

CHAPTER I

INTRODUCTION



1.1 The Problem

During the past two decades, industrialization has been growing very rapidly in Thailand. It is expected therefore that the rapid rate will be significantly increased in the future. These factories release various kinds of pollutants viz. trace toxic contaminants, dust, waste water and cosmetic agents etc. into the biosphere. The pollutants are also released from the burning of fossil, fuel and other human activities. The pollution of the air, soil and water as well as the contamination of micro-organism, plants, animals and human by these trace element are certainly growing into a serious threat and leading to heavy losses. Consequently it is very important to seek for a suitable indicator of exposure to trace element pollutants.

Man himself is a central target for these trace element pollutants, which normally occur in his body in relatively low concentration. It is possible that man himself is sometimes exposed or contaminated to levels giving rise to large-scale harmful somatic or genetic effects and sometimes lacking in essential elements. In order to judge if man or a population has been exposed to abnormal amounts of trace element pollutants, it is essential to determine the initial or baseline levels of various trace elements in normal population (or normal man).

According to the difficult determination of the trace elements distribution and composition of the whole body, and the different accumulation of trace elements in various tissues of man, it is necessary to find tissues or substrates with trace element compositions that are fairly reliable indicators of contamination and easy for chemical analysis.

Head hair is probably the most suitable for the above purpose because its growth reflects both the biomedical and the environmental history of an individual. The sampling and collection of hair is very convenient because it requires no special equipment or storage and does not cause any pain to the patient. Trace element concentrations in hair are also generally much higher than those in other tissue (1) and large samples are normally available, making the task for the analyst easy. Since hair is metabolically "dead", it can be stored without damage unit and it is also convenient to perform the analysis.

At present, not much work has been done on the investigation of trace elements in human hair. Actually, there is none in respect of the analysis of multi-elements with particular attention to the study of internal and external contaminations.

Neutron activation analysis is known as one of the promising techniques owing to its high sensitivity and adaptability to a wide variety of sample matrices. In addition, the principles and general features of the method have been thoroughly reviewed in the literature, viz. Bowen and Gibbons (2). Since, a suitable

neutron source in the form of research reactor Thai Research Reactor-1/Modification-1 (TRR-1/M-1) is readily available at the Office of Atomic Energy for Peace (OAEP), it is the purpose of this work to employ this technique for the investigation of multi-trace elements in human hair.

1.2 Objectives of the Study

1.2.1 To study both qualitatively and quantitatively of trace elements in human head hair of male, with respect to his occupations and residences, using Instrumental Neutron Activation Technique.

1.2.2 To differentiate the internal and external contamination of trace contaminants in hair samples.

1.2.3 To demonstrate that the obtained information can be used as an indicator in identifying the source of pollution.

1.3 Scope of the Investigation

1.3.1 About 180 head hair samples collected from men, who entered into the priesthood on April 1, 1980 will be analyzed. Each whole head hair was shaved off by a clean razor and kept in a separate clean plastic bag. Every donor was asked to kindly fill the form indicating age, occupation, residence and hair treatment.

1.3.2 Each sample was thoroughly washed three times with demineralize water to remove soap, and air-dried at room temperature in a laminar flow fumehood.

1.3.3 The individual sample from 1.3.2 was divided into two portions, the first portion was washed with acetone, water, water, water and acetone according to the IAEA recommended washing procedure (3) and named washed part, the latter was named unwashed part. Then, both of them were cut separately by clean stainless steel scissors.

1.3.4 The samples were analyzed qualitatively and quantitatively by using instrumental neutron activation analysis.

1.3.5 The reliability of this analysis was routinely tested with IAEA powered human hair reference material, HH-1.

1.4 The Benefit Obtained from the Study

1.4.1 The information obtained will be used for the establishment of the baseline levels of various trace elements in human head hair.

1.4.2 The information obtained will be used as an indicator for the identification of the source of pollution.

1.4.3 The information obtained will be used to preserve our environment as well as the health and welfare of our people.

1.5 Literature Search of Previous Work

1.5.1 Structure of the Hair

Hair is composed of a polymerized protein which is called keratins produced in the epithelial cells of higher vertebrates. These materials form the horny layer of the epidermis, nails, scales and feathers (4,5).

The hair shaft has a structure like a laboratory pressure tubing and consists of three regions (6).

1) The cuticle (a strong exterior layer) has a rather inhomogenous structure, being composed of a scaly keratinous part (the exocuticle) surrounding a zone consisting of cellular debris (the endocuticle). The cuticular scales overlap and move over one another, providing strong mechanical and chemical protection of hair against the environment. The cuticle regulates the absorption and adsorption of trace elements into the hair structure.

2) The cortex (bulk of the hair fibre) consists of an aggregate of filaments (called microfibrils) made up of low-sulphur proteins, embedded in a homogenous matrix composed of high-sulphur proteins (with amino acid such as cysteine). Although the diameter of these microfibrils has been found to be substantially constant along the hair shaft, the distance between adjacent microfibrils varies considerably, as does the content of high-sulphur proteins in the cortex.

3) The medulla (a central canal) or central portion of mammalian hair, is composed of vacuolated cells whose sulphur content is very low. This region of the hair shaft has an influence on its mechanical properties, since the fibrous content of the medullary cells is concentrated against the cell wall producing a kind of frame which increases the stiffness of the hair fibre.

1.5.2 Growth of Hair

Hair grows from structure in the epidermis called follicles (Fig.1.1). Protein is produced at the base of the follicles where cell division occurs, and as the developing hair shaft structure moves along the follicle to the skin surface, the protein polymerises to form keratin.

Hair growth from a given follicle is cyclic rather than continuous (7-11). Hair growth divides into three phases:

1) Anagen phase

In this phase, protein production and cell division occur vigorously in the follicle, keratinisation occurs in the canal leading to the surface, and the external hair grows in length as newly produced hair shaft emerges from the canal, at a rate which varies from individual to individual, but typically is 0.35mm. per day or about 1 cm. per month. This phase is typically of about 1,000 days length for human head hair.

2) Catagen phase

In this phase, growth slows to a stop, the root shrinks and becomes club-or bulb-shaped. This phase is typically of about 7-14 days length for human head hair.

3) Telogen phase

Approximately 100 days long for human head hair, the follicle is inactive; the external hair remains the length, attached to the scalp by the fact that the bulb-shaped root is

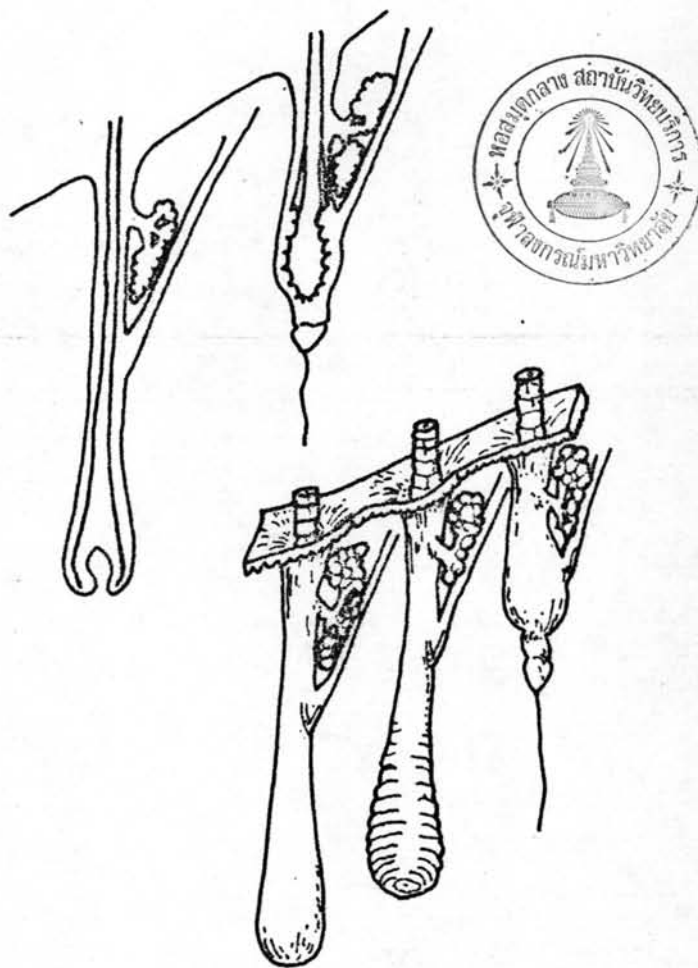


Fig.1.1 Hair Structure:

The diagram on the upper left shows a growing follicle, the one on the upper right a quiescent one. The lower diagram shows a follicle in anagen on the left, one in catagen in the middle and one in telogen on the right.

larger in diameter than the opening in the scalp surface. The hair will eventually be lost from the head, either by mechanical removal during the telogen phase or when a new hair is produced as the follicle resumes activity in the next anagen phase.

An average of 50-200 strands of hair are lost per day and the average person has about 100,000 strands of scalp hair (12).

1.5.3 Elemental Compositions of Hair

Early worker (12) had established water and ash content in hair, as well as Al, As, Br, Ca, Cl, Co, Cu, Fe, Mn, Ni, P, Pb, S, U and Zn contents. In 1977 (13), some 60 trace elements had been measured by a variety of techniques, e.g. neutron activation analysis, atomic absorption spectroscopy, etc.

Valkovic in 1977 (12) proposed to divide trace elements into three groups (Fig.1.2) that is:

- 1) elements which form the bulk of living matter:
H, Na, K, Mg, Ca, C, N, O, P, S, Cl
- 2) essential trace elements: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Si, Sn, Se, F, I and
- 3) nonessential trace elements (environmental pollutants): As, Hg, Pb, Cd, Ni, Se, Sb.

1.5.4 Trace Elements Distribution

The concentrations of many trace elements had been found to vary both down the length hair shaft (13-21) and across

H																		
													C	N	O	F		
Na	Mg												Si	P	S	Cl		
K	Ca		V	Cr	Mn	Fe	Co	Ni	Cu	Zn			As	Se	Br			
			Mo							Cd			Sn	Sb		I		
										Hg			Pb					



Elements which form the bulk of living matter



Essential trace elements



Toxic elements



FIGURE 1.2 : Periodic table of elements in hair

its diameter (22,23).

Many, but not all, metals increased in concentration with distance from the root and to an extent that varied with the subject (15). This was suggestive of a progressive contamination from the environment for hair exposed outside the scalp. Further, some patterns showed concentration peak (25) suggestive of the effect of dietary fluctuations, e.g. the case of Napoleon's hair. In the case of Napoleon's hair, Forshufrud and co-workers (24) found that As in Napoleon's hair showed an intermittent accumulation in sections. While Smith and co-workers (25), who examined the two samples of Napoleon's hair which were probably taken immediately after his death, found an increasing of As concentration at the distance near the scalp.

Some metals, such as Zn (13) and Co (26), had been found at least in some subjects, to exhibit little or no variation on correlation with distance from the root, perhaps suggest constant rate of incorporation through the root. Obrusnik and co-workers (16,17) agreed that Zinc variations along a single hair were rather small and all of trace elements (essential elements) in hair, Zn had the highest concentration. They suggested that both facts were probably due to relation of melanin pigment in hair and also suggested that the variations of element concentrations along the hair could be determined by measurement of element/Zn concentration ratios. Other elements, such as Na and Cl had higher concentration near the root and at the distal end, with reduced concen-

trations between, a circumstance attributed to equilibrium between removal by washing and addition from sweat (27).

Concentration patterns had been shown to be affected by cosmetic treatments and especially bleaching (13,15). Cu and Zn contents were suddenly reduced in bleached regions compared with elsewhere on the same shaft, suggestive of easy transport of ions out of the damaged shaft structure. Concentration patterns observed for Cu, Zn and As ions absorbed into hair (13,15,28), when hair was dipped into the higher concentration solution, indicated that the hair shaft structure was not homogenous in its capacity or affinity to bind such ions. This capacity increased with distance from the root for Cu and Zn, but decreased for As. Some regions of the shaft exhibited local increase of specific ions but not others. Bleaching increased absorption of Cu and Zn, but reduced that of As.

1.5.5 Trace Elements and Hair Colour

Relationship between trace elements in hair colour had not been carefully investigated. Kikkawa and co-workers (29) proposed the idea that the colour of the hair was related to its content of iron, cobalt, nickel, molybdenum, and copper. Low concentration of manganese was observed in white hair (30). It was found also that the dark colour hair had higher amount of Pb (14,32), Ti (14), Cu (14,30-31) and Ag (14) but had less amount of Cd (32). The comparison for trace element contents in blond, brown, black and red hair indicated that no significant differences were found

for Pb (33), As (32), Cr (32), Hg (33) and Se (32) but not for Mg, Zn, Cd and Cu (32). For nickel, no difference was discovered in relation to hair colour (33) but Schroeder and Nason (32) found that natural colour hair of females had more nickel in red hair than brown hair.

1.5.6 Trace Elements and Age

There was no significant change in levels of Sb (34), Zn, Mg, Co and Ni (32) with age. The decrease in level of Cd and Cu (32) with age was usually found in female but not in male (32-34). Ohmori and co-workers (34) reported that the content of As in hair of age ≥ 20 years as compared with age < 20 years was about 1.5 times. They also found less V concentration in age ≥ 20 years than in age < 20 years. In the case of mercury, there was no age difference correlation in that levels of hair in male, but there was a decline of Hg in female hair in subject to aged 57-70 years (33).

Imahori, et al (35) reported that there was a close relationship between mercury and selenium in hair and the concentration of mercury and selenium was related to age and sex. Mercury concentration in male showed an increasing trend with age while selenium concentration maintained constant level through all ages, both in male and female.

1.5.7 Trace Elements and Sex

It is difficult to summarize the effect of sex on levels of trace elements because age differences and distance

from scalp were not always considered. In general, there were higher levels of Cd (34), Co (32,34,36), Pb, Mg (32,36) and Ni (32) in female hair, but there were also reports of no difference between sexes for Cd (32-33), Cr (32,34,36), Cu (33,37), Pb (32-33), Hg, Ni (33), V (34) and Zn (32,37).

Arsenic was appreciably higher in male than in female hair in a population of over 1,000 but there was no significant difference between As levels in college age male and female (38). Ohmori and co-workers (34) studied in Japanese people and found more As in female than in male's hair.

Imahori, et al. (35) found that the bromine, calcium and magnesium content showed marked difference between male and female, especially in adult ages.

1.5.8 Uses of Human Head Hair

The content of trace elements in human head hair can be used for the study of biomedical and environmental effects. The major uses can be summarized briefly as follows:

1.5.8.1 Biological monitoring for correlation with environmental exposure gradient

Human hair has been analyzed to show correlation with exposure to environmental gradients of certain trace elements. These environmental gradients result from the production of high concentrations of one or more toxic elements from a single source or combined sources. These include gradients resulting from urban industrialized areas, refiners and petrochemical

complexes, smelters for Pb, Cd, As, Cu and Zn miners and special manufacturing or special uses of trace elements.

In 1979, Jenkins (38) reported that human hair had been found to be of value for correlating human exposure to environmental gradients for arsenic, antimony, cadmium, lead, mercury, nickel and vanadium for children and adult, and for chromium and tin for children only. Boron and copper in hair were not found to be correlated with environmental gradients.

1.5.8.2 Occupational and accidental exposure

People can be exposed to toxic elements as an occupational hazard or by an accident. In breathing and by touching or ingesting, workers undergo a long-term, low-level dose or a brief, high-level exposure. Accidents would include eating mercury-contaminated food (39-40), like fish and shellfish, bread made from treated seed or pigs fed contaminated grain. These doses may result in toxic symptoms or death.

Hair is usually suitable for chronic exposures over a long time. For studies immediately after acute exposures, urine and blood samples may be preferable, but sometime hair can be used. For example, Henley and co-workers (41) had determined the concentration of trace elements in the first two millimeter lengths of the root ends of human head hair of man who had short exposure. They found that the concentrations of trace elements in hairs should correlate well with the concentrations of trace elements in blood.

Al-Shahristani and Al-Haddad (39) indicated that the concentration of mercury in hair was correlated with illness. They found that people who had consumed Hg-contaminated grains but showed no symptoms had hair mercury concentration of from a few ppm to 300 ppm. While people with mild symptoms (slight tremor, mild ataxia and blurring of vision), moderate symptoms (partial paralysis, tunnel vision, difficulty in hearing, disarticulation) and severe symptoms (complete paralysis, loss of vision and hearing, loss of speech and coma) had hair mercury concentration of 120-600 ppm, 200-800 ppm and 400-1,600 ppm, respectively.

A study of persons exposed occupationally to mercury contamination (38,42), namely dentists, dental assistants, mercury smelter workers, tungsten refinery workers, industrial workers (mercury processing plant of pesticides) and tunafisherman. It was found that dentists, Hg smelter workers, and tungsten refinery workers had high levels of Hg in hair in comparison with hair of "controls" and also found that the Hg smelter workers inhaling mercury vapours were exposed to much higher risk of mercury poisoning than were workers breathing ore dust.

Arsenic in hair (22,38,43-44) had been studied for persons exposed to manufacture using of arsenic products, including people in mines and smelters. Comparisons had been made with unexposed "controls" showing significant differences.

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Wiesener and co-workers (45) and Neise (46) found that the hair of welders contained more Mn than that of normal people. The concentration of fluorine in the hair of people working with fluorine compounds was also enhanced. Gangadharn (47) reported that people working in / living near fossil fuel based station had higher Mn, Cd and people living in chemical area also had higher Cd and As than "normal" population.

Lead in hair (38) had been studied in relation to persons occupationally exposed to lead, including policemen, lead metal workers in battery and rayon manufacture, and printing office workers. It was found that lead workers, uranium miners, and printers showed high levels in comparison to "controls".

Chromium was found to be high in comparison to "controls" in plating worker (40) while the levels of Ag and Au were elevated in silver and gold worker and also in women wearing a significant amount of gold jewellery (40).

1.5.8.3 Identification of disease

Jekins (38) summarized that high levels of Sb, As, Pb, Ni and Hg in hair had been correlated with toxicity or poisoning in humans. High levels of Se, Hg and Cd caused loss of hair, Minamata disease and Itai-Itai disease, respectively, while high levels of V decreased cystine. Low levels of Cu had been associated with Menkes kinky hair syndrome. No correlations with excess or deficiency diseases or conditions

had been found in available reports for Ni, Cd, Co and Sn.

Low concentration of Zn in human hair was associated with Zn deficiency (11,12,48). Zn deficiency syndrome characterized by dwarfism, delayed epiphyseal closure, rough and hyperpigmented skin, and diabetes. After zinc administration or insulin treatment in the case of diabetes, the symptoms were relieved (e.g. the hair began to grow and the body length increased) and the hair Zn levels were raised.

1.5.8.4 Geographic distribution and variation of human being

There were significantly higher levels in human hair of As in Mexico and Chile due to nature high levels of As in water (38). Level of As in hair were high around Cu, Pb and Zn smelters or Cu and As mines in various countries (38). Higher or lower of Se were correlated with natural excesses or deficiencies in various region of North and South America (38) and these levels were fairly high in Venezuela (49) and Iraq(50).

Other differences, such as high levels of Hg, were correlated with high intake of Hg-contaminated fish (38), grain (39). Co, Cr, Ni, and Se were high in Venezuela Indians (38).

1.5.8.5 Historic trends in trace elements in hair

Comparison of the trace element concentrations in historic hair sample up to 200 years old (38) showed that there had been an increase in Cu, Ni, and V and a decrease in Sb, As, Cr. The significant decrease of arsenic was probably correlated with decrease in use of arsenical medicines and germicides and with

substitution of DDT and other pesticides for lead arsenate and Paris Green.

1.5.8.6 Forensic medicine

In forensic science, hair was used extensively to demonstrate, prove, and to date poisoning and exposure to various toxic element, especially arsenic, cadmium, chromium, lead, mercury, and nickel. The concentration along the length of the hair could be used to reveal the history of the poisoning. The pattern of concentration variation of metal, e.g. Hg along hair was shown to be more reliable criterion for hair individualization identification than average concentration values (39).

Smith and co-workers (24-25) examined the two samples of Napoleon's hair, taken immediately after his death, and found that As concentrations were increased. They suggested that Napoleon suffered from arsenic poisoning during his last days on St. Helena.

1.5.9 Washing Procedures

Various procedures and combinations (1,14,53) were studied including organic solvents, anhydrous alcohol, ethyl ether acetone, carbon tetrachloride and boiling water, soaps and detergents (ionic or non-ionic), EDTA (ethylene diamine tetraacetic acid) and dilute nitric acid. Each procedure showed the different effectiveness on the concentration of trace elements in hair. The next effectiveness was the different frequencies of washing. For these reasons, it was extremely important to find a suitable

washing procedure and frequencies of washing. In this connection, the International Atomic Energy (IAEA) organized a working group for this purpose. The proposed washing procedure(3) consisted of washing each hair sample in a clean container with five 10 minute contacted (with manual or mechanical shaking) with portions of acetone, water, water, water, and acetone, decanting off the wash liquid after each 10-minute wash. Although this washing technique had been used with quite satisfactory but there were many reports (47,51,54) indicated that losses of metals were still found, for example, Das, Hoede and Van der Sloot (51-52) discovered that there were small losses of Cu, Zn, Br, Sb, Au and Hg in hair while the levels of Cr, Fe, Co, Ag and Hf in hair dropped sharply by the first acetone wash but then remained approximately constant.

1.5.10 Sources of Trace Elements in Hair

Chittleborough (53) classified the origins of trace elements in hair into two sources:

1.5.10.1 Primary or endogenous sources

These sources were the matrix and connective tissue papilla, the sebaceous glands, the eccrine sweat glands, the apocrine glands and the epidermis. Trace elements might enter the hair in the following ways:

a) The matrix and connective tissue papilla (with its blood and lymph vessels) provided a "metabolic milieu" which included dissolved trace elements. These metals had considerable reactivity with the sulph-hydryl groups of follicular

proteins and combined with them forming mercaptides or metallo-proteins. The formation of keratin from these metallo-proteins in the matrix cell resulted in the incorporation of the metals into the hair structure.

b) The eccrine sweat glands secreted the eccrine sweat which contained water and salt of Na and K and some trace elements such as Cu, Fe, Mg, Mn, and Zn. The incorporation of metals in hair by contact with sweat (and sebum) was a distinct possibility in view of the prevalence of the S-S cross linkage in keratin and the known affinity of metals for sulphur in this form.

c) The sebaceous glands were the source of sebum (mixture of lipids and waxes) which might also contained trace elements, thus these elements were absorbed into the hair by contact with sebum.

d) The apocrine glands represented a special types of sweat gland that secreted a milky fluid, rich in lipids.

e) The epidermis when contaminated with sebum or sweat might come in contact with the hair.

1.5.10.2 Exogenous sources of trace elements included particulates in air, solutes present in water used for swimming and washing, and trace elements in soaps, shampoos, cosmetic agents, medications, etc.

In Thailand, there were about eight reports concerning trace elements in hair, such as Cu, Zn, Fe, Mn, Cr, Ni, and Cd.

In 1973, levels of 2-81 ppm lead were determined in hair samples from 99 healthy children, compared to levels of 139-2358 ppm lead in hair sample from 4 cases of chronic Pb intoxication by Kritalugsana and co-workers (55). They also reported that the determination of Pb in hair sample might be used to diagnose Pb poisoning, especially in children. In 1980, Menasveta and co-workers (56) reported that the levels of Pb in hair of people living in Pathumthani (Bangkok) were about 4 times higher than in hair of hill tribes, living in Chiangmai. Approximately 22% of people living in Bangkok had Pb in hair at level of more than 30 ppm which was a toxic level. They suggested that the two sources of this contamination were the Pb which happened from the burning of organo-lead compounds in fuel, and from **industrial operations** such as the manufacture of lead-acid batteries. They also found that the Pb concentrations in hair was related to persons occupationally exposed to lead, including traffic policemen, lead metal workers in battery and electrical manufacture, and children living in Pb-contaminated village, but was not related to sex. The relationship between the Pb-level in hair and age was not found in hill tribes but found in normal people, living in Bangkok (Pathumthani). The older people living in Bangkok, had higher Pb-level in hair than the younger had.

Benjanuvatra and Bennion (57) discovered that the Cr levels in children with diabetes were lower than in children without diabetes. This showed that the level of Cr in hair was



correlated with the diabetes.

Hambidge and co-workers (58) found that Zn concentrations in hair were correlated with age. But Gershoff and co-workers (59) who studied about trace minerals in Thai boys and girls with aged of 1½ to 7 years, living in 29 villages in Chiangmai, found that the different levels of the concentrations of Zn, Fe, Mg, and Cu were not related to the ages of the children. They reported that boys had higher levels of Zn, Fe, Cu, and Mg than girls and also found that the low incidence of sickness was found in children who had low hair copper levels.

In 1978, Suckcharoen and Nuorteva (60) reported that the mean mercury content in hair of both sexes was 2.3 ppm. in the unpolluted area and 2.9 ppm in the mercury-polluted TACSCO* area (people consumed Hg-contaminated fish 3-4 meals/week), while the mean mercury values of fishes, collecting near the caustic soda factory were 5-51 times higher than the mean for fish in unpolluted areas. They also found that the mercury content in human hair was significantly elevated in males but not in females living in the TACSCO area. The mercury content in hair of males in this area was about two times higher than in the control area (unpolluted area). Their reasons for this sex-based difference lied in the fact that they analyzed only the tip portions of the hair samples. The hair of males was shorter than females' hair so that the males' hair indicated more rapidly the elevation of the mercury content in the food.

*TACSCO- Thai Asashi Caustic Soda Co Ltd.