CHAPTER I
INTRODUCTION

Cadmium (Cd) is an environmental pollutant with insidious toxicity to humans, and may induce renal tubular dysfunction after long term exposure even at low levels (IPCS, 1992). For the general population, two main sources of Cd exposure from the environment are diet and tobacco smoke (Trzcinka-Ochocka *et al.*, 2004). According to the WHO (2001), food is a major source of Cd exposure. Food crops grown in soil containing Cd or that which is naturally rich in this metal, constitute a major source of non-workplace exposure other than cigarette smoke (Satarug *et al.*, 2004).

The Cd pathways from food to man are; soil-plant-animal-man, water-animal-man and soil-plant-man. Various plants can accumulate Cd from soil, e.g. crops, Nicotina sp. and especially rice because of its high rates of soil to plant transference (Kawada *et al.*, 1998). Rice is the main food for roughly half of the world's population. Human Cd toxicity caused by contaminated rice was first reported in Japan during the 1950s. These studies showed that subsistence rice farmers had become sick by drinking water contaminated with Cd and eating rice grown in paddy fields exposed to Cd by irrigation from the Jinzu River, which contained sewage (Osawa *et al.*, 2001). In rice-eating ethnic groups, such as the people of Thailand, Hong Kong, Taiwan, and Japan, half or more of their daily diet is rice. The latest data from Japan reported that the Japanese had the highest renal Cd levels in the world, and one third of the daily Cd intake comes from rice (Kawada *et al.*, 1998). Therefore, people living in Cd-contaminated areas are a high risk group for Cd-induced renal toxicity by consumption of foodstuff that is contaminated by Cd.

According to news from the Bangkok Post, reported on 28 Feb 2004, high levels of Cd contamination found in rice at Tak province could cause a high risk of renal malfunction and bone damage among villagers. This threat of Cd contamination was confirmed by the Pollution Control Department (PCD), with unusually high levels of Cd in local rice grains mentioned in its latest report. Samples of soil and water from three districts in Amphur Mae Sot have been found contaminated with Cd, allegedly as a result of zinc-mining by Tak Mining and Phadaeng Industry. The three districts are Phrathat Phadaeng, Mae Tao and Mae Ku known for its fragrant jasmine rice, which won national awards in 2002 and 2003. The two villages hit hardest by the contamination are Padeh and Mae Tao Mai in Phrathat Phadaeng districts (Sirisunthorn, 2004).

Tests of more than 30 rice grain samples from the riverside villages of Mae Tao Mai and Pha Dhe found Cd contamination exceeding the safe limit of 0.2 mg/kg, which is set by the Codex Committee on Food Additives and Contaminants, 2001 (Wangvipula, 2004). About 3,800 villagers from Mae Tao and Phrathat Phadaeng districts were tested for levels of Cd ingestion and related illnesses. About 5.6% of 850 people were found to have very high levels of Cd in their bodies, while 12.7% showed slightly high levels. Of those suffering from Cd contamination, five people had renal failure and 29 exhibited malfunctioning kidneys (The Nation, 2004). Health

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examination on 250 people at Phadeh village health center found toxic levels of Cd in the villagers, with an average blood Cd level of 3.58 ± 2.27 μg/l. Some exceeded the WHO value limit of 5 μg/l. The average urinary Cd level was 3.60 ± 4.92 μg/g creatinine, which exceeded the WHO maximum tolerable internal dose for the non-exposed population (2 μg/g Cr) (Matichon, 2004).

A Mae Sot hospital team conducted a large-scale health impact survey for 6,802 residents who lived in 12 villages of 3 districts: Phrathat Phadaeng, Mae Tao and Mae Ku, in Amphur Mae Sot, Tak province. The team investigated urinary Cd concentration levels (2004). The results showed a high rate of Cd exposed subjects, with 9.2 % of the subjects having urinary Cd of between 5 and 10 μg/g Cr, and 2.5% having higher than 10 μg/gCr. In Japanese Cd polluted areas, the reported prevalence of renal tubular dysfunction in subjects with urinary Cd was higher than or equal to 10 μg/g Cr in residents aged 50 years or older.

Therefore, people in the Cd-polluted area of Tak province, especially farmers who eat their own crops, may have renal dysfunction, due to high Cd exposure.

Physical and chemical properties of cadmium

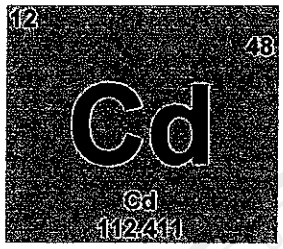
Cadmium (Cd) is a chemical element in the periodic table that has the symbol Cd and atomic number 48. It is a metallic element belonging, together with zinc and mercury, to group IIb in the periodic table. It is rarely found in a pure state. It is present in various types of rocks and soils and in water, as well as in coal and petroleum. Among these natural sources, zinc, lead, and copper ore are the main sources of Cd. Almost all Cd is obtained as a by-product in the treatment of zinc, copper, and lead ores. It is a naturally-occurring element in the earth's crust. It was first discovered in Germany in 1817. It is a soft, bluish-white metal which is easily cut with a knife. (see Figure 1) (WHO, 1992)

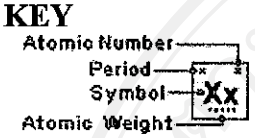
Some Cd compounds, such as Cd sulfide, carbonate, and oxide, are practically insoluble in water. There is, however, a lack of data on the solubility of these compounds in biological fluids, e.g. in the gastrointestinal tract and lung. These water-insoluble compounds can be changed to water-soluble salts in nature under the influence of oxygen and acids; Cd sulfate, nitrate, and halides are water-soluble. Most of the Cd found in mammals, birds, and fish is probably bound to protein molecules.

The speciation of Cd in soil, plants, animal tissues, and foodstuffs may be of importance for evaluation of the health hazards associated with areas of Cd contamination or high Cd intake. For example, although soil Cd levels in Shipham, United Kingdom, were found to be very much higher than in Toyama, Japan, Cd uptake by edible plants in Shipham was only a small fraction of that in Toyama (Tsuchiya, 1978 and Sherlock *et al.*, 1983).

Sources of human and environmental exposure to cadmium

Cd is released to the air, land, and water by human activities. Numerous human activities result in the release of significant quantities of Cd to the environment. The major sources of Cd release can be divided into three categories.





Symbol: Cd
Atomic Number: 48
Atomic Weight: 112.411
Element Classification: Transition Metal
Discovered By: Fredrich Stromeyer
Discovery Date: 1817 (Germany)
Name Origin: Greek: kadmeia [ancient name for calamine (zinc oxide)].
Density (g/cc): 8.65
Melting Point (°K): 594.1
Boiling Point (°K): 1038
Appearance: Soft, malleable, blue-white metal
Atomic Radius (pm): 154
Atomic Volume (cc/mol): 13.1
Covalent Radius (pm): 148
Ionic Radius: 97 (+2e)
Specific Heat (@20°C J/g mol): 0.232
Fusion Heat (kJ/mol): 6.11
Evaporation Heat (kJ/mol): 59.1
Debye Temperature (K): 120.00
Pauling Negativity Number: 1.69
First Ionizing Energy (kJ/mol): 867.2
Oxidation States: 2
Electronic Configuration: [Kr] $4d^{10}$ $5s^2$
Lattice Structure: Hexagonal (HEX)
Lattice Constant (Å): 2.980
Lattice C/A Ratio: 1.886

Figure 1 Characteristics of Cadmium (ATSDR, 1989).

One third of those activities are involved in mining, production, and consumption of Cd and other non-ferrous metals.

In general, the two major sources of contamination are the production and consumption of Cd and other non-ferrous metals, and the disposal of wastes containing Cd. Areas in the vicinity of non-ferrous mines and smelters often show pronounced Cd contamination. Increases in soil Cd content result in an increase in the uptake of Cd by plants; the pathway of human exposure to agricultural crops is thus susceptible to the increase in soil Cd. The uptake by plants from soil is greater at a low soil pH.

Processes that acidify soil (e.g. acid rain) may therefore increase the average Cd concentrations in foodstuffs.

Cd has a limited number of applications but within this range the metal is used in a large variety of consumer and industrial materials. The principal applications of Cd fall into five categories:

1. protective plating on steel

2. stabilizers for PVC
3. pigments in plastics and glass
4. electrode material in nickel-Cd batteries
5. a component of various alloys (Wilson, 1988).

Cd is used in electroplating, solder for aluminium, process engraving, Cd-nickel batteries and as a deoxidizer in nickel plating. Cd compounds are employed as television phosphors, and pigments in glazes, enamels, dyeing and printing (WHO, 1992).

Environmental levels of cadmium and human exposure

The major route of exposure to Cd for the non-smoking general population is via food; the contribution from other pathways to total uptake is small. Tobacco is an important source of Cd uptake in smokers. In contaminated areas, Cd exposure via food may be up to several hundred µg/day. Increased uptake can also occur as a consequence of food contamination and tobacco (WHO., 1992).

Kinetics and metabolism of cadmium in humans

Data from experimental animals and humans have shown that pulmonary absorption is higher than gastrointestinal absorption. Depending on chemical speciation, particle size, and solubility in biological fluids, up to 50% of the inhaled Cd compound may be absorbed. The gastrointestinal absorption of Cd is influenced by the type of diet and nutritional status. The nutritional iron status appears to be of particular importance. On average, 5% of the total oral intake of Cd is absorbed, but individual values range from less than 1% to more than 20%. Cd absorbed from the lungs or the gastrointestinal tract is mainly stored in the liver and kidneys, where more than half of the body burden will be deposited. With increasing exposure intensity, a growing proportion of the absorbed Cd is stored in the liver. Excretion is normally slow, and the biological half-time is very long (decades) in the muscles, kidneys, liver, and whole body of humans. The Cd concentrations in most tissues increase with age. Highest concentrations are generally found in the renal cortex, but excessive exposure may lead to higher concentrations in the liver. In exposed people with renal damage, urinary excretion of Cd increases and so the whole body half-time is shortened. The renal damage leads to losses of Cd from the kidney, and the renal concentrations of Cd will eventually be lower than in people with similar exposure, but without renal damage (WHO., 1992).

Toxicokintics

a. *Absorption (oral route)*:

Depending on the dietary intake and the iron status, it has been estimated that a European or an American adult absorbs Cd orally at an average rate varying between 1.4 and 25 µg/day (Elinder *et al.*, 1985 and Lauwerys *et al.*, 1982). The Cd absorption from the gastrointestinal tract is usually about 5%. However, it varies considerably and subjects with iron deficiency may absorb up to 10%.

For a given individual, the absorption following oral exposure to Cd is likely to depend on physiological status (age; body stores of iron, calcium, and zinc; pregnancy history; etc.) and, also, the presence and levels of ions such as Zn and other dietary components ingested with the Cd.

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b. *Distribution*

Most non-occupationally exposed people come into contact with Cd primarily through diet. Cd can be detected in virtually all tissues in adults from industrialized countries, with greatest concentrations in the liver and kidney. Average Cd concentrations in the kidney at birth are near zero , and rise roughly linearly with age to a peak (typically around 40-50 μg/g wet weight) between the ages of 50 and 60, after which kidney concentrations plateau or decline. Liver Cd concentrations also begin near zero at birth, increase to typical values of 1-2 μg /g wet weight by the age of 20-25, then increase only slightly thereafter.

Cd is widely distributed in the body, with the major portion of the body burden located in the liver and kidney. Liver and kidney Cd concentrations are comparable after short-term exposure, but the kidney concentration exceeds the liver concentration following prolonged exposure (30% of Cd body burden in the kidney).

The concentration of Cd in the liver of occupationally exposed workers generally increases in proportion to intensity and duration of exposures to values of up to 100 μg/g. The concentration of Cd in the kidney rises more slowly than in the liver after exposure and begins to decline after the onset of renal damage at a critical concentration of 160-285 μg/g

c. *Metabolism*

The most dangerous characteristic of Cd is its accumulation throughout a lifetime. Cd accumulates in the liver and kidneys and has a long biological half-life, from 17-30 years in males (Goyer, 1997). After uptake from the lung or gastrointestinal tract, Cd is transported in blood plasma initially bound to albumin, as shown in experimental animals. Cd bound to albumin is preferentially taken up by the liver. In the liver, Cd induces the synthesis of metallothionein and, a few days after exposure, metallothionein-bound Cd appears in the blood plasma. Because of its low molecular weight, Cd-metallothionein is efficiently filtered through the glomeruli and thereafter taken up by the tubules. Cd accumulates in the human kidney over on entire lifetime (Nordberg, 1992).

Metallothionein is an important transport and storage protein for Cd and other metals. Cd can induce metallothionein synthesis in many organs including the liver and kidney. The binding of intracellular Cd to metallothionein in tissues protects against the toxicity of Cd. Cd that is not bound to metallothionein may therefore play a role in the pathogenesis of Cd-related tissue injury.

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d. *Elimination*

Most Cd that is ingested or inhaled and transported to the gut via mucociliary clearance is excreted in the feces. Almost all fecal Cd represents material that was not absorbed from the gastrointestinal tract. Most absorbed Cd is excreted very slowly, with urinary and fecal excretion being approximately equal. Cd is also eliminated through hair and breast milk, but these routes are of limited importance for total excretion and do not significantly alter the biological half-time.

The placenta is only a partial barrier against fetal exposure to Cd. Several studies have shown that in the general population urinary Cd excretion increases with age, and this increase coincides with increased body burden. The amount of Cd excreted represents only a small fraction of the total body burden unless renal damage

is present. Following oral exposure, the major proportion of administered Cd is found in the feces, because absorption is low.

e. *Half-life*

Experimental and epidemiological evidence indicates strongly that the biological half-time in the whole body is extremely long (many years).

For the human kidney, the half-life ranged between 6 and 38 years, and for the human liver between 4 and 19 years (WHO, 1992).

Effects of cadmium on human

High ingestion exposure of soluble Cd salts causes acute gastroenteritis. Long-term occupational exposure to Cd has caused severe chronic effects, predominantly in the lung and kidney. Chronic renal effects have also been seen among the general population. The accumulation of Cd in the renal cortex leads to renal tubular dysfunction with impaired reabsorption of, for instance, proteins, glucose, and amino acids. A characteristic sign of tubular dysfunction is an increased excretion of low molecular weight proteins in urine. In some cases, the glomerular filtration rate decreases. Increase in urine Cd correlates with low molecular weight proteinuria, and the absence of acute exposure to Cd may serve as an indicator of renal effect. In more severe cases there is a combination of tubular and glomerular effects, with an increase in blood creatinine in some cases. For most workers and people in the general environment, Cd-induced proteinuria is irreversible. Urinary excretion of Cd is related to body burden, recent exposure, and renal damage. In people with low exposure, the urine Cd level is mainly related to the body burden. When Cd-induced renal damage has occurred, or even without renal damage if exposure is excessive, urinary excretion increases. Cd-exposed people with proteinuria generally have higher Cd excretion than those people without proteinuria. After high exposure ceases, the urine Cd level will decrease even though renal damage persists. The interpretation of urinary Cd is thus dependent on a number of factors (WHO, 1992).

Among other effects, disturbances in calcium metabolism, hypercalciuria, and formation of renal stones occur. High exposure to Cd, most probably in combination with other factors such as nutritional deficiencies, may lead to the development of osteoporosis and/or osteomalacia. There is evidence that long-term occupational exposure to Cd may contribute to the development of lung cancer, but observations from exposed workers have been difficult to interpret because of confounding factors. For prostatic cancer, evidence to date is inconclusive, but it does not support the suggestion from earlier studies of a causal relationship. At present, there is no convincing evidence for Cd being an etiological agent of essential hypertension. Most data speak against a blood pressure increase owing to Cd, and there is no evidence of an increased mortality rate due to cardiovascular or cerebrovascular disease. Data from studies on groups of occupationally exposed workers and groups exposed in the general environment show that there is a relationship between exposure levels, exposure durations, and the prevalence of renal effects (WHO, 1992).

Several studies have shown that in the general population urinary Cd excretion increases with age (Katagiri *et al.*, 1971; Tsuchiya *et al.*, 1976; Elinder *et al.*, 1978; Kowal *et al.*, 1979) and this increase coincides with the increased body burden.

Smokers have higher urinary excretion than non-smokers (Elinder *et al.*, 1978; Kowal *et al.*, 1979).

Acute Toxicity (oral exposures)

Doses of 20 to 30 mg/kg of Cd have resulted in human fatalities, but generally, fatal poisoning from Cd is rare. High doses of Cd are known to cause gastrointestinal irritation resulting in vomiting, abdominal pain, and diarrhea (ATSDR, 1989).

Following ingestion of Cd, an asymptomatic period of 0.5 to 1.0 hour may precede the onset of clinical signs. Depending on the severity of exposure, clinical signs of Cd poisoning following acute exposure include: nausea, vomiting, abdominal cramps, headache, muscle cramps, exhaustion, shock, and death (USAF, 1990).

Subchronic Toxicity

Because the toxic effects of Cd are functioned by a critical concentration being attained in a target organ, similar effects will occur following long-term exposure to low Cd levels and short-term exposure to high concentrations (Wang *et al.*, 1984). Consequently, renal and hepatic toxicity may occur if toxic Cd levels are attained in these organs even during subchronic exposure. A description of Cd-induced toxicity following oral exposure is presented in the following section, chronic toxicity. Generally, Cd is not as toxic via oral routes as via inhalation.

Chronic Toxicity

The most serious chronic effect of oral exposure to Cd is renal toxicity. This critical effect is characterized by tubular proteinuria resulting from renal tubular dysfunction. Friberg *et al.* (1974) estimated that this critical effect will not occur in humans until the Cd concentration in the renal cortex exceeds 200 μg/g.

Dietary intake of Cd has also been implicated in osteomalacia, osteoporosis and spontaneous fractures; conditions collectively termed "itai-itai" (ouch-ouch) disease and originally documented in postmenopausal women in Cd-contaminated areas of Japan (Friberg *et al.*, 1974).

Cd exposure has also been implicated in hypertensive disorders, a situation that is at present not thoroughly understood or verified (ATSDR, 1989).

Target organs

Kidneys: (Renal effects)

The kidney is considered to be the critical target organ for the general population as well as the occupationally exposed population. The cortex is the site within the kidney where the first adverse effect occurs.

Kidney damage is the initial site of damage in cases of low-level chronic inhalation exposure. Two distinguishing characteristics of Cd toxicity are the long half-life of Cd in the body (10-30 years) and the irreversible nature of renal dysfunction after both occupational and environmental exposure. When the exposure is chronic, Cd accumulates and increases body burden.

Long-term exposure to Cd causes renal tubular dysfunction with proteinuria, glucosuria, and aminoaciduria, as well as histopathological changes, in both experimental animals and humans. These are usually the first effects to occur after

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ingestion or inhalation exposure. In working environments with high Cd exposure levels, workers have also developed hypercalciuria, phosphaturia and polyuria, and some have suffered from renal colics due to recurrent stone formation. As the renal dysfunction progresses in severity, the glomeruli may also be affected and, in a few cases, the Cd-induced damage may lead to renal failure (WHO, 1992).

Bone tissues

Case studies indicate that calcium deficiency, osteoporosis, or osteomalacia can develop in some workers after long-term occupational exposure to high levels of Cd. Bone lesions (accompanied by renal damage) have also been reported in aged and malnourished women living in Cd-polluted areas in Japan. (Itai-Itai disease). Effects on the bone generally arise only after kidney damage has occurred and are likely to be secondary to resulting changes in calcium phosphorus and vitamin D metabolism. The daily intake via food and exposure levels in the air, which affect the bone, probably occur in the same range as that producing kidney effects (WHO, 1992).

Respiratory system

Long-term occupational exposure to high levels of Cd has been reported to cause emphysema and dyspnea in humans. The dose needed to produce these conditions is higher than that needed to produce renal effects. Chronic inflammation of the nose, pharynx, and larynx have been reported in some studies. Anosmia is a frequent symptom in Cd workers after prolonged exposure (WHO, 1992).

Reprotoxicity

The nervous system of embryo in animals appears to be a sensitive target. Limited evidence suggests that maternal Cd exposure may cause decreased birth weight in humans. Evidence is insufficient to determine an association between exposure to Cd and reproductive effects in humans (WHO, 1992).

Genotoxicity

No definitive conclusions can be drawn about the genotoxicity of Cd compounds. Data from experimental systems indicate that Cd, in certain forms, has genotoxic properties and it is reasonable to assume that these properties may also apply to other Cd compounds. It cannot be excluded, based on the available data (Forni *et al.*, 1990;1994) that Cd might exert genotoxic effects in populations exposed to Cd by inhalation (WHO, 1992).

Carcinogenicity

Several studies in humans have reported an excess risk of lung cancer in occupationally exposed cohorts. However, the evidence is limited rather than conclusive due to confounding factors (smoking, simultaneous exposure to other carcinogens). No studies have indicated that Cd may act as a carcinogen in the general population exposed by the oral route (WHO, 1992).

Overall, in view of the sum of data collected in genotoxicity tests, long-term animal experiments and epidemiological studies, it seems reasonable to consider Cd

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compounds as at least suspected human carcinogens (lung cancer). There is no evidence of an increased risk of prostate cancer in workers exposed to Cd compounds (WHO, 1992).

Other metals (Zn, Ca, P and Cu) and renal dysfunction

Metals are ubiquitous in the environment either naturally or anthropogenically. Both industry and agriculture have contributed considerably to the elevated concentrations of heavy metals through waste disposal, smelter stacks, atmospheric deposition, fertilizer and pesticide use, and the application of sewage sludge in arable land. As a result, an increased metal uptake by crops and vegetables grown for human consumption is often observed. It is well known that some metals, such as zinc (Zn), copper (Cu) and cobalt (Co) are essential at appropriate levels for most biological systems including that for humans, while others, such as cadmium (Cd) and lead (Pb) are highly toxic to plants, animals and humans. However, generally speaking, the excessive accumulation of most heavy metals and metalloids in soils and plants may pose serious health risks to humans and exert adverse impacts on the ecosystem itself (Granero and Domingo, 2002; McLaughlin *et al.*, 1999, 2000). For example, the accumulation of Cd in the human body (principally in the kidney and liver) can cause renal dysfunction and bone disease such as Itai-Itai (Nordberg, 1996). There are several studies demonstrating that environmental contamination with Cd has resulted in poor health among residents near smelters (Cai *et al.*, 1990; Nordberg *et al.*, 1997; Jin *et al.*, 2002). The major public health concern of Cd exposure in a general population is lifetime accumulation and possible renal damage through doses in the food chain, which have attracted much attention worldwide (Syers and Goldfeld, 2001). In a rat feeding study, Reeves and Chaney (2001) demonstrated that marginal mineral deficiencies in calcium (Ca) and iron (Fe) could readily enhance the body burden of Cd from the diet; and other natural competitors of Cd such as Zn, which is contained in food, can independently minimize Cd absorption. The effects on human health of mixtures of Cd, Pb and possibly other heavy metals in the environment have rarely been investigated. In recent years, the potential health risks of lifetime exposure to chemical mixtures have been considered as a pressing environmental health problem (Suk et al., 2002), particularly in developing countries where many local residents are living in the vicinity of small-scale industries, which often discharge waste containing chemical mixtures such as Pb, Cd and Zn (Yanez *et al.*, 2002). Yujing *et al.* (2005) focused on the contamination and health effects of the metal mixtures, because in a real environment exposure to mixtures of metals is ubiquitous in that Cd pollution is invariably being associated with Pb and Zn, etc. For this purpose, a dietary survey was taken for 3 groups in Nanning during October 2002. Samples of soils and plants (vegetables), and urine and blood of humans were measured for Cd, Fe, Cu, Zn, Ca and Pb, in addition to the urinary indicators of renal dysfunction. Albumin, NAG, β_2-MG and Retinol-binding protein (RBP) in urine were also measured. Results showed that soil contamination by metal mixtures had caused significant renal dysfunction in local residents living in the contaminated area, and the dose–response curve was somewhat altered by the mixed contamination of Cd and Pb as well as the intake of other minerals. Wu *et al.* (2001) showed urinary Ca as a biomarker of renal dysfunction in a general population exposed to Cd. An increase

in urinary Ca in early renal damage induced by Cd has been reported both in human and animal experiments. A significant dose-response relationship between the prevalence of hypercalciuria and the excretion of urinary Cd was observed, and a significantly increased prevalence of calciuria was found when excretion of urinary Cd exceeded 2 μg/g Cr. Because Cd can affect Ca^{2+} uptake by tubular cells, with decreased renal Ca^{2+} reabsorption, calciuria may reflect tubular cell damage caused by Cd. It was concluded that Cd exposure can result in increased excretion of urinary calcium in a general population and that there is asignificant dose-response relationship. Kido *et al.* (1993) showed that clearance methods were used to clarify the renal handling of Ca and phosphorus (P) in a population with renal dysfunction induced by exposure to environmental Cd. Seventy-six Cd-exposed subjects (32 men and 44 women) and 36 non-exposed subjects (18 men and 18 women) took part in this study. Fractional excretion of P was higher in the Cd-exposed subjects than in the non-exposed ones, while that of Ca was equal to that of the non-exposed subjects. The urinary excretion rates of Ca and P tended to be lower in the Cd-exposed subjects than in the non-exposed ones. The urinary excretion rate of Ca was closely related to creatinine clearance, while that of P was related to creatinine clearance and the percentage tubular reabsorption of phosphorus. It is thought that in Cd-induced renal dysfunction the urinary excretion of Ca depends on glomerular function, and no increased excretion of urinary Ca was observed by these clearance methods. It has also been clarified that the parallelism in the urinary excretion of Ca and Na persists in Cd-exposed subjects with renal dysfunction.

Detection of renal markers and cadmium toxicity

The first task in assessing renal damage caused by Cd toxicity is to confirm Cd exposure, most commonly carried out by measuring Cd in blood and urine. Cd in blood is believed to primarily reflect recent exposure, whereas Cd in urine correlates best with cumulative exposure indices and the whole-body burden of Cd, estimated by measuring tissue Cd. Cd levels in urine generally correlate with measures of renal effects in people exposed to Cd, and dose-response relationships have been observed for urinary Cd. Therefore, the amount of Cd in urine is commonly used as an indicator of Cd body burden (Mueller, 1993).

In addition, Jung *et al.* (1993) generally found better correlations between urine Cd levels and markers of renal effects than between blood Cd concentrations and renal markers.

The first detectable nephrotoxic effect of Cd is on increase in the excretion of proteins in urine, which cannot be detected by standard clinical proteinuria tests. The various classes of proteins that are measured in urine to assess the renal effects of Cd include:

(a) **Relatively high molecular mass enzymes**, which are not usually filtered through the glomerulus, originate primarily in the proximal tubular tissue. The enzymes that have proven to be most useful are alanine aminopeptidase (AAP; microsomal aminopeptidase) and the lysosomal enzyme, N-acetyl-β-D-glucosaminidase (NAG). Increased excretion of these enzymes is generally believed to indicate increased turnover of renal tubular tissue (Mueller, 1993).

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NAG is a lysosomal enzyme involved in the breakdown metabolism of glycoproteins. Increased NAG levels in urine are an early indication of renal disease and can serve as a valuable renal monitoring test in disorders such as nephritic syndrome, glomerulonephritis, drug abuse associated nephrotoxicity, diabetes-associated nephropathy, hypertension and urinary tract infections. Results in this study of Cd-exposed men showed that the high molecular mass enzymes, AAP and NAG, were increased at lower concentrations of urine Cd than were the low molecular mass proteins, retinol binding protein (RBP) and β_2-microglobulin (β_2-MG) (Mueller, 1993).

Jin *et al.* (1999) reported that urinary NAG isoenzymes were a biomarker of renal dysfunction caused by Cd in a non-occupational exposed population residing in a polluted area of China. The studied area was contaminated by industrial wastewater from a nearby smelter that discharged Cd-polluted wastewater into a river used for the irrigation of rice fields. There was a marked dose-dependent increase in the NAG of urine related both to urinary Cd and calculated Cd intake. It is concluded that urinary NAG could serve as a sensitive biomarker of renal dysfunction in Cd-exposed populations.

Jung *et al.* (1993) studied urinary protein and enzymes as the early indicators of renal dysfunction in chronic exposure to Cd, and tested the diagnostic sensitivity of various urinary analytes for detecting Cd-induced nephropathy at an early stage. They investigated subjects including healthy persons, and people who had been exposed to Cd either environmentally or occupationally. The study showed that urinary NAG and α_1-microglobulin (α_1-MG) could serve as screening markers to detect Cd-induced renal dysfunction at an early stage.

Kawada. (1995) investigated the renal effect indicators of exposure to Cd; NAG and others renal proteins. NAG is one of the recommended markers for detecting renal effects.

Moriguchi *et al.* (2003) studied the use of α_1-MG, β_2-MG, RBP and NAG, as markers of renal tubular dysfunction after environmental exposure to Cd, with special references to the effects of aging. Among the four tubular dysfunction markers, NAG showed the closest correlation with Cd, followed by α_1-MG and then β_2-MG, and RBP showed the least, although all the correlations were statistically significant. They concluded that NAG and α_1-MG were more sensitive in detecting Cd-induced tubular dysfunction than other markers in mass screening.

(b) **Intermediate molecular mass proteins** are normally filtered though the glomerulus in small amounts and reabsorbed primarily by the tubules. The protein that is most commonly represented by albumin and transferrin serves as an indicator of glomerular damage.

Albumin in urine may be an indication of kidney damage and if left untreated, can result in irreversible damage. Low albumin concentration in the urine (20-200 μg/min) or microalbuminuria is the earliest marker of renal damage. As a marker of kidney disease, microalbuminuria predates urinary albumin levels by approximately 5-10 years. Measurement of higher total urinary albumin levels (>200 μg/min) is important for conditions associated with an increased risk of renal failure such as diabetes, essential hypertension, nondiabetic renal disease, cardiac decomposition, pregnancy, and patients in pharmaceutical clinical trials (Mueller, 1993).

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(c) **Low molecular mass proteins** are normally filtered freely through the glomerulus and reabsorbed by the tubules. These proteins have been represented by β_2-MG, but because of stability problems it is being replaced in many studies by RBP and α_1-MG. Increased excretion of these proteins is considered an indication of damage to the tubular reabsorption mechanism. In this protein class, molecular mass may also be a factor. The molecular mass of β_2-MG and RBP is 11,700 and 20,600 Da, respectively. The α_1-MG, a glycosylated single chain protein, which has a molecular mass that varies from -29,000 to 33,000 Da, is characterized by charge heterogeneity (Mueller, 1993). Although α_1-MG excretion is known to be increased in tubular disorders, the possibility of its filtration rate varying and being affected by subclinical glomerular damage has not yet been investigated (Mueller, 1993).

The β_2-MG is a protein identical to the light chain of the HLA-A, -B, and –C antigen. It is expressed on nucleated cells, and found at low levels in the serum and urine of normal individuals. Free β_2-MG is filtered by the glomerulus and subsequently reabsorbed in the proximal tubular cells. Increased urinary excretion of β_2-MG is a sensitive indicator of renal insufficiency. The β_2-MG concentrations are increased in inflammatory diseases, some viral diseases, renal dysfunction, and autoimmune diseases. A number of publications are available which explain the interpretation of β_2-MG serum levels in assessing the status of individuals with various clinical conditions (Mueller, 1993).

The α_1-MG is a low molecular weight, pH stable glycoprotein. It has a molecular mass of 33,000 daltons and is synthesized by the hepatocytes and lymphocytes. It is almost entirely filtered in the glomeruli with approximately 99.8% of the re-absorption and catabolism taking place in the proximal tubules. Increased excretion of α_1-MG in tubular proteinemia is an indicator of reduced tubular re-absorption under normal glomerular filtration conditions. This form of proteinuria is typical for chronic interstitial nephropathy and acute and chronic tubular damage caused by endogenous and exogenous tubular toxins. In renal failure, the plasma levels of this microprotein increase from an early stage. The resultant protein hyperfiltration in the residual nephron causes increased renal excretion as re-absorption capacity is exceeded (overflow proteinuria). The α_1-MG can be used as a marker for the diagnosis of tubulointerstitial nephropathy. Acute and chronic forms of tubular insufficiency (all forms of primary and secondary Fanconi syndrome), heavy metal intoxication, nephrotoxic side effects of pharmaceuticals, and rejection reactions following kidney transplantation can also be excluded (Mueller, 1993).

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Objectives of the study

The objectives of this study were to investigate early Cd-induced renal dysfunction by measurement of various urinary renal markers and the dose-response relationship between Cd levels and renal markers in the urine samples of people living in the Cd-polluted area of Amphur Mae Sot, Tak Province, Thailand.

1.To detect early Cd-induced renal dysfunction by measurement of urinary renal markers [N-Acetyl-β-D-glucosaminidase (NAG), albumin, β_2-microglobulin (β_2-MG), α_1-microglobulin (α_1-MG), total protein, glucose, lysozyme, total amino nitrogen and creatinine].

2.To assess the correlation between Cd levels in urine and the above markers.

3.To assess the correlation of Cd levels and renal markers with other metals: Zn, Cu, Ca and P.

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