CHAPTER IV
DISCUSSION AND CONCLUSIONS

Cd is a cumulative toxin because of the lack of an active biochemical process for its elimination. It also has a long biological half-life of over 10 years. In the study area; the town was located in the north-western part of Thailand. There are several Amphurs and villages in Tak province and one of these, Amphur Mea Sot, was polluted from zinc mining activity at a nearby site. Soil and water along Huay Mae Tao in Mae Sot were contaminated with high levels of Cd. This high Cd contamination was also found in rice. Rice has been an almost exclusive factor to influence Cd body burden among the general population in Japan, China, Taiwan, and Thailand. Cd from rice accounted for approximately 30-40% of total dietary intake among the general population in Japan in the 1980s (Shimbo *et al.*, 2001). It is thus indicated that the main Cd exposure pathway in this study was the long-term consumption of homegrown rice polluted by Cd.

Table 1 shows the number of subjects in the study area (Mea Sot) classified by demographics and health data by means of a questionnaire given to each participant. Age of each subject can also represent the duration of exposure, because most subjects (99%) were born and had been working in this area. Confounding factors that could influence renal dysfunction were rice and water consumption, smoking habit and diseases related to renal dysfunction such as hypertension, anemia, urinary tract stone, renal or urinary tract disease, osteoporosis, diabetes and bone fracture. We used statistical tests to exclude confounding factors.

Table 2 and 3 show the results from urinary screening tests using urine strips and distribution of the subjects with positive urinary protein. The rate of positive urinary protein subjects was 33.48%. These results suggested that the subjects in Mae Sot with renal tubular disturbance already existed at a high rate.

In this study, all subjects were older than 40 years old. Their urinary Cd concentrations were over 5 μg/g Cr, which indicated Cd contamination (WHO criteria), in the first round of local hospital surveys in 2004. The subjects were selected equally between both sexes, and between 2 urinary Cd groups including those who had urinary Cd from 5-10 μg/g Cr and over 10 μg/g Cr in the 2004 survey. However, 52 subjects were found to have low urinary Cd concentrations (< 5 μg/g Cr). These findings might result from a change in consumption habits in this population, e.g. they might have quit eating polluted rice from their own rice field. Their urinary Cd concentrations in this study were lower than those reported previously. Another factor could be the contamination of metals from unclean containers, which might increase Cd concentration in the urine. The mean $\pm$ S.D. of the urinary Cd concentrations grouping this study was 3.95 ± 0.96, which indeed exceeds the WHO maximum tolerable value (2 μg/g Cr). On the other hand, the advantage of a urinary Cd concentration of below 5 μg/g Cr which was found in this study, was that NAG could be used as a biomarker in the case of a Cd concentration at that level.

Moreover, 28 subjects were found who had very high urinary Cd concentrations (>20 μg/g Cr). Therefore, Cd concentrations were classified into 4

group; 0-5, >5-10, >10-20 and >20 μg/g Cr instead of the 2 groups previously designed (Figure 5).

Urinary Cd is also used as an internal indicator of environmental exposure to Cd (Nogawa *et al.*, 1992; Nakashima *et al.*, 1997). An increase of urinary Cd reflects an increased body burden of Cd (Nogawa and Kido, 1993). Cd body burden increases with age, so this study classified urinary Cd concentration by age. It divided age into 4 groups to analyse the correlation of Cd concentration and age (Figure 6). Urinary Cd concentrations in all 4 age groups were approximately 10 μg/g Cr. When the subjects were divided into these age groups of below 50, 50-60, 61-70, and > 70 years old, urinary Cd concentrations showed no significant difference, but they tended to increase by age, except in the over 70 year old age group. While this result suggested that age might have affected urinary Cd concentrations in the studied subjects, the numbers of subjects in the group of over 70 year olds was lower than in the other groups, so the mean ± S.D. of Cd concentration was also below.

Cd body burden increased with age and was higher in women than men. In this study, there were slightly higher urinary Cd concentrations in women than men (Figure 7), which may be due to higher gastrointestinal uptake of Cd in women, since the absorption of oral Cd rises with decreasing iron stores (Flanagan PR *et al.*, 1978). Cd uptake rates can be as high as 20-30% in individuals with low iron stores (serum ferritin < 20 μg/L) and iron deficiency (Satarug *et al.*, 2004).

Food is the most important source of Cd exposure in the general non-smoking population in most countries (WHO., 1992). The correlation between urinary Cd concentrations and occupation was positive among the 224 participants of the polluted area, who were mainly farmers. They consumed rice from their own fields, which indicated that these farmers were in a high risk group for exposure to Cd (Figure 8). Cigarette smoking is also a major source of Cd exposure. Biological monitoring of Cd in the general population has shown that cigarette smoking may cause significant increases in blood Cd levels (Hossn E *et al.*, 1998). Figure 9 shows the urinary Cd concentrations and smoking status, which suggests no difference in Cd concentration between subjects who smoke and those who do not. This finding might result from the small number of sample size or wrong information given regarding smoking status.

Chronic exposure of Cd in foodstuffs and cigarette smoke has been associated with several diseases such as kidney tubular dysfunction, osteoporosis, diabetices, hypertension, urinary tract stone and increased cancer risk (Satarug *et al.*, 2005). Cd exposed subjects have also been associated with a increased prevalence of high blood pressure. A study in Belgium revealed that diabetic patients showed an increased susceptibility to renal toxicity of Cd (Buchet et al., 1990). The mean ± S.D. of Cd concentration in diseases such as hypertension, diabetes and urinary tract stone did not differ between subjects with and without evidence of these diseases (Figure 10). This result might suggest that the above diseases did not affect urinary Cd concentrations in this study.

Urinary Ca concentrations in this study tended to increased in proportion with age group (Table 4 and Figure 11). Urinary P, Cu and Zn concentrations in this study were non-proportional to the elevation of age (Table 5, 6, 7 and Figure 12, 13, 14).

Urinary excretion of NAG which is the most widely studied of all urinary enzymes, is another indicator of renal tubular damage (WHO, 1992). Although the enzymes in renal tubular cells cannot be filtrated at renal glomeruli because of their

high molecular weight, they are excreted into urine when renal tubular cells are destroyed due to some diseases or chemicals (Nakagawa *et al.* 1993). An increase of NAG has been reported among Cd exposed subjects in previous studies on targeted subjects in Cd polluted and non-polluted areas (Jin *et al* 2002). It has previously been reported that the excretion of NAG may be an even more sensitive indicator of Cd-induced renal tubular dysfunction than β_2-MG (WHO, 1992). However, the urine of itai-itai disease patients, whose renal tubular epithelial was too severely damaged to excrete enzyme, showed a smaller increase of NAG, in spite of a remarkable increase of low molecular proteins. This study found some subjects with NAG of more than 10 U/g Cr, which suggested proximal damage (Table 8), and the urinary NAG positive rates of the subjects with urinary Cd concentrations above 5 μg/g Cr were significantly higher than those in participants with Cd concentrations below 5 μg/g Cr (Figure 15). These results suggested that NAG is a good urinary marker for detecting the initial stage of renal damage, especially in subjects with low Cd exposure. Jung *et al.* (1993) also studied urinary excretion of several markers of tubular (including retinol binding protein and α_1-MG) and glomerular dysfunction in 171 subjects with a wide range of urinary Cd, and recommended a combination of NAG and α_1-MG for early detection of Cd-induced renal dysfunction in the subjects with urinary Cd < 5 μg/g Cr.

The target organ of Cd toxicity is the kidney, especially proximal renal tubules, where low molecular protein, glucose, amino-acids, and many elements, like Ca and P are absorbed after glomerular filtration. An index of renal tubular dysfunction or renal damage induced by Cd, Ca and P in urine can be used, but comparative evaluation studies are relatively scarce on urinary markers for a specific marker in epidemiological surveys. Only limited numbers of markers are accepted in the practice of mass screening. Among urinary low-molecular-weight proteins, β_2-MG is the most popular and useful index of proximal tubular dysfunction specifically induced by environmental Cd (Saito *et al.*, 1977; Nogawa *et al.*, 1979; Buchet *et al.*, 1990, Nordberg *et al.*, 1997). Urinary excretion of β_2-MG has a wide range, and reflects the degree of renal tubular dysfunction. This study found several Mae Sot residents with urinary β_2-MG of more than 1,000 μg/g Cr. In addition, some subjects had β_2-MG of more than 10,000 μg/g Cr (Table 9). These findings suggested the existence of severe renal tubular dysfunction. When a dose-response relationship is clarified between the urinary β_2-MG positive rate and urinary Cd in the study subjects, this study found that age was not an affected factor, which differed from the findings of Izuno *et al.*, (2000). Aging also contributes to the elevation of urinary β_2-MG. In non-exposed subjects, an increase of urinary Cd is observed in proportion to age because accumulation of Cd binding to metallothionein stores in the kidney for a long period of time, 10-30 years, in proportion to the total amount of Cd intake. However, in subjects living in Cd polluted areas for more than 30 years, the relationship between urinary Cd and age is not clear. The reason for this result might be that their total intake of Cd is too high, and stored Cd in their kidney exceeds the limit of accumulation in the organ. At the same time, when the amount of Cd stored in the kidney reaches maximum, renal damage occurs. Therefore, the urinary Cd concentration curve of the studied subjects who live in polluted areas for more than 40 years becomes plateau, and they have already suffered from kidney damage.

The α_1-MG is a low molecular protein with a molecular weight of 33,000, and it is bigger than β_2-MG (11,800). In comparison to β_2-MG, α_1-MG is stable at any urinary pH. Therefore, α_1-MG is a good marker in the case of acidic urine where β_2-MG is broken. Significant correlation between urinary α_1-MG and β_2-MG was reported in previous literature, and is concurrent to this study. Tohyama *et al.* (1986) proposed α_1-MG as an additional indicator of tubular dysfunction, as well as retinol binding protein and NAG. Ikeda *et al.* (1999) suggested that α_1-MG increased in association with an increase of whole blood Cd and urinary Cd. Dose response relationship between α_1-MG and Cd was also observed. In this study, subjects with urinary α_1-MG were found to have more than 15 μg/g Cr. In addition, some subjects had α_1-MG of more than 45 μg/g Cr (Table 10). This suggested the existence of severe renal tubular dysfunction.

Albumin and total protein are the markers of renal glomerular dysfunction, and are useful for clarifying complication of other kidney diseases like chronic glomerular nephritis in subjects with renal tubular dysfunction. Fels *et al.* (1994) recommended that a diagnostic approach to screen for nephrotoxicity, due to environmental hazards like Cd, should include not only proximal tubular markers but also the measurement of glomerular markers. This study found that some subjects had albumin and total protein of more than 30 and 150 mg/g Cr, respectively (Table 11 and 12). This suggested glomerular damage in the study subjects.

Amino urea nitrogen (AminoN) was also measured in this study because generalized aminoacid-uria is one of the characteristic symptoms of Fanconi syndrome and clinical diagnosis of itai-itai disease. Among the inhabitants of Mae Sot an increase of aminoN was observed with severe renal dysfunction. However, the levels found in this study were lower than those reported in Japanese inhabitants (Table 13).

Lysozyme (LYZ) is an enzyme in the cell. It functions as a digestive enzyme to dissolve foreign bodies such as the bacterial cell wall. It is one of the low molecular proteins, with a molecular weight of 14,500. Measurement of LYZ is an easy and useful method to detect the middle and high degree of renal tubular dysfunction. An increase of urinary LYZ has been observed, with a urinary β_2-MG of more than 10,000 μg/g Cr in Cd exposed Japanese subjects living in polluted areas (Piscator M., 1966). However, in this study, an increase of urinary LYZ in Mae Sot inhabitants was not seen (Table 14).

In this study, with levels of the urinary markers in subjects with a clinical history of hypertension and diabetes, albumin was higher than in subjects without these diseases. The positive association with higher correlation coefficients between urinary Cd and renal markers was observed as compared, with the analysis among all subjects including those with hypertension and diabetes.

Calcium and phosphorus, mainly excreted by the kidney, play an important role in maintaining the normal biological function of cells, and they are major components of the bone. It was shown that urinary Ca and P increased and could reflect an early renal dysfunction caused by Cd (Wu *et al.*, 2001). Elevated Ca and P are interesting as an early indicator of tubular dysfunction and in relation to the loss of bone minerals. Cd may cause demineralization of the skeleton and increase bone fragility. Moreover, low Ca consumption causes increased absorption, elevated retention and cumulation, and enhanced toxic action of Cd (Brzoska *et al.*, 1998).

The results of this study showed low Ca and P excretion in the urine samples of Mae Sot inhabitants, which was different from the data of Nakagawa *et al.* (1993), who reported that high Ca and P excretion related to high Cd concentration.

Aoshima *et al.*(1987) compared Ca urinary excretion, serum P and blood bases (alkalinity) in men and women living in the Cd polluted Jinzu River basin, Toyama in Japan reported urinary β_2-MG $\geq$ 1,000 μg/g Cr. Their investigations showed that hypophosphatemia and hypercalciuria were predominant in women despite a decrease in tubular reabsorption of Ca and P, with an increasing fractional excretion of β_2-MG. However, this study did not performed a 24 hour urine collection and measurement of serum Ca and P. Future studies should include Ca and P clearance using a 24 hour urinary collection. In Belgium, Staessen *et al.*(1999) conducted a prospective study on inhabitants exposed to environmental Cd, due to Zn smelter activity. They suspected that hypercalciuria observed in residents showing lower urinary Cd (a mean of 1 μg/g Cr) than Japanese subjects, causes a Ca negative balance, which might accelerate bone re-absorption and increase the relative risk of bone fractures.

In conclusion, the consumption of homegrown rice polluted by Cd resulted in an elevation of urinary Cd levels and renal dysfunction markers in Mae Sot residents. The excretion of urinary proteins as indicators of renal dysfunction increased in response to the elevation of Cd body load. Urinary biomarkers; β_2-MG, NAG, and α_1-MG are the sensitive and specific markers. β_2-MG has a wide range and increases in proportion to urinary Cd concentrations. NAG is the best marker for showing the differences between low (urinary Cd < 5 μg/g Cr) and high (urinary Cd > 5 μg/g Cr) Cd exposed subjects. Consequently, urinary NAG measurement is recommended for detecting minimum renal effects of Cd concentrations below 5 μg/g Cr. However, selection of markers depends upon the objectives of a study. In overall evaluation therefore, it is difficult to find a single marker to recommend for the detection of renal dysfunction caused by Cd exposure. Nevertheless, it should be noted that in cases of renal effects from Cd concentrations of over 5 μg/g Cr, β_2-MG, α_1-MG and NAG can be used in combination for renal dysfunction diagnosis.