An Assessment of Mercury Levels in Tuna Cans Sold in Windhoek Shops, Khomas Region in Namibia

Supervisor: Mr. Albert Samakupa, University of Namibia

A Report in the Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Natural Resources

Submitted to the Department of Fisheries and Aquatic Science, Faculty of Agriculture and Natural Resources, University of Namibia, in partial fulfillment of the requirement for the award of the degree of Bachelor of Science in Fisheries and Aquatic Science of the University of Namibia.

Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr. Albert Samakupa and has not presented elsewhere for the award of the degree. All the sources have been duly and appropriately acknowledged.

…….

12 November 2010

Emmanuel Captain Vellemu

(200715917)

Certification

This is to certify that this report has been examined and approved for an award of degree of Bachelor of Science in Fisheries and Aquatic Sciences of the University of Namibia.

Supervisor

_____

Head of Department

_____

Acknowledgments

My sincere gratitude should go to the Department of Fisheries and Aquatic Sciences for their continued support throughout this work together with the Head of Department. To my supervisor Mr. A. Samakupa, I would like to say thanks a lot for your unconditional assistance, corrections, concrete ideas right from the start to the end and guidance. Most importantly, I would like to say many thanks to Mrs B. Kachigunda, for help with the statistical analysis right from the design stage of the proposal. You have made this work possible. Special appreciations to Mr Ndunge from the Chemistry Department for your cordial and kind assistance on the use on the Atomic Absorption Spectrophotometer machine and all the analysis advice, Mr Kauvee, Mr Akawa and Ms Lempie for help with sample collection and lab work assistance including my colleague Malela for being with me throughout this work as a co-researcher. Special thanks to my sponsors the Royal Norwegian Government for financial support during the four year period of the programme.

Dedication

This work is dedicated to my parents and the family as a whole who taught me I can do anything only if I set my mind to it. I love you Mum and Dad.

ABSTRACT

There are various elements that are found in nature, including the marine environment. Some are essential whereas others are non-essential to living organisms. These metals are believed to be toxic when present in higher concentrations especially the non-essential ones such mercury (Hg). Lately, the environmental contamination by various chemicals such as heavy metals and persistent organochlorides has increased (Ikemoto *et al*., 2008). This poses major environmental and human health problems worldwide (Ensley, 2000). This study assessed mercury levels in the four common brands of imported tuna sold in Windhoek shops and to determine whether the levels are within the permissible limit of 1 ppm set by the Namibian Standards Institute (NSI). Therefore, according to the statistical tests done in this study it was concluded that there are no significant differences in mean treatments across the brands and cans even to that of the set limit of 1 ppm by NSI at 95% confidence interval. Nevertheless, although the individual mercury levels in cans do not pose an immediate danger, it is the accumulation of the mercury which might pose significant health problems more especially to children and pregnant woman. The problems associated with high mercury intake from consumption of tuna will be prone to these groups as is seen in the findings of this research paper.

CHAPTER ONE

INTRODUCTION

There are various elements that are found in nature, including the marine environment. Some are essential whereas others are non-essential to living organisms. In seafood many elements such as zinc (Zn) and copper (Cu) are present, and they are very important to humans at low concentrations (Frausto da Silva and Williams, 1993). However, most of these elements can actually be toxic when present at high concentrations (Oehlenschläger, 2002). In addition, some of these elements have been found not to have any functions, but can be toxic even at lower concentrations especially when exposed over a long period of time (Oehlenschläger, 2002). For example, mercury (Hg), lead (Pb), cadmium (Cd), and selenium (Se) are not required for metabolic activities, and can be toxic at higher concentrations (Clark, 1989). Other essential elements include calcium (Ca), potassium (K) as well as transitional metals such as iron (Fe) and copper (Clark, 1989).

The presence and concentration of heavy metals in aquatic environments depends on both natural and anthropogenic sources (Oehlenschläger, 2002). Some of these metals have been introduced in the aquatic environment through volcanic eruptions in marine environments (Gonzalez et al., 1998; Falconer, Davies and Topping, 1986). Heavy metal contamination of aquatic environments due to anthropogenic sources started to increase from the beginning of the industrial revolution (Nriagu, 1979). Lately, the environmental contamination by various chemicals such as heavy metals and persistent organochlorides has increased (Ikemoto *et al*., 2008). This poses major environmental and human health problems worldwide (Ensley, 2000).

Unlike many organic contaminants, most metals cannot be eliminated from the environment by chemical or biological transformation (Cunningham *et al.*1996).

In general, fish contain certain amounts of heavy metals since they live in aquatic ecosystems (Oehlenschläger, 2002). Usually, the concentration of such metals would depend on where the fish resides in the ecosystem. For example, in the open oceans fish would have the normal concentration of metals because there is no major pollution (Oehlenschläger, 2002). There are aquatic environments that are located closer to industrial activities such that fish found in these areas would normally contain heavy metal concentrations exceeding the natural concentration (Kalay *et al*., 1999; Dobson, 2000; Claisse *et al*., 2001; Prudente *et al*., 1997).

LITERATURE REVIEW

Heavy metals - Mercury:

There are elements that are classified as heavy metals and these include mercury, lead, arsenic and cadmium. Mercury is the only common metal that is liquid at room temperatures. It rarely occurs free in nature and is found mainly in compound forms (Clark, 1989). Mercury is widely used in many areas such as agriculture, medical (i.e. pharmaceutical) and instruments such as thermometers and barometers. It is also used in jewelry making, caring the back of mirrors, dental amalgams and printing as well as in the extraction of gold and silver (Fernandez, 2004). Although most of its medical uses have been discontinued, it is still used for industrial purposes such as in gold mining, paint, and battery manufacture **(**Peraza, *et. all*, 1998).

Mercury occurs in nature in three (3) forms; mercury (Hg), mercuric sulfide (HgS) and mercury (II) sulfide (HgS_2) (Clark, 1989). These inorganic forms of mercury can be converted by microorganisms into methylmercury $(CH_3Hg)^+$, or (MeHg) which is released from sedimentary

particles into the water and eventually accumulates in living organisms (Clarkson, Ballatori and Kerper, 1992). Most organisms have mercury in organic form but in fish nearly 90% of mercury is methylmercury (Fernandez (2004).

Mercury is known to accumulate in living organisms. Methyl mercury has the ability to build up with fish age leading to increased levels in old fatty fish species like tuna (Oehlenschläger, 2002). The concentration of organic methylmercury, which easily crosses through tissue barriers than inorganic mercury (Oehlenschläger, 2002), depends on the level that a species occupy in a food chain (Clark, 1989). Methyl mercury is more toxic as compared to inorganic mercury (Mozaffarian *et. al*., (2006). Most oceanic fish species such as tuna (*Thynus spp*) have high levels of mercury because they feed on other fish in the food chain (Oehlenschläger, 2002), and as a result accumulate large amounts of heavy metals in their flesh. The level of methylmercury in the organism increases at each step of the food chain (Clarkson, Ballatori and Kerper, 1992). The toxicity of mercury also depends on its form such as ionic, metallic or organic (Oehlenschläger, 2002).

Importance of fish consumption

Almost all the food that people consume have an influence on their health. In particular, fish is a very important source of proteins, vitamins, minerals and fatty acids. One of the most important fatty acid present in fish is omega – 3 fatty acid (Gochfeld and Burger, 2004). Omega-3 fatty acid is known to reduce risks associated with heart diseases such as stroke as well as mental problems related to depression and mental decline with age (Torpy, Lynm and Glass, 2006). In addition, fish is a good source of Docosahexaenoic acid (DHA), which is a specific omega-3 fatty acid that is beneficial for the brain development of infants, pregnant women, breast feeding

and child bearing mothers (Mozaffarian *et. al*., (2006). Compared to red meat, fish has negligible levels of saturated fats such as cholesterol (Torpy, Lynm and Glass, 2006). Studies also show that Omega-3 fatty acids decrease triglyceride levels, slow growth rate of atherosclerotic plaque, and lower blood pressure (Mozaffarian *et. al*., 2006).

Despite the fact that consumption of fish has beneficial effects, there are also negative aspects linked to consumption of fish. The accumulation of mercury in fish body tissues has several effects when consumed. Bjornberg *et al.* (2003) showed that there is a positive correlation between mercury levels in humans and fish consumption. According to Gochfeld and Burger (2004), methylmercury interferes with the architecture of the developing brain, disrupting microtubule assembly and interfering with the temporal sequencing of cell adhesion molecules that guide neuronal migration and connections. There are subtle effects on the developing nervous systems of infants (Torpy, Lynm and Glass, 2006). Pregnant women and those that plan to fall pregnant as well as breastfeeding mothers and very young children should avoid fish that have higher mercury content which include: shark, swordfish, king mackerel and golden bass (Torpy, Lynm and Glass, 2006). However, fish with low levels of mercury can still be consumed to supply these groups of people with the necessary nutrients.

Thus the health benefits of eating fish greatly outweigh the potential risks - especially when guidelines are used to reduce the small chance of being affected by such risks (Torpy, Lynm and Glass, 2006).

Study on concentration of mercury in canned fish

Several studies on mercury have been documented worldwide although more needs to be done. Gochfeld and Burger (2004) study indicated that white-style tuna had significantly more total mercury (mean 0.407 ppm) than light-style tuna (mean 0.118 ppm). In a single can of tuna, they found that the maximum mercury concentration was 0.997 ppm. Furthermore, their findings showed no significant differences in mercury levels by draining contents from the cans, style of packaging .i.e. those canned in oil or water and finally the fluid but significant variation was noticed among the years. The U.S. Food and Drug Administration (FDA) use 0.17 ppm in its risk assessment and public information as the recommended level of mercury in canned tuna.

In another study by Khansari *et.all* (2004), the average value for mercury in canned tuna fish was found to be 0.117 ppm from a range of 0.0369 ppm to 0.2618 ppm. The metal contents in the samples, expressed in $\mu g\ g^{-1}$ wet weight, varied from 0.20 ppm to 0.66 ppm with an average value of 0.29 ppm for mercury, from 0.09 ppm to 0.32 ppm based on Voegborlo *et.all*. (1999). Gochfeld and Burger (2001), found out that half of the samples in their analysis exceeded 0.50 ppm, the limit many states and countries set for safe human consumption. As for Namibia and South Africa, the recommended limit for mercury in canned tuna is 1mg/kg (L) = 1ppm (Saunderson, 2010; Pieter, 2010). Japanese tuna indicated mercury concentrations ranging from 50 to 120 μg g-1 in their internal organs (Ashraf, 2006).

Also, white tuna showed higher mercury levels (0.407 ppm) than light tuna (0.368 ppm) in a study conducted by Gochfeld and Burger, 2004. No significant differences in mercury levels were noted between tuna packed in oil or water, and concentrations were the same for whether

the contents were drained or undrained (Gochfeld and Burger, 2004). Nevertheless, the methods of preparation had little impact on methylmercury content in fish (Mozaffarian *et. al*., 2006).

PROBLEM STATEMENT

The environmental contamination by various chemicals such as heavy metals and persistent organochlorides has increased lately (Ikemoto *et al*., 2008). In fish, the methods of preparation have little impact on methylmercury content (Mozaffarian *et. al*., 2006). This means that chances of getting toxic mercury by consuming those species with high mercury levels regardless of any preparation method are likely to be high.

JUSTIFICATION

A thorough study on the presence of heavy metals particularly mercury in canned tuna is important as the element is known to be toxic if present in high amounts especially when limits are exceeded. The study of this nature is very much important for any individual with interest in consuming canned tuna especially pregnant woman and old age as well as those whose immune system is compromised. It also gives choices to consumers to select what specific brands of tuna to purchase based on the metal levels in the cans.

Currently, no literature on mercury levels in imported canned tuna has been documented in Namibia. Therefore, there was a need for this research study that will form a guideline for future investigations and even some adjustments in the fishing industry and the society as a whole at the same time some policies in the government be reviewed.

OVERALL OBJECTIVE

The overall objective of the study was to establish the safety of the imported canned tuna that can be purchased at various outlets in the city of Windhoek. This helped us to answer the question as to whether the tuna cans sold in Windhoek shops have mercury levels within the recommended limits set by the Namibian Standards Institute (NSI) at 1 ppm.

SPECIFIC OBJECTIVES

- To assess mercury levels in four different brands of canned tuna purchased in various outlets in Windhoek.

- To establish if the mercury levels in canned tuna found in shops around Windhoek fall within the recommended limit of 1 ppm.

RESEARCH HYPOTHESES

- There are significant differences in the mean mercury levels among the four canned tuna brands
- There are significant differences in the mean mercury levels between the canned tuna and the NSI recommended limit of 1ppm

CHAPTER TWO

MATERIALS AND METHODS

Sample collection

Samples of canned tuna were purchased from Shoprite, Checkers, Pick 'n' Pay and Woermanbrock shops. A total of 16 cans were sampled and purchased, consisting four different brands of canned tuna. During sampling, the date, location (shop) and specific tuna brands were recorded. All samples were collected on the same day, 15th September. The selection of sampling units was arbitrary as researchers heavily rely on personal judgment. From the shelves, four cans were picked from the right end, front middle, centre and left end of each shop. Hence, random selection of the samples was employed. The duration of this study was three months.

Sample preparation

Samples were opened to drain off the preservation liquid used (oil, brine and water) After that crucibles were thoroughly washed with a bit of nitric acid then rinsed with distilled water to remove excess dirt and labeled properly for identification. Then a known weight portion (about 30.0 grams) of the sample was obtained then placed in the crucibles for drying in the oven at 70^{o}C for 48 hours to remove excess liquid as much as possible. Thereafter, the following digestion method was employed in order to extract the mercury from the samples.

Digestion protocol for mercury analysis in the samples

Two digestion methods were employed during the analysis of the samples. The samples were first was digested following a heavy metal digestion protocol by Olowu, *et.al 2009* where by approximately 2.0 g of each sample was weighed using the analytical balance. Then they were placed in the clean crucibles and ashed in the furnace at 550^{o} C for 24 hrs. Then the samples were removed for cooling to cool off for 20 mins before dissolving the ash in 5 mL of concentrated nitric acid and made up to 25 mL volume with dionised water. Half the volumetric flasks used during this study investigation were A – grade type while the other half were B and C - grade flasks due to shortage. The working standards were prepared by the following general dilution formula;

$$\text{No. of moles for required volume} = \frac{\text{Required volume (ppm)} * \text{Required volume (ppm)}}{\text{Stock volume (ppm)}}$$

$$\text{i.e. Dilution formula} = M1V1 = M2V2$$

The field blank was prepared using the reagents that were used to make the concentration of the standards which was a 50 – 50 volume concentration of nitric acid and distilled water. Therefore, Atomic Absorption Spectrophotometer was used to determine the presence of total mercury in these samples. During this investigation, each brand of tuna was replicated four (4) times to increase precision and consistency.

Thereafter, a second digestion protocol was set up. The samples were first homogenized using a blender to obtain a dry powder, enriched in mercury content and also to get a more representative

sub – sample from the homogenized tissue. After that, a wet ashing method was done following (Oehlenschläger, 2002) protocol for identifying heavy metals in fish. This method was used to destroy the organic matrix in the sample since mercury has a tendency of forming compounds with other metals such as zinc. A combination of two acids was used (20 % nitric acid and 80 % sulphuric acid) at 60^{o}C using a heater for 2 hours. Then the samples were ready for spectrophotometer analysis once they cooled off. The working standards were prepared by the following general dilution formula;

$$\text{No. of moles for required volume} = \frac{\text{Required volume (ppm)} * \text{Required volume (ppm)}}{\text{Stock volume (ppm)}}$$

i.e. $\underline{\text{Dilution formula} = M1V1 = M2V2}$

The field blank was prepared using the reagents that were used to make the concentration of the standards which was an equal volume concentration of nitric acid, sulphuric acid and distilled water. Therefore, HACH DR 2700 Spectrophotometer was used to determine the presence of methylmercury in the samples.

During this investigation, each brand of tuna was replicated four (4) times to increase precision and consistency.

Another set of same samples were sent to an external laboratory (Ministry of Mines and Energy) for comparison interest.

Plate 1: Ashing the samples in furnace

Plate 2: Inserting correct lamp in the AA machine

Plate 3: HACH DR 2700 spectrophotometer

CHAPTER THREE

RESULTS

The following Table 1 shows the results for the second digestion method using the HACH Spectrophotometer.

Table 1: Summary statistics for the analysis

Brand	Mean (ppm)
Brand A	0.40
Brand B	0.50
Brand C	0.50
Brand D	0.30
Overall mean	**0.425**
Variance	**0.007396**
Standard deviation	**0.086**
Standard error of the mean	**0.021**
CV%	**20.149**

(Table 1) shows the summary statistics of the research investigation. According to the results, brand B and brand C share the same mean mercury concentration value (0.5ppm) while brand D showed a slightly lower mean mercury concentration (0.3 ppm) among the 4 brands including the commonly found brand A. Interestingly, the standard error of the difference, and the least significant difference indicated a zero value because there was no variation in the measurements done for the different replicates for each brand as seen in the coefficient of variation.

Below is a graph of the mean concentration of mercury in the four brands.

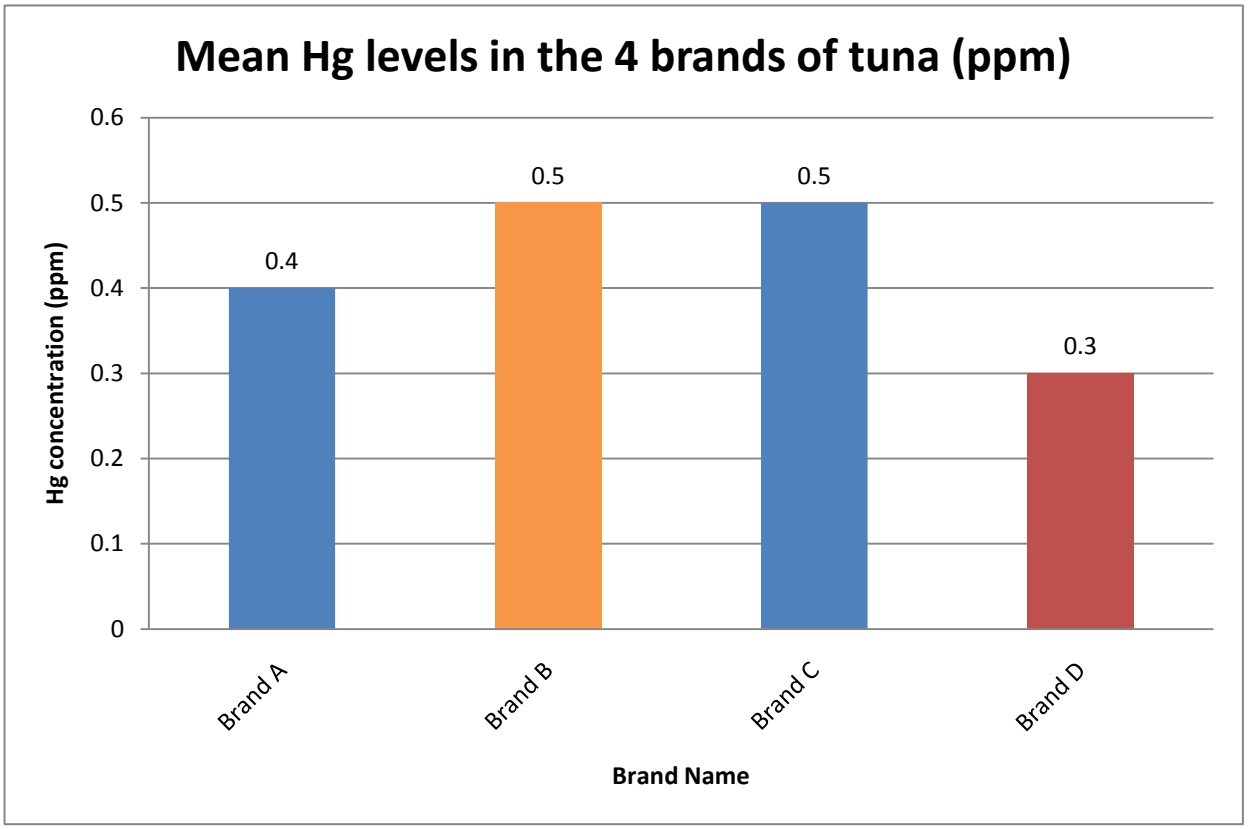

Figure 1: Mean mercury concentration for the 4 brands of tuna

Table 2: Sample results from an external laboratory (Ministry of Mines and Energy) showed results way below the machine's detectable limit of 10 ppm (see appendix 1)

CHAPTER FOUR

Discussion

From the results of this research investigation, a few items can be drawn for discussion looking at the outcome from the GENSTAT software analysis.

The initial analysis of the samples on the Atomic Absorption spectrophotometer showed values way below the machine detectable levels as seen in appendix Table 2. Similarly, mercury levels in tuna cans analysed by the Ministry of Mines and Energy showed limits way below the machine detectable point (10 ppm). The machine used was the XRF analyser and works with emitting X-rays.

Firstly, this could be due to the presence of mercury in very minute levels in the sample for possible detection point or perhaps no mercury presence in the samples.

The other reason was the lack of the mercury accessory on the AA machine specially designed to detect mercury. Another possible reason attributed to such outcome could be possible errors that might have risen during the sample preparation, standards and field blanks.

Nevertheless, no mercury level was detected on the AA spectrophotometer machine under this research study. However, this does not necessarily imply that there is no presence of mercury in tuna fish as it is a highly migratory and predatory fish also that is found at the top of the food chain such that it accumulates mercury in its body through a process called bioaccumulation (Clark, 1989).

Lastly, human and instrument errors might also attribute to such results to some extent as stated above. For example, the setup of the lab in relation to the position of the AA machine with no fume hood to extract any harmful gases during the analysis since these gases might interfere with the samples and the tests.

Secondly, another method was used to test the same tuna samples using the HACH 2700 spectrophotometer for argument sake that yielded results shown above in figure 1.

However, the results of the analysis showed a coefficient of variation of zero percent indicating that there was no variation in the mean concentration of mercury among the four samples of tuna under the investigation. Hence, it can be concluded that there was no significant difference in the means for the brands.

However, a brief discussion can be outlined for the same results based on the comparison with the Namibian Standards Institute recommended limit for mercury in tuna samples of 1 ppm. Firstly, the summary statistics table in the results clearly indicate that all mean values were almost 50 % way below the 1 ppm limit recommended by NSI. Nevertheless, it is important to note that Brand D had the lowest mean concentration of mercury of 0.30 ppm than all the three brands investigated. Also, Brand C and Brand B shared the same Hg level of 0.50 ppm which is higher than the other two with Brand A in the middle with an Hg level of 0.40 ppm.

Therefore, the analysis showed that there is no significant difference in mean mercury concentration between any of the cans in comparison with the NSI limit as seen in the results of the analysis. The graph above (figure 1) shows the results more clearly that this is true as all values were below 1 ppm with the two brands Brand C and Brand B topping by 0.50 ppm while Brand D has the lowest concentration of mercury (0.30 ppm). Hence, an interesting observation

could be that of the two brands having a higher concentration of mercury than the rest while Brand D yielded a very lower Hg concentration than the rest even lower than the commonly found Brand A.

High concentrations of heavy metals are only rarely found in fish muscle (Oehlenschläger, 2002). This could also attribute to the results obtained in this investigation. Pollution of the water bodies tends to increase the concentration of mercury in fish (Oehlenschläger, 2002). Hence, it can also be drawn here that perhaps the samples were from non contaminated areas to yield very low concentrations way below the acceptable limits.

LIMITATIONS TO THE STUDY

During the process of conducting this research study, a lot of challenges were experienced right from the start till the end. The main challenge was lack of the mercury VP 100 accessory on the AA machine that forced me to use the flame analysis instead of the cold vapour analysis that many scientists are using around the world. The sensitivity for AA machine is known to be very low for mercury that is quite a challenge to detect some levels in the samples with no VP 100 accessory on the machine

More metals for comparison sake would have been analysed also but the reagents for standards were limited. These would have made the study more beautiful to compare various metals in the samples other than mercury alone.

Conclusion

The tuna cans sold in Windhoek shops do not pose any health hazard to human because the levels are quite too low and way below the recommended limit of 1 ppm. These brands, A, B, C, and D also show no difference in the mean mercury concentrations. According to the statistical tests done it can be concluded that, there are no differences in mean treatments across the brands and cans even to that of the set limit of 1 ppm by NSI. However, based on this research investigation, the results would be extra unique given the technical knowledge of the AA machine at hand and using the A – grade materials for very accurate measurements since I was working with very low concentrations of these reagents and measurements such as ppm. Either way, this investigation has taught me learn a lot in as far as the AA machine is concerned. Though the individual mercury levels in cans do not pose an immediate danger, it is the accumulation of the mercury which might pose significant health problems more especially to children and pregnant woman. The problems associated with high mercury intake from consumption of tuna will be prone to these groups as mentioned in this report.

REFERENCES:

Ashraf, W. (2006). Levels of selected heavy metals in tuna fish: Docosahexaenoic acid (DHA) Darhran, Saudi Arabia.

Bjornberg, K.A., Vahter, M., Petersson-Grawe, K., Glynn, A., Cnattingius, S., Darnerud, P.O., Atuma, S., Aune, M., Becker, W., and Berglund, M., (2003). *Methylmercury and inorganic mercury in Swedish pregnant women and in cord blood*: influence of fish consumption. Environmental Health Perspective. 111, 637–641.

Burger, J., Gaines, K.F., Gochfeld, M., 2001. Ethnic differences in risk from mercury among Savannah River fishermen. Risk Anal. 21, 533–544.

Claisse, D., Cossa, D., Bretaudeau-Sanjuan, J., Touchard, G., and Bombled, B. (2001). Methylmercury in mollusks along the French coast, *Mar Poll Bull*, 42 (4), 329-332.

Clark .R.B. (1989). Marine Pollution; 2nd Edition: Oxford Science Publications. New York, USA.

Clarkson, T.W., Ballatori, N., and Kerper, L.E. (1992). Methylmercury transport across the blood-brain barrier by amino acid carrier: *American Journal of Physiol.* 262 (5pt. R761-R765).

Cunningham, B., Andres, S., Ribeyre F., Tourencq, J.N. and Boudou, A. (1996). Interspecific comparison of Cadmium and Zinc contamination in the organs of four fish

Dobson, J. (2000). Long term trends in trace metals in biota in the Fourth estuary, Scotland, 1981 – 1999, *Mar Poll Bull* 40 (12), 1214 – 1220.

Ensley, B.D. (2000) Rationale for use of Phytoremediation. *In*: Raskin, I. and Ensley, B.D. (ed.), Phytoremediation of toxic metals: *Using plants to clean up the environment*. John Wiley & Sons, New York. pp3-12.

Falconer, C.R., Davies, I.M., Topping, G. (1986). Cadmium in edible crabs (Cancer pagurus L.) from Scottish coastal waters, *Sci Total Environ 54, 73-183.*

Fernandez, G.L. (2004). Accumulation of Mercury and other heavy metals in some edible marine mollusks in Sibutad, Zamboanga del Norte. *9th National Convention on statistics* (NCS): Lantawan.

Food and Agriculture/World Health Organization (FAO/WHO) "*Evaluation of Certain Food Additives and the Contaminants Mercury, Cadmium and Lead*", WHO Technical Report Series No. 505, Geneva, 1972.

Frausto Da Silva, JJR., Williams, RJP., (1993). *The biological chemistry of the elements, The inorganic chemistry of life*, Oxford, Clarendon Press.

Gochfeld, M. and Burger, J. (2004). Mercury in canned tuna: white versus light and temporal variation. *Environmental Research* 96, pp 239–249. New Jersey, USA.

Gonzalez, F., Silva, M., Schalscha, E., Alay, F., (1998). Cadmium and lead in a trophic marine chain, *Bull environmental Contamination Toxicology,* 60, 112-8.

Ikemoto, T., Nguyen, P., Noboru, O., Iwata, A. ,Omori, K. Tanabe, S., Tuyen B. and Takeuchi, I. (2008) Biomagnification of Trace Elements in Aquatic Food Webs in the Mekong Delta, South Vietnam Using Stable Carbon and Nitrogen Isotope Analysis: *Arch Environment Contam Toxicol.54*:504-515.

Kalay, M., and Cahli, M., (1999). Heavy metal concentrations in fish tissues from the northeast Mediterranean sea, *Bull environmental Contamination Toxicology* 63, 673-81.

Khansari, F.E., Khansari, G.M., Abdollahi, E. (2004). Heavy metals content of canned tuna fish: The Journal of food chemistry, Volume 67, Issue 4, Pages 341-345.

Mozaffarian, D., and Rimm, E., (2006). Fish Intake, Contaminants, and Human Health, *Evaluating the Risks and Benefit:* The Journal of the American Medical Association: JAMA 296;1926:1885 – 1887.

Nriagu, J.O. (1979). Global Inventory of Natural and anthropogenic emissions of trace metals to the atmosphere, *Nature, (279),* 409-411.

Oehlenschläger, J. (2002). Identifying heavy metals in fish, *Safety and Quality issues in fish processing*, Cambridge, Woodhead Publishing Limited.

Olowu, R.A., Ayejuyo, O.O., Adewuyi, G.O., Adejoro , A.A.B., Denlovye, A.O., Babatunde and Ogundajo, A.L. (2009). Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons: Lagos, Nigeria.

Peraza, M.A., Fierro, F.A., Barber, D.S., Casarez, E., Rael, L.T. (1998). Effects of Micronutrients on Metal Toxicity. *Environmental Health Perspectives Supplements* Volume 106, Number S1, 1998.

Pieter, (2010). South African Bureau of Standards (SABS).

Prudente, M., Kim, E-Y., Tanabe, S., Tatsukawa, R. (1997). Metal levels in some commercial fish species from Manila Bay, The Philippines, *Mar Poll Bull*, 34, (8), 671-4.

Saunderson**, (**2010**).** Namibian Standards Institute **(**NSI**).**

Torpy, J.M; Lynm, C; Glass, R.M. (2006). Eating Fish: Health Benefits and Risks. The Journal of the American Medical Association: JAMA 296;1926:1884 - 1888

Voegborlo, R.B., El-Methnani, A.M., Abedin, M.Z. (1999). Mercury, cadmium and lead content of canned tuna fish.

Appendix

Table 1: Genstat Output for the analysis

Identifier	Values	Missing	Levels
Brand	16	0	4

Identifier	Minimum	Mean	Maximum	Values	Missing
Replicate	1.000	2.500	4.000	16	0
Concentration	0.3000	0.4250	0.5000	16	0

******* Analysis of variance *******

Variate: Concentration

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Brand	3	0.1100000	0.0366667		
Residual	12	0.0000000	0.0000000		
Total	15	0.1100000			

***** Tables of means *****

Variate: Concentration

Grand mean 0.42

Brand	1	2	3	4
	0.40	0.50	0.30	0.50

*** Standard errors of means ***

Table Brand

rep. 4

d.f. *

e.s.e. 0.000

*** Standard errors of differences of means ***

Table Brand

rep. 4

d.f. *

s.e.d. 0.000

*** Least significant differences of means (5% level) ***

Table Brand

rep. 4

d.f. *

l.s.d. 0.000

***** Stratum standard errors and coefficients of variation *****

Variate: Concentration

d.f.	s.e.	cv%
12	0.000	0.0

Summary statistics for Concentration

Number of observations = 16

Number of missing values = 0

Mean = 0.425, Median = 0.450, Minimum = 0.300, Maximum = 0.500

Lower quartile = 0.350, Upper quartile = 0.500, Standard deviation = 0.086

Standard error of mean = 0.021, Coefficient of variation = 20.149, Sum of squares = 0.110

Measured parameters (weight of the samples, wet and dried plus the mass of the crucibles)

Table A. Mass of crucibles in relation to the brand name of tuna

Crucible number	Replicate	Brand	Mass of crucibles (g)	Initial mass (g)	Dried mass
1	1	Brand A	30.0506	29.1333	1.8645
1	2	Brand A	23.1733	30.0506	1.8748
1	3	Brand A	31.0544	31.0544	2.0726
1	4	Brand A	29.1333	30.6506	1.8748
2	1	Brand B	30.4309	30.4568	2.0667
2	2	Brand B	31.4528	30.4309	2.0677
2	3	Brand B	30.9309	30.9309	2.2426
2	4	Brand B	30.4568	30.4919	2.0677
4	1	Brand C	29.2957	32.8303	2.0375
4	2	Brand C	33.8003	29.2957	2.2375
4	3	Brand C	29.2957	29.2957	2.0683
4	4	Brand C	32.8303	29.4950	2.2375
3	1	Brand D	32.9820	29.8138	2.0262
3	2	Brand D	28.8198	32.9820	2.0462
3	3	Brand D	32.9820	32.9820	2.1729
3	4	Brand D	29.8138	32.9920	2.0462

HACH DR 2700 Spectrophotometer readings

Table I: Readings from the spectrophotometer showing the number of replications with the corresponding measurement in parts per million (ppm).

Brand	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**	**Mean**
Brand A	0.4	0.4	0.4	0.4	0.4
Brand D	0.5	0.5	0.5	0.5	0.5
Brand B	0.3	0.3	0.3	0.3	0.3
Brand C	0.5	0.5	0.5	0.5	0.5

Mercury Calibration graph

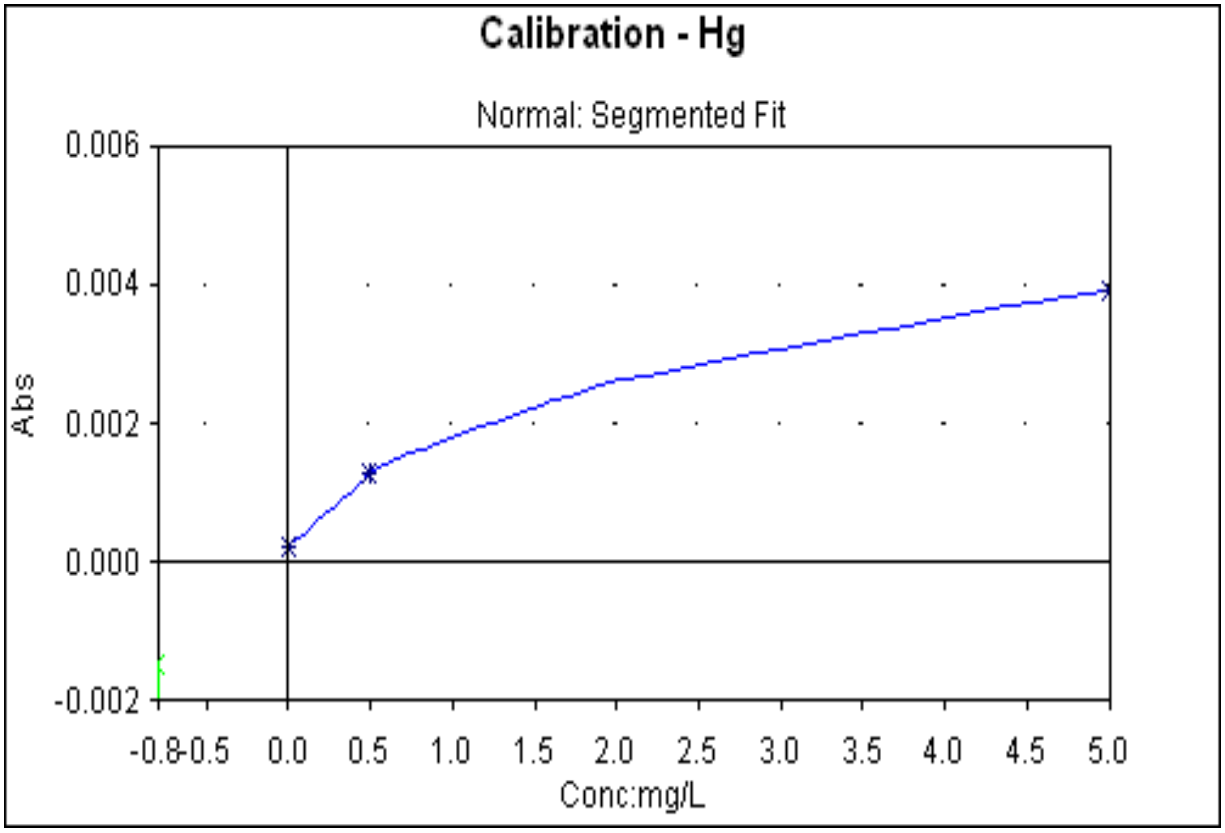

Fig.2: Mercury calibration graph for the first analysis on the AA Spectrophotometer.

Table J: Sample results from the AA spectrophotometer machine showing the absorbance and Hg concentration in the 4 tuna cans with the replicates.

SAMPLE ID	RESULT TYPE	SIGNAL	Rsd	CONC.	CORRECTED CONC.
		Absorbance	%	mg/L	mg/L
Blank	**Mean**	**0.0002**	**57.3764**	**0**	
Blank	Resample 1 of 3	0.0002			
Blank	Resample 2 of 3	0.0001			
Blank	Resample 3 of 3	0.0003			
Standard 1	**Mean**	**0.0012**	**39.8630562**	**0.5**	
Standard 1	Resample 1 of 3	0.0007			
Standard 1	Resample 2 of 3	0.0013			
Standard 1	Resample 3 of 3	0.0017			
Standard 2	**Mean**	**0.0039**	**8.72844696**	**5**	
Standard 2	Resample 1 of 3	0.0037			
Standard 2	Resample 2 of 3	0.0037			
Standard 2	Resample 3 of 3	0.0043			
Brand A	**Mean**	**-0.0002**	**198.793167**	**-0.16757**	**-0.167571783**
Brand A	Resample 1 of 3	-0.0003			
Brand A	Resample 2 of 3	-0.0003			
Brand A	Resample 3 of 3	0.0002			
Brand D	**Mean**	**-0.0010**	**36.0401802**	**-0.56298**	**-0.562975824**
Brand D	Resample 1 of 3	-0.0010			
Brand D	Resample 2 of 3	-0.0013			
Brand D	Resample 3 of 3	-0.0006			
Brand B	**Mean**	**-0.0013**	**22.5083904**	**-0.70759**	**-0.707589567**
Brand B	Resample 1 of 3	-0.0016			
Brand B	Resample 2 of 3	-0.0010			
Brand B	Resample 3 of 3	-0.0012			
Brand C	**Mean**	**-0.0015**	**27.56987**	**-0.79713**	**-0.797128558**
Brand C	Resample 1 of 3	-0.0016			
Brand C	Resample 2 of 3	-0.0018			
Brand C	Resample 3 of 3	-0.0010			

Table 2: External results from the Ministry of mines and Energy showing levels below machine sensitivity level (10 ppm)

SAMPLE	Hg (ppm)
Brand A	8.36
Brand A	6.29
Brand A	9.63
Brand A	< LOD
Brand B	2.45
Brand B	<LOD 6.4
Brand B	8.04
Brand B	5.29
Brand C	10.8
Brand C	11.35
Brand C	<LOD
Brand C	4.41
Brand D	< LOD
Brand D	3.67
Brand D	5.02
Brand D	2.4

Glossary

NSI:	Namibian Standards Institute
PPM:	Parts per million
Hg:	Chemical symbol for mercury
USFDA:	United States Food and Drug Administration
AAS:	Atomic Absorption Spectrophotometer
HACH 2700:	Type and model of spectrophotometer
AA:	Atomic Absorption
ANOVA:	Analysis of variance

Contents