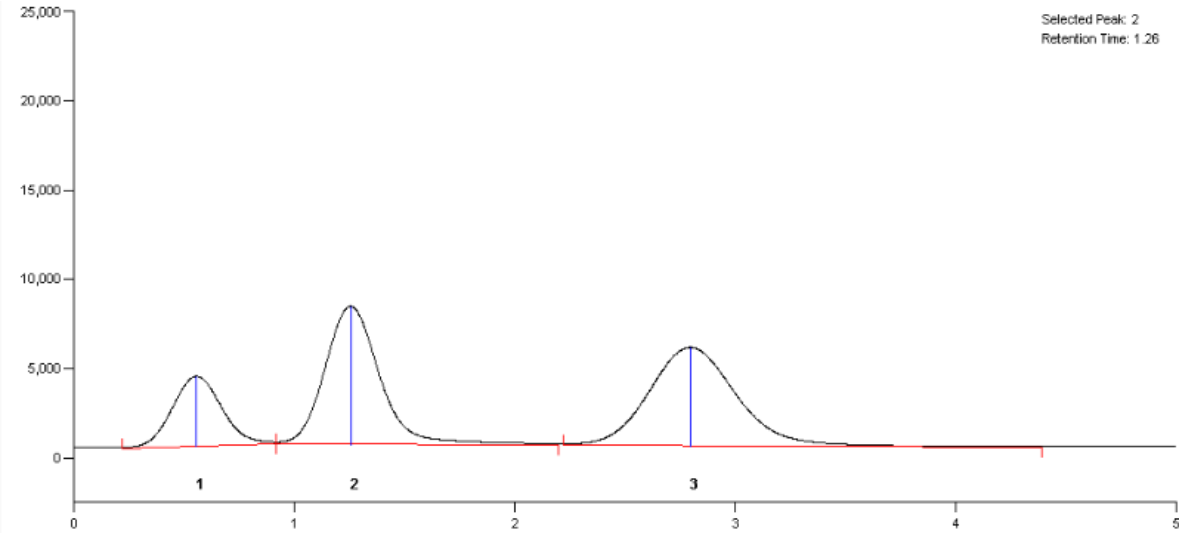

Figure 4.2: Chromatogram for GC-CVAFS Technique

4.2 Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection limit is the smallest concentration from which it is possible to deduce the presence of the analyte, while the limit of quantitation is the smallest quantity required to quantify the analyte with a reasonable degree of statistical certainty (Park et al., 2011). The calculation of LOQ using equation (4.1) and (4.2) respectively. Limit of detection (LOD) was 2.5 times lower than LOQ (Carbonel and Fernandez,2009).

$$\text{LOQ (ng/g)} = \frac{\text{Minimum concentration (ug/L) x Final dilution volume (ml)}}{\text{Sample Weight (g)}} \qquad (4.2)$$

$$\text{LOQ (ng/g)} = \frac{\text{Minimum concentration (pg) x Final dilution volume (ml)}}{\text{Volume analysed (ml) x sample weight (mg)}} \qquad (4.3)$$

In this study,Limit of Determination (LOD) and Limit of quantification (LOQ) as stated in table below.

Table 4.1: LOD and LOQ for Total and Methyl Mercury.

	Total Mercury, ng/g	**Methyl Mercury, ng/g**
LOD	**4**	**1.6**
LOQ	**10**	**0.64**

4.3 Determination of the Linearity of Calibration Curve

Linearity is the ability of a method to evoke test results which are directly proportionate to an analyte concentration within a working range. It is generally reported as the variance of the slope of the regression line. A mathematical linear regression equation, y = mx + C applied to the results should have an intercept and slope that gives a correlation coefficient. The confident level for correlation coefficient shall be > 0.990. The correlation coefficient can be calculated by using the formula 4.3.

$$R^2 = \left[\frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - x)^2 \sum (y - \bar{y})^2}} \right]^2 \qquad (4.3)$$

For total mercury, 5 -point calibration was performed before the samples were analyzed. The calibration curve was constructed based on the absorbance value of respective concentration with correlation of 0.9999 as in table 4.2**.**

Table 4.2 : Absorbance value for standard solution of total mercury

Total Mercury, ug/L	Absorbance
0.5	0.0091
2.0	0.0352
5.0	0.0854
10.0	0.1686
20.0	0.3374

In addition, methyl mercury calibration curve of peak height versus concentration was plotted with working range of 2 pg -1000 pg. Based on absorbance value, calibration curve with correlation coefficient of 0.9997 was obtained. Table 4.2 summarized the individual response towards respective standard.

Table 4.3: Absorbance value for standard solution of Methyl mercury

Methyl Mercury, pg	Peak Height
2	1088
5	2385
10	5078
50	24815
100	45795
250	116107
500	231775
1000	440851

The calibration curves for both total and methyl mercury were linear with the correlation coefficient of **0.999** and 0.9997 as stated above. Good linearity was achieved in both cases with correlation coefficients better than 0.990 for both parameter. Thus, the proposed method is able to produce acceptable linearity over the concentration range of 0.5 – 20 ug/L for total mercury and 2-1000 pg for methyl mercury.

4.4 Quality Control and Quality Assurance

The accuracy, reliability and consistency of both analysis was confirmed by using certified reference material (CRM) of different mercury levels which was analyzed in parallel with the samples. CRM analyzed were DORM-3 a reference product on fish protein and DOLT-4 a reference product on dog fish liver by National Research Council Canada (NRCC). The concentration of Total mercury and methyl mercury for both CRMs were determine and compared with the certified value.

Table 4.4: Analysis for total mercury in CRM

CRM	Analysed, ng/g (Total Mercury)	Certified value,ng/g (Total Mercury)
DORM-3 Recovery,%	381	382 ± 60
	99 %	
DOLT-4 Recovery, %	2489	2580 ± 22
	96 %	

Table 4.5: Analysis for methyl mercury in CRM

CRM	Analysed, ng/g (Methyl Mercury)	Certified value,ng/g (Methyl Mercury)
DORM-3 Recovery,%	349	355 ± 56
	98 %	
DOLT-4 Recovery, %	1318	1330 ± 120
	99 %	

The percentage recovery of CRM is calculated based on equation 4.4.

$$\text{Recovery of CRM} = \frac{\text{Analyzed value}}{\text{Certified value}} \times 100 \qquad (4.4)$$

Based on the analyzed result from analysis of CRM, recovery range is from 98 to 100 % recovery for both parameters. This indicates method employed for both total and methyl mercury is reliable, accurate and consistence although the digestion process is quite simple.

In this study, quality control measure included procedural blanks to eliminate contamination. In addition, to check the reproducibility of analysis, each of the sample were analyzed duplicate and the relative standard deviation (RSD) is varied between 0 – 10%. In order to determine the accuracy of the method, laboratory control spike (LCS) was done.

Laboratory control spike was done by spike known concentration of standard in to clean blank and undergo similar sample pretreatment procedure. Table 4.6 shows the recovery of all LCS analyzed for both total and methyl mercury.

Table 4.6: Recovery of laboratory control spike

	Total Mercury Recovery,%	Methyl Mercury Recovery,%
LCS-1	89 %	99 %
LCS-2	85 %	96 %
LCS-3	84 %	96 %

Based on the result, the recovery of LCS for both total and methyl mercury is less than 100%. This might due to losses during digestion process. For total mercury with about 86% recovery which is much lower than methyl mercury, after completion of digestion process, strong brown fume was observed. During mark up to a final volume procedure, brown fumes rush out furiously. This might be a reason for losses or mercury.

The recovery of methyl mercury shows a very good recovery compared to total mercury. This proves, slow digestion by methanolic potassium hydroxide is adequate for not decomposing all mercury compounds into total mercury. In addition, this result also indicate the derivatization process by sodium tetraethyl borate shows effectiveness in converting methyl mercury to volatile compounds and separation by packed column gas chromatography at optimum temperature appropriate to obtain good recovery for this analysis.

4.5 Total and methyl mercury in fresh water fish

Common Name	n	Total Mercury (ug/kg), mean ± s.d	Methyl mercury (ug/kg), mean ± s.d	Ratio of Methyl mercury /Total Mercury
Striped snake head	2	93 ± 2.67	92 ± 1.24	99%
Black Tilapia	2	28.9 ± 1.67	27.3 ± 2.42	94%
Short Barbel Pangas	2	18.9 ± 1.98	15.9 ± 2.13	84%
Red Tilapia	2	16.6 ± 2.87	3.71 ± 0.24	22%
Catfish	2	15.5 ± 1.34	12.4 ± 0.34	80%

Table 4.7: Total and methyl mercury result in fresh water fish

Table 4.7 shows a clear observation that different fish species have different level of mercury content. The result shows striped snakehead content highest concentration of total and methyl mercury. Striped snakehead had total and methyl mercury concentration 3 – 6 times higher than other type of fish because strip snakehead generally larger and consumed a mixed diet of smaller fish, snails, insect that already have certain concentration of mercury accumulate in their body. Thus, the bigger the fish, the higher mercury content due to bioaccumulation. In addition, mercury in fish also effected by living habitat.

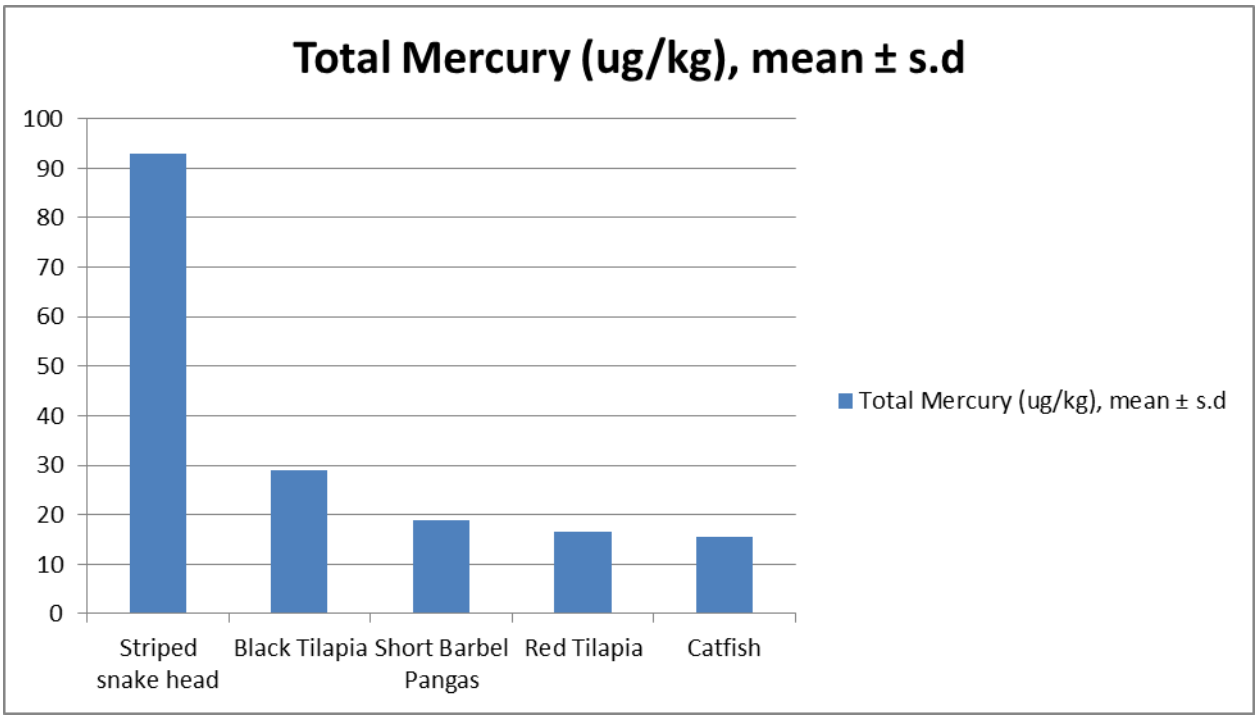

Figure 4.3: Concentration of Total Mercury in fresh water fish species

The result obtained from the experimental analysis of Total mercury in fish are compared with the regulation set in Malaysia Food Regulation,1985 which is the limit of Total mercury in food for human consumption is 0.5 mg/kg. Thus, based on the result on table 4.6, concentration of all fresh water fish species analyzed in this experiment lower than limit of regulation and it safe to be consumed.

In addition, methyl mercury also shows significant value in fresh water fish. Methyl mercury in fresh water has a wide range different, which range from 12.4 ± 0.34 to 84 ± 1.24. Striped snake head has highest level of methyl mercury compared to other type of fish with concentration of 84 ± 1.24.

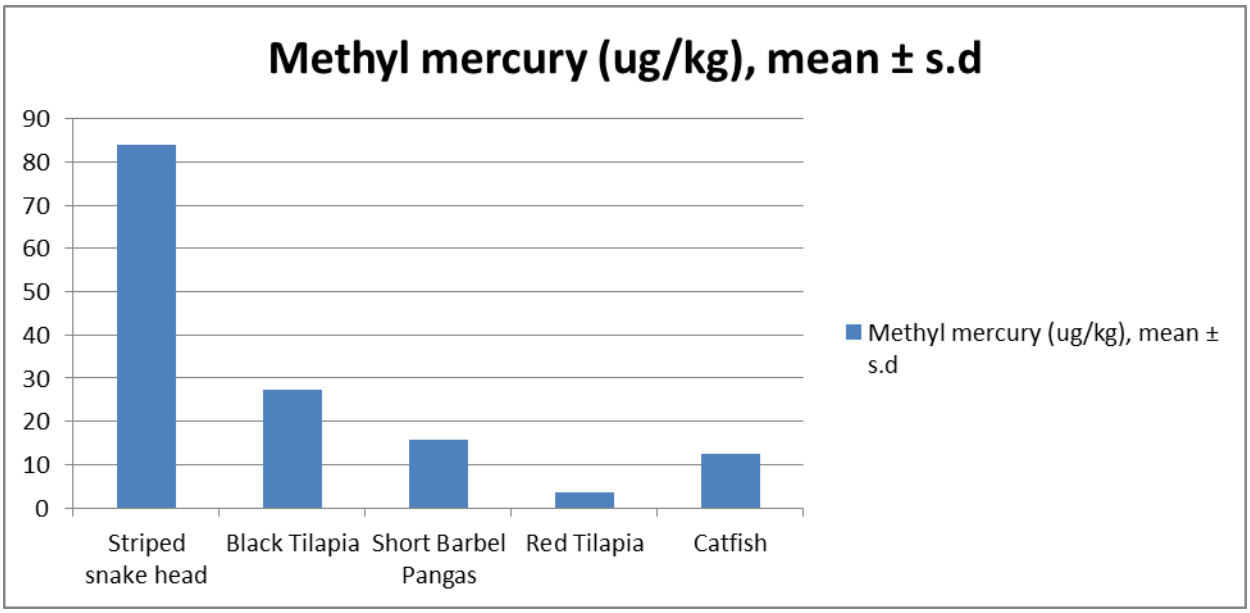

Figure 4.4: Concentration of Methyl Mercury in fresh water fish species

Based on recommendation by FOA/WHO, weekly consumption of fish with methyl mercury for adult dietary with body mass 60kg shall be 1.5 ug/kg (Hajeb et al, 2009). From the experimental result, all type of fresh water fish analyzed has exceeded the recommendation limit. However, that is only recommendation not a regulation. Red Tilapia contains lowest concentration of methyl mercury and this result agrees several studies done on tilapia species. Tilapia species generally red tilapia has lower content of mercury as they are fast-growing, lean and short-lived, with a primarily vegetarian diet, so do not accumulate mercury found in prey.

4.6 Total and Methyl mercury in Marine water fish

Table 4.8: Total and Methyl mercury result in Marine water fish

Common Name	n	Total Mercury (ug/kg), mean ± s.d	Methyl mercury (ug/kg), mean ± s.d	Ratio of Methyl mercury /Total Mercury
Wolf Herring	2	204 ± 3.56	146 ± 2.50	72%
Mackerel	2	34.1 ± 2.34	33.3 ± 0.53	98%
Snake Head	2	53.2 ± 1.65	51.8 ± 0.91	97%
Red Snapper	2	125 ± 5.69	120 ± 0.72	96%
Barramundi	2	116± 2.12	114 ± 3.15	98%

Table 4.8 shows result of Total and Methyl mercury content in marine water fish. As in figure 4.6, wolf herring content highest concentration of total mercury, 204 ± 3.56 and Methyl mercury 146 ± 2.50. Followed by Red Snapper, Barramundi, Snake Head and last but not least mackerel. The variation in both total and methyl mercury result is cause by several factors such as feeding habit and size of fish.

All type of fish analyzed for marine water species is predatory fish and that contribute to high mercury content in all marine water fish species. Methyl mercury appears to be effectively passed through the aquatic food web to the highest trophic level consumer in the community example predatory fish.

This is prove by study done by Li S et al,2009 suggesting that predatory species preferring benthic positions had higher total mercury concentrations than others suggesting that mercury accumulation is related to the interaction of feeding habit and habitat preference. In the study shows, fish that are bottom living and feed on other fish or aquatic animals are more likely at high risk of mercury exposure.

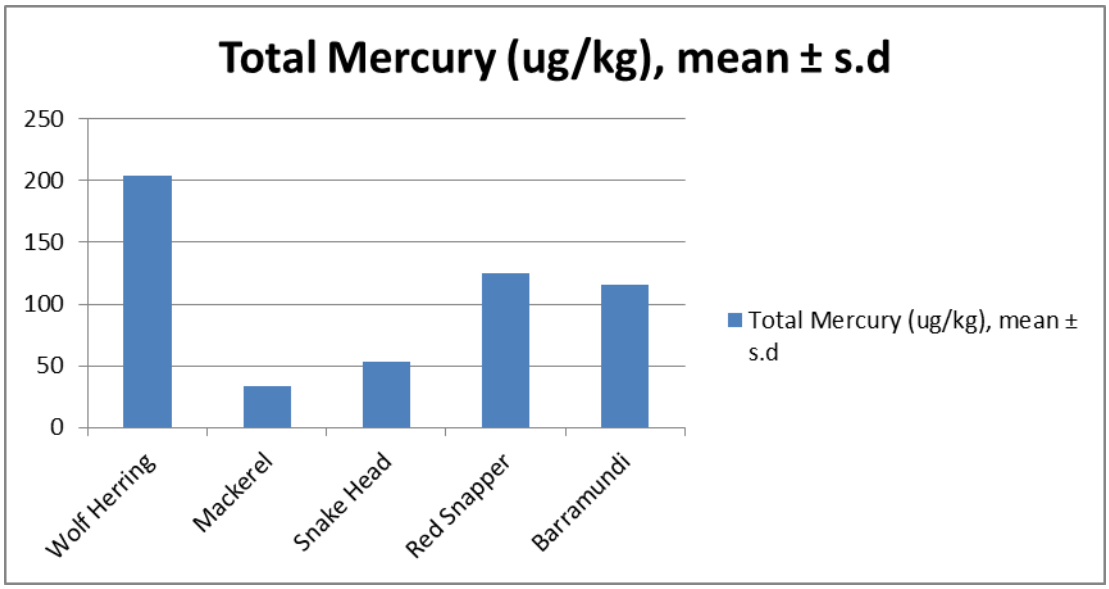

Figure 4.5: Concentration of total mercury on marine water fish species

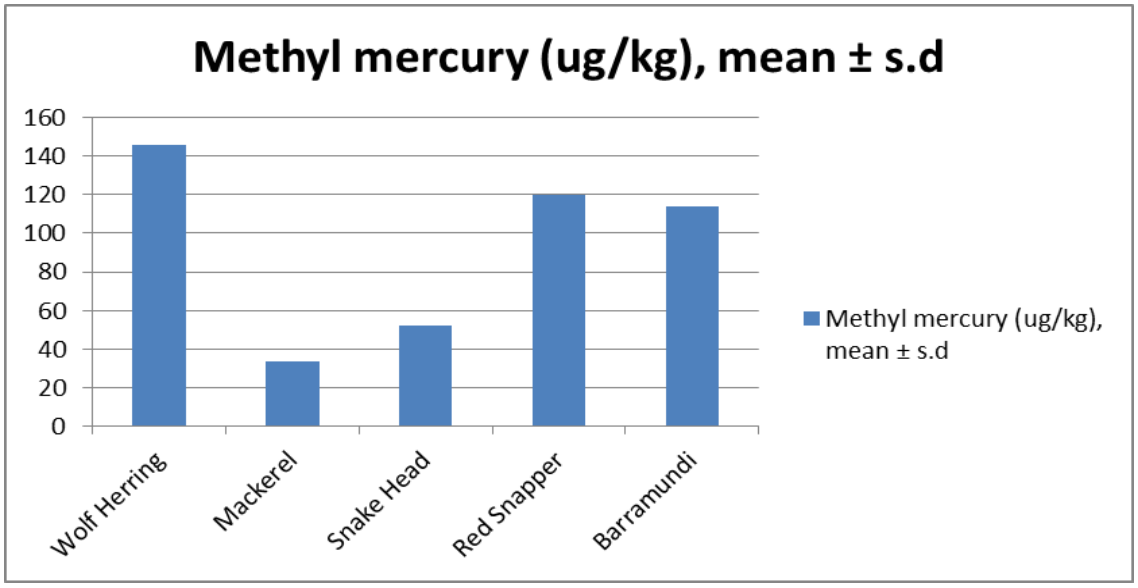

Figure 4.6: Concentration of Methyl Mercury in Marine water fish species

Barramundi has higher mercury concentration due to its feeding habit that consumed crustacean, zooplankton and shell fish including its own species that initially contain certain level of mercury thus, cause a faster bioaccumulation.

Among the predatory fish, mackerel shoe lowest total mercury concentration, 34.1 ± 2.34 and methyl mercury 33.3 ± 0.53. By observation, clearly mackerel smaller than barramundi in term of sizes.

Compared with the regulation by Malaysia Food Act and Regulation 1985, all marine water fish in this experiment is safe to be consumed as their concentration less than 0.5 mg/kg. For Methyl mercury, all fish species exceed the recommended limit by FOA/WHO; however, once again that is a recommendation not regulation.

4.7 Total and Methyl mercury in Fresh and Marine water

Table 4.9: Comparison Mercury concentration in Fresh and Marine water

Medium	Total Mercury (ug/kg),Average	Methyl mercury (ug/kg), Average
Fresh Water	34.6	28.7
Marine Water	107	93

From the table below, average concentration of total and methyl mercury in marine water higher than marine water. It is due to several factors. In study done by united state environmental protection agency, no more than 20% of the total mercury in a fresh water column exist as a methyl mercury complex. Once entering a water body, mercury can remain in water column or be lost from the lake through drainage water. In addition, mercury in fresh water can be lost due humic acid reduction of Hg(II) to elemental mercury or demethylation of methyl mercury mediated by sunlight. An amount will remain in dissolve gaseous state while most will volatilize.

On the other hand, mercury found in marine waters comprises a large reservoir of total mercury in planet. The conceptualization of ocean or marine water as mercury reservoir fitting as they served as sources of mercury to atmosphere as well as food chain. Based on research done by Fritzgerald 1996, total deposition of mercury to marine water was estimated at 10Mmoles/year, fresh water or river was estimated to be approximately 10% of this value.

The mercury deposited in the marine water may be transformed to methyl mercury as a result of both biotic and abiotic reaction. Higher in deposition of Mercury in the marine water system will result in enhanced food chain bioaccumulation and higher concentration of total and methyl mercury in marine fish.

The higher mercury concentration deposited in the water matrix, the higher possibility that methylation process occur and mercury transformed to methyl mercury which is most toxic state of mercury. Niagu 1979 estimated the global distribution of mercury and by far the largest repository is ocean sediment. This journal also estimated the ocean water contain 10^{13} g of mercury compared to fresh water on the order of 10^{7} g. This estimation prove that mercury in marine water is higher almost two times compared to fresh water. This will cause higher concentration of methyl mercury due to methylation process in marine water. Higher concentration of methyl mercury in marine water contribute to the cause why concentration of total and methyl mercury in marine water fish higher than fish in fresh water fish.

There are another factors that contribute to higher concentration of both total and methyl mercury in marine water fish compared to fresh water fish. Most reactive mercury in marine water system will formed dimethyl mercury which is less toxic than methyl mercury. At the same time, direct formation of methyl mercury from reactive mercury is also possible.

Unfortunately, dimethyl mercury unstable in marine waters and most of dimethyl mercury will decomposed to form methyl mercury. This process assumed to increase the concentration of methyl mercury in marine water system thus, increase marine fish mercury uptake and potentially posing risk to end consumer in food chain.

4.8 The Methyl Mercury to Total Mercury ratio in selected Fresh and Marine water fish species

Mercury is accumulated through food chain, especially in an aquatic medium where the concentration factors of a hundred and even thousand have been reported. Therefore, fish can have higher mercury content than other foods, but it difficult to give an average content because that depends on the fish species considered its age, size and condition of the water it lives (L.Aduma et al, 1995).

The ratio of methyl mercury to total mercury was practically determine in fish muscle due to its relative important for fish metabolism and also for human and wild life health (J. Ruales-Inzunza et al; 2007). The level of methyl mercury found in the variety of fish in this experiment varies from 22 – 98 % of total mercury. This agrees the assumption made by J.Ruales et al,2007 that 90% of total mercury in fish is methyl mercury.

From the assumption and analysis result, fish may constitute as the main source of methyl mercury in human. Since methyl mercury can across placenta barriers, infants exposed to methyl mercury before birth suffer brain damage, it bioaccumulation and magnification can be slower with consumption of smaller fish size and non-predatory fish species. Graph 4.5 and 4.6 shows the amount of methyl mercury as a function of total mercury in individual fish species.

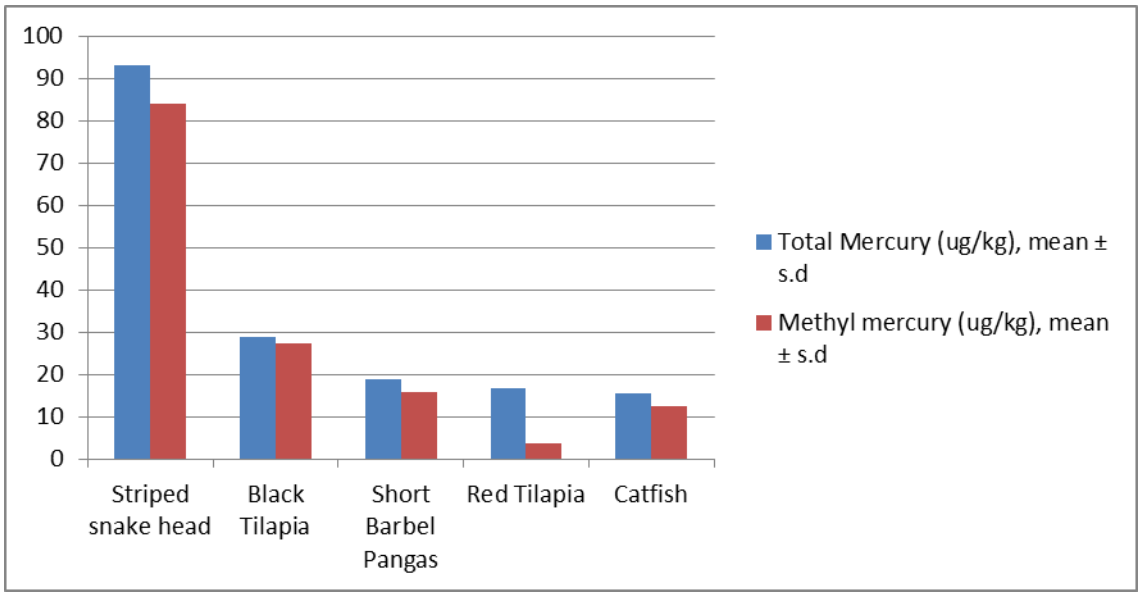

Figure 4.7: Methyl mercury to total mercury ratio for fresh water fish species

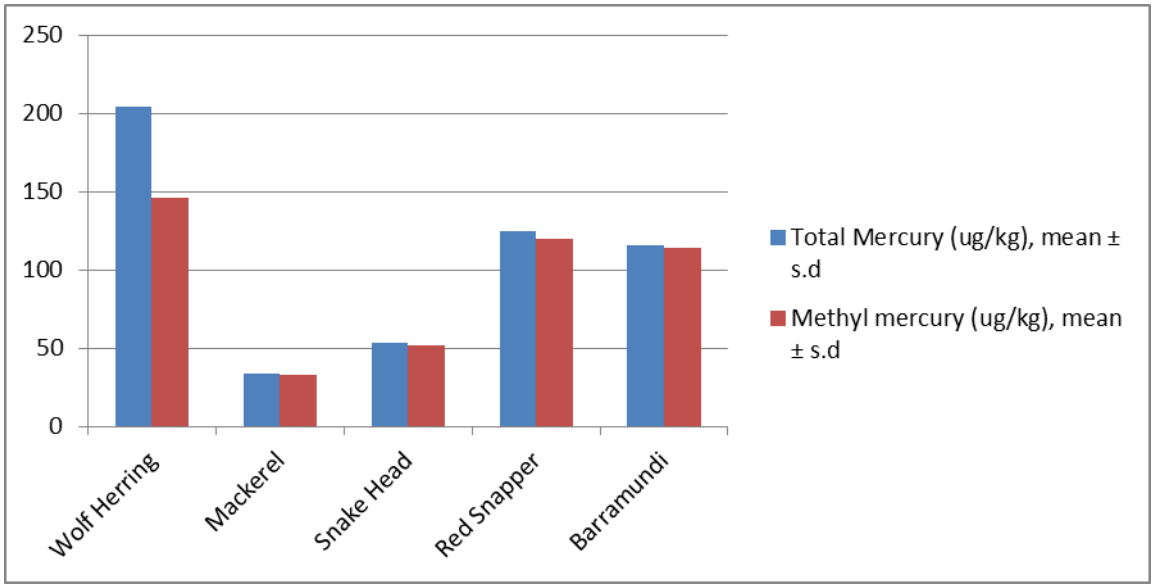

Figure 4.8 : Methyl mercury to total mercury ratio for marine water fish species

Concentration of methyl mercury to total mercury differs depending on the fish species. Based on the data from fresh and marine water fish, it shows methyl mercury accumulates depend on the aquatic system condition and the water chemistry, which respective to fish habitat. Larger, long – lived fish species at the upper end of food web typically have highest concentration of methyl mercury in a given water body. However, age was not determined while size can be observed while fish were purchased from a supermarket.

From the observation, among marine water fish, barramundi and red snapper are larger in size compared to mackerel and snakehead. Therefore, those species has high methyl mercury to total mercury ratio as in graph 4.6. In addition, for fresh water fish, stripped snake head is the biggest in size compared to other type of fish. Thus, it has highest methyl mercury to total mercury ratio among other fresh water species which is 99%.

In conclusion, analytical result for this experiment is agreeable with physical observation by size. Generally, the overall result shows average ratio of methyl mercury to total mercury around 84% and this finding similar with Bloom et al, 1991 stated that nearly 100% of the mercury found in fish tissue muscle is methyl mercury.

Chapter 5

Conclusion & Recommendation

Determination of total mercury and methyl mercury was successfully done in this study with limit of quantification of 10 ng/g and 0.64 ng/g. Reliability, accuracy and precision of digestion and analytical method is proven by determination of certified reference material, laboratory control spike and duplicate sample.

Certified reference material and laboratory control spike for total mercury meets a good recovery in average of 98% and 86%. Determination precision of pretreatment and analysis method done by using duplicate sample. In this experiment, all sample for total mercury have relative standard deviation in range of 0-5% which is less than 20% as per recommended by USEPA method.

For methyl mercury, sample pretreatment and detection method by cold vapor atomic absorption spectroscopy is suitable and adequate with good recovery of certified reference material and laboratory control spike of 99% and 97%. The relative standard deviation of duplicate samples also within the acceptable limit which is 0-5%.

All analyzed fish, fresh and marine water species purchased from commercial supermarket are safe for human consumption as it complies with Malaysian Food Act,1985. On the other hand, all of fish species analyzed have higher concentration of methyl mercury than recommended by FOA/WHO. However this is only a recommendation not a regulation.

Based on the study, marine water fish have higher total and mercury content than fresh water fish. Marine water fish have average of total and methyl mercury of 107 ug/kg and 93 ug/kg. On the other hand, concentration of total and methyl mercury for fresh water is only 34.6 ug/kg and 28.7 ug/kg. The result of marine water fish several times higher than fresh water fish. All these due to contribution of several factors as discuss in chapter 4, result and discussion.

In this study, ratio of methyl mercury to total mercury also have been examined and found that the average ratio of methyl mercury to total mercury for both fresh and marine water is 84% which is agreeable with studies done by Bloom et al,1991 state that nearly 100% of mercury found in fish muscle is methyl mercury.

As a recommendation, study on relation of water chemistry such as water ph and hardness with mercury accumulation in water system that will further contribute to the concentration of total and methyl mercury in fish muscle. In addition, the analysis on effect of mercury deposition in fresh water, marine water and sediment to concentration of mercury in fish could be carried out.

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