CHAPTER 3

RESULTS AND DISCUSSION

All analyses in this study were performed by (FIMH 400) Cold Vapor Atomic Absorption Spectrometry after acid digestion. In order to make the results reliable and reproducible, the optimum condition for analytical procedures and sample pre-treatment were investigated prior to analyze all samples. The following parameters were optimized.

3.1 Optimization the analytical reagents for mercury analysis by Perkin Elmer FIMH-400 Atomic Absorption Spectrometry

3.1.1 Effect of $NaBH_4$ concentration

The effect of $NaBH_4$, a reductant, concentration at 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 % (w/v) was examined. The results are shown in Figure 3-1 and Table D-1 in Appendix D. Increasing of peak height was observed when until concentration of $NaBH_4$ was 0.5% (w/v). However, student *t* test showed no significant different between 0.2 and 0.5% (w/v) $NaBH_4$. Thus, 0.2% (w/v) $NaBH_4$ was selected for further experiments. This concentration is also recommended in the manual procedure of Perkin Elmer FIMH 400.

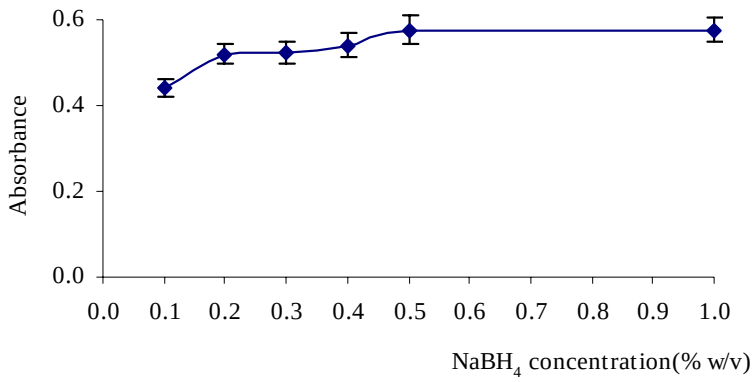


Figure 3- 1 Effect of $NaBH_4$ concentration on mercury analysis by Perkin Elmer FIMH 400 Cold Vapor Atomic Absorption Spectrometry

3.1.2 Effect of HCl concentration

The effect of HCl concentration as a carrier solution on peak height was examined. The results are shown in Figure 3-2 and Table D-2 in Appendix D. The maximum absorbances were observed when using 2-4% (v/v) HCl. Higher concentration of HCl showed decreasing in the absorbance. The 3% (v/v) HCl was selected to use as the carrier for further study.

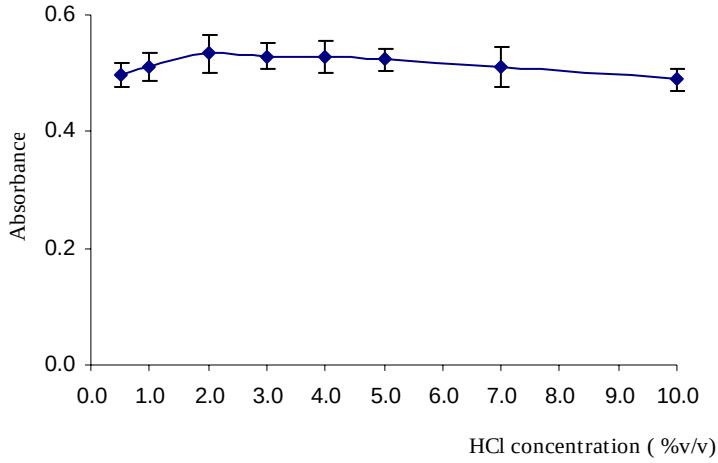


Figure 3- 2 Effect of HCl concentration on mercury analysis by Perkin Elmer FIMH 400 Cold Vapor Atomic Absorption Spectrometry

3.2 Optimization of the analytical procedures

3.2.1 Reagents for pre-cleaned process

Grease and dust on hair's surface have to be washed off prior to further analytical processes. There is no single methodology. Different laboratories use different washing procedures (Salmela *et al.*, 1981; Suzuki and Lenihan, 1988; Morton *et al.*, 2002). Deionized water, organic solvents, complexing agents, ionic and nonionic detergents and inorganic acids, were commonly used as washing solutions. In this study the effect of eight different washing agents were investigated. They were deionized water, acetone (an organic solvent), 1% w/v EDTA (a complex forming agent), 1% v/v Triton X-100 (a nonionic detergent), 1% v/v SLS (an inorganic detergent), 1% v/v baby shampoo (an inorganic detergent), 0.1M HCl and 1% v/v HNO_3 (inorganic acid). The results are shown in Figure 3-3 and Table D-3 in

Appendix D. Higher concentrations of mercury were found when using deionized water, 1% EDTA, 1% Baby shampoo, 1% HNO_3, than using acetone, 1% Triton-X 100, 1% SLS and 0.1M HCl as pre-cleaned reagent. The result from this study agreed with the work of Morton *et al.* (2002). He reported the loss of mercury and lead when washing hair sample with 0.1M HCl while mercury and zinc were only partially removed after washing for 8 hours with EDTA. The loss of mercury due to 1% EDTA washing did not observe in this study since the washing time is only 6 minutes. However, statistical paired *t*-tests were carried out for all washing agents and the results revealed no significant difference among these washing solutions. Comparing the washing solutions between 1% HNO_3 and deionized water, it found that % RSD of 1% HNO_3 is slightly higher than deionized water. Thus, in this study deionized water was selected for pre-washed process prior to sample digestion.

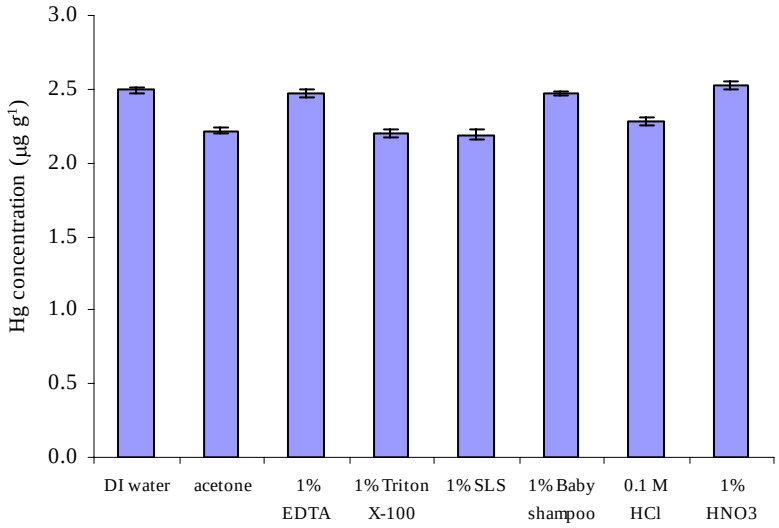


Figure 3- 3 Effect of pre-washed reagents to the analytical results by FIMH 400 Cold Vapor Atomic Absorption Spectrometry

3.2.2 Drying temperature

The effect of temperature using in drying process, 25°C, 60°C and 90°C, were studied. The results are shown in Figure 3-4 and Table D-4 in Appendix D.

A slightly depletion in mercury concentration was found when dried at 25°C. This may due to the sample was not completely dried which then affected the sample weight. The paired *t*-test was carried out for the drying at 60°C and 90°C, the result showed no significant difference at 95% confident limit. The drying temperature of 60°C was chosen for the whole study.

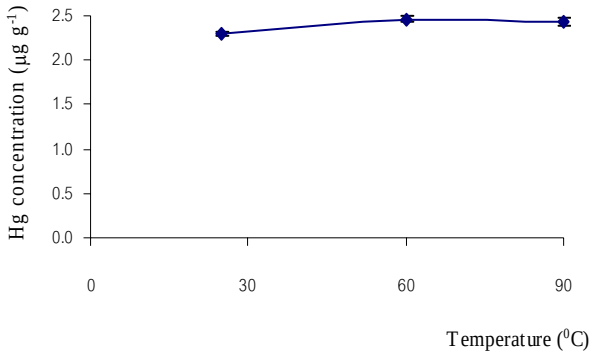


Figure 3- 4 Effect of drying temperatures on the analytical results by Perkin Elmer FIMH 400 Cold Vapor Atomic Absorption Spectrometry

3.2.3 Acid for digestion

As reproducible and accurate results will be given only digestion is completed. The efficiency of various was examined, HNO_3, HNO_3+H_2O_2, HNO_3+H_2SO_4 and HNO_3+$HClO_4$, for microwave digestion technique using in this studied.. The results are shown in Figure 3-5 and Table D-5 in Appendix D. No significant difference has been found at 95% confidence limit when *t*-test was used. Although stronger oxidant may be required to for biological samples breakdown organic compounds, H_2O_2, H_2SO_4 and $HClO_4$ do not show much influence in this study. Since, $HClO_4$ is posed to dangerous in some circumstances by possible explosion or fire the digestion (Wasiak *et al*., 1996), the mixture of HNO_3+H_2O_2 was chosen for the whole study.

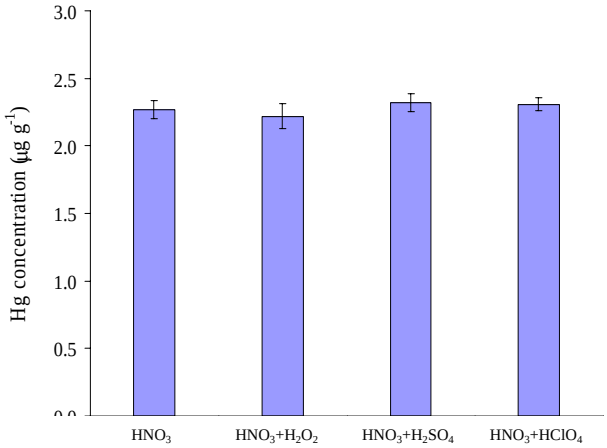


Figure 3- 5 Effect of acids using in digestion process on the analytical results analysis by Perkin Elmer FIMH 400 Cold Vapor Atomic Absorption Spectrometry

3.2.4 Digestion times

The microwave digestion technique is used in this study due to the advantage of rapid and effective decomposition (Loring and Rantala, 1995; Liang *et al.*, 2003). The digestion procedure is followed Loring and Rantala (1995), in which the digestion time of full power was set at 90 seconds. The effect of prolonged half power digestion time, after 90 seconds full power, was examined. The results are shown in Figure 3-6 and Table D-6 in Appendix D. The temperature of the digested solution is 85°C. Loss of mercury was found when prolonged digestion was over 15 minutes. The solution in a Teflon bomb was dried out after 30 minutes. In this study, the 90 seconds of full power and 5 minutes of half power digestion time were chosen. According to Chen *et al*. (2002), hair sample was digested at 90°C for 10 minutes while Caroli *et al.* (1998) digested hair sample for 18 minutes in microwave oven. Thus, the digestion method in this study provided shorter digestion time.

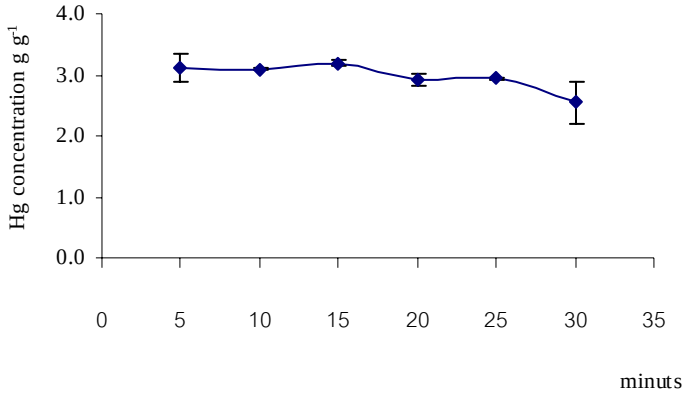


Figure 3- 6 Effect prolonged half power of digestion times, after 90 seconds full power, on analytical results by Perkin Elmer FIMH 400 Cold Vapor Atomic Absorption Spectrometry

3.3 Analytical Performance characteristics

3.3.1 Detection limit

The calculation of DL for Perkin Elmer FIMH 400 Cold Vapor Atomic AbsorptionSpectrometry was followed Equation 4 (see detail in Chapter 2, section 2.7.1). The standard deviation of 10 replicates of reagent blank was 0.004 μg L^{-1}. Thus, the limit of detection for total mercury by CVAAS was 0.012 μg L^{-1}. Detail is shown in Table 3-1.

Table 3- 1 Concentration of 10 replicates of reagent blank

Number	Reagent blank concentration (μg L^{-1})	Average	SD
1	0.001		
2	0.001		
3	0.002		
4	0.012		
5	0.010	0.004	0.004
6	0.006		
7	0.003		
8	0.003		
9	0.003		
10	0.003		

3.3.2 Linearity

Calibration curve using mercury standard solution in the range of 0-70 μg L^{-1} is shown the linearity as in Figure 3-8. Details are in Table D-8 (Appendix D).

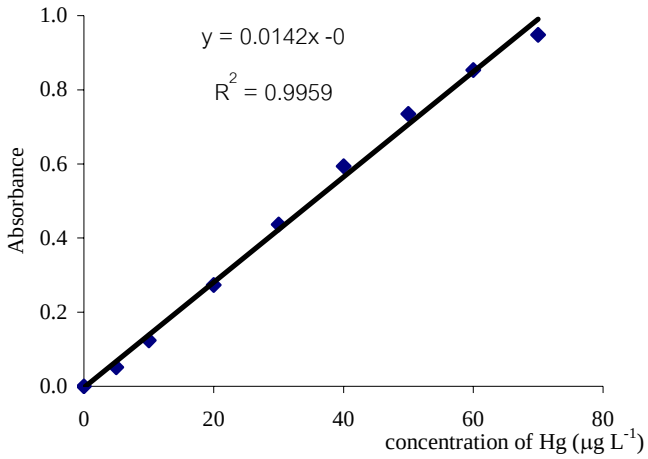


Figure 3- 7 Calibration curve

3.3.3 Recovery Test

The recovery test of hair sample spiked with standard mercury is shown in Table 3-2. Percent recovery was 98 % with 2%RSD.

Table 3- 2 The recovery tested

Sample + spiked	Concentration of Hg (μg L^{-1}) in sample	Determined concentration of Hg (μg L^{-1})		%recovery	
		1	2	1	2
Sample + 0.1 μg Hg	59.76	57.17	61.29	95.67	102.55
Sample + 0.2 μg Hg	69.76	67.60	68.34	96.90	97.97
Sample + 0.3 μg Hg	79.76	78.44	78.33	98.34	98.21
Sample + 0.4 μg Hg	89.76	85.57	85.40	95.33	95.14
Mean				97.51	
SD				2.26	
%RSD				2.32	

3.3.4 Accuracy

The accuracy of the proposed method was verified with certified reference material (BCR-397) human hair obtained from Institute for Reference and Measurement, Belgium. The concentration of total mercury obtained by this method was 11.5±0.3 μg g^{-1} while the certified value was 12.3±0.5 μg g^{-1} as presented in Table 3-1. This value shows no significant different by using *t*-test at 99% confident limit with a percent relative error of 2.7%.

Table 3- 3 Measurements of Hg concentration in certified reference material (BCR-397)

Repeated	Measured value (μg g^{-1})	Average ± SD	Certified value	% relative error	% recovery
1	11.5				
2	11.20	11.5±0.3	12.3±0.5	2.7	94.4
3	11.8				

3.3.5 Precision

The precision of the analysis was presented in the term of percentage of relative (%RSD) of 10 replication measurements of one sample. The %RSD was 2% (Table D-9 in Appendix D).

3.4 Quantification of the analyses: Standard addition method

In order to know whether the hair matrix would interfere with the measurement of total mercury by CVAAS technique, the slope of normal standard calibration curve was compared to a standard addition one. The slopes of these two methods were different as shown in Figure 3-7 and Table D-7 in Appendix D. Thus, the standard addition method was used to quantify the amount of mercury in all samples.

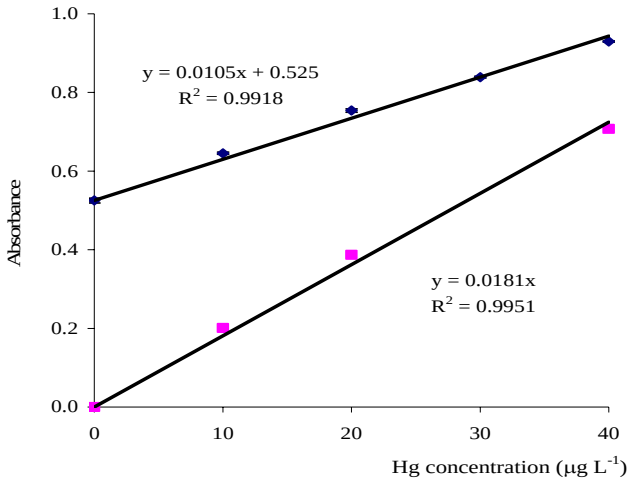
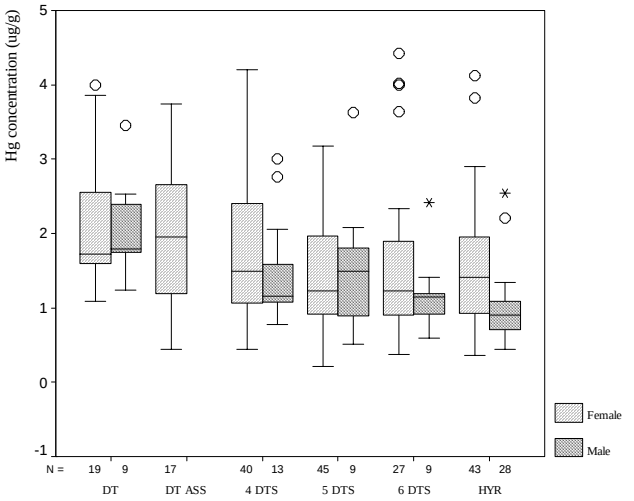


Figure 3- 8 Comparison standard calibration curve and standard addition curve for hair sample, ■ = calibration curve; ◆ = standard addition curve

3.5 Mercury concentration in the hair of dental personnel and Hat-Yai residents

The mercury level in hair of 189 exposed persons from Faculty of Dentistry, Prince of Songkla University and 71 non-exposed Hat-Yai residents were presented in Table D-11 in Appendix D. The average Hg concentration (range) in µg g^{-1} of dentists, dentist assistants, year-4 dentist students (DTS), Yr-5-DTS, Yr-6-DTS and Hat-Yai residents were 2.10±0.80 (1.08-4.00), 1.91±0.87 (0.44-3.75), 1.49±1.05 (0.37-4.42), 1.66±0.87 (0.44-4.21), 1.44±0.71 (0.25-3.18) and 1.32±0.75 (0.36-4.12), respectively (Table 3-3). It was found that mercury contents in the hairs of dental personnel were slightly higher than those of students and Hat-Yai residents. The difference of mercury concentration between male and female did not obviously observe. Saengsirinawin and Pringsulaka (1988) reported the mercury content in the hair samples collected from Bangkok dental personnel, the average Hg concentration in µg g^{-1} of dentists, dentist assistants, Yr-3-DTS, Yr-4-DTS, Yr-5-DTS, Yr-6-DTS and Dental technicians were 7.53±1.20, 10.12±0.84, 2.61±0.26, 5.18±0.58, 7.59±2.14, 10.49±3.20, 2.81±0.53, respectively which is much higher than this study in the dental personnel of Prince of Songkla University. This may due to the Bangkok dental

personnel used mercury drop in stead of amalgam capsule. The mercury vapor may release into the environment.



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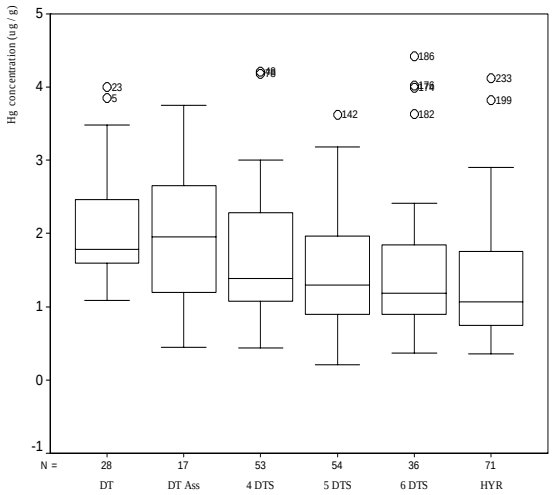

b

Figure 3- 9 Box and outliner plot presents Q_1, Q_2 and Q_3 of Hg level in different groups of dental personnel and Hat-Yai residents's hair. DT= dentist; DT ASS = dentist assistant; DTS = dentist student; HYR = Hat-Yai resident Q_1 = Quartile 1(25%), Q_2 = Quartile 2 (50%) and Q_3 = Quartile 3 (75%)
a = Mercury concentration in male and female
b = Mecury concentration in the average value for each group

Table 3- 4 The mercury concentration (μg g^{-1}) in hair of dental personnel and Hat-Yai residents

		Male	Female	Average
Dentist	Mean	2.03±0.60	2.14±0.89	2.10±0.80
	Range	1.24-3.45	1.08-4.00	1.08-4.00
	Median	1.76	1.85	1.78
	Number	10	18	28
Dentist Assistant	Mean	-	1.91±0.87	1.91±0.87
	Range	-	0.44-3.75	0.44-3.75
	Median	-	1.95	1.95
	Number	-	17	17
4^{th} year dental student	Mean	1.18±0.50	1.60±1.14	1.49±1.05
	Range	0.59-2.42	0.37-4.42	0.37-4.42
	Median	1.15	1.23	1.18
	Number	9	27	36
5^{th} year dental student	Mean	1.46±0.70	1.72±0.91	1.66±0.87
	Range	0.77-3.01	0.44-4.21	0.44-4.21
	Median	1.45	1.54	1.38
	Number	13	41	54
6^{th} year dental student	Mean	1.61±0.86	1.41±0.67	1.44±0.71
	Range	0.51-2.09	0.25-3.18	0.25-3.18
	Median	1.50	1.23	1.29
	Number	9	45	54
Hat-Yai residents	Mean	0.99±0.43	1.55±0.84	1.32±0.75
	Range	0.44-2.54	0.36-4.12	0.36-4.12
	Median	0.93	1.50	1.04
	Number	29	42	71

3.6 Statistical analyses

In order to investigate the effect of expose factors among the different categories of the independent variables of dental personnel as well as to compare the mean mercury levels of dental personnel group and Hat-Yai residents, the statistical analyses were performed. The information of exposed variables is gathered from questionnaires.

The effect of exposed factors on mercury content in dental personnel's hair

The exposure factors were classified under four categories; life style, work habit, dental filling and precautionary measures. The ANOVA ($P<0.05$) indicated the effect of those factors to the mercury concentration in hair.

3.6.1 Life style

The effect of life style in dental personnel was assessed by dividing into seven groups; (i) gender, (ii) religion, (iii) frequency of fish and seafood consumption, (iv) species of fish being consumed, (v) brands of shampoo, (vi) brands of conditioner, (vii) brands of hair color dye. The results of ANOVA *P* value and mean±SD of mercury for each group were presented in Table 3-4. The study revealed that the frequency of fish consumption slightly affected mercury accumulation in hair. A positive correlation between fish consumption and mercury levels in hair were also observed in several studies (Holsbeek *et al*., 1996; Babi *et al*., 2000; Olivero *et al*., 2002). In this study, no significant difference in mercury content among male and female dental personnel was found. Similar result had been reported by Olivero *et al. (*2002). The chemical hair treatment did not show any contribution to the mercury content in the hair samples collected in this study.

Table 3- 5 Life style

Factor number	Life style	Mean±S.D of Hg ($\mu g\ g^{-1}$)	*P* Value
1. Gender	Male	1.33±0.71	0.449
	Female	1.66±0.91	
2. Religion	Buddhism	1.59±0.88	0.417
	Islam	1.35±0.80	
	Christianity	1.19±0.64	
3. Frequency of fish and seafood consumption	1-6 times/week	1.57±0.81	0.058
	7-12 times/week	1.73±1.02	
	>12 times/week	1.28±0.60	
4. Species of fish being consumed	Mackerel	1.69±1.05	*
	Sea-bass	1.57±0.89	
	Sea mussel	1.09±0.51	
5. Brands of shampoo	Lavenus	1.36±0.62	0.272
	Rejoice	1.59±0.81	
	Pantene	1.69±0.97	
	Sunsilk	1.53±0.92	
	Clinic	1.57±0.93	
	Dove	1.15±0.45	
	Others	1.11±0.87	
6. Brands of conditioner	Lavenus	1.32±0.70	0.169
	Rejoice	1.92±0.86	
	Pantene	1.62±0.83	
	Sunsilk	1.61±0.84	
	Clinic	1.31±0.73	
	Dove	1.29±0.82	
	Others	1.75±0.93	
	Don’t use	1.60±0.98	
7. Brands of hair color dye	Natae	2.05±1.28	0.470
	Justmodern	2.04±1.09	
	Lolan	2.13±0.93	
	Nice and easy	2.05±0.00	
	Others	1.72±0.90	

* species of fish consumption chosen is more than one, therefore, SPSS can not calculate *P* value

3.6.2 Work habits

This study examined the work habit of dental personnel practice in their clinics. This includes number of working hour per week, years of practice and ventilation in the work place. The results presented in Table 3-5. Number of working hours per week and ventilation in the work place did not show any significant difference effect to the mercury concentration in the hair of dental personnel. Similar result was reported by Harakeh *et al.* (2002). The number of practice years revealed a

positive correlation with Hg level in hair (P=0.001). It was found that dental personnel who worked less than 4 years had less mercury in hair compare to those who worked in this area longer than 4 years. The results agree with the work of Scarlette *et al*. (1998).

Table 3- 6 Work habits

Factor number	Work habits	Mean±S.D of Hg ($\mu g\ g^{-1}$)	P Value
1. Number of working hours per week	0-10	1.62±0.83	0.446
	11-20	1.62±1.00	
	21-30	2.04±0.97	
	>30	1.79±1.03	
2. Years of practice	1-4	1.55±0.86	0.001
	>4	2.07±0.88	
3. Ventilation	Breeze	**	0.948
	Electric fan	**	0.994
	Air-condition	**	0.648

**the information from questionnaire can not calculate mean value but can group the answers to calculate P value

3.6.3 Dental filling

Three parameters in the dental filling category were (i) number of amalgams in the dental personnel's teeth, (ii) self preparation of amalgam and (iii) frequency of amalgam preparation per week as shown in Table 3-6. The result showed that none of those parameters influence in mercury level in hair. Similar result was reported by Bratel *et al*. (1997).

Table 3- 7 Dental filling

Factor number	Dental filling	Mean±S.D of Hg ($\mu g\ g^{-1}$)	*P* Value
1. Number of amalgams in the dental personnel teeth	1-2	1.62±0.88	0.925
	3-4	1.75±0.97	
	5-6	1.75±0.89	
	7-8	1.65±0.80	
	>8	1.65±1.01	
2. Self preparation of amalgam	Yes	1.60±0.99	0.599
	No	1.68±0.84	
3. Frequency of amalgam preparation per week	1-5	1.55±0.97	0.889
	6-10	1.70±1.29	
	11-15	1.67±0.40	
	16-20	1.00±0.27	
	>20	1.85±0.70	

3.6.4 Precautionary measures

The three precautionary measures consisted of using (i) gloves, (ii) mask and (iii) goggles (Table 3-7). The usage of gloves and mask were significant associated with a reduction in Hg concentration; *P*=0.031 and 0.053 respectively. Similar result was obtained by Haraken *et al* (2002). It was found that seventy percent of dental personnel of Prince of Songkla University used mask and gloves at all times. This practice provided a good protection against mercury as it can be up taken by inhalation and absorption through the skin.

Another precautionary measure was the frequency of using goggles. It was found only 17 % of dental personnel used goggles at all times. The analysis of the data revealed no significant effect of using goggles on Hg levels. .

Table 3- 8 Precautionary measures

Factor number	Dental filling	Mean±S.D of Hg ($\mu g\ g^{-1}$)	*P* Value
1. Frequency of using gloves	Always used	1.14±0.75	0.031
	Used sometimes	1.43±0.96	
	Did not use	1.73±0.85	
2. Frequency of using masks	Always used	1.69±0.83	0.053
	Used sometimes	1.27±0.79	
	Did not use	1.75±1.19	
3. Frequency of using goggles	Always used	1.92±0.89	0.119
	Used sometimes	1.55±0.72	
	Did not use	1.59±0.96	