

## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (ENGLISH)	v
ABSTRACT (THAI)	ix
TABLE OF CONTENTS	xiii
LIST OF TABLES	xvi
LIST OF FIGURES	xix
ABBREVIATIONS AND SYMBOLS	xxi
CHAPTER I INTRODUCTION	1
1.1 Principle, Theory, and Rationale	1
1.2 Cadmium polluted area in Thailand	6
1.3 Cadmium pollution in Japan and Itai-itai disease	8
1.4 Cadmium pollution in other countries	10
1.5 Environmental cadmium distribution to human	12
1.6 Cadmium toxicity	13
1.7 Cellular toxicity of cadmium	17
1.8 Cadmium osteotoxicity	20
1.9 Cadmium and calcium metabolism impairment	21
1.10 Cadmium and anemia	23
1.11 Cadmium and biomarkers	26
1.12 Hypothesis	31

1.13 Study Objectives	32
CHAPTER II MATERIALS AND METHODS	33
2.1 Instruments	33
2.2 Chemicals	33
2.3 Study area and sample population	35
2.4 Collection of urinary and blood samples	38
2.5 Determination of blood and urinary cadmium	39
2.6 Analysis of renal dysfunction markers	40
2.7 Determination of fractional excretion of calcium	44
2.8 Determination of bone markers	44
2.9 Determination of anemic biomarkers	46
2.10 Expression of the human fetal osteoblast genes after cadmium chloride treatment	47
2.11 Data analysis	51
CHAPTER III RESULTS	53
3.1 Population characteristics	53
3.2 Cadmium exposure	54
3.3 Exposure to cadmium, occupation and smoking status adjusted by age	54
3.4 Correlation of blood and urinary cadmium	55
3.5 Renal dysfunction biomarkers	55
3.6 Cadmium exposure VS renal and bone markers	56
3.7 Dose-response relationship between cadmium exposure and renal and bone markers	56

3.8. Gender dependent correlation between cadmium exposure, renal dysfunction and bone metabolism impairment	57
3.9 Anemia prevalence among cadmium polluted area inhabitants	59
3.10 Effect of cadmium on hFOB 1.19 mRNA expression	61
CHAPTER IV DISCUSSION AND CONCLUSIONS	91
REFERENCES	107
APPENDICES	132
Appendix A	133
Appendix B	136
Appendix C	139
CIRRICULUM VITAE	148

## LIST OF TABLES

Table		Page
1	Real time PCR primer sequence for osteoblast gene expression determination	51
2	Characteristics of 700 surveyed inhabitants living in a cadmium polluted area	63
3	Number of the study subjects classified by gender, occupation, smoking status and underlying diseases	64
4	Mean $\pm$ standard deviation of blood cadmium distribution according to age groups in both men and women	65
5	Mean $\pm$ standard deviation of urinary cadmium distribution according to age groups in both men and women	66
6	Relation between <b><u>blood cadmium</u></b> of the study subjects and three types of classified occupations and smoking status adjusted by age	67
7	Relation between <b><u>urinary cadmium</u></b> of the study subjects and three types of classified occupations and smoking status adjusted by age	68
8	Comparison of age, body mass index, blood cadmium, urinary cadmium and renal dysfunction markers between men and women of all study subjects living in cadmium polluted area	70

9	Comparisons of age, body mass index, urinary cadmium, blood cadmium, renal and bone markers between men and women aged 50 years and over, living in cadmium polluted area	71
10	Dose-response analysis of the concentrations of renal markers and bone markers to four levels of cadmium in 156 <b>men</b> aged $\geq 50$ years.	72
11	Dose-response analysis of the concentrations of renal markers and bone markers to four levels of cadmium in 256 <b>women</b> aged $\geq 50$ years.	73
12	Gender dependent correlations between urinary cadmium, bone markers and renal tubular dysfunction markers in the study subjects aged $\geq 50$ years	74
13	Multivariate regression analyses of age, sex, <b>blood cadmium</b> and renal tubular dysfunction markers on bone markers among cadmium polluted area inhabitants aged $\geq 50$ years	77
14	Multivariate regression analyses of age, sex, <b>urinary cadmium</b> and renal tubular dysfunction markers on bone markers among cadmium polluted area inhabitants aged $\geq 50$ years	78
15	Comparison of blood and urinary cadmium concentrations to blood indices in the study subjects	79
16	Mean and standard deviation of blood indices of the <b>anemic subjects</b> , both genders, in four groups of the subjects classified by concentrations of urinary cadmium (U-Cd) from $<2$ to $> 10$ $\mu\text{g/g Cr}$	80

17	Correlation between hemoglobin & hematocrit, and cadmium concentrations in blood and urine, renal markers, age and body mass index in all of the study subjects living in the cadmium polluted area	81
18	Relation between anemia prevalence in 5 age groups among the study subjects	83
19	Relation between anemia prevalence in <u>men</u> and urinary cadmium levels and renal dysfunction markers	84
20	Relation between anemia prevalence in <u>women</u> and urinary cadmium levels, and renal dysfunction markers	85
21	Relation between anemia prevalence and $\beta_2$ -MG, NAG levels after adjusted by means age and urinary cadmium	86
22	Relation between anemia prevalence and cystatin C levels after adjusted by means age and urinary cadmium	87

## LIST OF FIGURES

Figure	Page
1 Cadmium polluted area in Mae Sot District, Tak Province	36
2 The reaction of $\beta_2$ -MG in the enzyme immunoassay	41
3 The reaction of NAG in the colorimetric assay.	42
4 Reaction of serum cystatin C in the latex particle enhanced turbidimetric immunoassay	43
5 Histograms of blood cadmium concentrations distribution according to age groups in men and women who live in the cadmium polluted area.	65
6 Histograms of urinary cadmium concentrations distribution according to age groups in men and women who live the cadmium polluted area.	66
7 Correlation between blood and urinary cadmium in men and women who live in cadmium polluted area	69
8 The relationship between bone resorption and urinary cadmium among men and women whose age $\geq 50$ years from cadmium polluted area.	75
9 The relationship between bone resorption and impaired Ca reabsorption capacity among men and women	76
10 Correlation between urinary cadmium and hemoglobin in men and women	82

11	Morphology of hFOB cells treated with CdCl <sub>2</sub> concentrations 2.5, 5, 10, 20 and 40 μM compared to the untreated cells	88
12	Cytotoxicity of cadmium chloride in hFOB cells after 24 h treatment using MTT assay	89
13	Correlation of %hFOB cell viability between MTT and trypan blue exclusion assays after treatment with CdCl <sub>2</sub> solution	89
14	Expression of osteocalcin, typeI collagen and OPG genes of the hFOB cell after 24 hr treatment with CdCl <sub>2</sub>	90



## ABBREVIATIONS AND SYMBOLS

AAS	Atomic absorption spectrometer
$\alpha_1$ -MG	Alpha1 -microglobulin
$\beta_2$ -MG	Beta2 -microglobulin
$^{\circ}\text{C}$	Degree of Celsius
$\mu\text{g}$	Microgram
$\mu\text{M}$	Micromolar
AAP	Alanine aminopeptidase
Ab	Antibody
Ag	Antigen
Alb	Albumin
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
B-Cd	Blood cadmium
BCE	Bone collagen equivalent
BMI	Body mass index
C.I.	Confident interval
Ca	Calcium
$\text{Ca}^{2+}$	Calcium ion
Cd	Cadmium
$\text{CdCl}_2$	Cadmium chloride
cDNA	Complementary deoxyribonucleic acid
Col1A1	Collagen 1A1
Cr	Creatinine
Ct	Cycle threshold
Cu	Copper

Dex	Dexamethasone
dl	Deciliter
DMEM	Dulbecco's Modified Eagle Medium
DPD	Deoxypyridinoline
EIA	Enzyme immunoassay
Fe	Iron
FECa	Fractional excretion of calcium
g	Gram
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HCl	Hydrogen chloride
HClO <sub>4</sub>	Hydrogen perchlorate
Hct	Hematocrit
hFOB 1.19	Human fetal osteoblast like cell line 1.19
Hgb	Hemoglobin
HNO <sub>3</sub>	Nitric acid
IPCS	International Programme on Chemical Safety
kg	Kilogram
l	Liter
LC	Lethal concentration
LYZ	Lysozyme
M	Molar
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligram
min	Minute
ml	Milliliter
mRNA	Messenger RNA
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NS	Not significant
Na	Sodium

NAG	N-acetyl- $\beta$ -D-glucosaminidase
NaOH	Sodium hydroxide
nm	Nanometer
nmol	Nanomolar
NTx	N-terminal crosslink of type I collagen
OC	Osteocalcin
OPG	Osteoprotegerin
P	Phosphorus
Pb	Lead
PCD	Pollution Control Department
PCR	Polymerase chain reaction
pH	Power of hydronium ion
RANKL	Receptor Activator for Nuclear Factor $\kappa$ B Ligand
RBC	Red blood cell count
RBP	Retinol-binding protein
RDW	Red blood cell distribution width
RNA	Ribonucleic acid
RT-real time PCR	Reverse transcriptase real time polymerase chain reaction
S.D.	Standard deviation
S.E.	Standard error
S-Ca	Serum calcium
Std	Standard
U-Ca	Urinary calcium
U-Cd	Urinary cadmium
UV	Ultraviolet
VIS	Visible
WHO	World Health Organization
Zn	Zinc