ASSOCIATION BETWEEN MATERNAL FLUORIDE LEVEL AND PRETERM DELIVERY AND LOW BIRTH WEIGHT AMONG PREGNANCY AT ENDEMIC FLUORIDE AREAS IN LAMPHUN, THAILAND : A PREGNANCY- BIRTH COHORT STUDY

Mr. Nonthaphat Theerawasttanasiri

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Public Health
College of Public Health Sciences
Chulalongkorn University
Academic Year 2017

ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดา กับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน ประเทศไทย : การศึกษาไปข้างหน้า

นายนณธภัทร ธีระวรรธนะสิริ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาสาธารณสุขศาสตรดุษฎีบัณฑิต
สาขาวิชาสาธารณสุขศาสตร์
วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2560

Thesis Title	ASSOCIATION BETWEEN MATERNAL FLUORIDE LEVEL AND PRETERM DELIVERY AND LOW BIRTH WEIGHT AMONG PREGNANCY AT ENDEMIC FLUORIDE AREAS IN LAMPHUN, THAILAND: A PREGNANCY- BIRTH COHORT STUDY
By	Mr. Nonthaphat Theerawasttanasiri
Field of Study	Public Health
Thesis Advisor	Professor Surasak Taneepanichskul, M.D.

Accepted by the College of Public Health Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the College of Public Health Sciences

(Professor Sathirakorn Pongpanich, Ph.D.)

THESIS COMMITTEE

.....Chairman

(Associate Professor Ratana Somrongthong, Ph.D.)

.....Thesis Advisor

(Professor Surasak Taneepanichskul, M.D.)

.....Examiner

(Professor Sathirakorn Pongpanich, Ph.D.)

.....Examiner

(Assistant Professor Nutta Taneepanichskul, Ph.D.)

.....External Examiner

(Nanta Auamkul, M.D. M.P.H)

นณธภัทร ธีระวรรธนะสิริ : ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน ประเทศไทย : การศึกษาไปข้างหน้า (ASSOCIATION BETWEEN MATERNAL FLUORIDE LEVEL AND PRETERM DELIVERY AND LOW BIRTH WEIGHT AMONG PREGNANCY AT ENDEMIC FLUORIDE AREAS IN LAMPHUN, THAILAND: A PREGNANCY- BIRTH COHORT STUDY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. นพ. สุรศักดิ์ ฐานีพานิชสกุล, หน้า.

ความเป็นมา: การศึกษานี้มีวัตถุประสงค์เพื่อหาความสัมพันธ์ระหว่างระดับความเข้มข้นของฟลูออไรด์ในปัสสาวะมารดากับการคลอดก่อนกำหนด (preterm delivery) และทารกแรกคลอดน้ำหนักน้อย (low birth weight: LBW) และเพื่อเปรียบเทียบอุบัติการณ์ (Relative Risk: RR) การคลอดก่อนกำหนดและและทารกแรกคลอดน้ำหนักน้อยระหว่างกลุ่มไม่ได้รับสัมผัสปัจจัยเสี่ยง(Low-exposed) กับกลุ่มได้รับสัมผัสปัจจัยเสี่ยง(Exposed) กลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรดสูงในจังหวัดลำพูน จากการทบทวนวรรณกรรม งานวิจัยจำนวนมากยืนยันว่าฟลูออไรด์สามารถถ่ายโอนผ่านรกและการสัมผัสกับฟลูออไรด์ในระหว่างตั้งครรภ์อาจส่งผลให้เกิดภาวะคลอดก่อนกำหนดและทารกน้ำหนักแรกคลอดน้ำหนักน้อยได้ ฟลูออไรด์ในน้ำ (water fluoride : WF) ที่ระดับ 0.70 mg/L เป็นค่ามาตรฐานที่ยอมรับว่าป้องกันโรคฟันผุในขณะที่สามารถกควบคุมความเสี่ยงของโรคฟันตกกระ (fluorosis) ลำพูนเป็นหนึ่งในหกจังหวัดของประเทศไทยที่พบ WF>10.0mg/L และ> 50% ของครัวเรือนใช้น้ำที่มีฟลูออไรด์สูง อย่างไรก็ตามหลักฐานสนับสนุนระดับฟลูออไรด์ของมารดาที่สัมพันธ์กับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยยังไม่ได้มีการศึกษาในประเทศไทย

วิธีการศึกษา: ศึกษาในหญิงตั้งครรภ์ในพื้นที่จังหวัดลำพูนระหว่างเดือนกรกฎาคม2559 -พฤศจิกายน 2560 โดยการเลือกพื้นที่แบบเจาะจงใน อ.เมืองลำพูน อ.ป่าซางและอ.บ้านธิ ซึ่งเคยพบ WF>10.0 mg/Lและ LBW>7% โดยแบ่งกลุ่มประชากรที่ศึกษาตามระดับฟลูออไรด์ในน้ำ WF≤ 0.70 mg/L เป็นกลุ่มไม่ได้รับสัมผัสปัจจัยเสี่ยง (Low-exposed) และ WF>0.70 mg /L เป็นกลุ่มได้รับสัมผัสปัจจัยเสี่ยง(Exposed) กลุ่มตัวอย่างเป็นหญิงตั้งครรภ์จำนวน 141 คน อายุระหว่าง 20-35 ปี อาศัยอยู่ในพื้นที่ศึกษาตั้งแต่หนึ่งปีขึ้นไป ไม่มีพบความเสี่ยงของการตั้งครรภ์เมื่อมาฝากครรภ์ครั้งแรก อายุครรภ์น้อยกว่า 30 สัปดาห์เมื่อเข้าร่วมโครงวิจัย และวางแผนการคลอดที่ รพ.ลำพูน รพ.ป่าซาง หรือ รพ.บ้านธิ. โดยเก็บรวบรวมข้อมูลจากแบบสัมภาษณ์และเวชระเบียน การตรวจระดับฟลูออไรด์ในปัสสาวะขณะที่อายุครรภ์ น้อยกว่า 30, 31-33, และ34-36 สัปดาห์ การวิเคราะห์ระดับฟลูออไรด์โดยวิธี Total Ionic Strength Adjustment Buffer(TISAB) และ Ion Selective Electrode (ISE) โดยสูติแพทย์หรือแพทย์เป็นผู้วินิจฉัยการคลอดก่อนกำหนดและทารกน้ำหนักน้อย ใช้สถิติเชิงพรรณนาเพื่ออธิบายข้อมูลทั่วไปมารดาและทารกแรกเกิด สถิติการถดถอยโลจิสติกใช้เพื่อทดสอบความสัมพันธ์ระหว่างระดับฟลูออไรด์ในปัสสาวะมารดากับการคลอดก่อนกำหนดและทารกน้ำหนักแรกคลอดน้อย และเพื่อเปรียบเทียบอุบัติการณ์ของการคลอดก่อนกำหนดและภาวะ LBW ระหว่างประชาการทั้งสองกลุ่ม

ผลการศึกษา: ค่าเฉลี่ยฟลูออไรด์ในปัสสาวะ (maternal urine fluoride: MUF) เท่ากับ 1.27 (± .1.08) mg/L มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติของค่าเฉลี่ยระดับ MUFระหว่างทั้งสองกลุ่ม (p = .001) โดยกลุ่มได้รับสัมผัสปัจจัยเสี่ยงมีค่า MUF (1.65 ± 1.35) สูงกว่ากลุ่มไม่ได้รับสัมผัสปัจจัยเสี่ยง (0.98 ± .0.16) พบว่า ปัจจัยการบริโภคกาแฟ (p = .003), การฝากครรภ์ครั้งแรกเกิน 12 สัปดาห์(p = .033), การได้รับยา Triferdine(p = .033), การเคยมีประวัติการผ่าตัดคลอด (p = .037) และระดับฟลูออไรด์ในปัสสาวะมารดา (p = .040) ส่วนปัจจัยอายุมารดา (p = .006), การเพิ่มขึ้นของน้ำหนักขณะตั้งครรภ์ (p= .041), ระดับฟลูออไรด์ในน้ำประปาหมู่บ้าน (p = .048), การเคยมีบุตร (p = .049) และ ระดับฟลูออไรด์ในปัสสาวะมารดา (p = .049) มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการเพิ่มขึ้นของทารกแรกเกิดมีน้ำหนักน้อย อุบัติการณ์ของการคลอดก่อนกำหนด (RR) = 0.35 (95% CI = 0.0748 - 1.7129), และอุบัติการณ์ทารกแรกเกิดมีน้ำหนักน้อย (RR)= 1.53 (95% CI = 0.6188 - 3.8011)

สรุปผลการศึกษา ปัจจัยมารดามีระดับฟลูออไรด์ในปัสสาวะ (MUF) สูงเกิน 5 mg/L มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการเพิ่มขึ้นของทารกแรกเกิดน้ำหนักน้อย แต่ไม่มีมีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการคลอดก่อนกำหนด

สาขาวิชา สาธารณสุขศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2560 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5779162153 : MAJOR PUBLIC HEALTH
KEYWORDS: MATERNAL URINE FLUORIDE (MUF),PRETERM DELIVERY, LOW BIRTH WEIGHT, ENDEMIC FLUORIDE AREAS
NONTHAPHAT THEERAWASTTANASIRI: ASSOCIATION BETWEEN MATERNAL FLUORIDE LEVEL AND PRETERM DELIVERY AND LOW BIRTH WEIGHT AMONG PREGNANCY AT ENDEMIC FLUORIDE AREAS IN LAMPHUN, THAILAND: A PREGNANCY- BIRTH COHORT STUDY. ADVISOR: PROF. SURASAK TANEEPANICHSKUL, M.D., pp.

BACKGROUND: Many studies mention that fluoride can transfer through placenta and exposure to fluoride during pregnancy can risk to preterm delivery and low birth weight (LBW). Nowadays, water fluoride (WF) at 0.7 mg/L was recommended as standard level for good oral health to protect dental caries while controlling the risk of dental fluorosis. Lamphun is one of six provinces in Thailand where natural WF >10.0 mg/L were found, and >50% of households used water with high fluoride. However, the evidence advocate maternal fluoride level is associated with Preterm delivery and LBW have yet to be well studied in Thailand. This study aims to assess the association between maternal fluoride (MF) among pregnancy with preterm delivery and LBW. To compare the incidence (Relative Risk: RR) of preterm birth and LBW between Low-exposed and Exposed

METHODS: the cohort study on pregnancy-birth was conducted in Lamphun province between July 2016 to November 2017.Purpose sampling was used to select study areas of districts with WF >10.0 mg/L in the Mueang Lamphun, Pasang, and Ban Thi districts where WF >10.0 mg/L and LBW >7%. Village WF was used to classify study population group; WF≤ 0.70 ma/L as Low-exposed and WF>0.70 mg/L as Exposed. The 141-pregnant women age between 20 - 35 years, live in study areas within one year or more, have no risk at first ANC visit, gestational age < 30wks at first coming to the research and plan delivery at Lamphun, Pasang or Ban Thi hospital. Data will collect by interview questionnaires and medical records. Maternal urine at <30wks, 31-33wks, 34-36wks were collected to analyze fluoride level with Ion Selective Electrode (ISE) follow by TISAB. Preterm delivery and LBW were diagnosed by obstetrician or doctors. The descriptive statistics were used to describe general information of pregnant and newborn. Logistic regression will use to test the association between concentration level of fluoride in urine and preterm delivery LBW, and to compare the incidence of preterm delivery and LBW between the two study areas.

RESULTS: The average level of maternal urine fluoride (MUF) was 1.27 (± .1.08) mg/L. There was a strong significant difference of MUF (p=.001) between two groups, the MUF level of Exposed (1.65 ± 1.35) was higher than Low-exposed (0.98 ± .0.16). There were not suspected factors statistically significantly associated with Preterm delivery, whereas pregnant who have MUF level more than 0.5 mg/L (p = .003), was associated with increased of LBW. The RR of preterm delivery = 0.35 (95% CI = 0.0748 - 1.7129), and RR of LBW = 1.53 (95% CI = 0.6188 - 3.8011)

CONCLUSIONS: Our finding on MUF more than 0.5 mg/L was determined to increase of LBW but there was no associated with Preterm birth.

Field of Study: Public Health Student's Signature .....
Academic Year: 2017 Advisor's Signature .....

ACKNOWLEDGEMENTS

Undertaking my Ph.D. has been possible, I would like to take this opportunity to acknowledge sincere appreciation to the person who help, support or guidance me in the direct or indirect way of my study and research.

Firstly, I would like to greatly appreciate my thesis advisor Prof. Surasak Taneepanichskul and Doctor Robert S. Chapman (QE advisor) for the continuous support of my Ph.D. study and related research for his patience, motivation, and enormous knowledge. His guidance helped me during study research and writing of this thesis. I would like to thank the rest of my thesis committee: Assoc. Prof. Dr.Ratana Somrongthong, Prof. Dr.Sathirakorn Pongpanich, Asst. Prof. Dr.Nutta Taneepanichskul and Doctor Nanta Auamkul for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives. Also, I am gratefully acknowledged Prof. Dr. Andezhath Susheela Executive Director of the Executive Director of India's Fluorosis Research and Rural Development Foundation and Dr. Michael Connett, Executive Director of Fluoride Action Network who support and advise me about the fluoride study.

I greatly appreciate the scholarship support of Department of Health, Ministry of Public Health for tuition fee and allow full-time study. I am also gratefully acknowledged the funding for received towards my Ph.D. from the 90th Anniversary of Chulalongkorn University Research Study Scholarship Batch# 33 Round 3/2016, the funding received through National Research Council of Thailand (NRCT) for FY2017 Thesis Grant for Doctoral Degree Student to undertake my Ph.D. research study, the Scholarship for oral presentation in Salovakia of Graduate School of Chulalongkorn University and College of Public Health Science, Chulalongkorn University and Scholarship for oral presentation in Japan (full support) of Kyoto University.

Finally, I would like to thank all everyone who actively involved in this research including Lamphun Hospital, Pasang Hospital, Ban Thi Hospital, District Public Health Office Subdistrict and Health Promotion Hospital of Mueang Lamphun, Pasang, Banthi, Intercountry Center of Oral Health and mother-newborns dyads who participate in this study and made this study possible.

CONTENTS

Page

THAI ABSTRACT iv

ENGLISH ABSTRACT v

ACKNOWLEDGEMENTS vi

CONTENTS vii

LIST OF TABLES x

LIST OF FIGURES xii

CHAPTER I INTRODUCTION 1

1.1 Background and Rational 1

1.2 Research Question 5

1.3 Research Objectives 5

1.4 Research Hypothesis 6

1.5 Conceptual Framework 7

1.6 Benefit and Application 7

1.7 Operational Definition 8

CHAPTER II LITERATURE REVIEW 9

2.1 Water Fluoride and Safe Drinking Water 9

2.2 An Adequate Fluoride for Human 14

2.3 Metabolism of Fluoride 15

2.4 Biomarker of Fluoride 22

2.5 Analysis of Fluoride 26

2.6 Factors Effect to Fluoride Intake, Excretion and/or Fluoride-reduced Toxicity . 31

2.7 Effect of Fluoride 32

Page

2.8 Effect of Fluoride in Pregnant 38
2.9 Maternal and child health service in Thailand and Lamphun 45
2.10 Risk Factor of Premature Delivery and/or LBW 51
2.11 Research on Preterm Delivery and LBW and Fluoride 62
2.12 GIS Technology and Application 69
CHAPTER III METHODOLOGY 73
3.1 Research Design 73
3.2 Study Area 74
3.3 Study Population 75
3.4 Sample Size Calculation 76
3.5 Sampling technique 79
3.6 Measurement Tools 80
3.7 Data Collection 84
3.8 Data Analysis 93
3.9 Ethical Consideration 95
CHAPTER IV RESULT 96
4.1 Water Fluoride and Village Water Supply System 97
4.2 GIS Data and Geo-graphical of Endemic Fluoride Areas 102
4.3 Baseline Demographic and Characteristics 110
4.4 Clinical Characteristic of Pregnant Women before Delivery and Newborn 115
4.5 Comparison of Maternal Urine Fluoride Level between the two groups. 119
4.6 Association of MUF Level and Preterm Delivery. 120
4.7 Association of MUF Level and LBW 121

Page

4.8 Comparison Incidence of Preterm Delivery and LBW between the two groups 122

CHARPTER V DISUSIONS 124

5.1 WF, Endemic fluoride areas GIS and Geo-visual maps 124

5.2 Comparison of MUF between the two groups 125

5.3 Association of MUF level with Preterm delivery and LBW 127

5.4 Incidences of Preterm delivery and LBW 130

CHAPTER VI CONCLUSIONS AND RECOMMENDATIONS 132

6.1 Conclusions 132

6.2 Benefits and Strength of Study 133

6.3 Limitations 134

6.4 Recommendations 135

..... 140

REFERENCES 140

APPENDICS 154

VITA 184

LIST OF TABLES

page

Table 2- 1 Standard of Fluoride level in Drinking water. 12

Table 2- 2 Groundwater Quality Standards for Drinking Purpose 13

Table 2- 3 Recommendation of adequate fluoride intake 14

Table 2- 4 Units Normally Used for Measuring Fluoride 30

Table 2- 5 Component of OBIMIN-AZ and Triferdine 150 49

Table 2- 6 Percentage of villages with high Fluoride in drinking water in Upper North, Thailand 2010 66

Table 4- 1 Percentage of water sample by types and fluoride level 100

Table 4- 2 Percentage of water sample and household with unsafe drinking water 101

Table 4- 3 Percentage of the village with an endemic area and household use of unsafe drinking-water by district 103

Table 4- 4 Percentage of the village with an endemic area and Percentage of Households using unsafe drinking-water by sub district. 104

Table 4- 5 Baseline demographic and Clinical characteristic of pregnant women ... 113

Table 4- 6 Clinical characteristic of Pregnant women before Delivery 116

Table 4- 7 Clinical characteristic of Newborn 118

Table 4- 8 Comparison of MUF level beteen Low-Expose and Expose. 119

Table 4- 9 Univariate and Multivariate Logistic Regression Analysis of Preterm delivery 121

Table 4- 10 Univariate and Multivariate Logistic Regression Analysis of Low Birth weight 122

Table 4- 11 Comparison Incidence of Preterm delivery between two groups..... 123

Table 4- 12 Comparison Incidence of LBW between two groups..... 123

LIST OF FIGURES

page

Figure 1- 1 Conceptual Framework 7

Figure 2- 1 Fluoride Belt in the world and Country with endemic fluorosis 10

Figure 2- 2 Fluoride metabolic in human 16

Figure 2- 3 Absorption and distribution of fluoride in human 20

Figure 2- 4 pH and urine fluoride excretion in human 22

Figure 2- 5 Spectrophotometer Instrument (Orion 4 Star) 27

Figure 2- 6 Ion Selective Electrode(ISE) Method Instrument (Thermo Scientific Orion 4-Star Benchtop Plus pH/ISE Meter) 28

Figure 2- 7 Ion Selective Electrode(ISE) Method Instrument 29

Figure 2- 8 Transfer and accumulate of fluoride from placenta to fetus 40

Figure 2- 9 Maternal and child health handbook 45

Figure 2- 10 The new WHO antenatal care model 46

Figure 2- 11 Risk assessment criterion of pregnant women at 1-st ANC visit 47

Figure 2- 12 Basic components of ANC visit in Thailand 48

Figure 2- 13 Diagnose of Congenital hypothyroidism (CHT) 51

Figure 2- 14 Situation of Low Birth Weight in Global 63

Figure 2- 15 Situation of LBW and Preterm delivery in Upper North 2013-2015 64

Figure 2- 16 Situation of Fluoride in drinking water in Thailand, 2010 66

Figure 2- 17 Map of fluoride distribution in Lamphun (2014), ... 67

Figure 3- 1 Study design 73

Figure 3- 2 Percentage of village classified by level of fluoride concentration in underground water in Mueang Lamphun Province, Thailand 74

Figure 3- 3 Map of study areas: Meuang Lamphun, Pasang and Ban Thi district 75

Figure 3- 4 Installation Smart System Info Free GPS for Android mobile 83

Figure 3- 5 Geolocation (GPS) measurement by Smart System info (free mobile app.)..... 83

Figure 3- 6 Study design and time of data collection 84

Figure 3- 7 *Data collection and Sample* Requirement..... 84

Figure 3- 8 Screening and Procedures for pregnant women in the research study... 88

Figure 3- 9 Follow up Card for communicate among research assistant and follow up the pregnant women. 89

Figure 3- 10 Umbilical Cord Blood Collection 92

Figure 4- 1 Process of village water supply sampling..... 97

Figure 4- 2 Five types of Village water supply system 98

Figure 4- 3 Levels of fluoride found in different water sources..... 100

Figure 4- 4 Unsafe drinking water in different water sources. 102

Figure 4- 5 The average of water fluoride in sub district level..... 106

Figure 4- 6 GIS data and Google Map showed separate layer (A: one layer) and Mixed (B: Mixed of Layer 5 and 9) 108

Figure 4- 7 GIS data and Google Map showed Mixed of Layer 6, 7 and 8..... 109

Figure 4- 8 Flow of the study participants. 110

ACRONYMS AND ABBREVIATIONS

Abbreviation	Description
ANC	Antenatal care
BW	Body weight
°C	Degrees Celsius
cm	Centimeter
df	Degrees of freedom
dm^3	Cubic decimeter
g	Gram
hr	Hour
ISE	Ion Selective Electrode
IU	International unit
kg	Kilogram
KML	Keyhole Markup Language
KMZ	Zipped file containing one or compressed KML files
L	Liter
LBW	Low birth weight
m	Meter
m^2	Square meter
m^3	Cubic meter
µg	Microgram
mg	Milligram
µL	microliter
µmol	Micromole
min	Minute
mL	Milliliter
mol	Mole
n	Number
ppb	Parts per billion
ppm	Parts per million

Abbreviation	Description
ppt	Parts per trillion
SD	Standard deviation
SE	Standard error, standard error of the mean
sec.	Second
TISAB	Total ionic strength adjustment buffer
U	Unit
V	Volt

CHAPTER I

INTRODUCTION

1.1 Background and Rational

Fluorine is a natural element, it found about 0.3 g/kg of Earth's crust and widely distributed in rocks, coal, clay, and soil. Fluorine is rarely found in the natural as an elemental state but usually occurs in ionic forms as negative or positive electric charge, [1] or combined with other chemicals in a wide variation of minerals, including apatite, cryolite, fluorspar, hornblende, mica and a number of pegmatites such as tourmaline and topaz. [2]

Fluoride has both effects. it's beneficial in reducing the incidence of dental caries. However the adverse effects caused by consumption of groundwater and/or foods growth by water with high fluoride level, or food processed or cooked with a high fluoride water. [3] Consumption water with high fluoride concentration for 10 to 12 years can increase the risk of fluorosis. Children are most at risk to dental fluorosis and people age above 45 years are more vulnerable to skeletal fluorosis. [4]

At present, an appropriate water fluoride intake is recommended for good oral public health to afford the best balance of defense from dental caries as well as controlling the risk of dental fluorosis. World Health Organization [5] recommend intake of fluoride between 0.05 to 0.07 mg/kg-BW/day are accepted, whereas intakes above 0.1 mg/kg-BW/day increase the risk of dental fluorosis. The American Dental Association

Council on Scientific Affairs [6] recommend an optimal fluoride in well water is 0.7 mg/L (ppm), as well as U.S. Public Health Service [7], and also Food and Drug Administration of Thailand. [8]

In Thailand, the safe drinking water is water with fluoride less than or equal 0.7 mg/L Intercountry Centre for Oral Health and Department of Groundwater [9] have surveyed safe drinking water of village in 2010 found that areas with safe drinking water of village less than 5 0 % of total surveys were in Lamphun, Tak, Suphan Buri, Phetchaburi, Samut Songkhram and Samut Sakhon. The study of C. Joon Chuah and colleague in 2014 [10] reported that in Lamphun areas there are 35% of more deep wells contained water with at least 1.5 mg/L fluoride compared with 7% of shallow wells.

Nowadays, insufficient researchers prove that fluoride transplacental to impair fetus [11] and exposure to high fluoride during pregnancy can risk to preterm delivery and low birth weight (LBW).[12-21]. The studied as below;

M. Diouf et al [22] studied fluoride and LBW found that LBW related with pregnant live in endemic areas and water fluoride level consumption, the level of fluorosis related with incidence of LBW adjusted for gender, consanguinity, anemia, hypertension and mothers with moderate dental fluorosis (level 4 of Dean's Index, 100% of surface teeth are affected and attrition) were 3.75-times higher delivery with LBW babies than controls (25.9% vs 6.9%).

Sastry M Gurumurthy et al. [23] studied on maternal fluoride serum, and adverse fetal outcomes found that fluoride level in serum of pregnant is associated with adverse fetal outcomes. There are significant negative associations between maternal serum fluoride level and cord serum fluoride level with birth weight, gestational age, and APGAR score. Also, small negative associations between birth weight, APGAR score and gestational age with placenta fluoride concentrations in the maternal, fetal and peripheral surface. However, significant positive correlations were observed when the maternal serum fluoride was more than 1 mg/L; there was 10.58-times higher risk for LBW, 8.65 times higher risk for preterm delivery and 3.8-time higher risk for low APGAR score. When the cord serum fluoride was more than 0.22ppm, there was 2.76-times increased risk of LBW, 4.6-time higher risk for preterm delivery and 2.5-time higher risk for low APGAR score. Hence increasing of mother fluoride serum would be risk of preterm delivery, LBW and poor APGAR count.

Preterm delivery and LBW [24, 25] are the great Global problem on women's and child's health, including Thailand. Preterm delivery refers to a baby born before 37 weeks of pregnancy complete and LBW refer to newborns weight less than 2,500 grams. WHO [25] estimated that 15 million babies or more than 10% are born with preterm delivery every year and 15 % to 20 % of them are LBW, these numbers are increasing. Almost one million of premature babies die due to problems of preterm birth, several survivors face a lifetime of disability, including learning disabilities, visual

and hearing problems and prematurity is leading cause of Global death in children less than 5 years.

The incidence of LBW in Thailand in the 1980s was about 12%, it decreased and remained at 8% to 9% during 1990 to 2000. However, the target of LBW has been set to less than 7% since 1991. The survey of Multiple Indicator Cluster Survey (MICS) in 2006 reported that incidence of LBW was 9.2% with no difference of housing regional and education of mother significantly but a small difference between poor and rich family (8.5% vs 10% respectively). In some remote areas, the prevalence of LBW is very high.[26]

There are several studies about health effects of fluoride in endemic fluoride areas, most of them studied on dental fluorosis. Fluorosis was found in every region of Thailand, but the prevalence was highest (61%) in Northern, furthermore, concentrations of fluoride in drinking water and urine of samples of local people in Northern were found to be the highest compared to the other regions (Leatherwood et al., 1965). Also studied on dental fluorosis in pregnancy, many studies reported high incidences of fluorosis in Northern Thailand including Lamphun causing by consumed of high fluoride drinking water. [10]

However, lacking data studied on fluoride level in pregnancy and newborn. Moreover, the evidence advocate maternal fluoride level is associated with Preterm delivery and LBW have yet to be well studied in Thailand

1.2 Research Question

1) What are the water fluoride (WF) levels in villages water supplies?

2) How to identify the endemic fluoride areas and normal areas in Lamphun?

3) What are the levels of maternal urine fluoride (MUF) among pregnancy in Exposed and Low-exposed group?

4) Is there different of MUF between Exposed and Low-exposed group?

5) What are the association between MUF level and preterm delivery?

6) What are the association between MUF level and LBW?

7) Is there different of incidence of preterm delivery and LBW between the normal area and endemic area?

1.3 Research Objectives

1.3.1 General Objectives

The aims of this study were measured villages water fluoride and identity into normal and endemic area by geographical mapping information (GIS), measure and compare maternal urine fluoride (MUF) among pregnancy and compared MUF level between Exposed and Low-exposed group. assess the association between MUF with preterm delivery and LBW, to compare the incidence of preterm delivery and LBW between 2the two groups.

1.3.2 Specific Objectives

1) To measure WF level of villages water supplies.

2) To identify village with WF level into normal area and endemic areas and d present by GIS technology and geo-visual map on Google Maps

3) To measure the level of MUF among pregnancy.

4) To compare the MUF level between the Exposed and Low-exposed group.

5) To assess the association between MUF and preterm delivery.

6) To assess the association between MUF and LBW.

7) To compare the incidence of preterm delivery and LBW between the Exposed and Low-exposed group.

1.4 Research Hypothesis

1) The level of MUF in Exposed was higher than Low-exposed group.

2) The level of MUF associated with preterm delivery.

3) The level of MUF associated with LBW.

4) The incidence of preterm delivery and LBW in Exposed were higher than Low-exposed group.

1.5 Conceptual Framework

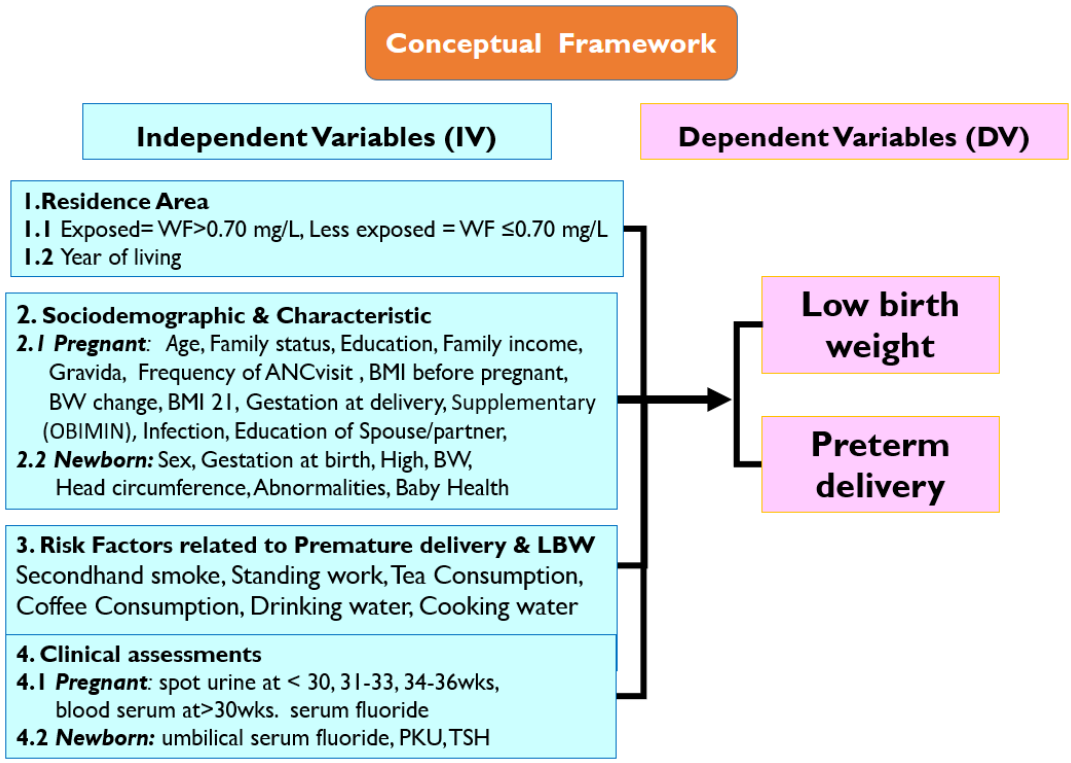


Figure 1- 1 Conceptual Framework

1.6 Benefit and Application

1) The results of this study provided the water fluoride level of village water supplies as the Geographic information system (GIS) and Geo-visual map of endemic fluoride areas. This is useful for health policy authorities, local governments, and villagers and enables collaboration to resolve these issues

2) The findings of this study provided the evidence of fluoride level among pregnant women, newborn, and the incidence of preterm delivery and LBW in an endemic area and normal area.

3) The finding of this study would be useful for an environmental health concern to reducing exposed to fluoride and prevent adverse health effect in vulnerable groups.

4) This study can applicable to endemic fluoride areas in other regional part of Thailand.

1.7 Operational Definition

1) Normal area defines as areas with fluoride level of water supply ≤0.70 mg/L

2) Endemic Areas define as areas with fluoride level of water supply > 0.70 mg/L

3) Exposed group refers to pregnant women who live in endemic areas**.**

4) Low-exposed group refers to pregnant women who live in normal areas

5) Preterm delivery refers to pregnant delivery earlier 37 weeks of pregnancy are completed.; [25]

6) Low birth weight (LBW) refer to newborns weighing less than 2,500 grams, with the measurement taken within the first hours of life. [24]

7) APGAR score refers to a quick test performed on a baby at 1 and 5 minutes after birth, Apgar test is done by a doctor, midwife, or nurse.[27]

8) Newborn refers to a baby at delivery and under 28 days of age [24]

CHAPTER II

LITERATURE REVIEW

2.1 Water Fluoride and Safe Drinking Water

2.1.1 Natural water fluoride

Fluorine has strong tendency to acquire a negative charge, it is one of most reactive and electronegative of all chemical elements.[1]. Fluorine as the elemental state is rarely found in natural, generally found as ionic forms or combined with other chemicals in a wide variation of minerals, including apatite, cryolite, fluorspar, hornblende, mica and a number of pegmatites such as tourmaline and topaz. [2]

Natural Water with high fluoride are typically found at the bottom of high mountains and in areas with geological sediment of the marine with fluoride belts. The Geographical of ***fluoride belts*** [2] are from the Syrian Arab Republic through Jordan, Egypt, Libyan Arab Jamahiriya, Algeria, Morocco, and Rift Valley. Another belt is one stretching from Turkey through Iraq, Islamic Republic of Iran, and Afghanistan to India, northern Thailand, and China, similar areas can be found in Americas, China and Japan. [28], showed in Figure 2-1

Fluorides can be reserved up from land and store in plants, or they may be placed on the upper parts of the plants. The concentration of fluoride accumulate in plants depends on the kind of plant, the type of soil, and level and form of fluoride in the soil. There are well-known that tea plants can accumulate fluoride in leaves.

Animals that eat plant contain with fluoride may accumulate fluoride. However, mostly fluoride accumulates in the bones or shell more than in eatable meat. [29].

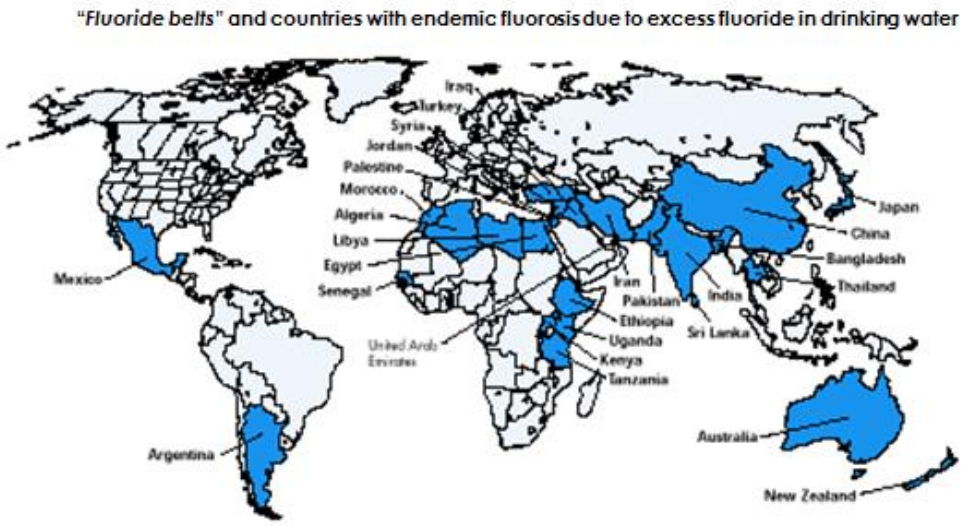


Figure 2- 1 Fluoride Belt in the world and Country with endemic fluorosis
Source http://rampages.us/mediatyphoon/wp-content/uploads/sites/4468/2014/12/Unicef_fl_map.gif

Fluoride does not removed from the water when it is boiled or freezing, except distillation system, but this system is usually used in laboratories, it is not appropriate and expensive for the household.[30, 31]

2.1.2 Fluoridation and safe drinking water

According *to WHO International Standards for Drinking-water (*1958 to 1963) studied and found that consumption of drinking water with high fluoride at 1.0 mg/L to 1.5 mg /L could increase to dental fluorosis in some children and finally effect to skeletal impairment in both adults and children. Fluoridation of 1.0 mg/L fluorine recommends resisting the progress of dental caries in children. The International

Standards of the *Guidelines for Drinking-water Quality (GDWQ)*, was. publish by WHO in 1984. [3, 29]

The first recommendation fluoride value of 1.5 mg/L was in 1993, although the Guidelines determined that there was no indication needed to be revised guideline fluoride value of 1.5 mg/L, but in areas with high natural fluoride levels, the guideline value may be difficult to accomplish and most important to consider climatic conditions, volume of water intake and intake of fluoride from other sources.[31]

The guidance of the American Dental Association Council on Scientific Affairs (ADA, 2010) recommends fluoride level is 0.7 mg/L (ppm) and also recommended by the Pollution Control Department [32] and Food and Drug Administration of Thailand. [33]

WHO [5] has suggested the level of fluoride in drinking water should be adjusted to between 0.5 mg/L to 1.0 mg/L(ppm), depending on the climate. U.S.PHS guidelines [7] mention the maximum acceptable fluoride level in drinking water should be lowered to 0.7 ppm. to prevent the risk of convincing fluorosis due to unknown, and unintentional expose to fluoride from multiple collective sources.

At the present, fluoride of 0.7 mg/L (ppm) is recommended to be an “optimal” level to public health for community water fluoridation to prevent dental caries while can control the risk of dental fluorosis. [5, 6, 8, 34]. The US.EPA recommends that well water should be verified microorganisms and other elements including fluoride level in the minimum for once every three years. [35]

The Standard of Fluoride level in Drinking water is showed in Table 2-1.

Table 2- 1 Standard of Fluoride level in Drinking water.

Source	Fluoride level	Reference
Community water fluoridation	0.7 mg/day	US. PHS (2015)
Community water	0.7 mg/L (ppm)	WHO (2014)
Drinking water	0.7 mg/day	ADA (2010)
Drinking water, bottled water	0.7 mg/L (ppm)	FDA (2010), PDC (2009)

Source: Applied from the US. PHS (2015), WHO (2014), ADA (2010), Thai FDA (2010), PDC (2009)

2.1.3 Safe drinking water

Water safety and quality are fundamental to human development and well-being [3] Providing access to safe water is one of the most effective instruments in promoting health and reducing poverty. Since 1958, WHO has been the authority on drinking water quality, with its Guidelines for Drinking-water Quality (GDWQ) being used as the scientific basis for standard setting in most countries.

In Thailand, the Notification of Ministry of Natural Resources and Environment [36] for Groundwater Quality Standards for Drinking Purpose was published in May 21, B.E.2552 (2009) and Department of Health, Ministry of Public Health (2010)[37]. It's composed of twenty-three parameters, including physical, chemical, toxic elements and bacterial characteristics, showed in Table 2.-2

Table 2- 2 Groundwater Quality Standards for Drinking Purpose

Property	Parameter	Unit	Standard	
			Optimal Value	Max. allowable
Physical	1. Color	Pt-Co	5	15
	2. Turbidity	JTU	5	20
	3. pH Value	-	7.0 - 8.5	6.5 - 9.2
Chemical	4. Iron (Fe)	mg/l	≤ 0.5	1.0
	5. Manganese (Mn)	mg/l	≤ 0.3	0.5
	6. Copper (Cu)	mg/l	≤ 1.0	1.5
	7. Zinc (Zn)	mg/l	≤ 5.0	15.0
	8. Sulphate (SO4)	mg/l	≤ 200	250
	9. Chloride (Cl)	mg/l	≤ 250	600
	10. Fluoride (F)	mg/l	≤ 0.7	1.0
	11. Nitrate (NO3)	mg/l	≤ 45	45
	12. Total hardness as CaCO3	mg/l	≤ 300	500
	13. Non Carbonate hardness as CaCO3	mg/l	≤ 200	250
	14. Total dissolved solids	mg/l	≤ 600	1,200
Toxic	15. Arsenic (As)	mg/l	none	0.05
	16. Cyanide (CN)	mg/l	none	0.1
	17. Mercury (Hg)	mg/l	none	0.001
	18. Lead (Pb)	mg/l	none	0.05
	19. Cadmium (Cd)	mg/l	none	0.01
	20. Selenium (Se)	mg/l	none	0.01
Bacterial	21. Standard Plate Count	Colonies/cm^3	≤ 500	-
	22. Coliform Bacteria	MPN/100 cm^3	≤ 2.2	-
	23. E.Coli		none	-

Notes: Pt-Co = Platinum Cobalt Scale, JTU = Jackson Turbidity Unit, MPN = Most Probable Number

Source: Notification of the Ministry of Natural Resources and Environment, B.E.2552 (2008) issued under the Groundwater Act, B.E.2520 (1977) which was published in the Royal Government Gazette, Vol. 125, Special Part 85D dated May 21, B.E.2552 (2009).

Notification of the Department of Health, ministry of Public Health B.E 2553(2010)

2.2 An Adequate Fluoride for Human

The American Dental Association [38] mention that the minimum adequate of total ingestion intake is determined to 3.0 mg/day for women including during pregnancy and 0.05 mg /kg-BW/day for infants and children over 6 months. The adequate intake of minimal safe level exposure dose of 0.01 mg/kg-BW/day for infants and 0.05 mg/kg-BW/day for other age groups., maximum estimated exposure [39] for infants, 0.17 mg/kg-BW/day, 0.19 mg/kg/day for children and 0.10 mg/kg/day for adults.

WHO [5] recommend fluoride intakes between 0.05 and 0.07 mg/kg-BW/day are accepted, whereas intakes above 0.1 mg/kg-BW/day increase the risk of dental fluorosis. showed in Table 2-2

Table 2- 3 Recommendation of adequate fluoride intake

Group of people	Fluoride level	Reference
Infant to less than 6 months	0.01-0.17 mg/kg/day	ATSDR (1993); FNB (1997), NRC (2001), ADA (2015)
children	0.01 - 019 mg/kg/day	ADA (2015)
Women and pregnant	3.0 mg/day	ADA (2015)
Adult	0.05 - 0.10 mg/kg/day	ADA (2015)
Accept	0.05 and 0.07 mg/kg-BW	WHO (2014)
Risk	above 0.1 mg/kg-BW/day	WHO (2014)

Source: Applied from ATSDR (1993), FNB (1997), NRC (2001), ADA (2015), WHO (2014)

Juntarawijit, C. (2003) studied Health Risks of Fluoride Exposure in Drinking Water revealed that WF level which is suitable for Thai people should be 0.5 mg/L. This result could be explained that the size of body in Thai people was lower than the western people. Moreover, the climate change temperature and behavior of water consumption were different between the two group. Thai people generally have low body weight but consume a lot of water due to high of weather temperature. Thus, the study suggested that the optimal WF level in tap water should be 0.5 mg/L.

2.3 Metabolism of Fluoride

The overall metabolism of fluoride in human can be divided into 3 steps: absorption, distribution, and excretion, showed in Figure 2-2

1) Exposure and Absorption

1.1) ***Fluoride Exposure:*** After intentional or unintentional intake, mostly fluoride is absorbed in the gastrointestinal tract or enters the body through the lungs. After ingestion, within minutes we can detect increased plasma fluoride levels, which mean that fluoride is readily absorbed from the stomach. The peak level usually occurs during the first hour; it can be less than 30 min. Then the plasma level will rapidly decline due to bone uptake and urinary excretion. If the amount of fluoride ingested is small.

Approximately 75% to 90% of the fluoride ingested is absorbed from the gastrointestinal tract, with higher proportions from waters than from objects. The half-time for absorption is approximately 30 minutes, the peak plasma concentrations usually occur within 30 to 60 minutes. Absorption across the oral mucosa is limited and probably accounts for less than 1 % of the daily intake. Absorption from the stomach occurs freely and is inversely related to the pH of the gastric contents, and most of the remaining fluoride that enters the intestine will be absorbed quickly. High concentrations of dietary calcium and other cations that form insoluble complexes with fluoride can decrease fluoride absorption from the gastrointestinal tract.

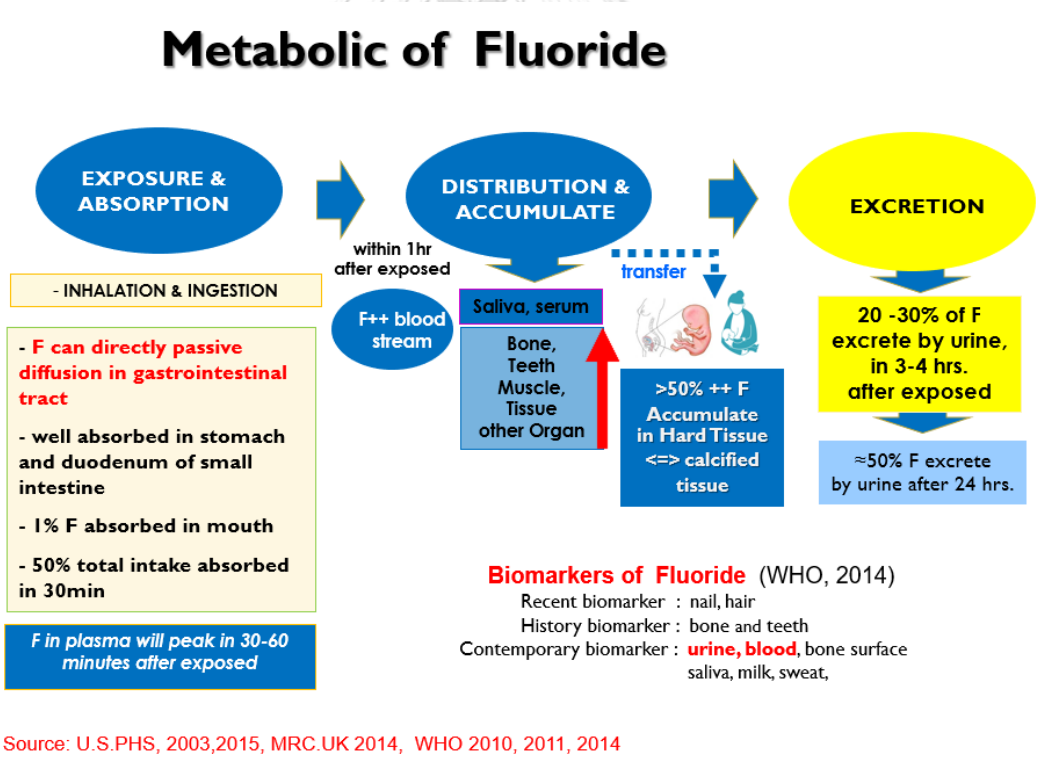


Figure 2- 2 Fluoride metabolic in human

Source : Applied from [3, 5]

Plasma is the central compartment where the ions pass before being distributed and eliminated. Roughly 50% of the absorbed fluoride is excreted in the urine during the following 24 hours. Most of the remaining 50% will become associated with calcified tissue showed in Figure 2-2

Human are exposed to fluoride from natural and/ or human activity sources. The consumption of food products, drinking water and other beverages is normally the main route of exposure. High intakes of fluoride have been well-known in geographical areas worldwide with fluoride belt where the surrounding environment is high natural fluoride. [40]

1.2) Source of fluoride Exposure: Word Health Organization [41] mention about source of fluoride can release to environment and people exposure to fluoride in indifferent way as below;

(1) Natural sources: Fluoride is commonly spread in Earth's crust, generally as the minerals cryolite, fluorspar and fluorapatite. Some regions have greatly high concentrations of fluoride. Fluoride can occur in the air as gaseous form, complicated to particles or in aerosols that can be transported by wind over long distances before being dropped. Fluoride can also be moved by water, naturally complexed with aluminum, but it is immovable in the soil.

***(2) Industrial processes*:** Most of the fluoride in urban release from aluminum smelters industrial approximate 10%. Another main source of fluoride release to environmental from chemicals in Agriculture such as phosphate fertilizer production

and pesticides. Phosphate fertilizers or fluoride-containing pesticides were polluted fluoride into the soil. Other sources comprise of glassworks, drain fumes and the production of metals such as nickel, steel, copper and, bricks, ceramics and adhesives. Hydrogen fluoride is used in the semiconductor industry and in commercial laundries which highly soluble in water with very acid.

(3) ***Drinking water*** in many countries, groundwater naturally contains high fluoride levels, therefore intake of fluoride in drinking water exceeds than food. The intake is determined by fluoride level in water and daily water consumption. Water fluoridation has been adopted by several countries as a cost-effective public health to prevent dental caries. The dental health benefits are obtained when the concentration of fluoride in drinking water is not over than 0.7 mg/l.

(4) Food. is the primary source of fluoride, but Levels of fluoride is commonly low in meat, fruit and vegetables and small of fluoride in all foodstuffs.

Aod and colleague (1997) [8] study fluoride in vegetable found that the concentration of fluoride was between 0.1mg/L to 0.6 mg/L, the level of fluoride depend on the type of vegetable. Chinese Chives (gui-chai) is highest of fluoride (0.53 mg/L) and Winged bean (tùa poo) Is the lowest of fluoride (0.02 mg/L)

However, fluoride levels can be high in bones of animal and fish such as salmon and sardines or tea leaves, drinking of brick tea can lead to high fluoride consumption.

(5) Dental care products and supplementary. In many countries, dental care products can contain with fluoride. WHO and American Dental Association

Foundation [5, 6] recommend fluoride level of toothpaste, mouthwash, mouth rinse can contain between 1.0 to 1.5 mg F/gram and based on estimates of an average ingestion of 0.5 grams. Dental products are one more major source of fluoride intake, mostly in children. Using toothpaste in children age 2 to 5 years old could result in intake of 0.50 - 0.75 mg F/use and 0.4 - 1.2 mg F/use in children age 7 to 13 year. Fluoride in mouthwash could give 0.2 to 0.4 mg F/use. Fluoride tablets and topical gels are extra sources of fluoride exposure [4]. In Thailand, FDA recommend fluoride in toothpaste was not more than 1,100 ppm [8], 250 – 2,400 ppm in mouthwash and 0.25 mg/tab , 1 mg/tab in fluoride tablet.

Factors affecting fluoride intake: Several factors that may influence fluoride intakes, such as special dietary habits, socioeconomic status, and environmental conditions; these factors may be unknown, the study population should be selected at random. Where the conditions are known (e.g. in regions with a high water fluoride), a careful selection of the study population must be made from multiple sites.

2) Distribution and Accumulate

After ingestion within minutes, fluoride will rapidly absorb from the stomach and increase in plasma. The peak level usually occurs during the first hour or it can be less than 30 min. Then plasma level will rapidly decline due to bone uptake and urinary excretion. If the amount of fluoride ingested is small (not more than a few mg), the plasma level will return to normal within 3-6 hours. It was showed in Figure 2-4

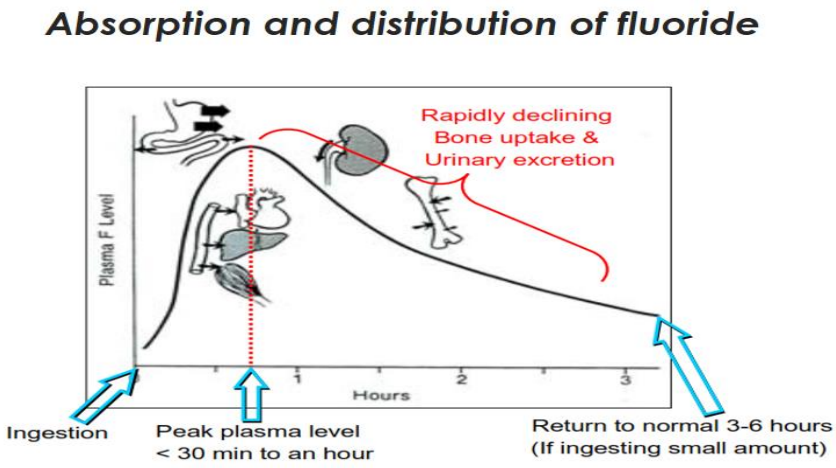


Figure 2- 3 Absorption and distribution of fluoride in human

Source: Applied from [3, 5] & [42]

The distribution of fluoride concentrations is in plasma or extracellular fluid and intracellular fluid of most soft-tissues. Intracellular fluoride concentrations are lower, but they change proportionately and simultaneously with those of plasma, exception of the kidney, which concentrates fluoride within the renal tubules, tissue-to-plasma fluoride ratios are less than 1.0. Almost 50% of absorbed fluoride is taken up by the calcified tissues. Fluoride is an avid calcified tissue seeker.

There are two general forms of fluoride in human plasma. The ionic form, detectable by the ion-specific electrode, is the one of interest in dentistry, medicine, and public health. Ionic fluoride is not bound to proteins, to other components of plasma, or soft tissues. The other form consists of several fat-soluble organic fluorocompounds, which can be contaminants derived from food processing and packaging. The concentration of ionic fluoride in soft and hard tissues is directly related to the amount of ionic fluoride intake, but that of the organic fluorocompounds is not

provided that water is the major source of fluoride intake, fasting plasma fluoride concentrations of healthy young or middle-aged adults, expressed as micromoles per liter (μmol/l), are roughly equal numerically to the fluoride concentrations in drinking water, expressed as milligrams per liter (mg/l). Plasma-fluoride concentrations tend to increase slowly over the years.

3) Excretion

Some diuretics (mannitol or saline) increase fluoride excretion because the diuretic dilutes the tubular fluid, thus increases pH. In conclusion, tubular reabsorption of fluoride is primarily related to urinary pH and only secondarily related to urinary flow rate. urinary pH.

Usually the pH of the interstitial fluid is relatively high, about 7, so Hydrogen Fluoride (HF) dissociates and releases fluoride ions. Fluoride ions can then diffuse into capillaries and return to plasma. As a result, less fluoride is excreted when tubular fluid and urine is acidic. When tubular fluid becomes more alkaline [43], the fraction of fluoride in HF form is less, and not many Hydrogen Fluoride diffuse into the interstitial fluid. Thus, less fluoride diffuses into capillaries and return to plasma. The large amount of ionic fluoride which cannot diffuse through the tubular membrane is left in the renal tubule to be excreted. As a result, more fluoride is excreted when tubular fluid and urine is alkali.

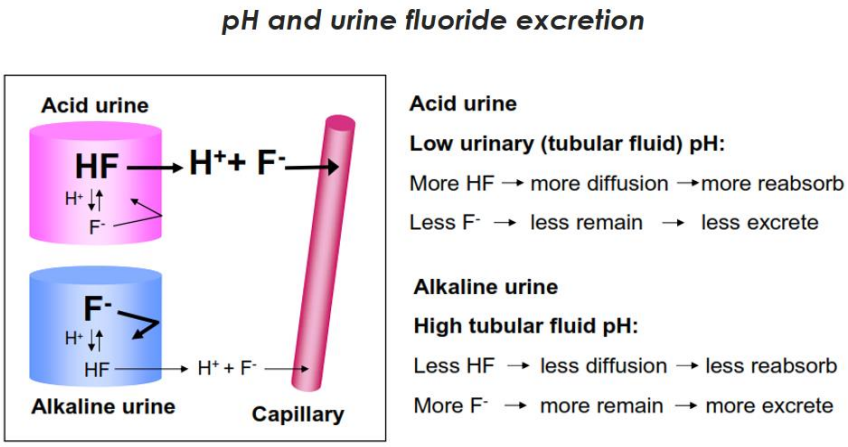


Figure 2- 4 pH and urine fluoride excretion in human

Source: Applied from [3, 5] & [42]

2.4 Biomarker of Fluoride

Total fluoride exposure can be monitored by evaluating fluoride level in plasma, urine or ductal saliva. The amount of fluoride in these biological liquids is indicative of the level of total fluoride exposure. After intake of fluoride – whether through drinking water, dietary components or fluoride supplements – the plasma fluoride level will rise immediately, but it starts to decrease within 30–60 minutes, and reverts to the original level within 3–6 hours [5].

WHO: [5] recent reviews have considered the suitability biomarkers for fluoride exposure. The biomarkers at present are contemporary biomarkers, present or very recent biomarker and historical biomarker. As below:

1) Contemporary Biomarkers

There are several fluids used to determine concentration of fluoride in various partitions of body [40]. The contemporary biomarkers of fluoride are in urine, plasma

serum, saliva, bone surface, breast milk, sweat. Some of these are readily accessible and useful for determining the current exposure of fluoride. The values achieved are not direct measure of fluoride accumulation in body but indicate the burden of body because incomplete to defined relation between fluoride concentrations in bone and extracellular fluids.

Serum fluoride level can be used as a biomarker for assessment to indicator of fluoride intake into the human. [44]. Moreover, serum fluoride level use to controlling and prevention of fluorosis in each area.

The stable distribution of fluoride concentrations is in plasma and soft-tissues. The Intracellular are lower fluoride concentrations, but they change proportionately and simultaneously with those of plasma, exception of the kidney, which concentrates fluoride within the renal tubules, tissue-to-plasma fluoride ratios are less than 1.0. Almost 50% of absorbed fluoride is taken up by the calcified tissues. Fluoride is an avid calcified tissue seeker.

WHO [5] recommend urinary fluoride is a widely accepted biomarker of recent fluoride exposure and has frequently been used as an indicator of fluoride exposure from drinking water. The concentration of urinary fluoride excretion is related to plasma, but variations in urinary flow and pH. The 24-hour collection of urine is more representative for fluoride level in urine but the measurements on early morning, second void urine samples can also be used to assess fluoride excretion. [45]

2) Recent Biomarkers

The concentrations of fluoride in nails and hair appear related to intake over longer periods of time. The fluoride level indicates the reflection of the average plasma fluoride concentrations over time. Nails grow at about 0.1 mm/day so the average level of fluoride intake over a 1-3-week period can be estimated. Fluoride in hair could be used to estimate intake over longer periods. Refinements of the sampling methods for these human tissues and improved testing technologies are needed. Additional research should clarify the physiological factors that can influence fluoride uptake and accumulation in these tissues.

Nalini Parimi and colleague [46] studied hair as biomarker of fluoride exposure. The samples of hair were taken from occipital subject to analysis fluoride by a fluoride ion electrode. The result found that the level of water fluoride concentration correlated with levels of fluoride concentration in hair.

3) Historic Biomarkers

The body burden of fluoride is best reflected in the calcified tissues, though enamel is not the tissue of choice because most of its fluoride was taken up during tooth formation. After tooth eruption, exposure to widely fluctuating concentrations of fluoride in the oral cavity significantly affects fluoride levels in the surface layers of enamel, where the highest concentrations of fluoride are found. Bone fluoride concentrations are much better indicators of long-term fluoride exposure and body burden, though fluoride is not uniformly distributed throughout bone. For example,

cancellous bone has higher fluoride concentrations than does cortical bone. The fluoride concentrations of dentine are similar to those of bone and, as in bone, they tend to increase over the years provided that fluoride intake does not decline. Dentine, especially coronal dentine, maybe the best marker for the estimation of chronic fluoride intake and the most suitable indicator of the body burden. The tissue does not normally undergo resorption, it is more easily obtained than bone, it seems to continue accumulating fluoride slowly throughout life, and it is permeated by extracellular fluid. Dentine is usually protected from exposure to fluoride in the oral cavity by the covering enamel or cementum.

4) Fluorosis as a Biomarker

Epidemiological studies by Dean and colleagues in the 1930s clearly demonstrated the relationship between dental fluorosis in humans and the level of fluoride in water supplies. These and other studies have shown that there is positive relationship between level of fluorosis, plasma, bone fluoride levels and concentration of fluoride in drinking water. These studies suggest that fluorosis can be used as a biomarker for level of fluoride exposure, though dental fluorosis is a reflection of fluoride exposure only during the time of enamel formation. Increasing of fluorosis in both fluoridated and non-fluoridated communities has been used to indicate level exposure to fluoride in the drinking water. Then fluorosis was using as a biomarker

2.5 Analysis of Fluoride

2.5.1 TISAB Analytical Method

A Total Ionic Strength Adjustment Buffer *(TISAB)* is analytical methods to detect and measure fluorides in metabolites and biomarkers of exposure in people or other fluorides media.

TISAB is used to adjust samples and standards to the same ionic strength and pH; this allows the concentration, rather than the activity, to be measured directly and often read directly off a meter. The pH of the buffer is about 5, a level at which fluoride is the predominant fluorine-containing species. The buffer contains cyclo-hexylene-dinitrilotetraacetic acid, which forms stable complexes with Fe(III) and Al(III), thus removing interferences by freeing fluoride ions form complexes with these ions (NIOSH 1994; Schamschula et al. 1985; Tusl 1970).

2.5.2 Analytical method and Instrument

1) Ultraviolet visible spectroscopy (**UV-Vis)** or **ultraviolet visible spectrophotometry** (**UV/Vis)**

Spectrophotometric methods are widely used in the determination of fluoride because of advantages such as simplicity, convenience, accuracy and reproducibility. [47]. This method refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet visible spectral region. It uses light in the visible and adjacent (near-UV and near-infrared ranges).

Figure 2- 5 Spectrophotometer Instrument (Orion 4 Star)

Source: Applied from [48]

The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. [49] Spectrophotometer is an instrument to measures absorbance and it might use either filters, a monochromator or spectrometer for absorbance wavelength selection.

2) Ion Selective Electrode (ISE)

The Ion Selective Electrode (ISE) method is the most widely used method for determining fluoride levels in the environmental media. ISE methods are simple to perform and have good accuracy and sensitivity. Fluoride-specific electrodes are commercially available. The method detects only free fluoride ions in solution.

Because of the inherent restriction of this technique, several approaches have been recommended to prepare the sample for analysis.

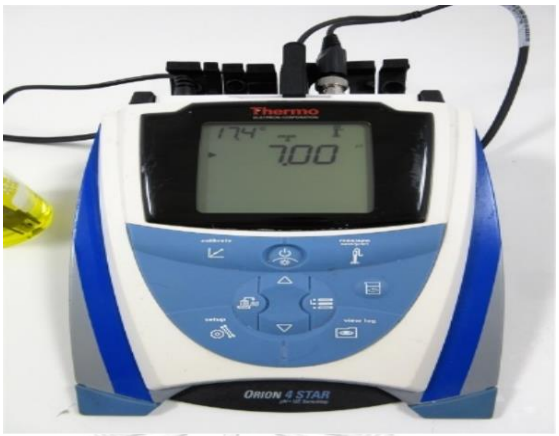


Figure 2- 6 Ion Selective Electrode(ISE) Method Instrument (Thermo Scientific Orion 4-Star Benchtop Plus pH/ISE Meter)

Source: Applied from [48]

Ion Selective Electrodes (ISE) are membrane electrodes that respond selectively to ions in the presence of others. These include probes that measure specific ions and gases in solution. The most commonly used ISE is the pH probe Other ions that can be measured include fluoride, bromide, cadmium, and gases in solution such as ammonia, carbon dioxide, and nitrogen oxide.

The use of Ion Selective Electrodes in environmental analysis offer several advantages over other methods of analysis. First, the cost of initial setup to make analysis is relatively low. The basic ISE setup includes a meter (capable of reading millivolts), a probe (selective for each analyte of interest), and various consumables used for pH or ionic strength adjustments.

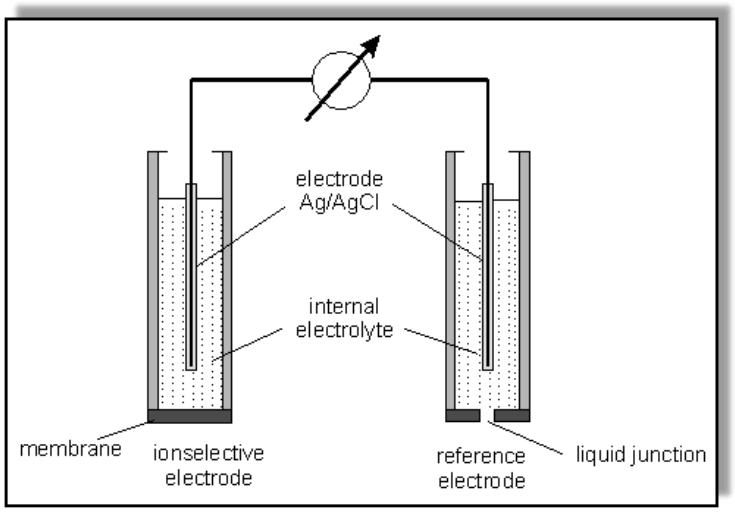


Figure 2- 7 Ion Selective Electrode(ISE) Method Instrument

Source: Applied from College of Arts and Sciences, New Mexico State University (2006)

The expense is considerably less than other methods, such as Atomic Adsorption Spectrophotometry or Ion Chromatography. ISE determinations are not subject to interferences such as color in the sample. There are few matrix modifications needed to conduct these analyses. This makes them ideal for clinical use (blood gas analysis) where they are most popular; however, they have found practical application in the analysis of environmental samples, often where in-situ determinations are needed and not practical with other methods. A large number of indicator electrodes with good selectivity for specific ions are based on the measurement of the potential generated across a membrane. Electrodes of this type are referred to as ion-selective electrodes. The membrane is usually attached to the end of a tube that contains an internal reference electrode. This membrane electrode and an external reference electrode are then immersed in the solution of interest. Since the potentials of the

two reference electrodes are constant, any change in cell potential is due to change in potential across the membrane.

2.5.3 Units of Measuring Fluoride and Analytical method

WHO [5] recommend urinary fluoride is a widely accepted biomarker of recent fluoride exposure and has frequently been used as an indicator of fluoride exposure from drinking water. The concentration of urinary fluoride excretion is related to plasma, but variations in urinary flow and pH. The 24-hour collection of urine is more representative for fluoride level in urine but the measurements on early morning, second void urine samples can also be used to assess fluoride excretion. [45]

The standards for urinary fluoride concentration (mg/L; ppm) for all ages, for time-controlled urine collections; lower and upper margins show in Table 2-4

Table 2- 4 Units Normally Used for Measuring Fluoride

Medium	Unit	Equivalent
Water	1 ppm	1 mg/L
Plasma	Mol/L	0.019 mg/L
Bone ash	1 ppm	1 mg/kg
	1%	10,000 mg/kg

Source: applied from [50]

2.6 Factors Effect to Fluoride Intake, Excretion and/or Fluoride-reduced Toxicity

Factors affecting fluoride intake: Several factors that may influence fluoride intakes, such as special dietary habits, socioeconomic status, and environmental conditions; these factors may be unknown [51]. Thus, study population in the research should be selected at random. Where the conditions are known (e.g. in regions with a high water fluoride), a careful selection of the study population must be made from multiple sites.

Factor affecting fluoride excretion: *high vegetable intake diet* can influence on urinary fluoride excretion. In people who consuming diets high in vegetables, urine excretion is more alkaline than urine excreted by those consuming a diet high in meats. Higher alkalinity can affect in excretion of a higher proportion of ingested fluoride. Thus, people with high vegetable intake will relatively with high fluoride excretion, it does not certainly imply a high fluoride intake [5], showed in Figure 2-4

Factors Effect to Fluoride-induced Toxicity: The study of Verma, R. J and Sherlin, D. M. (2011) [52, 53] found that intake of *vitamin C* (50 mg/kg body weight) and *vitamin E* (2 mg/0.2 ml olive oil/animal/day) from day 6 to 19 of gestation along with NaF significantly ameliorates NaF-induced reductions in body weight, feed consumption, absolute uterine weight (only with vitamin E treatment) and number of implantations. As compared with NaF-treated alone, the total percentage of skeletal and visceral abnormalities were significantly lowered in fluoride plus vitamin C treated

animals. Vitamin E was less effective. These findings suggest that vitamin C significantly reduced the severity and incidence of fluoride-induced embryotoxicity in rats.

Also, the study on vitamin D (2 ng/0.2 ml olive oil/animal/day) treatment significantly ameliorated the fluoride-induced reductions in body weight, feed consumption and absolute uterine weight. As compared with fluoride-treated alone, the total percentage of skeletal and visceral abnormalities observed in fetuses was significantly lowered in fluoride plus vitamin D-treated animals. These findings suggest that vitamin D treatment significantly reduced the severity and incidence of fluoride-induced embryotoxicity. The ameliorative effect of vitamin D against skeletal and visceral abnormalities could be due to stimulation of intestinal absorption of calcium and phosphate, thus raising the plasma calcium and phosphate concentrations.[52, 53]

2.7 Effect of Fluoride

2.7.1 Effect on Human

Fluoride has both effects. it' s beneficial in decreasing the incidence of dental caries, however the adverse effects were cause by intake with height fluoride level or high fluoride water for long time.[5]. The US EPA mention the Reference Dose (RfD) of fluoride in the Integrated Risk Information System (IRIS) is 0.06 mg/kg/day [54]. The lethal dose of fluoride in adult approximately to 5 - 10 g (32 - 64 mg/kg-BW); the minimum dose of 5 mg/kg-BW has been considered that could be leading to acute health effects. Gessner, Whitford and et al (1994) [55] reported that case of death due

to acute fluoride poisoning resulting from drinking water was approximately to have invested approximately 17.9 mg/kg-BW.

1) Acute toxicity

Acute effects of fluoride occurred by intake high fluoride, the violence is from mild to severe, or even death. Fluoride intake is a direct cellular poison, which binds calcium and interferes with the activity of proteolytic and glycolytic enzymes. Ingested fluoride reacts with gastric acid to produce hydrofluoric acid in the stomach.

The acute toxicity of fluoride depends on type of composite that was ingested. Generally, inorganic fluorides salt solution is more toxic (e.g., sodium fluoride) than unsolvable fluoride. (e.g., calcium fluoride) (WHO, 1984). The effects including nausea, vomiting, abdominal pain, diarrhea, fatigue, drowsiness, coma, convulsions, cardiac arrest and death seizures and muscle spasms may also occur. [55, 56] Death due to respiratory paralysis is a possibility. Severe tissue damage, respiratory effects, cardiac arrest and deaths have been noted in case reports of individuals exposed accidentally to hydrofluoric acid through dermal contact (Buckingham, 1988; Upfal & Doyle, 1990; Bordelon et al., 1993).

The gastrointestinal effects occurred following amounts of fluoride ingestion, the acute toxic arise from the corrosive action of hydrofluoric acid, which is produced within the acidic environment of the stomach (Spak et al., 1990; Whitford, 1990; Augenstein et al.,1991). Damage to the gastric mucosa (e.g., hemorrhage, loss of epithelium) has also been observed in human volunteers administered acidulated

phosphate fluoride gels (Spak et al., 1990) or sodium fluoride solutions (Spak et al., 1989). Some individuals may be unusually hypersensitive to stannous fluoride, as manifested by ulcerations in oral cavity after topical treatment (Razak & Latifah, 1988).

Cardiac arrest following accidental exposure to high levels of fluoride has been attributed to the development of hypocalcemia and/or hyperkalemia (Cummings & McIvor, 1988; Augenstein et al., 1991; ATSDR, 1993). The acute effects of fluoride upon the central nervous system may be due to fluoride-induced hypocalcemia and the inhibition of cellular enzymes (Augenstein et al., 1991). Respiratory effects (e.g., hemorrhage, pulmonary edema, tracheobronchitis, shortness of breath) have been observed in individuals following inhalation of hydrogen fluoride (Dayal et al., 1992; ATSDR, 1993).

The acute effects of fluoride inhalation are severity of respiratory tract irritation, with cough, suffocate and pulmonary edema. Severity of burns or continued visual failings could result from eye and skin expose, this exposure can be deadly [57].

2) Chronic toxicity

Fluoride could fast spread to intracellular and extracellular water of tissues by systemic circulation; however, in people and laboratory animals, about 99% of total body burden of fluoride is retained in teeth and bones. In teeth and skeletal, fluoride is combined into the crystal lattice.[40]. Adverse effects of fluoride occurred by repeated or prolonged exposure intake high fluoride, the violence is follows:

2.1) Effect on teeth and bone: The well-known occurrence of a strong affinity between fluoride and biological apatite is based on the ease of chemical substitution of hydroxyl component of calcium hydroxyapatite by fluoride. Pure fluorapatite contains approximately 3.7% fluoride; up to about one-third of the total hydroxyl ions in enamel can be replaced by fluoride ions. Normal human apatite tissue, i.e. bone and tooth tissue, never approximates to pure fluorapatite, although fluoride substitution varies considerably, being dependent on the tissue-fluoride environment at the time of calcification. Once formed, the apatite/fluorhydroxyapatite remains chemically stable until the tissue is resorbed, remodelled, or otherwise metabolized. A small amount of fluoride increase is possible by diffusion and adsorption of fluoride to the crystal structure.

2.2) Fluoride in teeth: The fluoride content of tooth tissues reflects the biologically available fluoride at the time of tooth formation; in the bulk of the enamel, once formed, it remains constant, in contrast to the fluoride levels in bone, which continue to accumulate throughout life. Post-eruptive change is reflected in the outer layer of enamel owing to diffusion of fluoride from the oral environment (i.e. saliva, ingested materials, dental plaque and therapeutic applications). The pulpal surface of dentine also shows post-eruptive change, with an increasing of fluoride are related to the final stages of dentine formation as well as to physiologically stimulated secondary dentine. Most fluoride in enamel is a historical catalogue of the prevailing environmental levels of fluoride available at the time of tooth development; it is

unlikely to reflect contemporary or other post-eruptive periods. The characteristics of fluoride distribution in teeth are a relatively high concentration of 500-4000 mg/kg in surface enamel (approximately 50 m) and a lower concentration (50-100 mg/kg) in deep enamel.

S Takizawa (2014) [58] studied source of F intake in various foodstuffs, meat, vegetables, and rice in Lamphun found that rice prepared with cooking water from the piped water supply by washing several times and being soaked overnight before being steamed, contained the highest amount of fluoride. When soaking sticky rice in water with F concentration 0–15 mg/L for 24 hours, there was a strong correlation between the F level in the soaking water and the F in the rice, the maximum F content of 10 mg/kg of rice (wet weight). However, fluoride in the rice cooked in households was slightly less than the rice samples used in the soaking experiments. The correlation of fluoride between cooking water and cooked rice was not as strong. The fluoride level of cooked rice is not affected only WF by cooking water for soaking, but also by cooking methods and soaking time.

2.7.2 Effect on Laboratory Animal

In 1995, Mullenix and collogue [59] studied neurotoxic effects of fluoride in laboratory rats. The result showed that rats given drinking fluoride water are increasing of plasma fluoride concentrations in range. The suffer neurotoxic effects differ allowing to dose of fluoride when given to the rats - as adult or young animals, or through

placenta before birth. Rats exposed before birth were born hyperactive and remained so during their lives. Rats exposed as young or adult animals showed depressed activity. Then in 1998, Guan and colleague [60] had studied as Mullenix to make more understanding in the mechanism underlying the effects and found that some key chemicals in membrane of brain cells were substantially dwindling in rats given fluoride when compared to rats did not get fluoride.

2.6.3 Effect on Environment

Waugh reported that the US EPA identify fluoride as one of the twelve critical pollutants [20]. The US Agency for Toxic Substances and Disease Registry [56] identify fluoride is one of the priority list of hazardous substances that pose a risk to environment and human health because It only change its form and cannot be shattered in the environment; it can. The US Center for Disease Control and Prevention [35] mention fluoride is one of Ten Great Public Health Achievements in US between 1900 to 1999.

The European Commission Scientific Committee on Health and Environmental Risks [61] mention that fluorides in water is associated with numerous elements existing in each type of water, mostly with aluminum in freshwater, magnesium and calcium in seawater, and resolve into the residue where they are strongly attached to sediment particles. When dropped on land, fluorides are toughly retained by soil, establishing tough associations with soil components. Discharge removes only a small amount of fluorides from soils.

2.8 Effect of Fluoride in Pregnant

2.8.1 Metabolism

Fluoride metabolism and level of fluoride in pregnant woman is changing with the progress of pregnancy follow the gestational age. [62]. The critical period of fetus developing will progress and related with preterm delivery at 10, 28 and 33 weeks of gestational age. [63]

When gestational age 10 weeks or little more, the embryo is now called a fetus, which means 'offspring'. In this gestational age, the process of bone is forming, fetus is about 3.1 centimeters long from crown to rump and weighs less than 4 grams. She may be small, but she's very active, swallowing fluid and kicking her new limbs. Fluoride is useful and essential for process of bone developing of fetus in this period. Fluoride balance in infants can be positive or negative during the early months of life, depending on whether intake is sufficient to maintain the plasma concentration that existed at the time of birth [64].

At 28 weeks of gestational age, fetus weighs a little more than 1kg, may measure up to 38 centimeters from top to toe. In this time the developing of bone is more complete, the little of fluoride enough for developing of bone.

At 33 weeks of gestational age, fetus weighs about 2kgs and measures up to 44 centimeters from head to toe. She may already be getting ready for birth by turning upside-down.

The study of Deena B.Thomas et al conducted in Mexico city in 2016 [65] found that mean urinary fluoride levels was 0.91 mg/L, and it were not statistically different across three stages of pregnancy. Fluoride levels correlated across the stages of pregnancy studied, with stronger correlations between neighboring stages. Urinary fluoride changed as pregnancy progressed with levels increasing until ~23 weeks and then decreasing until the end of pregnancy.

J Opydo-Szymaczek and M Borysewicz-Lewicka studied a case control on fluoride in non-pregnant and pregnant women in Poland (2005) [63] where the level of F in drinking water ranges from 0.4 to 0.8 mg/L. They found that mean fluoride concentrations in fasting morning urine samples (1.28 mg/L) and 24-hour collections (1.340 mg/L) of the same woman do not differ significantly ($p>0.05$). The mean fasting morning urinary F levels in the 28th week of pregnancy were significantly lower ($p<0.01$) than in the 33rd week (0.653 mg/L vs. 0.838 mg/L, respectively). In the control group F values were higher than in the study group (mean = 1.300 mg/L) ($p< 0.01$)

2.8.2 Transplacental fluoride

At the present, many studies reported that fluoride can cross the placenta and is transferred from mother to fetus [66]. Fluoride is eliminated from the body primarily in the urine. In infants, about 80–90% of a fluoride dose is retained; in adults, the corresponding figure is approximately 60%. These values can be altered by alterations in urinary flow and urinary pH. [40, 67-72].

Moshe Ron et al (Israel) [73] study fluoride concentration in amniotic fluid, fetal cord and maternal plasma found that Maternal and fetal plasma fluoride were not different significantly. In the older gestation age group, fetal cord plasma fluoride was higher than maternal plasma significantly. An amniotic fluid fluoride at term was higher than mid-trimester pregnancy, significantly. This higher concentration may imply higher fetal urinary excretion of fluoride at term due to the lower appropriation of fluoride as the process of bone calcification is more complete.

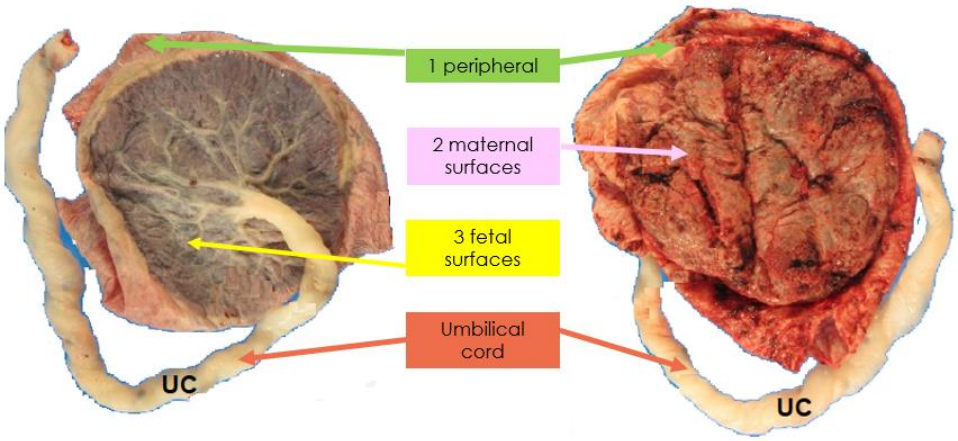


Figure 2- 8 Transfer and accumulate of fluoride from placenta to fetus
Source: Applied from [23]

Basha, P. M. et al. (2011) [11] studied rats on exposure to high fluoride (100 and 200 ppm) to assess neurotoxic potential of fluoride in discrete areas of the brain in terms of lipid peroxidation and the activity of antioxidant enzyme system. The rats were given fluoride through drinking water (100 and 200 ppm) and maintained subsequently for three generations. Fluoride treatment significantly increased the lipid peroxidation and decreased the activity of antioxidant enzymes viz, catalase,

superoxide dismutase, glutathione peroxidase, glutathione S-transferase, and glutathione level in first-generation rats and these alterations were more pronounced in the subsequent second and third-generation rats in both the doses tested. Decreased feed and water consumption, litter size and organ (brain) somatic index, marginal drop in body growth rate and mortality were observed in all three generations. Decreased antioxidant enzyme activity and increased malondialdehyde levels found in the present study might be related to oxidative damage that occurs variably in discrete regions of the brain. Results of this study can be taken as an index of neurotoxicity in rats exposed to water fluoridation over several generations.

Gurumurthy Sastry, et al, [68] study role of placenta to combat fluorosis in endemic fluorosis area found the difference accumulated of fluoride level in placenta surface. The peripheral surface was 2-times higher than maternal serum and 6-times higher than cord blood. The maternal surfaces and fetal surfaces were 3- time higher than cord blood.

2.8.3 Effect on Preterm delivery

The recent research mentioned that exposure to fluoride during pregnancy can cause of preterm delivery, LBW. [12-21] Fluoride can transfer to placenta and damage the brain of the offspring (Basha P, et al., 2011), A systematic review shown that exposure of fluoride associated with a decrease of human intelligence (IQ). [74] It is well known that thyroid hormone takes role of development of the Central Nervous

System (CNS) during the pregnancy (Nandi Munshi and Taplin, 2015). Some evidence report that fluoride can defeat thyroid activity (Peckham S, et al., 2015).

The retrospective cohort study of Rachel Hart and colleague [75] conducted in women with births live in Singleton US between 1993 and 2002, whereas water fluoride concentration area with more than 1.0 mg/L and area with fluoride concentration less than1.0 mg/L. The annual incidence of preterm birth in US was about 10%. The result found fluoride level associate with preterm birth, area with more than 1.0 mg/L are associate with increased risk to preterm birth 6.34% and area with fluoride concentration less than1.0 mg/L risk to preterm birth 5.52%.

2.8.4 Effect on Low Birth Weight

A. K. Susheela, [19, 76, 77] reported that fluoride causes serious damage to gastrointestinal (GI) mucosa by destroying microvilli resulting in non-absorption of nutrients from the diet. Fluorosis effected to developed hypothyroidism and anaemia by destroy erythrocytes, thereby contributing to loss of haemoglobin which results in anaemia. Breymann review that haemoglobin alone is insufficient to guide management of pregnant women with iron deficiency and anaemia. In Nepal, iron and folic acid supplementation reduced the incidence of low birth weight by 16%. Supplementation of 14 micronutrients including iron, folic acid and zinc reduced low birth weight by 14%, thus confirming no added advantage of multiple micronutrients over iron and folic acid. Other factors need to be investigated, and one such factor is fluoride intake.

Fluoride causes serious damage to the gastrointestinal (GI) mucosa by destroying microvilli resulting in non-absorption of nutrients from the diet. Fluoride is also known to destroy erythrocytes, thereby contributing to loss of haemoglobin which results in anaemia

M. Diouf et al [22] studied fluoride and LBW found that LBW was associated with pregnant living in endemic areas and water fluoride level consumed, the level of fluorosis associated with the incidence of LBW adjusted for gender, consanguinity, anemia and hypertension. Mothers with moderate dental fluorosis (level 4 of Dean's Index, 100% of surface teeth are affected and attrition) were 3.75-time higher delivery with LBW babies than controls (25.9% vs 6.9%).

Mina Aghaei et al (2015) [20] Fluoride is a toxic chemical and it is a risk factor for thyroid hormone production in children when the exposure to fluoride occurs during intrauterine growth period [19], and effects on pregnant women by causing anemia, leading to premature delivery and new born with low birth weight [76]. The study of

A. K Susheela. (2015) [77] in India found that anaemia and prevent infant and maternal mortality to a large extend through interventions, primarily addressing elimination of fluoride and promotion of a nutritive diet. The crux of the problem lies in fluoride deranging the GI mucosa resulting in non-absorption of nutrients. Upon fluoride withdrawal, mucosa regenerates and enhances haemoglobin production.

Fagin's report in Scientific American 'on second thoughts about fluoride' during 2008 is a warning to all concerned as he has revealed the risk of fluoride causing disorders affecting teeth, bone, brain and thyroid gland. As early as 1979, US dairy scientists have reported that thyroxine and triiodothyronine in serum decreased with increasing urinary fluoride in cattle. Cattle affected with fluorosis developed hypothyroidism and anaemia. Thyroid hormone status in married women prior to conception may therefore be required to be assessed.

Sastry M Gurumurthy et al [23] studied and found that maternal serum fluoride level are associated with adverse fetal outcomes. There are negative significant correlations between maternal serum fluoride and cord serum fluoride with birth weight, gestational age and APGAR score. Also, small negative correlations between birth weight, APGAR score and gestational age with placenta fluoride concentrations in maternal, fetal and peripheral surface. However, positive significant correlations were observed when the maternal serum fluoride was more than 1 ppm, there was 10.58-time higher risk for LBW, 8.65 times higher risk for preterm delivery and 3.8-time higher risk for low APGAR score. When the cord serum fluoride was more than 0.22ppm, there was 2.76-time higher risk for LBW, 4.6-time higher risk for preterm delivery and 2.5-time higher risk for low APGAR score. Thus, increasing of mother fluoride serum will risk of preterm delivery, LBW and poor APGAR count.

2.9 Maternal and child health service in Thailand and Lamphun

2.9.1 Antenatal Care (ANC) in Thailand

The maternal and child health service in Thailand was originally established in 1918, and the first official maternal and child health (MCH) handbook was published in 1985. (Figure 2-9). Since then, the handbook has been a major feature of the Thai MCH service and an important instrument for improving the MCH service in Thailand [78].

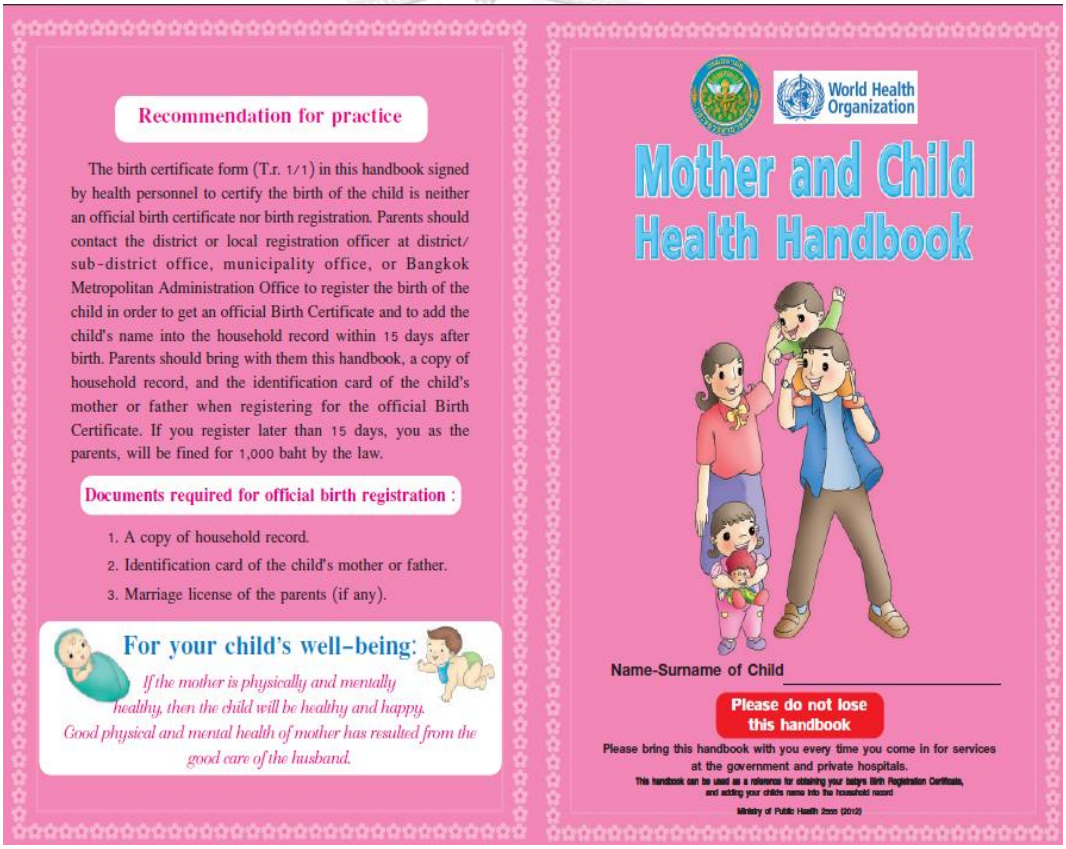


Figure 2- 9 Maternal and child health handbook

***Source*:** Mother and Child Health Handbook, Bureau of Health Promotion, Department of Health, Ministry of Public Health B.E.2555 (2012)

Accordingly, the handbook is an important tool to help ensure that clients obtain all basic MCH services. During 1989 to 2008, the MCH book has been periodically reviewed, revised and updated to cover essential MCH developments and to meet the evolving needs of both providers and users.

At present the MCH book was updated following *the new WHO Antenatal Care Model* (Figure 2-10) recommended by the World Health Organization (WHO,2002) [79].

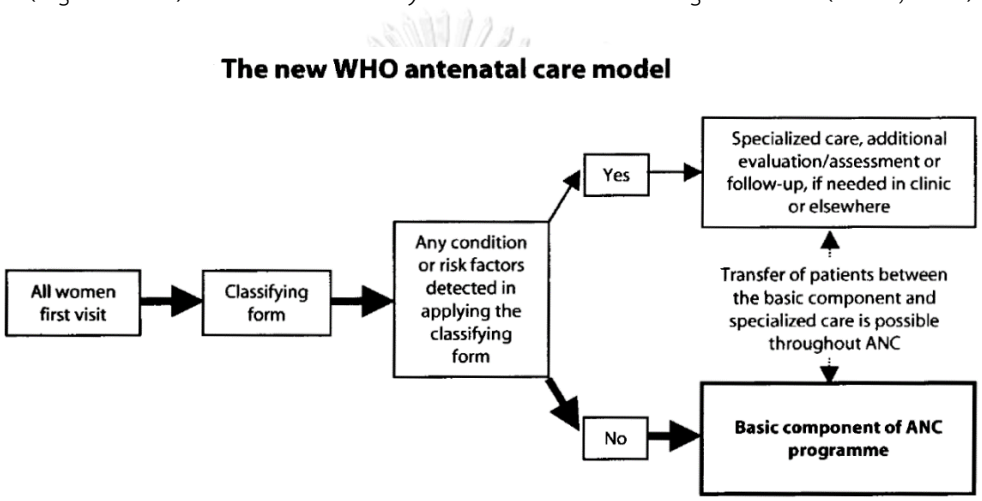


Figure 2- 10 The new WHO antenatal care model

Source: *UNDP/UNFPA/WHO/World Bank Special Program of Research, Development and Research Training in Human Reproduction*, WHO (2002)

The risk assessment criteria for pregnant woman at the 1st ANC visit (Figure 2-11) are used to determine pregnant women into two groups: those eligible to receive routine ANC or basic component (Figure 2-10), and those who need special care based on their specific health conditions or risk factors. The criteria are contained 18 checklist questions that require binary responses (yes/no). They cover the patient's obstetric history, their current pregnancy and general medical conditions. Women who answer 'yes' to any of the 18 questions would not be eligible for the basic component of the

new WHO antenatal care model; they should receive care corresponding to the detected condition

Risk Assessment Criteria for Pregnant Woman at the 1st ANC Visit

(assessed by health personnel)

Item	Criteria for Assessment	No	Yes
	Past History		
1.	Stillbirth or neonatal death (first 1 month)		
2.	3 consecutive abortions		
3.	Having baby with birth wieight < 2,500 g.		
4.	Having baby with birth wieight > 4,000 g.		
5.	Hospitalized for hypertension treatment during pregnancy or toxemia of pregnancy		
6.	Undergone surgery of reproductive system organ such as myoma, mypmecomy, cervical cerclage, etc.		
	Current History		
7.	Multifetal pregnancy		
8.	Age < 17 years (up to EDC)		
9.	Age > 35 years (up to EDC)		
10.	Rh Negative		
11.	Vaginal bleeding		
12.	Pelvic myoma		
13.	Diastolic pressure ≥90 mmHg		
14.	Diabetes		
15.	Kidney disease		
16.	Heart disease		
17.	Drug addiction, alcohol addiction		
18.	Other diseases of internal medicine such as anemia, thyroid, SLE, tc. (please specify).....		

If any of the responses fall into "Yes", the new approach of pregnancy care is not applicable to the pregnant woman and special care and/or additional assessment should be employed.

Figure 2- 11 Risk assessment criterion of pregnant women at 1-st ANC visit

***Source*:** Mother and Child Health Handbook, Bureau of Health Promotion, Department of Health, Ministry of Public Health B.E.2558 (2015)

The timing for five ANC visit program

Accordingly, the new WHO Antenatal Care Model (2002), schedule of 5 ANC visit are following;

1-st ANC visit: should be in the first trimester of pregnancy around less than or equal week 12 of gestation age

2-ndt ANC visit: should be scheduled week 18 ± 2 of gestation age

3-rd 1-st ANC visit: should take place in or around week 26 ± 2 of gestation age

4-tht ANC visit: should be between weeks 32 ± 2 of gestation age

5-th ANC visit: should take place in around week 38 ± 2 of gestation age

Checklists of Service Inclusion by Gestational Age

(recorded by health personnel)

1st Visit, Date.....(should be before 12 weeks)	Weeks				
	<12	20	26	32	38
1. Check classifying form, no high risks		■	■	■	■
2. Check weight, height, blood pressure		■	■	■	■
3. General physical examination		■	■	■	■
4. Urine exam (Multiple dipstick) for protein, sugar, asymptomatic bacteria		■	■	■	■
5. Transfer to the doctor for lung and heart sounds exam		■	■	■	■
6. Pelvic exam (may postpone to the 2nd visit)		■	■	■	■
7. Test for Hb/Hct/OF/DCIP (every gestational age) and test for VDRL, Anti HIV, blood gr, Rhtyping, HbsAgr		■	■	■	■
8. Give the 1st dose of tetanus toxoid vaccine		■	■	■	■
9. Give iron and/or folic, and iodine supplementation		■	■	■	■
10. Give advice in case of emergency with abnormal signs, with telephone number for emergency contact		■	■	■	■
2nd Visit, Date.....(20 weeks)					
1. Check weight, blood pressure	■				
2. Pelvic exam (in case not performed at the 1st visit)	■				
3. Ultrasound exam (if applicable)	■				
4. Give iron, iodine, calcium supplementation	■				
5. Give the 2nd dose of tetanus toxoid vaccine (at least 1 month interval of the 1st dose)	■				
6. Give post-test counseling for the blood result and abnormal signs, with telephone number for emergency contact	■				

3rd Visit, Date.....(should be before 26 weeks)	Weeks				
	<12	20	26	32	38
1. Check weight, blood pressure	■	■			
2. Test urine for protein, sugar	■	■			
3. General physical examination, check for anemia, edema	■	■			
4. Pregnancy exam, estimate gestational age, measure uterine height, Listen to fetal heart sound	■	■			
5. Give iron, iodine, calcium supplementation through the pregnancy period	■	■			
6. Advice mother to observe fetal movement	■	■			
7. Give advice in case of emergency with abnormal signs, with telephone number for emergency contact	■	■			
4th Visit, Date.....(32 weeks)					
1. Test for Hb/Hct, VDRL. Anti HIV	■	■	■		
2. Give advice about delivery, breastfeeding plan, contraception	■	■	■		
5th Visit, Date.....(38 weeks)					
1. Check fetal position, if breech presentation, refer to ECV or CS	■	■	■	■	
2. Record in ANC booklet, remind of bringing it along when coming for delivery	■	■	■	■	
3. If delivery does not occur at 41 weeks of gestation, give advice to come to the hospital	■	■	■	■	

Figure 2- 12 Basic components of ANC visit in Thailand

Source: Mother and Child Health Handbook, Bureau of Health Promotion, Department of Health, Ministry of Public Health B.E.2558 (2015)

2.9.2 Antenatal Care (ANC) in Lamphun

The antenatal care (ANC) service of government hospital in Lamphun was following the Nation ANC service guideline under Mother and Child Health Board (MCH Board) of Lamphun province. However, the supplementary support for pregnant women are different in each hospital and depend on contracting unit of primary care (CUP) of district level.

There are two supplementary to support the pregnancy; OBIMIN-AZ and Triferdine 150.

Table 2- 5 Component of *OBIMIN-AZ and Triferdine 150*

OBIMIN-AZ	Triferdine 150
iodine (as K iodate) 0.2 mg,	Iodine 0.15 mg
folic acid 1 mg,	folic acid 0.4 mg
Fe fumarate 200 mg,	
	Iron 60.81 mg
vit A 5,000 IU,	
vit C 75 mg,	
vit D 400 IU,	
vit E 10 IU,	
vit B1 2 mg,	
vit B2 3 mg,	
vit B6 2.5 mg,	
vit B12 3 mcg,	
nicotinamide 20 mg,	
Ca lactate 250 mg,	
Zn (as Zn sulfate) 20 mg	

Source: Applied from Monthly Index of Medical Specialities (MIMS)

2.9.1 Newborn Screening for PKU and CHT In Thailand

The Newborn Screening Program [80] for Phenylketonuria (PKU) and congenital hypothyroidism (CHT) was started as pilot project in 1993 and it has been established as a national health policy since 1996 by the Department of Medical Sciences, Ministry of Public Health. At present, a nationwide program is covered newborns around the country.

Phenylketonuria (PKU) is caused by phenylalanine hydroxylase deficiency characterized by elevated plasma levels of phenylalanine and its metabolites. In untreated cases, there is gradual development of irreversible severe mental retardation, seizures, microcephaly and hypopigmented hair and skin (Nyhan and Ozand, 1998; Scriver and Kaufman, 2001; Smith and Lee, 2001; Rezvani, 2007). [81]. The incidence of PKU was 1:327,740 compare to other countries ranges was 1:10,000 to 1:70,000 (AAP, 1996; Aoki, 2003; Jiang et al, 2003). Whereas incidence of PKU in the Thai population is 2.22: 100,000 live births [82]. It is a rare disease in Thailand [81].

Congenital hypothyroidism (CHT) is a condition that, if left untreated, can cause lifelong human suffering because of severe mental retardation and deficiency of growth. The incidence of CHT was 1: 3000-4000 life births [83] .

Infants with PKU appear normal at birth. Diagnosis of PKU by clinical criteria is usually made later when the infant is a few months old after the development of irreversible brain damage [81]. First screening cut off for CHT (TSH ≥ 25 mU/l) and PKU ≥4mg/dl are abnormal.

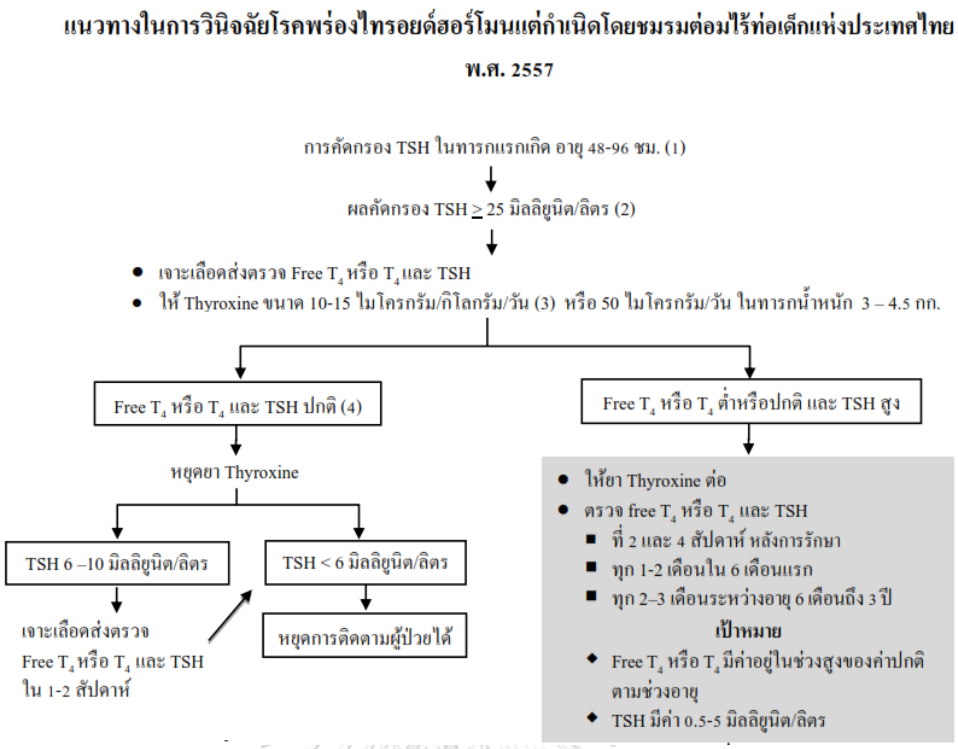


Figure 2- 13 Diagnose of Congenital hypothyroidism (CHT)

Source: Thai Society for Pediatric Endocrinology Division of Endocrine & Metabolism, Department of Pediatrics, Faculty of Medicine Siriraj Hospital

2.10 Risk Factor of Premature Delivery and/or LBW

2.10.1 Risk Factors of Preterm Delivery

1) Definition

Gestational Age: The duration of gestation is measured from the first day of the last normal menstrual period. **[84]** Gestational age is expressed in completed days or completed weeks.

Preterm refer to gestational age of less than 37 completed weeks (i.e. less than 259 days)

Term refer to gestational age of 37 to less than 42 completed weeks (i.e. 259 to 293 days)

Post Term refer to gestational age of 42 completed weeks or more (i.e. 294 days or *more).*

Perinatal Period refers to commences from 22 weeks (154 days) of gestation age (the time when the birth weight is 500 g) and ends at 7 completed days after birth.

Neonatal Period refers to the period of less than 28 days after birth. Early neonatal period refers to the period before 7 days of age. Late neonatal period refers to the period from completion of 7 days up to 28 days of life.

2) Diagnose of Preterm Deliver:

The best estimate of gestation depends on judgement, based on all the historical, ultrasound and baby examination data, the estimate as entered in the database is most accurate. The obstetrician or doctor will determine and diagnose follow definition by ICD-10 Code ***O60.13*** [25]. There are 3 level of preterm delivery, based on gestational age of pregnancy;

(1) extremely preterm refer to labor less than 28 weeks.

(2) very preterm refer to labor at 28 to less than 32 weeks

(3) moderate preterm refer to labor at 32 to less than 37 weeks

3) Risk Factors of Preterm delivery

The Bureau of Health Promotion, Department of Health, Ministry of Public Health, Thailand [85, 86] specify the Indicator to assess risk of pregnancy at first ANC were past and current history of pregnancy as follow;

3.1) Past History

(1) Stillbirth or neonatal death (first 1 month)

(2) Consecutive abortions

(3) Having baby with birth weight < 2,500 g.

(4) Having baby with birth weight > 4,000 g.

(5) Hospitalized for hypertension treatment during pregnancy or toxemia of pregnancy.

(6.) Undergone surgery of reproductive system organ such as myoma, myomectomy, cervical cerclage, etc.

3.2) Current history

(1) Multifetal pregnancy

(2) Age < 17 years or Age > 35 years (up to EDC)

(3) Rh Negative

(4) Vaginal bleeding

(5) Pelvic myoma

(6) Diastolic pressure ≥90 mmHg

(7) Illness with one diseases or more, such as of Diabetes, Kidney, Heart diseases

(8) Drug addiction or alcohol addiction

(9) Other diseases of internal medicine such as anemia, thyroid, SLE, etc. (please specify)

Nipunporn and colleague, Bureau of Health Promotion, Department of Health [85, 86] recommend that the most important risk factor of premature later pregnancy and factors involve of Preterm delivery and pregnant complication as follow;

(1) Maternal age over 35 years or less than 17 years.

(2) Inflammatory conditions, such as mother's vagina bacterial vaginosis.

(3) Mothers infected with bacteria Group B streptococcus.

(4) Mothers with cavities and / or inflammation of the gums.

(5) Mother has a severe septicemia.

(6) Congenital uterine abnormalities such as congenital uterine tissue disorders, abnormal uterine shape.

(7) Expansion of the uterus, such as twin pregnancy, hydramnios or uterine fibroid embolization (UFE).

(8) Rupture of amniotic sac before actual labor.

(9) Diseases of blood vessels, such as heart disease, high blood pressure.

(10). Drinking alcohol, smoking and drug use among pregnant women.

Chumnijarakij T. et al (1992) [87] studied risk approach for maternal risk factors for LBW newborn in Thailand mention that maternal obstetrical risk factors for LBW included: vaginal bleeding during early pregnancy (RR = 3.28), maternal hypertension (RR = 3.48), convulsion during pregnancy (RR = 3.29), no prenatal care or less than 4 visits, cigarette smoking (RR = 2.04), coffee or tea drinking during pregnancy(RR = 2.16), and repeated induced abortions (RR = 2.16).

Ajchara [88] mention that the risk factors of preterm delivery following:

(1) Stress: occurred by single women who is low socioeconomic status, anxiety, depression, life events (divorce, separation, death), abdominal surgery during pregnancy

(2) Occupational fatigue: upright posture, use of industrial machines, physical exertion, mental or environmental stress

***(3) Excessive or impaired uterine distention*:** multiple gestation, polyhydramnios, uterine normally or fibroids, diethystilbestrol.

***(4) Cervical factors*;** History of second-trimester abortion, History of cervical surgery, Premature cervical dilatation or effacement

(5) Infection; Sexually transmitted infections, Pyelonephritis, Systemic infection, Bacteriuria, Periodontal disease

(6) Placental pathology placenta previa, abruption, vaginal bleeding

(7) Miscellaneous; previous of preterm delivery, substance abuse, smoking, maternal age (<18 or >40 years), African-American race, poor nutrition and

low body mass index, inadequate prenatal care, anemia (hemoglobin <10 g/dL), excessive uterine contractility, low level of educational, achievement, genotype

(8) Fetal factors; congenital anomaly, growth restriction

2.10.2 Low Birth Weight (LBW) and Risk Factors

1) Definition

Low birth weight (LBW): refer to babies are newborns weighing less than 2,500 grams, which the measurement taken within the first hours of life. The diagnosis of. LBW was assesses by doctors or obstetrician follow definition of ICD-10 Code ***P07.00*** [24]. There are 3 level of LBW based on weight; [24]

(1) Low Birth Weight (LBW) refer to birthweight less than 2500 g.

(2) Very Low Birth Weight (VLBW) refer to birthweight less than 1500g.

(3) Extremely Low Birth Weight (ELBW) refer to birth weight is less than 1,000 g.

2) Causes of LBW

Prematurity and intrauterine growth retardation (IUGR) are two main causes of LBW. LBW is often used as a proxy indicator to quantify the magnitude of IUGR in developing countries because valid assessment of gestational age is generally not available.

2.1) ***Premature*** There are many reasons for premature delivery, however, in many cases the cause is unknown. Reasons include high maternal blood pressure, acute infections, multiple births, hard physical work, or stress. The word *preterm* may

also be used to describe these infants.

2.2) ***Intrauterine Growth Retardation(IUGR)*** Intrauterine growth retardation is a subtype of LBW of extraordinary importance to developing countries. IUGR is a condition where fetal growth has been constrained. An inadequate nutritional environment *in utero* can be one reason for this constrained growth. IUGR is usually assessed clinically when the fetus is born by relating the size of the newborn to the duration of the pregnancy using the 10^{th} percentile of a reference population. A small size for gestational age indicates IUGR, or the inability of the fetus to reach its growth potential. Infants diagnosed with IUGR may be:

(1) LBW at term (‡ 37 weeks gestation and < 2500 g);

(2) preterm (<37 weeks gestation and weight less than the 10^{th} percentile)

(3) IUGR at 37 weeks gestation and weight less than the 10^{th} percentile with a birthweight ‡ 2500 g. (de Onis et al., 1998, Eur J Cl Nutr 52(S1))

Thus, because not all preterm infants are IUGR, LBW among preterm infants overestimates poor growth due to nutritional causes; and because some IUGR infants weigh more than 2500 g (the third classification), LBW at term underestimates the overall magnitude of the IUGR problem.

2.3) ***IUGR-LBW:*** in developing countries IUGR affects about two-thirds of infants born with LBW; the remaining one-third of these LBW infants are born preterm, some of whom are also affected with IUGR. (Arifeen, 1997) IUGR-LBW is used in some publications to refer only to IUGR infants who are LBW at term. IUGR infants born at

term (‡37 weeks) with LBW (<2500 g) are referred to in this publication as LBW at term.

2.4) *Small for Gestational Age (SGA)*: SGA infants have birthweights below a given low percentile cut-off for gestational age. SGA and IUGR are not strictly synonymous: some SGA infants (e.g., those born to short mothers) may represent merely the lower extreme of the "normal" fetal growth distribution, while other infants who meet the criteria for "appropriate for gestational age" may have actually been exposed to one or more growth-inhibiting factors. In individual cases, however, it is usually very difficult to ascertain whether or not the observed birthweight is the result of restricted *in utero* growth; classification of an infant as IUGR is thus based on the established cut-off for SGA. (WHO, 1995, Report No. 854)

2.5) *Undernutrition*: In this report the term undernutrition refers collectively to stunting, underweight, wasting, low body mass index, and fetal growth retardation – conditions of inadequate nutrition.

2.6) *Malnutrition*: In this report the term malnutrition refers to both undernutrition and overnutrition – conditions of both deprivation and excess.

2.7) *Chronic Energy Deficiency(CED)*: *"A steady state at which a person is in an energy balance although at a cost either in terms of increased risk to health or as an impairment of functions and health."* A BMI <18.5 kg/m in adults indicates CED. (James et al., 1988)

3) *Risk Factors of LBW*

Isranurug et al (2007), a cohort study conducted in 2007 shown that factors significant contributors to LBW were maternal age (<20 and >35 y; RR = 1.85, 95% CI:1.47- 2.33), maternal height (<145 cm; RR = 2.29, 95% CI: 1.57-3.34) and pregnancy weight gain (<10 kg, RR = 1.67,95% CI: 1.24-2.26). Teenage pregnancy had higher rates of LBW (15.5%) compared to older mothers (8.8%).[26]

Khunpradit S. [89] mentioned that preterm birth (Prematurity) and intra-uterine growth restriction (IUGR) are two main causes of LBW in developing country, IUGR is great cause. While in developed country, LBW cause by preterm delivery are caused by hypertension, infection, hardworking, multi pregnant, stress or anxiety.

3.1) Pregnant Complication

Pregnant complication refers to health problems that occur during pregnancy, which would be mother's health, the fetus's health or both, (CDC, 2006). The Pregnant complications could be cause to LBW and/or preterm delivery. Most common pregnant complications which serious for medical issues [62] are below.

(1) Miscarriage is the loss of a pregnancy in the first 20 weeks. About 10 to 20 % of known pregnancies end in miscarriage, and more than 80 % of miscarriages happen before 12 weeks. Most first-trimester miscarriages are believed to be caused by chromosomal abnormalities in the fertilized egg that keep the embryo from developing.

Vaginal spotting or bleeding is usually the first sign of miscarriage, the ultrasound will order by healthcare practitioner to see what's going on in the uterus and possibly do a blood test.

(2) Pre-eclampsia is a serious condition that affects about 5 % of pregnant women. The diagnosed of preeclampsia will have high blood pressure *and* protein in urine or liver or kidney abnormalities after 20 weeks of pregnancy. Most expectant mothers who get preeclampsia develop mild symptoms near their due date, and they and their babies do fine with proper care, but it can progress quickly, and severe preeclampsia can affect many organs and cause serious or even life-threatening problems. Women whose preeclampsia is severe or getting worse need to deliver early.

(3) Low amniotic fluid (oligohydramnios) The amniotic sac fills with fluid that protects and supports developing of baby. When there's too little fluid, it's called oligohydramnios. Low levels of amniotic fluid usually occur in their third trimester. When pregnant this happen, caregiver will follow pregnancy closely to be sure baby continues to grow normally. If it near the end of pregnancy, labor will be induced.

(4) Gestational diabetes during pregnancy. Diabetes during pregnancy develops similar of other types of diabetes. Gestational diabetes effects on cells to use sugar (glucose) and causes of high blood sugar that can affect pregnancy and baby's health. Any pregnancy complication is concerning, but there's good news. Expectant moms can help control gestational diabetes by eating healthy foods, exercising and, if necessary, taking medication. Controlling blood sugar can prevent a difficult birth and

keep pregnancy and baby healthy. In gestational diabetes, blood sugar usually returns to normal soon after delivery. But if you've had gestational diabetes, you're at risk for type 2 diabetes. You'll continue working with your health care team to monitor and manage your blood sugar

(5) Ectopic pregnancy When a fertilized egg implants outside the uterus, it's an ectopic pregnancy. One in 50 pregnancies is ectopic. Because the clear majority of ectopic pregnancies occur in a fallopian tube, they're often called "tubal" pregnancies. It's important to catch this type of pregnancy early because the growing embryo could rupture fallopian tube and cause internal bleeding that can be fatal. Since there's no way to transplant an ectopic pregnancy into the uterus, ending the pregnancy is the only option.

(6) Placenta Previa Placenta Previa is lying unusually low in uterus, next to or covering cervix. It isn't usually a problem early in pregnancy, but if the placenta remains dangerously low as your pregnancy progresses, it can cause bleeding, which can lead to other complications and may require you to deliver early. The location of placenta will be checked during mid-pregnancy ultrasound exam, but only a small percentage of women who have placenta previa in mid-pregnancy still have it when they deliver their baby. Placenta previa is present in up to 1 in 200 deliveries. women who have placenta previa when they give birth must deliver by cesarean section.

A.K. Susheela et al (2014) [77] Anemia in pregnancy and low birth weight babies is a problem prevalent in India and other nations. This communication reports the results of a novel initiative to address the issue. The uniqueness of the strategy lies in withdrawal of fluoride, consumed through a variety of sources including water. The study was conducted in 2 Delhi Government hospitals; screened 3262 pregnant women visiting Antenatal Clinics (ANCs). Women upto 20 week pregnancy with hemoglobin (Hb)11.0 – 5.0 g/dl; with urine fluoride level (UFL) > 1.0 mg/L and not suffering from any ailment(s) selected. Total 481 pregnant women, grouped into sample (n=234) and control (n=247), through a computerized random sampling procedure. The sample group introduced to, two interventions (1) removal of fluoride from ingestion (2) counseling based intake of essential nutrients through dairy products, vegetables and fruits. No intervention was introduced to control group. Both groups received supply of iron and folic acid (100 mg iron + 500 µg folic acid) through ANCs. Both groups monitored for UFL and Hb until delivery during their visits to ANC. BMI recorded initially and prior to delivery. Birth weight of babies and other details recorded from Labour room register. Results reveal in sample group women, UFL decreased in 152/234 (65.0%). An increase in Hb upon practise of interventions recorded in 182/234 (77.7%). Body Mass Index enhanced. The percentage of pre-term deliveries

2.11 Research on Preterm Delivery and LBW and Fluoride

2.11.1 Preterm Delivery and LBW

Preterm delivery and LBW [25] are the great problem on women's and child's health of Global, including Thailand. Preterm delivery refers to baby born before 37 weeks of pregnancy complete and LBW refer to newborns weight less than 2,500 grams. WHO (2015) estimated 15 million babies or more than 10% are born with preterm delivery yearly, 15 % to 20 % of them are LBW, these number are increasing. Almost 1 million of premature babies die due to problems of preterm birth, several survivors face to lifetime of disability, comprising learning disabilities, visual and hearing problems. Prematurity is primary cause of Global death in children less than 5 years.

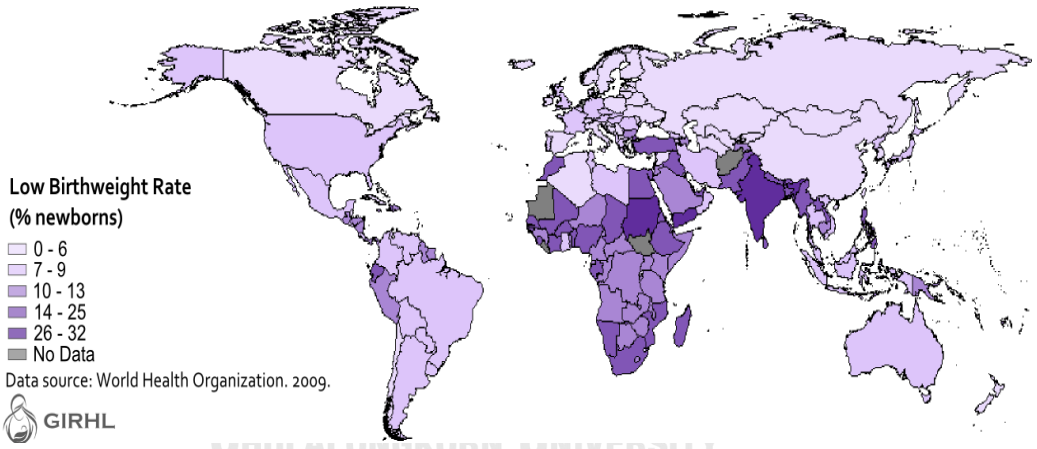


Figure 2- 14 Situation of Low Birth Weight in Global

Source: WHO (2009) http://www.girhl.org/stillbirth_lowbirthweight

In Thailand, the National rate of LBW was set as less than 7 % since 1991, but incidence rate is higher and remained at 8% to 9% during 1990 to 2000 until now [86]. The National incidence rate of Preterm delivery (2012) was 12% of live births [90]. The survey of Multiple Indicator Cluster Survey (MICS) in 2006 reported that prevalence of LBW was 9.2% with no significant difference by housing regional area and maternal

education but small difference between poor and rich family (10% vs 8.5%, respectively). In some remote areas, the prevalence of LBW is very high. In 2015 the incidence rate of LBW was 8.24% [91].

LBW is one of women's and child's health problem in Lamphun. It was the highest of upper North in 2015. Trend of LBW in Lamphun was increasing between 2013 to 2015 from 10.98% to 11.27% and 50.27% LBW case (5.67% of total) and cause by preterm birth and 49.73% from SGA or IUGR, showed in Figure 2-13

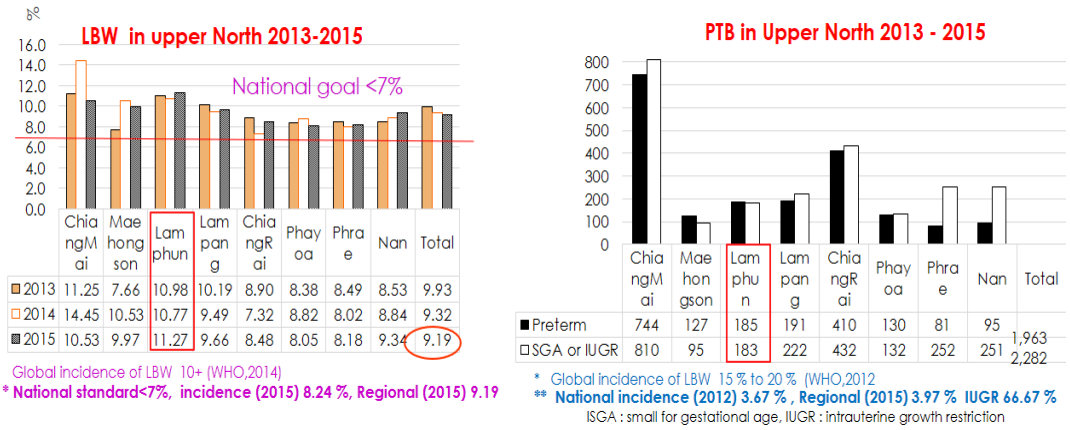


Figure 2- 15 Situation of LBW and Preterm delivery in Upper North 2013-2015

Source: Report of Health Promotion Center Region1 Chiang Mai (2015), Department of Health.

2.11.2 Study on fluoride effect in Thailand

Northern and western Thailand were the most areas of high drinking water fluoride level which the geographical of fluoride belt passed through, including Chiang Mai Chiang Rai, Mae Hongsorn, Lamphun, Lampang, Phayoa, Kanchanaburi, and Ratchaburi, [1, 92]. The water contamination with fluoride has found higher than 10

mg/L in underground water, surface water and also has found high fluoride in bottled water. The adverse health effect on people would occur by consume fluoride by drinking and cooking water.

In 2010, Intercountry Centre for Oral Health (ICOH) and Department of Groundwater [9] studied about *safe drinking water* in Thailand (water with fluoride less than or equal 0.7 mg/L, FDA. 2010, PCD. 2009). The study was conduct in 77 provinces, drinking water of villages were surveyed and found that areas with safe drinking water of village less than 50% of total surveys were 6 provinces compose with Tak, Suphan Buri, Phetchaburi, Samut Songkhram and Samut Sakhon, including Lamphun, showed in Figure 2-14

Lamphun is greater fluoride area in Upper North, the reported from the survey found that fluoride in drinking water between 1.5 mg/L to more than 10 mg/L, showed in Table 2-5.

The study of C. Joon Chuah and colleague [10] reported that in Chiang Mai there are 31% of the shallow wells contained of fluoride risk levels (≥1.5 mg/L) compared with the 18% observed in the deep wells. However, in Lamphun site, 35% of more deep wells contained water with at least 1.5 mg/L fluoride compared with 7% of shallow wells, showed in Figure 2-15

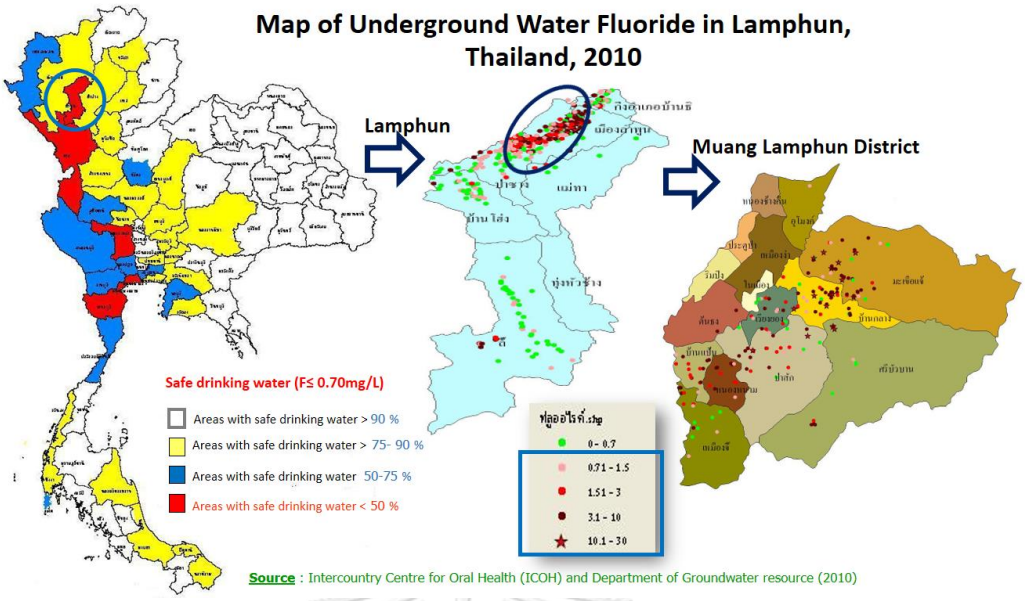


Figure 2- 16 Situation of Fluoride in drinking water in Thailand, 2010

Source: Intercountry Centre for Oral Health (ICOH) and Department of Groundwater Resource Department (2010)

Table 2- 6 *Percentage of villages with high Fluoride in drinking water in Upper North, Thailand 2010*

Province	Total survey	Low		Optimal		High					
		F < 0.50 mg/l		F 0.50 - 1.5mg/l		1.5 - 4.0 mg/l		> 4.0mg/l		> 10.0mg/l	
		villages	%	villages	%	villages	%	villages	%	villages	%
Chiang Mai	1,641	1,171	71.36	313	19.07	132	8.04	25	1.52	0	0
Maehongson	133	123	92.48	8	6.02	1	0.75	1	0.75	0	0
*** Lamphun	1,440	939	65.21	254	17.64	130	9.03	97	6.74	20	1.39
Lampang	139	124	89.21	8	5.76	0	0	1	0.72	6	4.32
ChiangRai	319	293	91.85	15	4.70	5	1.57	1	0.31	1	0.31
Phayoa	174	122	70.11	29	16.67	22	12.64	1	0.57	0	0
Phrae	147	132	89.80	11	7.48	3	2.04	1	0.68	0	0
Nan	222	216	97.30	4	1.80	2	0.90	0	0	0	0
Total	**4,215**	**3,120**	**74.02**	**642**	**15.23**	**295**	**7.00**	**127**	**3.01**	**27**	**0.64**

Source: Intercountry Centre for Oral Health (ICOH) and Department of Groundwater Resource Department (2010)

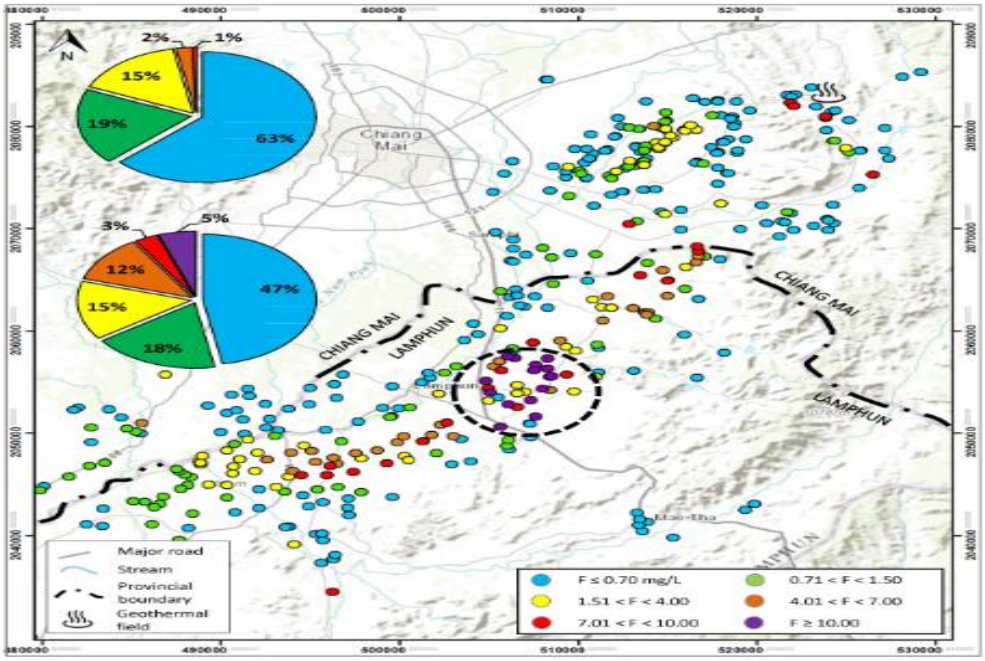


Figure 2- 17 Map of fluoride distribution in Lamphun (2014),

***Source*:** applied from C. Joon Chuah et al (Fluoride: A naturally-occurring health hazard in drinking-water resources of Northern Thailand 2014)

There are some studies of health effects of fluoride on pregnancy in Thailand, most of them study about dental fluorosis. Somsak Chuckpaiwong et al [16] studied about fluoride analysis of human milk in remote areas of Thailand found that average fluoride concentration in breast milk was 0.017 ppm. There was no difference in breast milk fluoride concentration between regions and there are not relationship between breast milk fluoride content and fluoride concentrations in either drinking water or water for domestic.

The study of S. Chaiwong and colleague on blood, urine fluoride, iodine, and blood thyroid functions tests in pregnant women at 1-st trimester of gestational age in

in Phayao found that mean urinary fluoride (UF) was less than 0.2 μg/L, urinary iodine was positively correlated with urinary fluoride and thyroid stimulating hormone (TSH), and negative correlation with free triiodothyronine (FT3). There are negative correlation between free triiodothyronine (FT3) and thyroid stimulating hormone (TSH). [93]

The retrospective study of M. Namkaew and P. Wiwatanadate [94] on exposure to water fluoride and chronic pain in high fluoride area (F> 0.7 mg/L) and low fluoride area (F≤ 0.7 mg/L) found that there is association between the average daily fluoride intake and lower back pain (OR = 5.12; CI = 1.59–16.98). The OR of back pain between high fluoride area and low fluoride area was 1.58, (CI =1.10–2.28, RR = 1.22 CI =1.14–1.31). However, there were no relationships between the average daily fluoride intake and chronic leg and knee pains (skeletal fluorosis).

Most of fluoride research studied on dental fluorosis. Fluorosis were found in every region of Thailand, but highest prevalence was found in Northern (61%), moreover, drinking water and urine samples of people were highest of fluoride level found in Northern compared to other regions (Leatherwood et al., 1965) [95]. Additional to the studied on dental fluorosis in pregnancy found that high incidences of fluorosis in Northern Thailand including Lamphun causing by consumed of high fluoride drinking water (Chuah et al, 2016) [96].

However, there is lacking study on the evidence of fluoride level among pregnancy and umbilical cord of newborn. Moreover, preterm delivery and low birth

weight have drawn recent attention but have yet to be well studied on adverse effects of fluoride in pregnant women.

2.12 GIS Technology and Application

Geographic information systems (GIS) have become a popular tool in public health, and environmental public health applications [97]. By tracking the sources of health outcomes, government agencies and public health professionals can identify the geo-graphical and geospatial patterns of at-risk populations and targeting interventions.

GIS technology can be used to estimate exposures to individuals in cross-sectional, case-control, and cohort research. Regularly the most difficult, expensive, and time-consuming characteristic of environmental health studies is obtaining accurate exposure information. A GIS can combine information contained in existing databases and/or data that can be computerized to estimate exposure levels, for example, to agricultural pesticides, to individuals residing or working within defined geographic regions. [98]

A GIS database consists of geospatial referencing data such as geographic coordinates (e.g., latitude/longitude), addresses, and postal codes as well as attributes that can be linked to map layers by a common identifier, or geocode [99]. GIS can collect data including geographic areas of epidemic disease incident, epidemic data and statistics, of patients who have epidemic disease at hospitals, different species of

bacteria in each area, high risk areas and surveillance zones that must eliminate source of disease etc. [100]

Nowadays, numerous public health agencies are using the resource integration and analytic capabilities of GIS to create analytical and descriptive solutions. Applications of the GIS and Geo-visual maps are illustrated integrating and presenting this data presents a challenge [101]. The modern GIS provided Dynamic maps, images, and advanced spatial functions in GIS. The GIS applications are already making health information accessible through the Web [102].

Applications of the GIS and Geo-visual maps are illustrated through an in-depth discussion of specific case studies in public health research at the university

GIS plays a critical role in determining where and when to intervene, and thereby improving the quality of care, in-creasing accessibility of service, finding more cost-effective delivery modes, and preserving patient confidentiality while, at the same time, satisfying the needs of the research community for data accessibility[103].

2.12.1 Google Earth and Google Maps

Google Earth [104] is a computer program that reduces a 3D representation of Earth based on satellite imagery. The program maps composed of superim-posing satellite images, aerial photography, and GIS data onto a 3D globe, allowing users to see cities and landscapes from various angles. Users can explore the globe by entering addresses and coordinates, or by using a keyboard or mouse. The program can also be downloaded on a smartphone or tablet, using a touch screen or stylus to

navigate. Users may use the program to add their own data using Keyhole Markup Language and upload them through various sources, such as forums or blogs. Google Earth is able to show various kinds of images overlaid on the surface of the earth and is also a Web Map Service client.

Google Maps [105] is a web mapping service developed by Google. It was launched in February 2005 to offers satellite imagery, street maps, 360° panoramic views of streets (Street View), real-time traffic conditions (Google Traffic), and route planning for traveling by foot, car, bicycle (in beta), or public transportation. Google Maps program designed by Lars and Jens Eilstrup Rasmussen at Where 2 Technologies. In October 2004, the company was acquired by Google, which converted it into a web application. After additional acquisitions of a geospatial data visualization company and a realtime traffic analyzer [106].

Google Maps' [106] satellite view is a "top-down" or "birds eye" view; most of the high-resolution imagery of cities is aerial photography taken from aircraft flying at 800 to 1,500 feet (240 to 460 m), while most other imagery is from satellites. Much of the available satellite imagery is no more than three years old and is updated on a regular basis. Google Maps uses a close variant of the Mercator projection, and therefore cannot accurately show areas around the poles.

Nowadays, Google Maps are an extensive tool in public health, and environmental health research [97]. It has not only become a part of everyday life, but it also has become an extremely important platform for storage and management GIS

data undertaking a range of research areas and enabling new ways to do research [99] e.g. environmental health, public health research [100, 102], epidemiology information on maps [107].

2.12.2 GADM database of Global Administrative Areas

GADM database of Global Administrative Areas: GADM [108] is a spatial database of the location of the world's administrative areas (or administrative boundaries) for use in GIS and similar software. Administrative areas in this database are countries and lower level subdivisions such as provinces, district and sub districts. The GADM describes where these administrative areas are (the "spatial features"), and for each area it provides some attributes, such as the name and variant names. The boundary data by country and support whole world.

The data are available as shapefile, ESRI geodatabase, RData, and Google Earth KMZ format. Shapefiles can be used for most mapping and "GIS" software. You can download a free program such as Q-GIS or DIVA-GIS. The RData files can be used in *R* with the 'SP' package loaded.

This dataset is freely available for academic use and other non-commercial use. Redistribution, or commercial use is not allowed without prior permission. You are free to create maps and use the data in other ways for publication in academic journals, books, reports, etc. The GADM current version is 2.8 (November 2015). Version 3 is expected to be available in August 2017.

CHAPTER III

METHODOLOGY

3.1 Research Design

This research analytical Cross-sectional Study and Prospective Cohort Study.

The Cross-sectional Study on water fluoride and endemic fluoride areas were conducted from July 2016 to January 2017. The pregnancy-birth cohort study was conducted from July 2016 to November 2017 in Mueang Lamphun, Pasang and Ban Thi District, Lamphun province, Thailand. The study was conducted on pregnant women gestation age less than 30 weeks until delivery. All information of the participants during ANC to delivery with diagnosed of delivery, and the information of newborn with diagnose of LBW were collected from questionnaires and medical record at Lamphun, Pasang and Ban Thi hospital, Lamphun province.

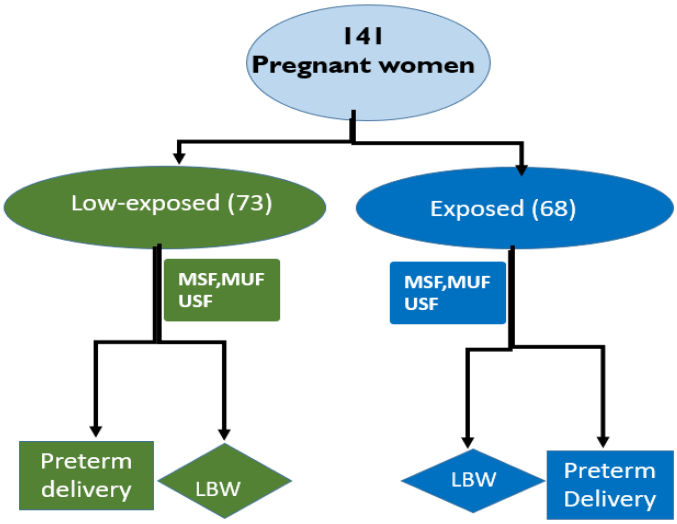


Figure 3- 1 Study design

3.2 Study Area

Lamphun province is subdivided into eight districts (Amphoe), 51 sub-districts (Tambon) and 575 villages (Muban). The residential areas are sub-divided into two areas, areas of Municipality (Thesaban) and areas of Tambon Administrative Organizations *(TAO).* There are 12 municipalities and 47 TOA. [109].

The survey of ICOH [9], in 2010 found that Lamphun was one of six province has found WF higher than 10.0 mg/L and more than 50% of households used unsafe drinking water. There were more than 240 villages (17%) have water with high fluoride (1.5 mg/L to more). Showed in Figure 3-2.

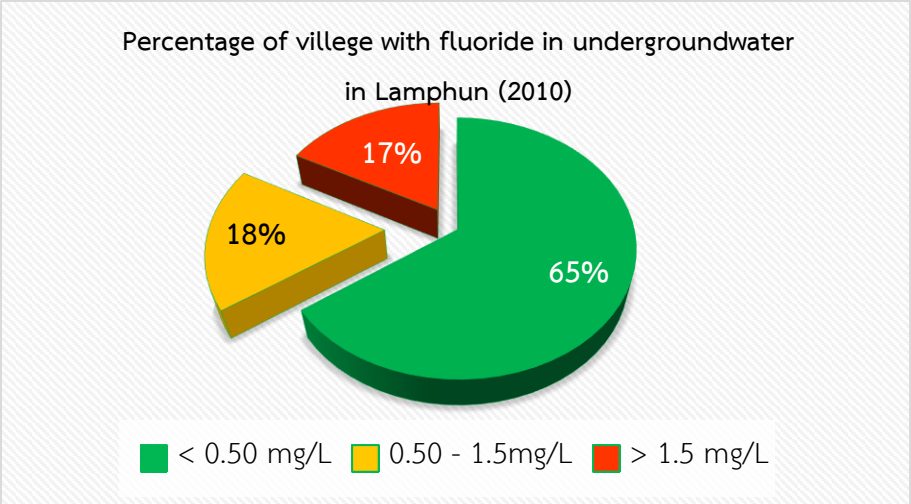


Figure 3- 2 Percentage of village classified by level of fluoride concentration in underground water in Mueang Lamphun Province, Thailand

Source: Intercountry Centre for Oral Health (ICOH) and Department of Groundwater Resource Department (2010)

Refer to the standard water fluoride level [31-33], this study used 0.7 mg/L of WF as a cut point to classify villages via WF level into two groups.

1) The Low-exposed group was pregnant women who live in village with WF ≤ 0.7 mg/L or Normal areas.

2) The Exposed group was pregnant women who live in village with WF > 0.7 mg/L or Endemic fluoride area.

Thus, the study areas were conducted in Meuang Lamphun, Pasang and Ban Thi District, Lamphun province Thailand. Showed in Figure 3-3.

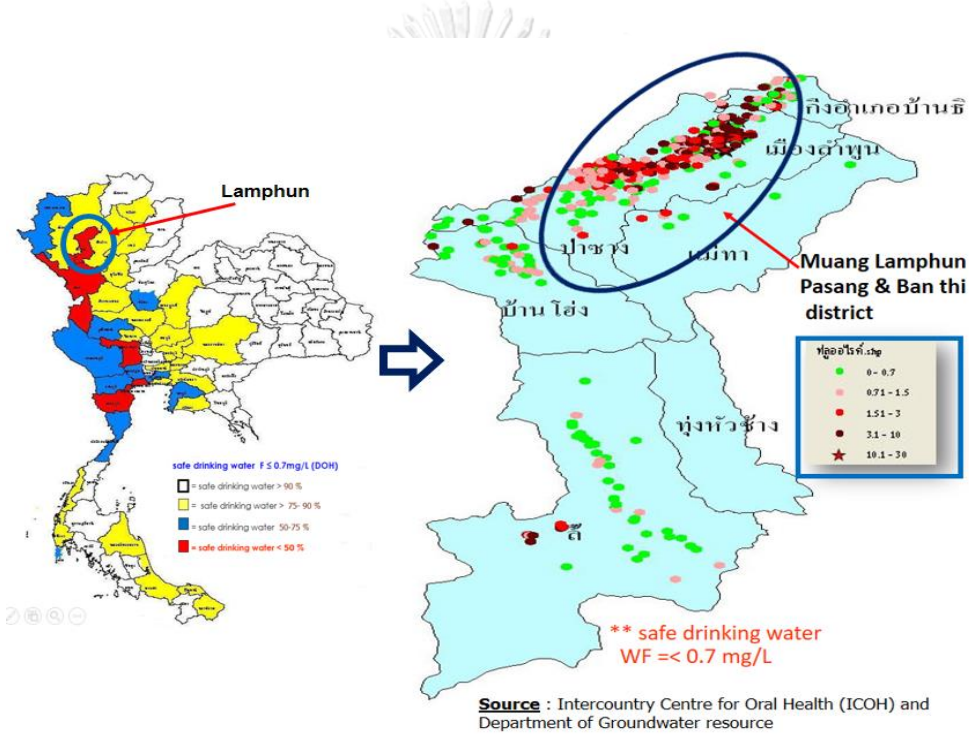


Figure 3- 3 Map of study areas: Meuang Lamphun, Pasang and Ban Thi district
Source: Applied from Intercountry Centre for Oral Health (ICOH, 2010)

3.3 Study Population

The study population were pregnant women live in 26 subdistricts and 303 villages in Meuang Lamphun, Pasang and Ban Thi District. The participants who meet the inclusion and exclusion criteria were selected to this study.:

1) Inclusion criteria

(1) Pregnant women age between 20 to 35 years.

(2) Residence in Mueang Lamphun, Pasang or Ban Thi district for one year or more.

(3) Gestational age was equal or less than 30 weeks at 1-st data collection.

(4) Have No risk criteria of history and current pregnancy at fist ANC visit following the new WHO antenatal care model, 2002.

(5) Plan to delivery at Lamphun, Pasang or Ban Thi hospital.

(6) Agree to participate in this study with informed consent

2) Exclusion criteria

(1) Multifetal pregnancy.

(2) Incomplete with data collections.

3.4 Sample Size Calculation

3.4.1 Water sample

In this study, public water supply of village (water plants) were selected by purposive. The researcher wants to evaluate WF of village water supply that still using of villages people. The number of sample size was summarized by number of water supply that support village people of the three districts. The total of village of three subdistricts were 303 villages, but village water supplies were 439 of water plants. This is because of some villages have more than one source of water supply.

The most visible geographical of the Northern Thailand's are surrounded by mountain chains, high mountains, a central basic, and an upland plateau. Areas. Then, most people face to lacking water supply sources, including Lamphun province. The water supplies sources and water supply organization supporter in Lamphun are difference between rural and town.

In rural areas, there is low housing density. The water supplies are supported by village committee or Subdistrict Administration Organization (SAO). Some villages do not have their owned water supply system (water plants) because they are separated by the original villages, when the original villages were divided into 2 or 3 new villages and the new village still use water supply support from the original village. Some villages have 2-3 or more sub-villages and they are very far apart. Then village committee or SAO built the water plant to support the village people. Some villages have more than one sources of water supply system e.g. village tap, water dispenser or bottled water.

On the other hand, in the town areas, there is high density of households and people are supported water supply by waterworks. For example, in *Nai Mueang sub district, Mueang Lamphun district*, there are 17 villages. The main source of water supply which delivery by the pipe to every households was only provincial waterworks authority waterworks. However, there are a lot water dispensers and bottled water (local brand) to support households.

The sampling of village water supply as a representative of village water supply of village cannot use in this study. Because the fluoride level of villages water supplies in each area is not same and the effect of fluoride depend on dose of exposed to people in a different dose the concentration, dosed and effect of.

Therefore, the number of village water supplies of 303 villages in three districts were 439 samples

3.4.2 Pregnant women

This study apply the formula of Chow S, Shao J & Wang H. [110] and use Powerandsamplesize online program [111] to calculate the sample size.

$$n_A = \kappa n_B \text{ and } n_B = \left(\frac{p_A(1-p_A)}{\kappa} + p_B(1-p_B)\right)\left(\frac{z_{1-\alpha/2} + z_{1-\beta}}{p_A - p_B}\right)^2$$

$$1-\beta = \Phi\left(z - z_{1-\alpha/2}\right) + \Phi\left(-z - z_{1-\alpha/2}\right) \quad , \quad z = \frac{p_A - p_B}{\sqrt{\frac{p_A(1-p_A)}{n_A} + \frac{p_B(1-p_B)}{n_B}}}$$

Refer to the Health Data Center (HDC) of Lamphun Provincial Health Office [112]. The in 2014 to 2016 shown that the proportion of LBW in normal area and endemic area were 0.66 and 0.85 respectively. In this study, we want to test the different proportions of two groups. We perform a two-sample test to determine whether the proportion in group A (pA) is different from the proportion in group B (pB). The hypotheses are

H_0 : pA – pB = 0 , H_1 : pA – pB ≠ 0

Where **K**= nA /nB is the matching ratio

Φ is the standard Normal distribution function

Φ^{-1} is the standard Normal quantile function

α is Type I error, β is Type II error, meaning $1-\beta$ is power

κ = 1, pA = 0.66 , pB = 0.85

alpha =0.05, beta = 0.20

Sample size: Exposures = 80, Low-exposed = 80

Sample size by using a continuity correction:

Exposed = 90, Low-exposed = 90

Finally: Exposed = 113, Low-exposed = 113

However, amount of the subjects were be added with 25 % [113] of the calculate sample size in order to avoid the problem of subject loss follow up or dropout. The final sample size was 113 pregnant women per group, totally 226 pregnant women.

3.5 Sampling technique

The study areas of this study were purposively selected every villages of districts where have found WF higher than 10.0 mg/L and average WF of district equal or higher than 1.50 mg/L.

The pregnant women were selected by convenience sampling with inclusion and exclusion of the study protocols

3.6 Measurement Tools

3.6.1 Questionnaires and Data collection form

The questionnaires in this study were developed from the guideline of criteria for classifying and checklist of the new antenatal women for the basic component at first ANC visit following the new WHO antenatal care model (2002) [79]. A structural interview was used to collect information of the pregnant women at first time data collection of the study. Then the information of the participant during ANC until delivery, postpartum, newborn information were collected by a Medical record form. The analysis of maternal and newborn fluoride, and village water supplies were used data collection form that design for this study to collection the data.

Content Validity: the questionnaires were created following the study objectives by research. The content and construction of questionnaires were approved for validity by five experts, including expert of College of Public Health Sciences, Chulalongkorn University, experts of environmental health, obstetrician, dentists and public health. The questionnaires were revise and organize following comment results of expertise., and the score Index of Item-Objective Congruence (IOC) of each questionnaire item were between 0.5. and 1.0. [114].

1) Questionnaires: were divided into 3 parts to use for data collection

Part 1 Residence Area: the questionnaires were composed residence area and year of living in the village of participant

Part 2 Sociodemographic & Characteristic: the questionnaires were composed of

(1) Pregnant: Age, Family status, Education, Family income, Gravida, Frequency of ANC visit, BMI before pregnant, BW change, BMI 21, Gestation at delivery, Supplementary (OBIMIN), Infection, Education of Spouse/partner,

(2) Newborn: Sex, Gestation at birth, High, BW, Head circumference, Abnormalities, Baby Health

Part 3 Risk Factors related to Premature delivery & LBW: the questionnaires were composed of Secondhand smoke, standing work, Tea Consumption, Coffee Consumption, Drinking water, Cooking water

Part 4. Clinical assessments the questionnaires were composed of

(1) ***Pregnant***: spot urine at < 30, 31-33, 34-36wks, blood serum at>30wks. serum fluoride

(2) ***Newborn:*** umbilical serum fluoride, PKU, TSH

2) Medical record forms and clinical assessment: was designed to collect medical record data of ANC, postpartum and newborn as following;

2.1) ANC and Postpartum: the data composed of History & medical record**:** Gravida, LMP, EDC, pregnant high, Body weight before pregnant, Body weight before delivery, Gestational age of 1-st ANC, Frequency of ANC visit, History of cesarean delivery, infection during pregnant, Hematocrit. The clinical assessments were composed of request and laboratory form of serum and urine for fluoride examine.

2.2) Newborn: the data composed Gestational age at birth, Sex, Body weight, Height, Head circumference, APGAR score at 1 and 5 minutes, type of delivery, Congenital abnormalities, Baby Health, TSH level, PKU level. The clinical assessments were composed of request and laboratory form of umbilical serum fluoride (USF) examine.

3) Village water supply and Geolocation survey form: was designed to record the information and survey the geolocation (GIS) of the villages water supply.

3.6.2 Instrument for Analyze fluoride

The instrument for analyze fluoride in this study was *Thermo Scientific Orion*, model 4-star benchtop. The method analysis was Ion Selective Electrode(ISE) using ion-selective electrodes, which is the standard method for the examination of water and wastewater by ORION model 4-star (16th edition28) after use of a total ionic strength adjustment buffer.

3.6.3 Geolocation measurement Application

This study used ***Smart System Info [115]*** - *a* free Global Positioning System (GPS) application for an android mobile device to measure the location of water supply plant location of the village water supply. The process to install application was showed in Figure 3-4.

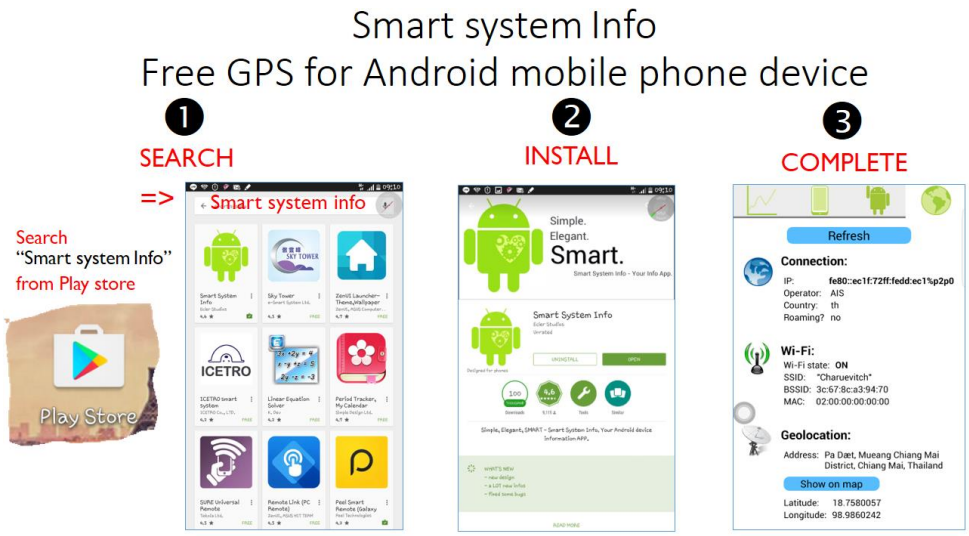


Figure 3- 4 Installation Smart System Info Free GPS for Android mobile

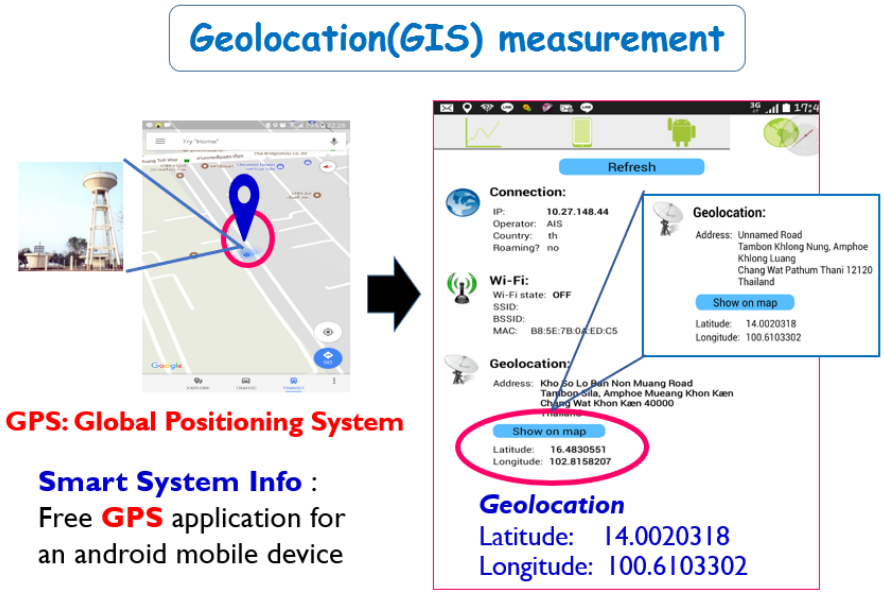


Figure 3- 5 Geolocation (GPS) measurement by Smart System info (free mobile app.)

3.7 Data Collection

The data collection was conducted from July 2016 to November 2017. The procedures and instruments was showed in Figure 3-6 and 3-7.

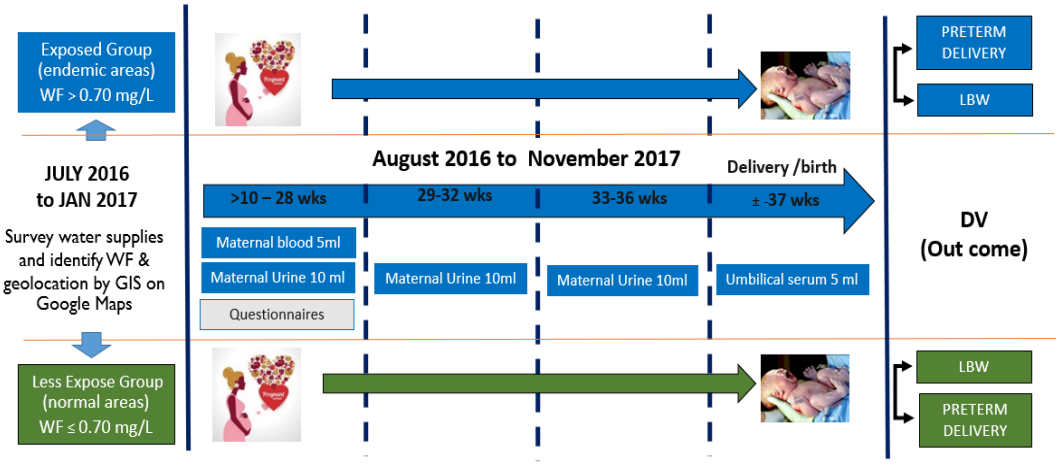


Figure 3- 6 Study design and time of data collection

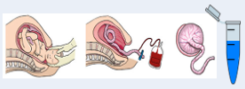
Sample	Requirements	Method	Test/Standard	LAB
Village Water supply 	30ml water supply and Geolocation (GIS) of 303 villages water supply plants	Collect and send with room temperature to LAB follow by TISAB	ISE instrument F (≤ 0.7mg/L)	ICOH
Maternal Serum 	5ml of pregnant blood at vein in gestation age at 1-st ANC (=<30wks)	Centrifuge speed 2500 rpm for 10 min separate serum into Eppendorf tube and Keep in frozen at -20 °C until measure follow by TISAB	ISE instrument F (1.0 mol/L)	ICOH
Maternal Urine (spot urine) 	10ml of pregnant urine for 3 times 1)1-st ANC =<30 wks 2) 31-33 wks 3) 34-36 wks	keep in 2-8 °C until measure follow by TISAB	pH (5.5 – 7.0) Cre (29 - 226 mg/dL) ISE instrument	Lamphun hosp. Pasang hosp. Ban Thi hosp. ICOH
Umbilical cord Serum 	5ml of umbilical cord blood at birth	Centrifuge speed 2500 rpm for 10 min, separate serum into Eppendorf tube and Keep in frozen at -20 °C until measure follow by TISAB	ISE instrument F (≤ 0.7mg/L)	ICOH

Figure 3- 7 *Data collection and Sample* Requirement

3.7.1 village water supply information and Geolocation

The Cross-sectional survey on water fluoride and endemic fluoride areas were conducted from July 2016 to January 2017. In this study, the researcher was cooperated with three District Public Health Office in Mueang Lamphun, Pasang and Ban Thi; 30 Health Promotion Hospital in 26 Sub District and three Department of Health Promotion of three hospital in study areas. Health Center. We have meeting and inform them about this research. They were educated about the process of water sample collection, explained about the survey form and process of measuring geolocation (GPS) of water supply plant by *smart inflo system*

For the number of household using water of each water supply. The data were summarized from the list of water supply using member. We reference this number from the administration of water supply. e.g village tap and waterworks, we received the data from village committee, community committee, municipality and Sub District Administration Organization (SAO). For bottled water (local brand), we collect the data from the bottled water owner.

In this study, the researcher has concern very much about number of household using water of each type of water supply. Because it means the number of people who expose to hjgh WF or safe drinking water. The result of WF level of village was used to identify the suady areas and woulde be effect from village people. If the

number is incorrect, the priority of endemic fluoride area and percentage of household that imply to people who face to fluoride effect is incorrect, too.

1) The information and location of village water supply:

The survey was conducted by the health officer of Health Promotion Hospital in 26 Sub District. The questionnaire of village water supply (water plant) survey were composes of Name of village, village number, sub district, district, amount of using household what's village number or village name that is the source of water supply for village use, and latitude and longitude and note for village that has more than one water plant or dose not has village water supply.

2) Water sample:

The 439-water sample of village water supply were collected with geo-location (GIS) to analyze level of fluoride by trained sub district health officer and collected at District Public Health of Mueang Lamphun, Pasang and Ban Thi before sent to analyses at CIOH Chiang Mai. The material and process to collect the water samples was following; [48];

1) Plastic bottles volume 30 cc was used to collect the water sample.

2) Clean faucet mouth and open valve to drain water for a few minutes.

3) Wash the bottle with water sample that was collected 2 to 3 times.

4) Collect water sample 30 cc and cover tightly.

5) Written detail of water sample and label on the bottle.

6) Keep water sample bottles in box without preserve and send within one day to laboratory of ICOH Chiang Mai for analyze of fluoride level.

7) ISE method will be used to analyze level of fluoride follow by TISAB refer to provision technical advice of WHO Oral Health Program 2014.

3.7.2 Data of pregnant women and Newborn

The pregnancy-birth cohort study was conducted from July 2016 to November 2017 in Lamphun, Pasang and Ban Thi hospital, Lamphun province. The procedure of data collection was showed in Figure 3-8

1) Questionnaires

The 226 pregnant women were recruited to this study with the protocol of inclusion and exclusion criteria. The recruited pregnant women were interviewed by questionnaires with full of four parts when gestational age equal or less than 30 weeks. The 1-st data collection information of pregnant was collected at from July 2016 to June 2017. The information of pregnant was collected only one time at first after receiving adequate research information and sign consent form.

Screening and *P*rocedures Card: the researcher must have designed Screening and *P*rocedures card for relevant person who assist in the study. The pregnant women were screened following the Protocol of inclusion & exclusion criteria of participants selection by trained ANC nurses. The procedures was showed in (Figure 3-8).

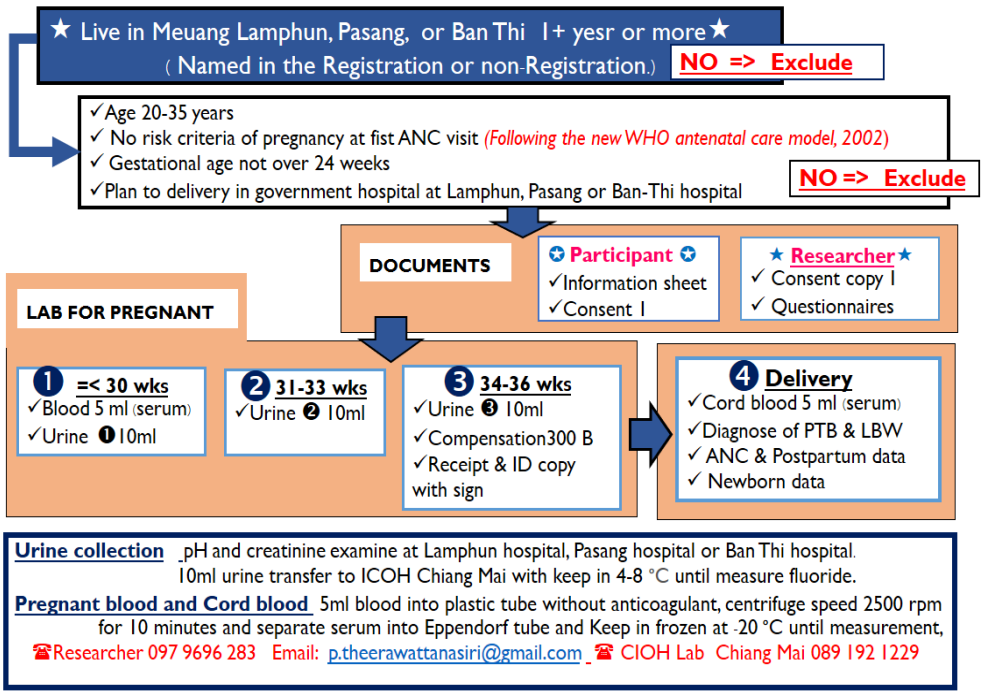


Figure 3- 8 Screening and Procedures for pregnant women in the research study

Follow up Card: During data collection, the researcher has designed the follow up card used for communication and make understanding among relevant assistant researcher of the department of hospital e.g. ANC Department, Laboratory Department. Labour Department or other Department and among hospital in study when pregnant women have serviced among the hospital by Health Referral system. (Figure 3.9

Data of pregnant women were starting to collect at gestation age less than 30 weeks (about 24 – 30 weeks) until delivery and then collecting postpartum and newborn data The diagnose of delivery and LBW were diagnosed by or obstetrician doctor.

Figure 3- 9 Follow up Card for communicate among research assistant and follow up the pregnant women.

3) Maternal Blood Serum of pregnant women

The maternal blood serum was collected to analyze level of fluoride for one time at gestational age equal or less than 30 weeks with normal services ANC programs of hospital. The process of maternal blood serum sample collection was showed in Figure 3-4 and 3-5. The process [44] as following; below.

1) Maternal blood serum was collected at arm vein of pregnant by laboratorial with 5 ml, then put into plastic tube without anticoagulant.

2) Centrifuge speed with 2,500 rpm for 10 minutes and separate serum into Eppendorf tube and Keep in frozen at -20 ℃ temperature until measurement, this process was conducted by laboratory of Lamphun, Pasang or Ban Thi hospital,

3) The maternal blood serum was sent to ICOH Chiang Mai.

4) ISE method was used to analyze level of fluoride following TISAB refer to provision technical advice of WHO Oral Health Program 2014.

4) Spot urine of pregnant women

The urine of pregnant women was collected to analyze level of fluoride for three times at gestational age 24-30, 31-33, and 34-36 weeks. The process of collection was conducted with normal services ANC programs of hospital.

In this process, urinary pH and creatinine were examined to confirm urinary excretion of fluoride concentration urinary pH and creatinine use to kidney function within an individual, an estimate of 24-hour urinary excretion of fluoride can be calculated by multiplying the ratio of urinary fluoride to urinary creatinine (F: Cr ratio) with creatinine reference values. The mean 24-hour urinary creatinine value of 15 mg/kgBW/day (with 5^{th} and 95^{th} percentiles of 8 and 22 mg/kgBW/day) has been reported as the standard urinary excretion of creatinine [5]. The urine pH and urine Creatinine will be doing by process of laboratory of Lamphun hospital

The process of spot urine collection [44] was showed in Figure 3-4 and 3-5 The process as following; below.

1) The urine bottles volume size 10 ml with label of pregnant code and area code were prepare for all pregnant.

2) The pregnant women were explained to collect the urine by herself from laboratorial. The urine was collected after little urinated and sent the urine bottle to laboratory of Lamphun, Pasang or Ban Thi hospital.

3). The spot urine was examined for pH and creatinine at the hospital.

4) The urine sample bottles were kept in cool box at 4-8 °C and transfer to analyze level of fluoride at laboratory of ICOH Chiang Mai.

5) ISE method used to analyze level of fluoride following TISAB refer to provision technical advice of WHO Oral Health Program 2014.

5) Umbilical Cord Serum collection

The umbilical cord serum was collected by nurse at Labour room clinic. The method of umbilical cord serum sample collection as below [44]

1) After the baby was delivered, the umbilical cord was clamp and cut, while waiting for the placenta to be deliver done, the residual umbilical cord blood was collected from the umbilicus vein by nurse.

2) 5 ml of umbilical cord blood was collected, then put into plastic tube without anticoagulant and centrifuge speed with 2,500 rpm for 10 minutes

4) The umbilical cord serum was sent to laboratory of ICOH Chiang Mai.

5) ISE method was used to analyze level fluoride of umbilical serum following TISAB refer to provision technical advice of WHO Oral Health Program 2014.

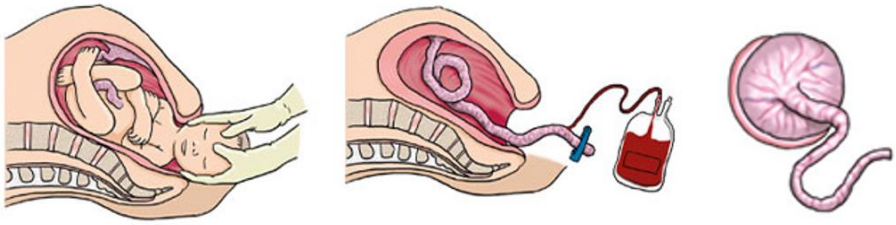


Figure 3- 10 *Umbilical Cord Blood Collection*

Source: http://www.scbb.com.sg/donate/collection/Pages/Home.aspx

The process of cord blood collection would be complete with a few minutes when the placenta was expelled. Umbilical cord serum of newborn would be collect one time at birth by healthcare providers of labour department of Labour of Lamphun, Pasang or Ban Thi hospital, showed in Figure 3-5 and Figure 3-8

6) Postpartum and Newborn information

The information of postpartum and newborn were collected from medical record by medical record form which researcher design for this study. The data were record by the nurse after the pregnant delivery. showed in Figure 3-4 and Figure 3-5.

3.8 Data Analysis

This study was used IBM SPSS Statistics Version 22 (Windows) support by Chulalongkorn University to record and analyze the data. following.

3.8.1 WF, Endemic fluoride areas, GIS data and Google Map

1) Microsoft Excel was used to create a GIS database and interpret the data of village water supply, geolocation of water supplies, and the fluoride levels.

2) The Smart System Info [115]; a free global positioning system (GPS) application for Android mobile device, was used to measure the geolocation of the water supplies.

3) The data boundaries of province, districts, and subdistricts of the Lamphun, were allowed and downloaded from the Global Administrative Areas GADM [108]

4) Google Earth [104] and Google Maps [106]: Google Earth was used to create and manage the geo-visual map layers as the Keyhole Markup Language (KML) and Keyhole Markup Language Zipped (KMZ) files. Then the data of all layers were export as KML or KMZ files for imported and presented in Google Maps

5) Type of water supplies was classified as village taps, waterworks, village filters, water dispensers, or bottled water.

6) A level of WF >0.7 mg/L was used to identify unsafe drinking water and endemic areas. Descriptive statistics were used to describe the results. Because the WF value was not evenly distributed, the median rather than the mean was used for calculating the average of the WF level. Percentages were used to analyze villages in

endemic areas and/or household exposure to high fluoride water and to determine unsafe drinking water in each of the subdistrict

7) WF level of water supplies were classified into five groups; (1) WF: ≤0.7 mg/L, (2) WF: 0.71–0.99 mg/L, (3) WF: 1.0–4.0mg/L, (4) WF: 4.1–10.0 mg/L, and (5) WF: 10.1–13.0 mg/L, respectively. The blue legend was used in the maps and dark blue was used to indicate levels of fluoride.

8) Water was classified as water with fluoride ≤0.7 mg/L, which was considered to be safe drinking water (green legend), and WF >0.7 mg/L, which was considered as unsafe drinking water (red legend).

9) Average WF of subdistricts were as follows: WF ≤0.7 mg/L (green), WF between 0.71 and 0.99 mg/L (yellow), WF between 1.0 and 0.4 mg/L (orange), and WF >4.0 mg/L (red).

10) Percentages of villages with endemic fluoride areas and household exposed to fluoride was given a red legend, with the intensity of the color range increasing from light to dark red depending on the level.

3.8.2 Diagnosis of Preterm Delivery and LBW

1) Preterm delivery. The obstetrician or doctor will determine and diagnose follow definition by ICD-10 Code ***O60.13*** [25].

2) Low birth weight (LBW): newborns weighing was measured within the first hours of life. LBW will assess by doctors follow definition by ICD-10 Code ***P07.00*** [24]**.**

3.7.3 Descriptive Statistics

1) Baseline data and clinical data: the comparison of demographic and clinical characteristics of pregnant women and newborns between Exposed and Low-exposed.

(1) Continuous data were presented as mean and standard deviation (mean ± SD). Data were compared by independent t-test.

(2) Categorical data were presented as frequency and percentage (n, %). Data were compared by chi-square test

(3) p-value of less than 0.05 was determined as statistical significant.

2) Independent t-Test was used to compare difference of fluoride level in urine of maternal and umbilical cord serum between Exposed and Low-exposed group.

3) Binary Logistic Regression use to assess the association between level of fluoride maternal urine and preterm delivery and LBW

4) Relative Risk or Risk Ratio (RR) was used to compare incidence of preterm delivery and LBW between Exposed and Low-exposed group

3.9 Ethical Consideration

This study, the protocol was approved by the Ethical Research Review Committee of Lamphun Hospital (Ethic LPN 50/2559) and The Ethics Research Review Committee of Research Involving Human Research participants, Health Science Group Chulalongkorn University, (COA no. 154/2016). All study subjects were provided an adequate study information before decision and signed consent form to participate in the study. They could withdraw or refuse to participate at any used in this study.

CHAPTER IV

RESULT

This study was conducted in Mueang Lamphun, Pasang and Ban Thi district, Lamphun province from July 2016 to November 2017.

A cross-sectional surveyed on water fluoride level of village water supply and identified endemic fluoride area were completed within 7 months from July 2016 to January 2017.

The cohort study of 226 pregnant women who were recruited to participate in this study was conduct July 2016 to November 2017.

The results of the study were presented in seven parts as the followings

4.1 Water Fluoride Analyses and Endemic Fluoride area

4.2 GIS data and Geo-graphical of Endemic fluoride areas

4.3 Analyses of fluoride level among pregnancy and newborns.

4.4 Comparison of urinary fluoride between Exposed and Low-exposed group

4.5 Association of maternal fluoride level and preterm delivery.

4.6 Association of maternal fluoride level fluoride level and LBW.

4.7 compare the incidence of preterm delivery and LBW between the Exposed and Low-exposed group.

4.1 Water Fluoride and Village Water Supply System

The study areas of this study were purposively selected of 303 villages in Mueang Lamphun, Pasang and Ban Thi District, Lamphun province Thailand. The 439 of water samples was collected to analyses fluoride level at CIOH Chiang Mai. The WF of village water supply was used to classify endemic fluoride area and Normal areas, the process was showed in Figure 4-1.

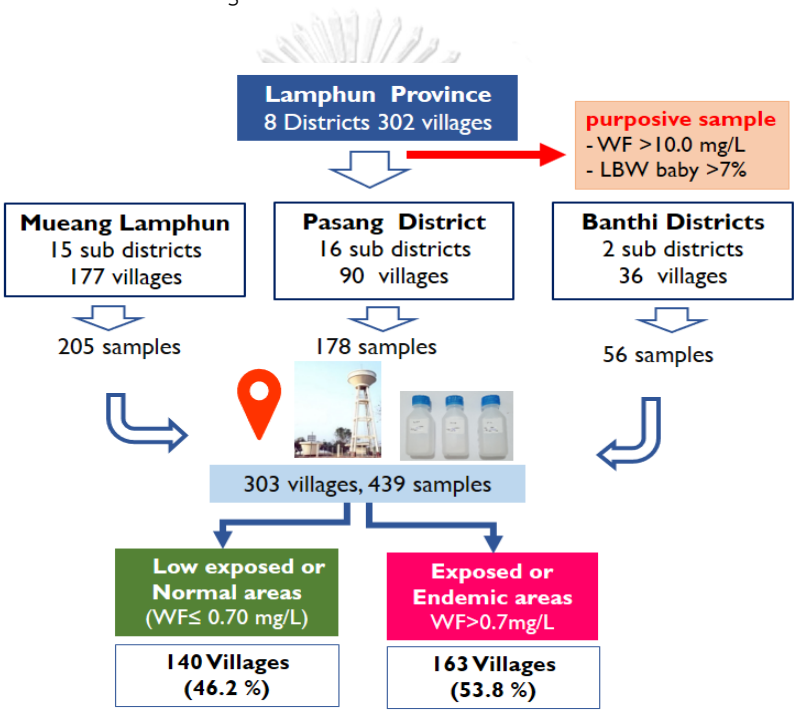


Figure 4- 1 Process of village water supply sampling

4.1.1 Village water supply system

There are two primary sources of water supply in Lamphun; the groundwater and surface water systems. The villages water supplies were composed of five types such as village taps (81.6%), village filters (12.5%), water dispensers (2.7%), bottled water (2.1%) and waterworks (1.1%), respectively, showed in Figure 4-2

(1) *Village taps*, in which most of the water is sourced from groundwater. The treatment system itself depends on the budget of administrations. Some village taps have the same treatment system as the waterworks, others include parts of the treatment process, and a smaller number only have water which is pumped to a storage tank and distributes through a pipeline. The administration system is made up of the local municipal government, Subdistrict Administration Organization (SAO), and a village or community-based committee.

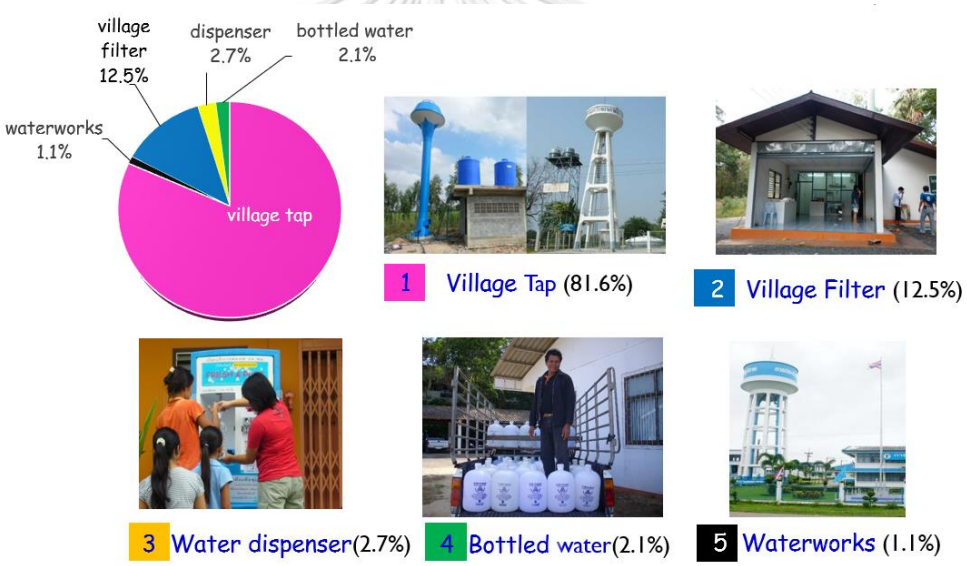


Figure 4- 2 Five types of Village water supply system

(2) *Village filter*: This water is sourced from village taps. The administration system is operated by village or community-based committees. The treatment systems consist of reverse osmosis (RO), ultraviolet, or ozone treatments. Households can access water by purchasing and transporting water in containers to their home by themselves, although some villages offer a home delivery system.

(3) *Water dispensers*: the water is sourced from village taps or groundwater in the form of shallow wells. Most are set up as private businesses. The treatment systems include RO, ultraviolet or ozone treatments. Households can access water by purchasing containers and bringing them home themselves.

(4) *Bottled water* (local brands): this water is sourced from groundwater. Most are set up as private businesses. The treatment systems include RO, ultraviolet or ozone treatments. After treatment the water will be kept in 20-liter plastic buckets or plastic bottle sized 750 ml and delivered to the home of customers.

(5) *Waterworks:* the source of water comes from the Mae Kuang river and it's operated by the provincial w*aterworks* authority. The treatment systems consist of rapid mixing, slow mixing, sedimentation filtration, chlorination, or storage tanks, and distribution occurs through a system of pipelines. This method is only used in the Mueang Lamphun district.

4.1.2 WF level and Unsafe drinking water

Table 4-1 present percentage of water sample by types and fluoride level. The WF range of village water supplies was 0.10–13.60 mg/L. The maximum fluoride level was 13.60 mg/L in village taps (Table 4-1). The average (median) WF was highest in water works (0.82 mg/L), village taps (0.61 mg/L), bottled water (0.38 mg/L), village filters (0.26 mg/L) and water dispensers (0.18 mg/L), respectively (Figure 4-3).

Table 4- 1 Percentage of water sample by types and fluoride level

Type of water	Number of sample		Fluoride Level (mg/L)		
	(n=439)	%	Minimum	Maximum	Median
Water supply					
village tap	358	81.6	0.10	13.60	0.61
waterworks	5	1.1	0.26	0.83	0.82
Drinking-water					
village filter	55	12.5	0.10	3.99	0.26
water dispenser	12	2.7	0.10	12.30	0.18
bottled water	9	2.1	0.10	3.08	0.38
Total	**439**	**100**	**0.10**	**13.60**	**0.54**

Abbreviations: mg/L– milligram per liter

Data were presented as mean ± standard deviation (mean ± SD) and frequency (n, %)

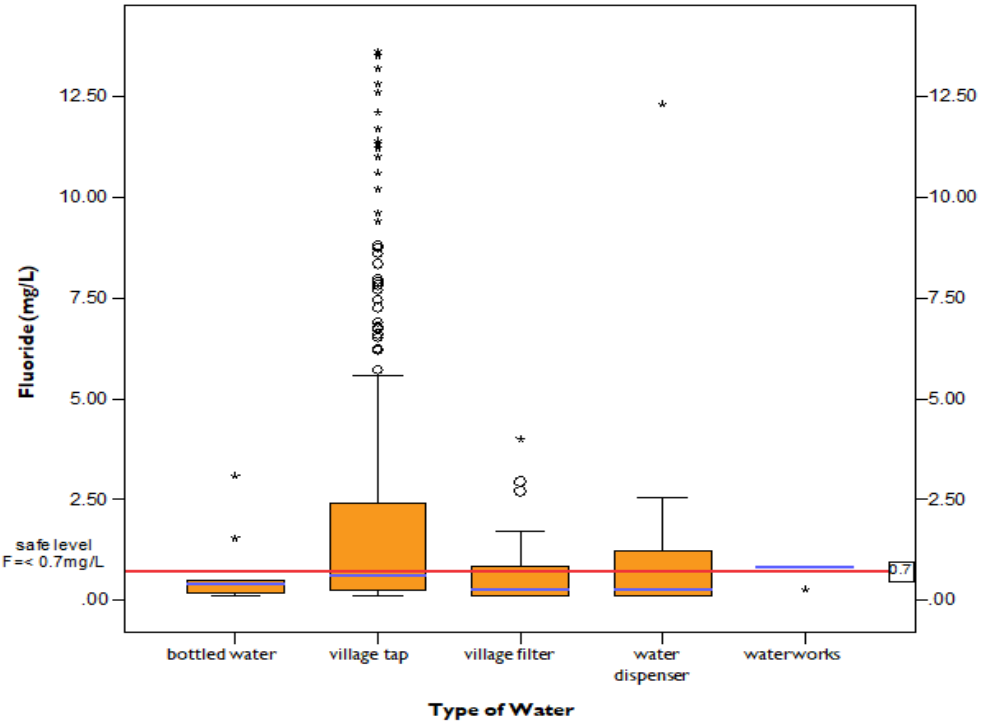


Figure 4- 3 Levels of fluoride found in different water sources.

Table 4-2 present percentage of water sample and household with unsafe drinking water. Of the 439 samples, 44.2% (194) contained unsafe drinking water. Unsafe drinking water was the highest for village taps (37.8%), followed by village filters (4.1%), waterworks and water dispensers (0.9%), and bottled water (0.5%). Of 79,807 households, 79.0 % used water from village. Overall 45.6% of household had unsafe drinking water and 34.9% used unsafe drinking water from village taps.

Overall 45.6% of households use unsafe drinking water. The highest percentage was waterworks (100%), village tap (44.1%) village filter (24.4%) bottle water (23.1%) and water dispenser (15.2%) respectively (Figure4-4)

Table 4- 2 Percentage of water sample and household with unsafe drinking water

Type of water	Water Sample (n=439)		Household*	
	Total (n, %)	Unsafe drinking water (n, %)	Total (n, %)	Unsafe drinking water (n, %)
Water supply				
village tap	358 (81.6)	166 (37.8)	63,073 (79.0)	27,842 (34.9)
waterworks	5 (1.1)	4 (0.9)	6,096 (7.6)	6,096 (7.6)
Drinking-water				
village filter	55 (12.5)	18 (4.1)	9,068 (11.4)	2,213 (2.8)
water dispenser	12 (2.7)	4 (0.9)	1,163 (1.5)	177 (0.2)
bottled water	9 (2.1)	2 (0.5)	407 (0.5)	94 (0.1)
Total	**439 (100)**	**194 (44.2)**	**79,807 (100)**	**36,422 (45.6)**

Note: Data were presented as frequency and percentage (n, %)

Source: amount of water sample by the survey, amount of household by water using registration of village committee, municipality, Subdistrict Administration Organization or drinking water business owner.

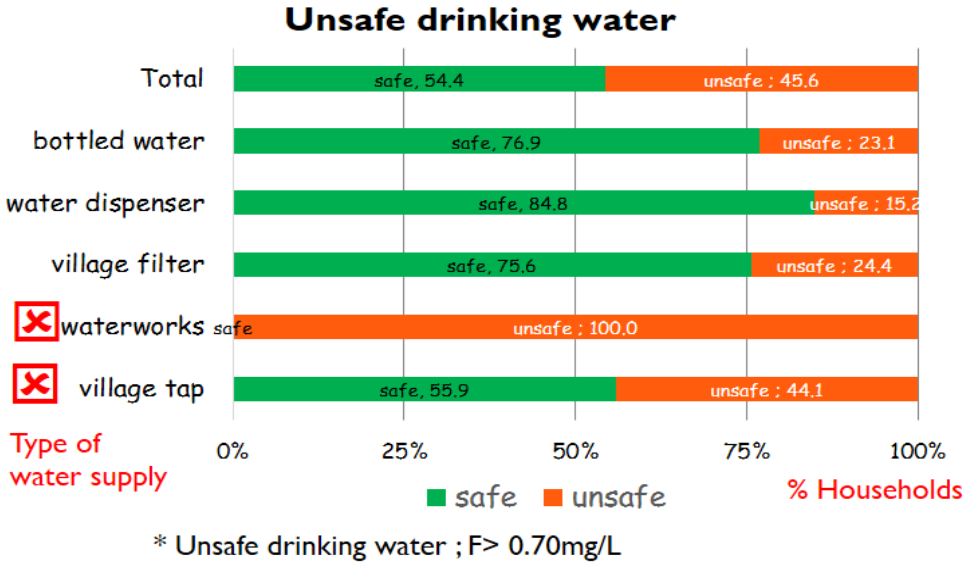


Figure 4- 4 Unsafe drinking water in different water sources.

4.2 GIS Data and Geo-graphical of Endemic Fluoride Areas

4.2.1 Endemic fluoride areas

Table 4-3 present Percentage of the village with an endemic area and household use of unsafe drinking-water by district. Overall, of 303 villages, 53.8% could be considered endemic areas (Figure 4-4), and of 79,807 households, 45.6% used unsafe drinking water. The percentage of villages with endemic areas were high in district of Ban Thi (75.0%), Mueang Lamphun (53.7%) and Pasang (45.6%) respectively. The percentage of a households using unsafe drinking water was highest in the Ban Thi (63.9%), Mueang Lamphun (49.8%) and Pasang (31.9%) districts.

Table 4- 3 Percentage of the village with an endemic area and household use of unsafe drinking-water by district

District	Village (n=303)		Household	
	Total	Endemic area (n, %)	Total	Unsafe Drinking water (n, %)
Muaeng Lamphun	177	95 (53.7)	46,404	23,121 (49.8)
Pasang	90	41 (45.6)	25,162	8,032 (31.9)
Ban Thi	36	27 (75)	8,241	5,269 (63.9)
Total	**303**	**194(44.2)**	**79,807**	**36,422 (45.6)**

Note: Data were presented as mean ± standard deviation (mean ± SD) and frequency (n, %)
Source: amount of water sample by the survey, amount of household by water using registration of village committee, municipality, Subdistrict Administration Organization or drinking water business owner.

Table 4-4 present Percentage of the village with an endemic area and Percentage of Households using unsafe drinking-water by sub district. Of a total of 303 villages, 54% (163) were endemic areas. Of a total of 26 sub districts, three sub districts, namely Makhuea Chae, Ban Klang, and Pa Sang (which including two sub districts) were all endemic villages. All households in the Ban Paen and Nai Mueang sub districts had unsafe drinking water.

Table 4- 4 Percentage of the village with an endemic area and Percentage of Households using unsafe drinking-water by sub district.

District / subdistrict	Range (mg/L)	Median (mg/L)	Villages (n=303)		Household	
			Total (N)	Endemic areas (n, %)	Total	Unsafe Drinking water (n, %)
Mueang Lamphun						
Makhuea Chae [c]	1.10-13.50	7.89	21	21 (100)	4,547	4,387 (96.5)
Ban Klang [c]	3.90-12.80	7.90 [b]	12	12 (100)	2,409	1,879 (78)
Ban Paen [c, d]	1.10-6.24	3.43	9	9 (100)	1,885	1,885 (100)
Nai Mueang [d]	0.82-0.83	0.83	17	17 (100)	6,096	6,096 (100)
Pa Sak	0.10-13.60 [a]	1.97	18	12 (66.7)	3,846	2,520 (65.5)
Nong Nam	0.10-7.26	2.44	9	6 (66.7)	696	523 (75.1)
Wiang Yong	0.40-7.70	1.86	8	5 (62.5)	1,659	1,008 (60.8)
Mueang Chi	0.10-5.40	0.35	14	5 (35.7)	2,877	1,315 (45.7)
Umong	0.18-2.23	0.48	11	3 (27.3)	3,717	1,302 (35)
Si Bua Ban	0.10-4.45	0.35	12	3 (25)	5,323	942 (17.7)
Mueang Nga	0.10-0.85	0.27	10	1 (10)	4,236	479 (11.3)
Ton Thong	0.12-0.96	0.43	9	1 (9.1)	3,279	752 (22.9)
Pratu Pa	0.10-0.33	0.14	11	0 (0)	1,868	0 (0)
Rim Ping	0.10-0.21	0.10	10	0 (0)	2,731	0 (0)
Nong Chang Khuen	0.13-0.38	0.15	6	0 (0)	1,235	0 (0)
Total	**0.10-13.60**	**0.40**	**177**	**95 (53.7)**	**46,404**	**23,088(49.8)**

Table 4-4 Percentage of the village with an endemic area and Percentage of Households using unsafe drinking-water by sub district. (Cont.)

District / subdistrict	Range (mg/L)	Median (mg/L)	Villages (n=303)		Household	
			Total (N)	Endemic areas (n, %)	Total	Unsafe Drinking water (n, %)
Pasang						
Pa Sang [c]	2.71-4.15	3.99	5	5 (100)	2,290	1,525 (66.6)
Pak Bong	0.58-1.59	1.19	5	4 (80)	2,185	1,459 (66.8)
Mae Raeng	0.18-3.45	1.02	11	7 (63.6)	2,921	1,307 (44.7)
Ban Ruean	0.27-1.84	0.96	8	5 (62.5)	2,230	758 (34)
Tha Tum	0.10-1.30	0.63	14	7 (50)	4,092	869 (21.2)
Nakhon Chedi	0.16-2.94	0.68	13	5 (38.5)	3,080	473 (15.4)
Nam Dip	0.10-3.40	0.51	17	6 (35.3)	2,916	617 (21.2)
Makok	0.10-3.32	0.33	9	2 (22.2)	2,808	350 (12.5)
Muang Noi	0.16-0.39	0.26	8	0 (0)	2,640	0 (0)
Total	**0.10-4.15**	**1.35**	**90**	**41 (45.6)**	**25,162**	**7,358 (29.2)**
Ban Thi						
Huai Yap	0.61-8.80	1.23	16	13 (81.2)	5,424	2,813 (51.9)
Ban Thi	0.12-8.60	4.42	20	14 (70)	2,817	2,274 (80.7)
Total	**0.12-8.80**	**0.62**	**36**	**27 (75)**	**8,241**	**5,087 (61.7)**
Total	**0.10-3.60**	**0.54**	**303**	**163(53.8)**	**79,807**	**35,533(44.5)**

Note [a] maximum WF level, [b] highest average WF level, [c] sub district where every village were endemic areas. [d] sub district where every village and every household used unsafe drinking-water.

Abbreviations: mg/L, milligram per liter

Figure 4-5 present the average of water fluoride in sub district level. The maximum WF level was 13.60 mg/L in Pasak, with the highest average fluoride level of 7.90 mg/L observed in Ban Klang (7.90 mg/L) in the Mueang Lamphun district (Figure 3). Of a total of 26 subdistricts, 50% were endemic fluoride areas. In five subdistricts all villages were endemic fluoride areas, namely Makhuea Chae, Ban Klang and Pa Sang, and in those, two subdistricts, namely Ban Paen and Nai Mueang, every household used unsafe drinking water (Figure 4-5).

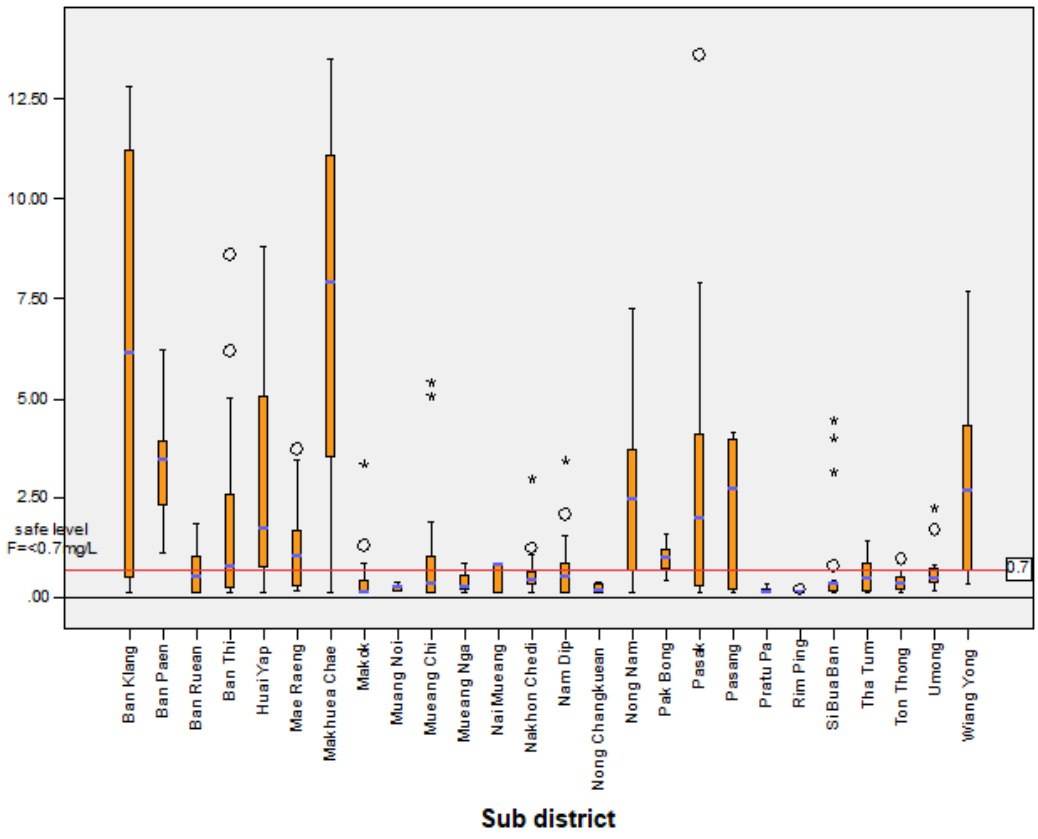


Figure 4- 5 The average of water fluoride in sub district level.

4.2.2 GIS & Geo-visual on Google Maps

The data of 439 village water supplies (water plants) of 303 village, including general information and geolocation (GPS) were collected and created as GIS data by Microsoft Excel and analyses data were present on Google Map as Geo-visual map of 10 layers, following;

Layers of geo-visual maps

(1) ***Study area:*** composed of the boundaries of Lamphun province, Mueang Lamphun, Pasang and Ban Thi districts

(2) ***A sub district of the Mueang Lamphun District:*** composed of the 15 sub-district boundaries of *the* Mueang Lamphun District.

(3) ***A sub district of the Pasang and Ban Thi Districts:*** composed of 9 sub district boundaries of Pasang and 2 sub district boundaries of Ban Thi District.

Layer of GIS data of Water fluoride level

(4) Types of Water supplies. composed of GIS data 439 water supplies plant location and classified of 5 type of water supplies system.

(5) Fluoride levels of water supplies: composed of GIS data 439 water supplies plant location and classified to 5 level of water fluoride.

(6) Unsafe drinking water in the Mueang Lamphun district: composed of the legend present safe drinking water and unsafe drinking water in Mueang Lamphun.

(7) Unsafe drinking water in the Pasang and Ban Thi districts. : composed of the legend present safe drinking water and unsafe drinking water in Pasang and Ban Thi districts.

Layer of geo-visual maps of fluoride analysis

(8) Average water fluoride (WF) level of sub district (Median): composed of the average of WF in sub district level in Mueang Lamphun, Pasang and Ban Thi districts.

(9) Percentage of household exposed to fluoride in sub district: composed of the map areas present percentage of household used unsafe drinking water in district level in Mueang Lamphun districts.

(10) Percentage of village with Endemic Fluoride in sub district: composed of the map areas present percentage of household used unsafe drinking water in district level in Pasang and Ban Thi districts.

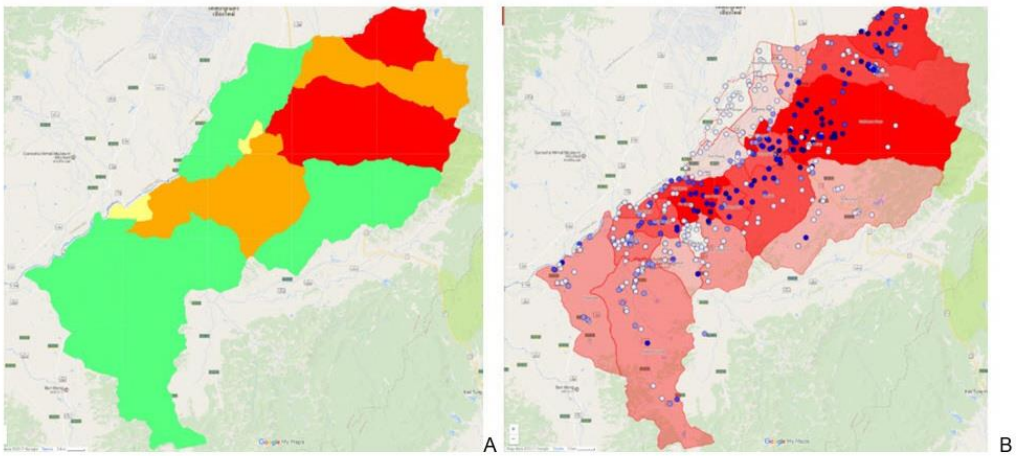


Figure 4- 6 GIS data and Google Map showed separate layer (A: one layer) and Mixed (B: Mixed of Layer 5 and 9)

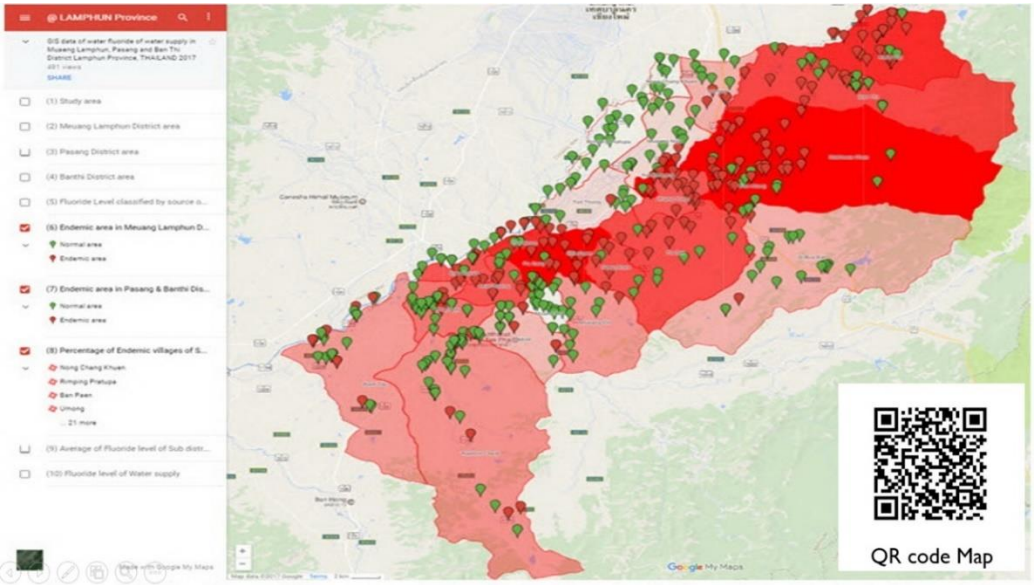


Figure 4- 7 GIS data and Google Map showed Mixed of Layer 6, 7 and 8

The geo-visual map and GIS data can be used separately, or the layers can be mixed together. An example of this shown in Figures 4 and 5.

The relevant agencies or internet user can access to the GIS data and Google Maps through their web browsers or smart phone devices from anywhere.

The GIS data of water and geo-visual map were presented in Google Maps, and are available at;

https://drive.google.com/open?id=1mi4Pvomf5xHZ1MOjK44pdp2xXFw&usp=sharing.

4.3 Baseline Demographic and Characteristics

The study was conducted from August 2016 to November 2017This study and. The 226-pregnant women age between 20 to 35 years were recruited to participate and start first data collection; 113 of participants in Exposed and 113 of Low-exposed group. After the 3-rd times of data collection, the 197participants were remained to continue, 6 abortions, 12 did not complete 3 times data collection, 5 moved ANC and lost to follow up. The 141 pregnant women were completed data collection until delivery, consist of 73 pregnant women in Low-exposed and 68 in Exposed group. Flow of the study population was demonstrated in Figure 4-8

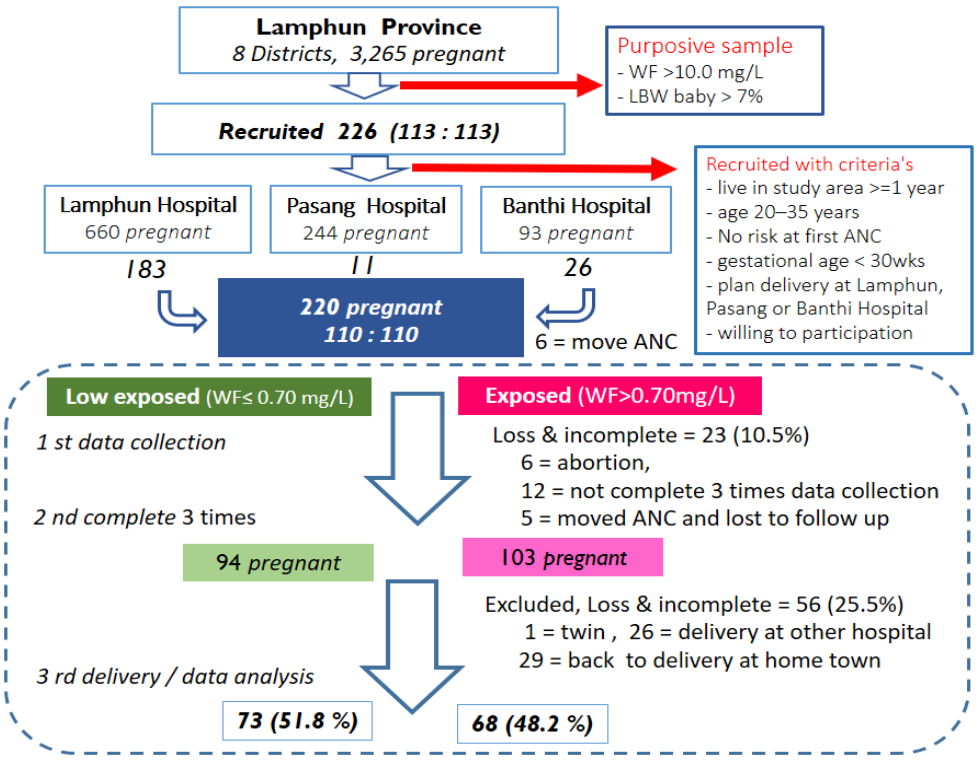


Figure 4- 8 Flow of the study participants.

Table 4-5 present baseline demographic and Clinical characteristic of pregnant women following:

Residence areas:

There was a strong significant difference WF level of village water supplies between Low-exposed and Exposed group (p = .000). Overall the average WF level of village water supply was 2.74 (± 3.52) mg/L, in Low-exposed was 0.39 (± 0.26) mg/L. and in Exposed group was 5.26 (± 3.65) mg/L. There was a no significant difference Year of living between Low-exposed and Exposed group (p = .67). Overall the average Year of living was 4.05 (± 1.21) years, in Low-exposed group was 4.00 (± 1.46) years, and in Exposed group was 4.10 (± 1.445.26) years

Demographic and characteristic of Pregnant women:

Of the 141 pregnant women, 51.8% (73) were Low-exposed 48.2% (68) were Expose group. There was a no significant difference education of pregnant women (p=.67), education of partner/souse (p=.56), family status (p=.56), family income (p=.56) between Low-exposed and Exposed group.

Current history:

There was a no significant difference average about Age between Low-exposed and Exposed group (p=.45). The average age of pregnant women (± SD) was 27.31 (± 4.35) years, in Low-exposed group was 27.58 (± 4.35).00 (± 1.46) years, and in Exposed group was 27.03 (± 4.37) years.

For weight and High, there was a significant difference Hight (p=.006), Body weight before pregnant (p=.02) of pregnant women between Low-exposed and Exposed group (p=.006). The average of Hight (meters) in Low-exposed (1.58 ± 0.59) was higher than Exposed (1.56 ± 0.56), the average of Body weight before pregnant in Low-exposed (55.50 ± 10.53) was higher than Exposed (51.724 ± 8.60). However, there was no significant difference of BMI (p=.17), between the two groups.

Past history. Most of them (74, 52.5%) have been history of delivery and a few (4.3%) have history of Cesarean section. However, there was no significant difference about history of pregnant have been delivery (p=.74) and Cesarean section (p=.21), between the two group.

Risk Factors to Premature delivery & LBW

There was a no significant difference of Drinking water, (p=.66), Cooking water (p=.22), Tea consumption (p=.49), Coffee consumption (p=.49), Standing work (p=.60) between Low-exposed and Exposed group. The average of height in Low-exposed. But There was a significant difference of secondhand smoke between Low-exposed and Exposed group (p=.02), The percentage of secondhand smoke in Low-exposed (38, 63.3%) was higher than Exposed group (22, 36.7%).

Table 4- 5 Baseline demographic and Clinical characteristic of pregnant women

Variables	Pregnant Women			p-value
	Total (n=141), %	Low-exposed (n=78), %	Exposed (n=72), %	
Residence areas				
Village WF	2.74 ± 3.52	.39 ± 26	5.26 ± 3.65	.000 ***
Year of living	4.05 ± 1.21	4.00 ± 1.46	4.10 ± 1.44	.67
Demographic and characteristic				
Number of pregnant	141 (100)	73 (51.8)	68 (48.2)	
Education of pregnant				.67
primary school	36 (25.5)	16 (44.4)	20 (55.6)	
secondary school	42 (29.8)	21 (50.0)	21 (50.0)	
High school/vocational	49 34.8)	28 (57.1)	21 (42.9)	
certificate / higher	14 (9.9)	8 (57.1)	6 (42.9)	
Education of partner/souse				.56
primary school	36 (25.5)	17 (47.2)	19 (52.8)	
secondary school	45 (31.9)	24 (53.3)	21 (46.7)	
High school/vocational	47 (33.3)	23 (48.9)	24 (51.1)	
certificate / higher	13 (9.2)	9 (69.2)	4 (30.8)	
Family status				
live together	141 (100)	73 (51.8)	68 (48.2)	
Family income				.56
5000 - 10000	36 (25.5)	17 (47.2)	19 (52.8)	
10001 - 15000	45 (31.9)	24 (53.3)	21 (46.7)	
15001 - 20000	47 (33.3)	23 (48.9)	24 (51.1)	
> 20001	13 (9.2)	9 (69.2)	4 (30.8)	
Clinical characteristic of Pregnant women				
Current				
Age	27.31 ± 4.35	27.58 ± 4.35	27.03 ± 4.37	.45
Height (m)	1.57 ± .59	1.58 ± 0.59	1.56 ± 0.56	.006 **

Table 4.5 Baseline demographic and Clinical characteristic of pregnant women**(cont.)**

Variables	Pregnant Women			p-value
	Total (n=141)	Low-exposed (n=78)	Exposed (n=72)	
Current				
BW before pregnant (kg)	53.68 ± .9.80	55.50 ± 10.53	51.724 ± 8.60	.02 *
BMI before pregnant	21.82 ± 3.74	22.24 ± 4.08	21.38 ± 3.29	.17
1-st ANC > 12 wks. (Yes)	46 (32.6)	30 (65.2)	16 (34.8)	.02 *
Gestation age 1-st ANC	11.7 ± 4.5	12.2 ± 4.1	11.1 ± 4.1	.16
Past history				
Have been pregnant (Yes)	74 (52.5)	37 (50.0)	37 (50.0)	.74
No	67 (47.5)	36 (53.7)	31 (46.3)	
History of Cesarean (Yes)	6 (4.3)	5 (83.3)	1 (16.7)	.21
No	135 (47.5)	68 (54.4)	67 (49.6)	
Risk Factors to Premature delivery & LBW				
Drinking water				.66
tap water	23 (16.3)	13 (56.5)	10 (43.5)	
bottled water	118 (83.7)	60 (50.8)	58 (49.2)	
Cooking water				.22
tap water	30 (21.3)	19 (63.3)	11 (36.7)	
bottled water	111 (78.7)	54 (48.6)	57 (51.4)	
Tea Consumption (Yes)	2 (1.4)	2 (100)	0 (0)	.49
Coffee Consumption (Yes)	2 (1.4)	2 (100)	0 (0)	.49
Standing work (Yes)	16 (11.3)	7 (43.8)	9 (56.3)	.60
Secondhand smoke (Yes)	60 (42.6)	38 (63.3)	22 (36.7)	.02 *

Note [a] Fisher's Exact Test (n less than 5),

(m) = meters, (kg) = kilogram, (mg/L) = milligram per liter ,

Data were presented as mean ± standard deviation (mean ± SD) and frequency (n, %)

* p < .05, ** p < .01, *** p < .001

4.4 Clinical Characteristic of Pregnant Women before Delivery and Newborn

4.4.1 Clinical Characteristic of Pregnant Women before Delivery

Table 4-6 resent Clinical characteristic of Pregnant women before Delivery following;

There was strong significant difference of maternal urine fluoride (MUF) between the two groups (p=.001). The average of MUF of population was 1.27 (± .1.08), in Low-exposed was 0.98 (± .64) mg/L and in Exposed was (1.59 ± 1.35)

There was significant difference of fluoride creatinine ratio (MUF/Cr ratio) between the two groups at gestation age of pregnancy less than 30 week (p=.019), 31-33 week (p=.008), and 34-36 week (p= .017). The average of MUF/Cr ratio in Exposed group was increasing when gestation of pregnancy increased 0.223 (± .19) mg F/g Cr, 0.223 (± .18) mg F/g Cr and 0.245 (± .24 mg) F/g Cr respectively.

There was no of Hematocrit (Hct, %) between the two groups. The average Hematocrit (%) of pregnant was 35.16 ± 2.87, in the low-exposed and Exposed were 35.02 ± 2.56 and 35.31 ± 3.19.55 respectivly.

There was significant difference BW Change (p=.004) between the two groups. The average of BMI Change of pregnant were 13.50 (± .9.80) kg, the average of BMI Change in Low-exposed and Exposed were 14.64 (± 4.93) and 12.27 (± 4.55) kg.

There was significant difference of average BMI 21 before delivery (p=.015) of the two group. The average BMI 21 before delivery of population was 21.82 (± 3.74). in Low-exposed was 22.24 (± 4.08) and in Exposed was 21.38 (± 3.29)

There was significant difference of Gestation of delivery/birth(p=036) between the two group. The average of Gestation of delivery/birth of newborn was 38.58 ± 1.58 weeks, in Low-exposed and Exposed was 38.31 (± 1.51) ,38.87 (± 1.62) weeks

However, there was no significant difference of urine pH, creatinine, Infection, ANC < 5 visits, Average of ANC visit and Supplementary between the two groups.

Table 4- 6 Clinical characteristic of Pregnant women before Delivery

Variables	Pregnant Women			p-value
	Total (n=141)	Low-exposed (n=78)	Exposed (n=72)	
Average MUF (mg/L)	1.27 ± .1.08	.98 ± .64	1.59 ± 1.34	.001 **
MUF/Cr ratio -1 (mg/L)	.189 ± .17	.157 ± .14	.223 ± .19	.019 *
MUF/Cr ratio -2 (mg/L)	.189 ± .15	.156 ± .11	.223 ± .18	.008 **
MUF/Cr ratio -3 (mg/L)	.203 ± .20	.154 ± .141	.245 ± .24	.017*
Creatinine -1 (mg/L)	7.17 ± 4.64	6.88 ± 4.65	7.48 ± 4.65	.44
Creatinine -2 (mg/L)	7.44 ± 4.84	7.19 ± 4.65	7.72 ± 5.26	.51
Creatinine -3 (mg/L)	7.42 ± 4.22	7.18 ± 3.91	768 ± 4.56	.49
Urine pH -1	6.78 ± .63	6.80 ± .65	6.76 ± .60	.70
Urine pH -2	6.84 ± .59	6.84 ± .56	6.83 ± .64	.96
Urine pH -3	6.82 ± .52	6.86 ± .49	6.77 ± .56	.33
Hematocrit (%)	35.16 ± 2.87	35.02 ± 2.56	35.31 ± 3.19	.55
Infection (Yes)	5 (0.7)	3 (60)	2 (40)	.29
DM	1 (0.7)	1 (100)	0 (0)	
GDM1	1 (0.7)	0 (0)	1 (100)	
HBAg+	3 (2.1)	2 (66.7)	1 (33.3)	
BW Change (kg)	13.50 ± .9.80	14.64 ± 4.93	12.27 ± 4.55	.004 **
BMI 21 before delivery	21.82 ± 3.74	22.24 ± 4.08	21.38 ± 3.29	.015 *
ANC < 5 visits (Yes)	43 (30.5)	27 (62.8)	16 (37.2)	.10
Average of ANC visit	4.55 ± 0.78	4.42 ± 0.85	4.68 ± 0.68	.055
Gestation of delivery/birth (wk)	38.58 ± 1.58	38.31 ± 1.51	38.87 ± 1.62	.036 *
Supplementary				.39
OBEMIN	56 (39.7)	26 (46.4)	30 (53.6)	
Triferdine	85 (60.3)	47 (55.3)	38 (44.7)	

Note Fisher's Exact Test (n less than 5), (mg/L) = milligram per liter, (mg) = milligram, (g) = gram, (mg/dl) = milligram per deciliter, MUF = maternal urine fluoride, F/Cr ratio = Fluoride Creatinine Ratio (mg F/ g Cr), Data were presented as mean ± standard deviation (mean ± SD) and frequency (n, %) * p < .05, ** p < .01, *** p < .001

4.4.2 Clinical characteristic of Newborn

Table 4-7 present Clinical characteristic of Newborn following: Overall there were no significant difference Clinical characteristic of newborn.

There was no significant difference of Sex of newborn (p=.74) between the two groups. There were 42 of boys (56.0%), 62 of girls (44.0%), 53.2% (42) was boy in Low-exposed, 46.8% (37) was in Exposed and 50.0% (31) of girls in both groups.

There was no significant difference of Newborn weight (p=.99) between the two groups. Overall Newborn weight was 2978.92 (± 407.50) g. The average of Newborn weight in Low-exposed was 2979.12 (± 381.35) g and in Exposed was 2978.71 (± 436.69) g.

There was no significant difference of Newborn high (p=.67) between the two groups. Overall Newborn high was 50.60 (± 2.21) cm. The average of Newborn high in Low-exposed was 50.60 (± 2.20) cm and in Exposed was 50.76 (± 2.23) cm.

There was no significant difference of Head circumference (p=.37) between the two groups. Overall Head circumference was 33.37 (± 1.32) cm. The average in Low-exposed was 33.47 (± 1.31) cm and in Exposed was 33.47 (± 1.31) cm.

There was no significant difference of APGARR score 1 (p=.51) APGARR score 5 (p=.51) and between the two groups. Overall APGARR score 1 was 9.99 (± 0.84). The average of APGARR score 1 in Low-exposed was 10.0 and in Exposed was 9.99 ± 0.12. Overall APGARR score 5 was 9.77 (± 0.57). The average of APGARR score 1 in Low-exposed was 9.77 (± 0.57) and in Exposed was 9.71 (± 0.52).

There was no significant difference PKU and TSH between the two groups. Overall average PKU was 1.13 (± 0.33), in Low-exposed and Exposed were 1.15 (± 0.35) and 1.11 (± 0.31) mU/ml respectively. The average TSH was 4.86 (± 6.47) mg/dl, in Low-exposed and in Exposed was 3.97 (± 2.50) and was 5.69 (± 8.61) respectively

Table 4- 7 Clinical characteristic of Newborn

Variables	Pregnant Women			
	Total (n=141)	Low-exposed (n=78)	Exposed (n=72)	p-value
Type of delivery				.70
Normal delivery	70 (49.6)	34 (55.3)	36 (44.7)	
Cesarean section	61 (43.3)	33 (54.1)	28 (45.9)	
Vacuum extraction	10 (7.1)	6 (60.0)	4 (40.0)	
Sex (boy)				
boy	42 (56.0)	42 (53.2)	37 (46.8)	.74
girl	62 (44.0)	31 (50.0)	31 (50.0)	
Newborn Weight (g)	2978.92 ± 407.49	2979.12 ± 381.35	2978.71 ± 436.69	.99
Newborn high (cm)	50.60 ± 2.21	50.60 ± 2.20	50.76 ± 2.23	.67
Head circumference (cm)	33.37 ± 1.32	33.47 ± 1.31	33.26 ± 1.33	.37
APGARR score 1	9.74 ± 1.32	9.77 ± 0.57	9.71 ± 0.52	.51
APGAR score 5	9.99 ± 0.84	10.0	9.99 ± 0.12	.30
PKU (mU/ml)	1.13 ± 0.33	1.15 ± 0.35	1.11 ± 0.31	.11
TSH (mg/dl)	4.86 ± 6.47	3.97 ± 2.50	5.69 ± 8.61	.51

Note Fisher's Exact Test (n less than 5), F:C ratio = Fluoride : Creatinine Ratio

(cm) = Centimeters, (mg/L) = Milligram per liter, (kg) = Kilogram, (g) = gram

Data were presented as mean ± standard deviation (mean ± SD) and frequency (n, %)

* p < .05, ** p < .01, *** p < .001

4.5 Comparison of Maternal Urine Fluoride Level between the two groups.

According to Table 4-8 below, there was strongly significant difference (p=001) between of MUF level between the two groups. The average of MUF level was 1.27 (± .1.08) mg/L, the average MUF level in Low-exposed was 0. .98 (± .64) mg/L, the average MUF level in Exposed 1.560 (± 1.34) mg/L.

Table 4- 8 Comparison of MUF level beteen Low-Expose and Expose.

Variables	Pregnant Women			
	Total (n=141)	Low-exposed (n=78)	Exposed (n=72)	p-value
Average MUF (mg/L)	1.27 ± 1.08	.98 ± .64	1.60 ± 1.34	.001 **
Fluoride Creatinine Ratio				
MUF/Cr ratio -1 (mg/L)	.189 ± .17	.157 ± .14	.223 ± .19	.019 *
MUF/Cr ratio -2 (mg/L)	.189 ± .15	.156 ± .11	.223 ± .18	.008 **
MUF/Cr ratio -3 (mg/L)	.203 ± .20	.154 ± .141	.245 ± .24	.017*
MUF by urine test 1-3				
MUF1 (mg/L)	1.216 ± 1.23	.903 ± .80	1.551 ± 1.50	.019 *
MUF2 (mg/L)	1.28 ± 1.15	1.051 ± .85	1.533 ± 1.38	.008 **
MUF3 (mg/L)	1.338 ± 1.44	.998 ± .77	1.703 ± 1.85	.017*
Average MUF group (cut point (.5 mg) a*				
Low MUF (=<0.5 mg/L)	23 (16.3)	14 (60.9)	9 (3891)	.234
High MUF >0.5 mg/L	118 (83.7)	59 (50.0)	59 (50.08)	
Average MUF group (cut point (1.0 mg) b*				
Low MUF (=<0.5 mg/L)	78 (55.3)	49 (62.8)	29 (37.8)	.003*
High MUF >0.5 mg/L	63 (44.7)	24 (38.1)	39 (61.9)	

Note a* reference to Juntarawijit C (Thailand, 2003), b*reference to *Rachel Hart et al, (US,2009)* (cm) = Centimeters, (mg/L) = Milligram per liter, Data were presented as mean ± standard deviation (mean ± SD) and frequency (n, %) * p < .05, ** p < .01, *** p < .001, a

When comparison MUF by low and high group (cut point at 1.omg/L). There was a strongly significant difference (p=.003) of MUF between the two groups. We found that 55.3% (78) of pregnant women were have low MUF level (MUF ≤1.0 mg/L) and 44.7 % (63) were have high MUF level (F>1.0 mg/L). In the low MUF group (F≤1.0 mg/L), 62.8% (49) was found in Low-exposed and 38.1% (24) were found in Exposed. In high MUF group (>1.0 mg/L), 61.9% (28) was found in Exposed and 38.1% (24) was found in Low-exposed.

There was significant difference of fluoride creatinine ratio (MUF/Cr ratio) between the two groups at gestation age of pregnancy < 30 week (p=.025), 31-33 week (p=.006), and 34-36 week (p= .004). The average of F/Cr ratio in Exposed group was increasing when gestation of pregnancy increased 0.225 (± .196) mg F/g Cr, 0.226 (± .192) mg F/g Cr and 0.264 (± .250 mg) F/g Cr respectively.

4.6 Association of MUF Level and Preterm Delivery.

Table 4-9 present Univariate and Multivariate Logistic Regression Analysis of Preterm. Data of 141 pregnant women who completed data collection were analyzed. Univariable analysis of suspected factors risk to preterm delivery with a *p*-value less than 0.2 to 0.5 and factors that researcher reference in previous study such as MUF more than1.0 mg, 1-st ANC more than 12 wk, Supplementary, History of cesarean, and BW change

A Binomial logistic regression analysis was done to adjust for those factors. We found that there are no factors significantly associated with preterm delivery

Table 4- 9 Univariate and Multivariate Logistic Regression Analysis of Preterm delivery

Variables	**Univariate** *analysis*		*Multivariate analysis*	
	OR (95% CI)	**p-value**	**OR $_{adj.}$(95%CI)**	**p-value**
MUF >1.0 mg [a*]	1.37 (0.31, 5.97)	.675	1.45 (0.30, 6.96)	.642
1-st ANC >12 wk.	0.42 (0.10, 1.74)	.230	0.51 (0.12, 2.21)	.364
Supplementary	2.68 (0.61, 11.70)	.190	4.21 (00, 1.70)	..100
History of cesarean	0.27 (0.03, 2.67)	.265	0.12 (.00, 179)	.125
BW change	0.93 (0.79, 1.09)	.370	0.91 (0.78, 1.07)	.265

Note a*reference to *Rachel Hart et al, (US,2009)*

* p < .05, ** p < .01, *** p < .001

4.7 Association of MUF Level and LBW.

Table 4-10 present Univariate and Multivariate Logistic Regression Analysis of Low Birth weight. Data of 141 newborns who was collected umbilical serum and whose mother completed data collection were analyzed. Univariable analysis of suspected factors risk to LBW with a *p*-value less than 0.2 to 0.5 and factors that researcher reference in previous study such as MUF more than 0.5 mg/L, BW before pregnancy, BW change during pregnancy, 1-st ANC more than12 wk., History of delivery, and Supplementary

A Binomial logistic regression analysis was done to adjust for those factors. We found that only MUF more than 0.5 mg/L was significantly associated with LBW (*p=.003)*

Table 4- 10 Univariate and Multivariate Logistic Regression Analysis of Low Birth weight

Variables	**Univariate** *analysis*		*Multivariate analysis*	
	OR (95% CI)	**p-value**	**OR $_{adj.}$ (95%CI)**	**p-value**
MUF (>0.5 mg/L)	6.45 (2.16, 19.30)	.001**	6.16 (1.85, 20.50)	.003*
BW before pregnancy	0.96 (0.90, 1.02)	..178	0.98 (0.92, 1.04)	.523
BW change	0.92 (0.82, 1.03)	.143	0.86 (0.78, 1.01)	.061
1-st ANC >12 wk.	0.78 (0.27, 2.27)	.647	1.81 (0.45, 7.34)	.404
History of delivery	2.2 (0.78, 6.40)	.137	1.73 (0.54, 5.13)	.356
Supplementary	1.41 (0.51, 3.90)	.511	1.67 (.55, 5.13)	.368

Note a* reference to Juntarawijit C (Thailand, 2003)

* p < .05, ** p < .01, *** p < .001

4.8 Comparison Incidence of Preterm Delivery and LBW between the two groups

4.8.1 Incidence and Comparison Risk Ratio of Preterm Delivery

Table 4-11 present the comparison incidence of preterm delivery between two groups. The incidence of preterm delivery and the relative risk (or risk ratio) were used to compare the risks for preterm delivery the two groups. The incidence of preterm delivery in Exposed and Low-exposed were 8.23 % VS 2.94%. The relative risk (or risk ratio) of preterm delivery was 0.35. this study pregnant women who live in endemic fluoride areas had 0.35 times the risk of preterm delivery compared to pregnant women who live in Normal areas.

Table 4- 11 Comparison Incidence of Preterm delivery between two groups

Group	Preterm (n)	Normal (n)	Total (n)	Cumulative Incidence
Exposed	2	66	68	2/68 = 2.94 %
Low-expose	6	67	73	6/73 = 8.23 %

Relative risk (Risk Ratio) = 2.94 / 8.23 = 0.35

95% CI = 0.0748 - 1.7129, Z-test = 1.286, p-value = 0.1983

4.8.2 Risk Ratio of LBW

Table 4-11 present the comparison incidence of LBW between two groups. The relative risk (or risk ratio) was used to compare the risks for Low birth weight in the two groups. In this study pregnant women who live in endemic fluoride areas had 1.53 times the risk of give Low birth weight baby compared to pregnant women who live in Normal areas.

Table 4- 12 Comparison Incidence of LBW between two groups

Group	LBW (n)	NW (n)	Total (n)	Cumulative Incidence
Exposed	10	58	68	10/68 = 14.71 %
Low-expose	7	66	73	7/73 =9.59 %

Relative risk (Risk Ratio) = 14.71 / 9.59 = 1.53

95% CI = 0.6188 - 3.8011, Z- test = 0.923, P-value = 0.3558

CHARPTER V

DISUSIONS

5.1 WF, Endemic fluoride areas GIS and Geo-visual maps

Our findings showed that nearly half of household in Lamphun used water with high fluoride, and mostly was village tap water sourced from groundwater. The maximum level WF was 13.6 mg/L – up to 19 times of safe drinking water (0.70 mg/L). This finding support previous studies of the International Water Association (IWA) in 2006 [1], Inter Country for Oral Health (ICOH) [9] and Leatherwood (1965)[95] which reported that Northern Thailand had the areas with the highest level of fluoride in the groundwater. and cooking. The study of Chuah et al [10, 96] reported that groundwater in Lamphun contained fluoride with a concentration of at least 1.5 mg/L.

However, village WF level cannot determine fluoride exposure of population. To determine fluoride, should examine the total intake of fluoride from all sources in plasma, urine or ductal saliva to indicate level of fluoride exposure of individual.

In addition, some of village water supplies were improved the quality and remove fluoride, such as village filter, water dispenser and bottled water. However, the examination of water sample still found higher of WF than >0.70 mg/L. These finding would be implying that the management and quality control about village committee or business owner were not good. For dispenser and bottled water, the water quality should be sticky control and monitoring by safe drinking water law

(Groundwater Quality Standards for Drinking Purpose under the Groundwater Act, B.E.2520), in case of the offender should be punishment.

The result of GIS data and Geo-visual map on Google Maps shown the distribution of endemic fluoride areas located following the geoligal *fluoride belt.* Similar to the study of the WHO in2006 [116], Kongpun et al [92], Chuah Chong Joon [96]and C. Joon Chuah et al [10]. These would be useful for relevant agencies including local government, health policy authorities, village community administrators and village people for collabotion to resolving the problem. These woulde be make a better understanding the distribution of fluoride belt and enable to discide on the best way to provide water supply and safe drinking water for the community.

5.2 Comparison of MUF between the two groups

Based on the findings average MUF, MUF group and fluoride creatinine ratio (F/Cr ratio). The average of MUF level in Exposed was higher than Low-exposed (1.59 ± 1.34 mg/L vs 0.98 ±.64) mg/L. When comparison by MUF group (cut point at 1.0mg/L). The percentage of pregnant in low MUF level (F≤1.0 mg/L) in Low-exposed was more than Exposed (62.8% vs 38.1%) whereas high MUF level (F>1.0 mg/L) in Exposed was more than Low-exposed (61.9% VS 38.1%). The average F/Cr ratio were increasing when gestation of pregnancy increased at gestation < 30-week, 31-33 and 34-36week; (0.225 ±.196, 0.226 ±.192, and 0.264 ±.250 mg F/g Cr) respectively. The F/Cr ratio n in Exposed were higher than Low-exposed.

Similar to the previous study of J Opydo-Szymaczek and M Borysewicz-Lewicka (2005) [69] in Polan found that mean of fluoride level in fasting morning urine and 24-hour collections of the same woman do not differ and mean urinary fluoride levels in the 33rd week were were 0.838 mg/L and 28th week of pregnancy were 0.653 mg/L. Deena B.Thomas et al (2016) [117], fluoride levels correlated across the stages of pregnancy, with stronger correlations between neighboring stages. Urinary fluoride use changed as pregnancy progressed with levels increasing until ~23 weeks and then decreasing until the end of pregnancy.

Although average MUF level in Exposed were significantly higher than Low-exposure. However, some of pregnant in Low-exposed were high of MUF (>1.0 mg/L). Our finding confirm the WHO (2014), exposure to fluoride in human depend on behavior of consumption of individual through drinking water, dietary components, fluoride supplements or other.

Then, pregnant women living in endemic fluoride areas have chance exposing to high fluoride but its depend on behavior consumption. To determine level of exposure have to examine fluoride level in plasma, urine or ductal saliva of individual.

In Addition, to determine the WF result in LBW and PTB, should be assese the level of fluoride exposure of individual and examined the association in depth.

5.3 Association of MUF level with Preterm delivery and LBW

Our study found there were not any factors significant association between with preterm delivery (p=0.40). Similar to study of Rachel Hart et al, 2009[75], A.K Susheera 2015[76]).

However, our finding found that there are some factors significantly associated with preterm delivery at *p*-value of < 0.05 such as coffee consumption (p= .003), 1-st ANC >12 wk. (p= .033), Supplementary (Triferdine) (p= .033) and history of cesarean section (p= .037)

Our findings showed that LBW was associated with increasing of village WF level (p = .048) and MUF level (p = .049). This findings support the previous study of M. Diouf et al [22] which found the incidence of LBW were 3.75-time higher delivery with LBW babies than controls (25.9% vs 6.9%).

However, our finding found that there MUF more than 0.5 mg/L significantly associated with LBW (p= .003), These findings support the previous studied of A. K. Susheela, [19, 76, 77] reported that fluoride causes serious damage to gastrointestinal (GI) mucosa by destroying microvilli resulting in non-absorption of nutrients from the diet. Fluorosis effected to developed hypothyroidism and anaemia by destroy erythrocytes, thereby contributing to loss of haemoglobin which results in anaemia. Breymann review that haemoglobin alone is insufficient to guide management of pregnant women with iron deficiency and anaemia. In Nepal, iron and folic acid supplementation reduced the incidence of low birth weight by 16%. Supplementation

of fourteen micronutrients including iron, folic acid and zinc reduced LBW by 14%, thus confirming no added advantage of multiple micronutrients over iron and folic acid. The study of Sastry M Gurumurthy et al (India 2011) reported that MSF >1ppm was association with 10.58 times of LBW and 8.65 times of preterm delivery. WHO (2014) [5] reported that about 50% of fluoride excreted by urine

The finding in our study was contrast the study Sastry M Gurumurthy et al. The MUF in pregnant women were strong significant difference between the two groups (MUF (mg/L) = 1.27 ± .1.08, Low-exposed = 0.98 ± .64 mg/L and Exposed was =1.59 ± 1.34 mg/L p= 0.001). we can imply that pregnant women in Low-exposed group have MSF at least 1.96 mg/L (ppm) or two times of fluoride in study of Sastry M Gurumurthy et. However, there are no effect of fluoride on LBW and preterm delivery in the Low-exposed and Exposed. This is might be because of the National ANC program in Thailand support Supplementation including Iodine, iron and folic acid (Triferdine). More over another Supplementation composed of nine vitamins including viatamin C and vitamin D. The study of Verma, R. J and Sherlin, D. M. (2011) [52, 53] found that intake of *vitamin C and vitamin D* significantly reduced the severity and v*itamin D* incidence of fluoride-induced embryotoxicity treatment significantly ameliorated the fluoride-induced reductions in body weight, feed consumption and absolute uterine weight

The previous studies have reported poor nutritional during pregnancy status and small maternal body weight can affect weight of newborn (WHO, 2006).

Underweight of pregnant women were 1.64 times risk of increasing LBWN (Han et al, 2011); this was not seen in our study. In our study, low weigh beforepregnancy and BMI were not significantly associated with LBW (adjusted OR=0.749; 95% CI: =0.475-1.182, p=0.215). High BMI before pregnancy was also not associated with a LBWN. Weight gained during pregnancy was more important than BMI before pregnancy risk for LBW in our study. Women with inadequate weight gain in our study, no matter their BMI before pregnancy, were 3.4 times more likely to have a LBW (adjusted OR=3.357; 95% CI: 2.114-5.332,)

The study of Sananpanichkul, P. and S. Rujirabanjerd [118] studied preterm delivery and LBW in Northern Thailand reported that the frequency of ANC visits was significantly associated with normal weight baby (NWB) (Odds ratio=11.04) (Chiang Mai LBW Study Group et al, 2012). Mumbare et al (2012) reported frequency of ANC was associated with a 4.98 times greater risk of LBW.

Although ANC visit does not control by socioeconomic status and environmental factors that contribute to a LBW, it worth in indicated factors, such as smoking, consumption of alcohol, drug use, and poor diet. If confounding factors can be identified, the risk for delivering a LBW may be reduced or eliminated through prenatal counseling. Pregnant women who do not have at least 5 ANC visits may not benefit from the provided pregnancy educational sessions about maternal and fetal health. The study did not find an association between ANC less than 5 visits and LBW (adjusted OR=0.964; 95% CI: 0.666-1.395, p=0.844) even though only two-thirds of

pregnant women in the study had at least 5 ANC visits. This unexpected finding may be due to confounding factors not controlled in the study. The study concluded that preterm delivery, being a primiparas women and inadequate weight gained during pregnancy for the maternal BMI were all significantly associated with increased risk of LBW

Traisathit, P., et al (2014) [119] studied relation between behavioral of pregnant women living in fluoride areas and iodine deficiency condition in 2011. Results show that, during pregnancy, if pregnant used iodine salt, it will decrease of risk to iodine deficiency and risk of LBW. In our study, we do not ask this issue and do not test iodine inserum.

5.4 Incidences of Preterm delivery and LBW

Our finding showed that the incidence of preterm delivery in Exposed group was higher than Low-exposed group (8.23 % vs 2.94%). Our finding was contrast the previous retrospective cohort study of Rachel Hart and colleague in the US [75] which found that preterm delivery in high fluoride areas (WF **≥**1.0 mg/L) was higher than Low fluoride area (WF <1.0 mg/L) was 6.34% vs 5.52%

From our finding 1-st ANC visit >12 wk. (p = .033) was statistically associated with increased of Preterm delivery (*Obj. 3*) and From baseline data, there is a different of 1-st ANC visit >12 wk. (p = .02) between the two groups, the percentage of pregnant women have 1-st ANC visit in Low-exposed group was higher (65.2%) > than Exposed

group (34.8%). *Therefore* 1-st ANC visit >12 week was a factor influence to preterm delivery in Low-exposed group.

The relative risk (or risk ratio: RR) of preterm delivery was 0.35 (RR <1)., it can explain that pregnant women who live in endemic fluoride areas had 0.35 times risk of preterm delivery compared to pregnant women who live in normal areas. Whereas pregnant women who live in endemic fluoride areas had 1.53 times the risk of give Low birth weight baby compared to pregnant women who live in Normal areas.

The relative risk (RR) was 1.53 (RR <1), The Incidence of LBW in Exposed and Low-exposed were 14.71 % vs 9.59%. Our finding supported the study of M. Diouf et al (2012); LBW was associated with pregnant living in endemic areas and WF level consumption. It can imply that pregnant women who live in Endemic fluoride areas had 1.53 times risk to give LBW baby compared to pregnant women who live in Normal areas.

CHAPTER VI
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In summary, WF of village water supplies were 0.10-13.60 mg/L, the maximum WF was 13.60 mg/L (~19 times safe drinking water), found in village tap. More than half (53.8%)of villages (N=303) were endemic fluoride areas, 45.6% (N=70,807) of households use unsafe drinking water, mostly used village tap (44.1%). Fifty percent of sub districts(N=26) have average WF(mean) higher than standard level. 5 sub districts where every villages were endemic fluoride areas and 2 of those where every households of every villages used unsafe drinking water. The result of GIS and Geo-visual maps shown the distribution of endemic fluoride areas located following the geological of fluoride belt.

The mean MUF in Exposed was higher Low-exposed significantly (p = .001) and mean MUF at gestational age 34-36week >31-33 week >less than 30week (p = .025, p = .006, p = .004)

Our finding found that, there was strong significant difference of MUF (p=.001) between two groups, the MUF level of Exposed (1.65 ± 1.35) was higher than Low-exposed (0.98 ± .0.16).

There were statistically significantly associated of MUF level (p = .040), coffee consumption (p = .003), 1-st ANC visit >12 wk. (p = .033), supplementary of Triferdine

(p = .033) and history of cesarean (p = .037) with increased of Preterm delivery, whereas village WF level (p = .048), MUF level (p = .049), age of pregnant women (p = .006), body weight change during pregnancy (p= .041),) and history of delivery (p = .049) were increased of LBW respectively.

The Relative Risk (RR) of preterm delivery was 0.35 (95% CI = 0.0748 - 1.7129), and RR of LBW was 1.53 (95% CI = 0.6188 - 3.8011)

6.2 Benefits and Strength of Study

Our findings are provided WF of village water supplies and endemic fluoride areas as the GIS with Geo-visual map. This is useful for health policy authorities, local governments, and villagers and enables collaboration to resolve these issues. In addition, to educate people in endemic fluoride areas to prevent and reduce fluoride exposure in drinking water.

In this study, GIS technology and Google Maps were used to present the findings that will be useful for the relevant agencies or internet users to access and distribute information through web browsers or smart phone devices from anywhere.

In addition, the findings provided the evidence of MUF level among pregnant women, the incidence of preterm delivery and LBW in endemic fluoride areas. These would be useful for an environmental health concern to reducing exposed to fluoride and prevent adverse health effect in vulnerable groups. This study can applicable to endemic fluoride areas in other regional part of Thailand.

6.3 Limitations

There were probably uncertainties and limitations in this study that need mention including;

1) The Smart System Info program for measuring GPS was suitable only for an android phone device. The dislocation of GPS data caused by the measuring process is based on the brand and model of the mobile phones as well as on the signal strength of the network providers. To reduce error in the result, it should be possible for the application to be refreshed for a new area to access the correct location.

2) The boundary data in this study was version 2.8, November 2015 which is resulted in an incorrect overlap of geographical boundaries on Google Maps. Any new version should be updated accordingly.

3) A free version of Google Maps supports for 10 layers per one map. For more than 10 layers researcher should be managed and separated into more than one map.

4) To accessibility real-time service of GIS data and Geo-visual on Google Maps, user need to access by internet. The maps display depends on signal strength of network providers and would be out of service when at rural areas. However, user can download offline maps when was in rural area or without network signal.

5) The human errors would be occurred, such as pregnant woman would be forgot date of last menstrual period (*LMP*) which be affected to the predict date of pregnant delivery (EDC), gestational age calculation and data analysis.

6) In this study, the maternal serum fluoride (MSF) and umbilical serum fluoride (USF) have been collected and examine but we cannot find fluoride level (0 mg/L). The result of MSF was affect by the overlap period time of data collection and the gestational diabetes mellitus (GDM) screening project. The result of UF was affect by during time waiting be for delivery of pregnant women, due to during that most of the have GDM project was.

6.4 Recommendations

6.4.1 Health Policy Authority, Local Government or village committee

Our finding found that village filter, water dispenser and bottled water were high of fluoride which should be monitoring and control by the safe drinking water law [36, 37, 120] following the Notification of the Groundwater Quality Standards for Drinking Purpose B.E.2552 (2009) and Department of Health, Ministry of Public Health (2010). To reduce fluoride exposure from village filter, water dispenser and/or bottled water. The involve agencies authority including health authority, local government, village committee, business owner should be sticky monitor and control the quality of water by safe drinking water law following the Notification of the Groundwater Quality Standards for Drinking Purpose B.E.2552 (2009) and Department of Health, Ministry of Public Health (2010)[37], in case of the offender should be punishment.

Although some village filter, water dispenser and bottled water that are improving water quality to reduce fluoride exposure following the drinking water law.

However, a lot of local people cannot pay for all their water uses. Most of them stilled use waterworks or village tap for drinking and cooking. Under Sustainable Development Goal target 6.1 calls for universal and equitable access to safe and affordable drinking water by the UN General Assembly [121] the explicitly recognized the human right to water and sanitation. Everyone has the right to sufficient, continuous, safe, acceptable, physically accessible, and affordable water for personal and domestic use. Thus, local government as Municipality, Subdistrict Organization (SAO) or village committee should be take authority and roll to support safe drinking water for village people

The Health Policy Authority, Policy maker of Local government, village committee including healthcare volunteers could be use GIS and Geo-graphical map that present the distribution of endemic fluoride areas to plan for finding safety land for water supply plant, health education for reducing fluoride exposure and adverse health effect of village people in endemic fluoride areas.

6.4.2 Village people

One issue must concern for people at endemic fluoride area for the unintentional fluoride intake by cooking water with high fluoride.

Most people in Lamphun well known that water fluoride is primary cause of fluoride intake in human and they use bottled water for drinking instead ground water. However, Most of them do not know fluoride does not removed from the water when it is boiled or freezing, except distillation system.[30, 31], they think fluoride would be destroy by heat, whereas it will be increasing both fluoride level with toxicity. Thus,

many household used waterworks or village tap for cooking and some of them improved water quality by heat for drinking. In Northern Thailand, most people eat strictly rice more than plated rice. The process of sticky rice cooking was soak in water several hours or overnight before streamed.

S. Takizawa et al (2014) [58] studied on fluoride level in various foodstuffs, meat, vegetables, and rice in Lamphun found that the major source of fluoride intake was identified as cooking water. Most people use pipe water washing and soaked the rice. The pipe water come from groundwater contained the highest amount of fluoride. The experimental soaking sticky rice with WF at l 0–15 mg/L for 24 hours, there was a strong correlation between the WF level in the soaking water and the F in the rice. The maximum fluoride level of 10 mg/kg of rice (wet weight). However, fluoride in the rice cooked in households was slightly less than the rice samples used in the soaking experiments. The correlation of fluoride between cooking water and cooked rice was not as strong. The fluoride level of cooked rice is affected by not only the level of cooking water for soaking, but also by cooking methods and soaking time.

The recommendation for people that do not use water with high WF level for cooking, preparing, or cooking groundwater for cooking, and, at least, to use other waters such as bottled water for rice soaking. Shortening the rice soaking time can also lower the F absorption by rice.

Thus, the recommended and educated on this issue would be useful and reduce fluoride exposure in the population.

6.4.3 Future research

For future research, tor study on fluoride exposure and health effect in mother and newborn. The researcher should be concern about appropriate biomarker that able to identify fluoride exposure. The excretion urine fluoride which *WHO* [5] recommend urinary fluoride is a widely accepted biomarker of recent fluoride exposure and has frequently been used as an indicator of fluoride exposure from drinking water should be considered. However, the tools for urine collection in newborn should be suitable would be use such as urine collectors.

Due to fluorides can reserved up from land to store and accumulate in any parts of plants depend on the kind of plant. The accumulate in animals were by ingestion water or eaten plants growth with fluoride, mostly in the bones or shell more than in eatable meat. [29]. However, the study of fluoride on type of food in term of local plant or animal with high fluoride in endemic areas were have a few evidences, result of the study would be useful to educate people to reduce fluoride exposure from local food.

Although this study has added up the study population with 25% of the calculate sample size to avoid the problem of subject loss follow up or dropout. However, finally the percipients had decided to change the hospital to delivery which reduce amount of complete data and affect to result analysis. Then the researcher should be concern with cohort study that take long time follow up the participants.

For future research, study on exposure of fluoride and effect in mother and newborn. The researcher should be concern about the biomarker which able to identify fluoride exposure. The excretion urine fluoride might be considered be an appropriate biomarker. However, urine collection in newborn should be use proper tools such as urine collectors to collect the urine sample.

REFERENCES

1. IWA, *International Water Association (IWA, WHO): Fluoride In Drinking-Water*. 2006, TJ International (Ltd), Padstow, Cornwall, UK. 144.
2. WHO, *Fluorides and Oral Health* 1994(Report of a WHO Expert Committee on Oral Health Status and Fluoride Use): p. 38.
3. WHO, *Guidelines For Drinking-Water Quality*. 4th ed ed. WHO Library Cataloguing-in-Publication Data, ed. UN-Water. 2011: WHO. 541.
4. N Gouri Pratusha, et al., *Fluoride Toxicity – A Harsh Reality.* International Research Journal Of Pharmacy 2011: p. 79-85.
5. WHO, *Basic Methods For Assessment Of Renal Fluoride Excretion In Community Prevention Programmes For Oral Health 2014*, R.J. Baez, P.E. Petersen, and T.M. Marthaler, Editors. 2014, WHO. p. 83.
6. ADA. *Oral Health Topics: Fluoride Supplements, Facts About Fluoride*. 2016 [cited 2016 6 Feb]; Available from: http://www.ada.org/en/member-center/oral-health-topics/fluoride-supplements#dosschedule.
7. Service, U.S.P.H., *Recommendation For Fluoride Concentration In Drinking Water For The Prevention Of Dental Caries.* Public Health Reports 2015. **Vol. 130** p. 14.
8. Thailand, F.a.D.A.o., B.o.D. Health, and I.o.N.M. University, *Situation Of Fluoride Concentration In Bottled Water.* 2008. 85.
9. Intercountry Centre for Oral Health (ICOH). *Research & Report*. 2010 [cited 2016 7 Feb]; Available from: http://203.157.65.18/doh_info/web/uploads/pdf_961/YdhP47N86DNgcbjbzF1mu4XS5o5wKNYx6GiLlXn0lN7jvZp8KYX2-1LMnEAAP0AcN0GNU2uiYDtwNLCHcO0zqTUzp43r2dVnlAiV.pdf.
10. Chuah, C.J., et al., *Fluoride: A Naturally-Occurring Health Hazard In Drinking-Water Resources Of Northern Thailand.* Sci Total Environ, 2016. **Vol. 545-546**: p. 266-79.
11. Basha, P.M., P. Rai, and S. Begum, *Evaluation of Fluoride-Induced Oxidative Stress in Rat Brain: A Multigeneration Study.* Biol Trace Elem Res, 2011. **Vol. 142**(3): p. 623-37.

12. John D. MacArthur, *Fluoride, Premature Birth And Impaired Neurodevelopment: A Global Review Of Recent Laboratory, Clinical, And Ecological Evidence That Fluoride Is A Significant Risk Factor For Premature Birth And Long-Term Neurological Disabilities.* Townsend Letter: The Examiner of Alternative Medicine, 2013.
13. Brzezinski, A., et al., *Fluoride Metabolism In Pregnant Rats.* Proc Soc Exp Biol Med, 1961. **Vol. 108**: p. 342-5.
14. Maheshwari, U.R., et al., *Fluoride Balances In Pregnant And Nonpregnant Women.* J Occup Med, 1981. **Vol. 23**(7): p. 465-8.
15. Lardon, J.P. and R. Vincent, *Fluoride, And Pregnancy. Should Fluoride Be Prescribed For Pregnant Women?* Rev Odontostomatol (Paris), 1986. **Vol. 15**(1): p. 31-5.
16. Chuckpaiwong S., et al., *Fluoride Analysis Of Human Milk In Remote Areas Of Thailand.* Southeast Asian Journal of Tropical Medicine and Public Health, 2000. **Vol. 31 No.3**(3): p. 583-586.
17. Sawa, R., et al., *Fetal Plasma Hypoxanthine Level In Growth-Retarded Fetuses Before Labor.* Journal of Clinical Endocrinology and Metabolism, 1997. **Vol. 82 No. 12**(12): p. 4028-4031.
18. Magalhaes, D., G. Matheus, and C. Martinelli, *Excessive Fluoride Administration In Pregnant Rats And Its Effect On The Maxillary Bones And Teeth Of The Offspring (Author's Transl).* Arq Cent Estud Fac Odontol UFMG (Belo Horiz), 1978. **Vol. 15**(1-2): p. 113-21.
19. A.K. Susheela, et al., *Effective Interventional Approach To Control Anaemia In Pregnant Women.* Current Science, 2010. **Vol. 98**(10): p. 1320-1330.
20. Aghaei, M., et al., *Effect Of Fluoride In Drinking Water On Birth Height And Weight: An Ecological Study In Kerman Province, Zarand County, Iran.* Research Fluoride, 2015. **Vol. 48(2)**(2): p. 160-168.
21. Chlapowska, J. and J. Opydo-Szymaczek, *Dietary And Hygienic Aspects Of Fluoride Exposure In Pregnant Women.* Ann Acad Med Stetin, 2004. **Vol. 50**(Suppl 1): p. 19-22.

22. M. Diouf, et al., *Pregnant Women Living In Areas Of Endemic fluorosis In Senegal And Low Birth Weight Newborns: Case–Control Study.* Revue d'Epidémiologie et de Santé Publique, 2012. **Vol. 60**: p. 103–108
23. Gurumurthy Sastry M, et al., *Association Of Higher Maternal Serum Fluoride With Adverse Fetal Outcomes.* International Journal of Medicine and Public Health, 2011. **Vol. 1**(2): p. 13-17.
24. WHO, *Guidelines On Optimal Feeding Of Low Birth Weight Infants In Low-And Middle-Income Countries*. 2011.
25. WHO. *Preterm Birth*. Fact sheet 363 2015 [cited 2016 18 Feb 2016]; Available from: http://www.who.int/mediacentre/factsheets/fs363/en/.
26. Winichagoon, P., *Thailand Nutrition In Transition: Situation And Challenges Of Maternal And Child Nutrition.* Asia Pacific Journal of Clinical Nutrition, 2013. **Vol. 22**(1): p. 6-15.
27. American Academy of Pediatrics—Committee on Fetus and Newborn. *Committee on Obstetric Practice: APGAR Score* 2015 [cited 2015 October 20]; Available from: https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Obstetric-Practice/The-Apgar-Score.
28. UNICEF. *UNICEF Tap Project*. Fluoride Belt in The world and Country with endemic fluorosis 2014 [cited 2015 Dec 15]; Available from: http://rampages.us/mediatyphoon/2014/12/07/voluntourism-unicef-and-advertising/.
29. WHO Expert Committee on Oral Health Status and Fluoride Use, *Fluorides And Oral Health.* 1994: p. 1-37.
30. Michael A. Lennon , et al., *Fluoride.* n.d.
31. J. Fawell, et al., *WHO: Fluoride In Drinking-Water*. 2006: WHO.
32. Pollution Control Department of Thailand. *Standards Quality of Drinking Water*. 2009 [cited 2016 October 20]; Available from: http://www.pcd.go.th/info_serv/reg_std_water01.html.
33. Food and Drug Administration of Thailand. *Standards Quality of Drinking Water*. 2010 [cited 2015 October 22]; Available from:

http://food.fda.moph.go.th/law/data/announ_fda/066Explain_Notification316(2553).pdf.

34. US.PHS, *U.S. PUBLIC HEALTH SERVICE RECOMMENDATION FOR FLUORIDE CONCENTRATION IN DRINKING WATER FOR THE PREVENTION OF DENTAL CARIES.* Public Health Reports 2015. **130**(Reports and Recommendations): p. 14.
35. U.S.CDC. *Community Water Fluoridation*. 2016 March 18, 2015 [cited 2016 Feb 7]; Available from: http://www.cdc.gov/fluoridation/faqs/wellwater.htm.
36. Ministry of Natural Resources and Environmen. *Drinking Water Quality Standard in Thailand*. 2009 [cited 22016 November 20]; Available from: http://www.pcd.go.th/info_serv/reg_std_water01.html.
37. Department of Health Laboratory Center, D.o.H. *Water quality criteria*. 2015 [cited 2017 22 Dec]; Available from: http://rldc.anamai.moph.go.th/index.php?option=com_content&view=article&id=29&Itemid=512.
38. Patsouri, K., *Re-Examining The Fluoride Intake During Pregnancy: A Necessity Or Not For The Incoming Member.* Journal of International Dental and Medical Research (JIDMR), 2015. **Vol. 1** (ISSUE 6): p. 150-156.
39. Viswanathan, G., et al., *Mapping Of Fluoride Endemic Areas And Assessment Of Fluoride Exposure.* Sci Total Environ, 2009. **Vol. 407**(5): p. 1579-87.
40. WHO, *Fluorides: Environmental Health Criteria 227*, ed. R. Liteplo, et al. 2002: World Health Organization. 290.
41. WHO, *Inadequate Or Excess Fluoride: A Major Public Health Concern.* 2010(Preventing Disease Through Healthy Environments): p. 5.
42. Student.ahc.umn.edu. *Dent 5302 Topics In Dental Biochemistry*. 2009 [cited 2016 Feb 15]; Available from: http://student.ahc.umn.edu/dental/2012/5302/2009-L6-3Apr.pdf.
43. Viswanathan, G., et al., *Determining the optimal fluoride concentration in drinking water for fluoride endemic regions in South India.* Science of the Total Environment, 2009. **407**: p. 5298–5307.

44. Kantawong, F., et al., *Serum Fluoride Level Of Children In Omkoi District, Chiang Mai Province.* Bulletin of Chiang Mai Associated Medical Sciences, 2014. **47 No.3**(Vol. 47 No. 3 September 2014 185): p. 185-191.

45. Biolab Medical Unit, *Fluoride.* Nutritional and Environmental Medicine, London England, 2012.

46. Parimi, N., et al., *Hair As Biomarker Of Fluoride Exposure In A Fluoride Endemic Area And A Low Fluoridated Area.* Int J Trichology, 2013. **Vol. 5**(3): p. 148-50.

47. Barghouthi, Z. and S. Amereih, *Spectrophotometric Determination Of Fluoride In Drinking Water Using Aluminium Complexes Of Triphenylmethane Dyes.* Water SA, 2012. **Vol. 38**(4): p. 543-548.

48. Chamnanprai, S. *Analysis Of Fluoride Concentration In Drinking Water*. [ppt] 2000 [cited 2016 Feb 18]; Available from: http://slideplayer.in.th/slide/2149799/.

49. Wikipedia.org. *Ultraviolet–Visible Spectroscopy*. 2016 30 March 2016, [cited 2016 Marh 31]; Available from: https://en.wikipedia.org/wiki/Ultraviolet%E2%80%93visible_spectroscopy.

50. NRC and The National Academies Press (NAP), *Fluoride In Drinking Water: A Scientific Review Of EPA's Standards.* 2006: p. 467.

51. WHO, *Provision of Technical Advice: TISAB*, R.J. Baez, P.E. Petersen, and T.M. Marthaler, Editors. 2014, WHO. p. 67-78.

52. Guna Sherlin, D.M. and R.J. Verma, *Vitamin D Ameliorates Fluoride-Induced Embryotoxicity In Pregnant Rats.* Neurotoxicol Teratol, 2001. **Vol. 23**(2): p. 197-201.

53. Verma, R.J. and D.M. Sherlin, *Vitamin C Ameliorates Fluoride-Induced Embryotoxicity In Pregnant Rats.* Hum Exp Toxicol, 2001. **Vol. 20**(12): p. 619-23.

54. Paul Connett, James Beck, and H. Spedding Micklem, *The Case Against Fluoride: How Hazardous Waste Ended Up In Our Drinking Water And The Bad Science And Powerful Politics That Keep It There*. 2010: Chelsea green publishing, white river junction, Vermont. 374.

55. Gessner BD, et al., *Acute fluoride poisoning from a public water system.* The New England Journal of Medicine, 1994. **330 No.2**(Research & Review Articles on Disease & Clinical Practice).

56. ATSDR, *Toxicological Profile For Fluorides Hydrogen Fluoride And Fluorine*. 2003.

57. World Health Organization, *Inadequate or Excess Fluoride: A Major Public Health Concern.* 2010.

58. S Takizawa, et al., *Fluoride Absorbtion by Rice From Cooking Water*, in *XXXIInd Conference Of The International Society For Fluoride,Fluorosis Prevention: From Research At The Cellular Level To Mitigation November 25–28, 2014* 2014: Chiangmai, Thailand.

59. Phyllis J. Mullenix, et al., *Neurotoxicity Of Sodium Fluoride In Rats.* Neurotoxicology and Teratology, 1994. **Vol. 17 No. 2**: p. 169-177.

60. Zhi-zhong Guan, et al., *Influence Of Chronic Fluorosis On Membrane Lipids In Rat Brain.* Neurotoxicology and Teratology, 1998. **Vol. 20, No. 5**: p. 537-542.

61. SCHER. *Fluoridation*. 2016 [cited 5 Feb 2016 Public Health]; Available from: http://ec.europa.eu/health/scientific_committees/opinions_layman/fluoridation/en/l-3/1.htm#0.

62. BabyCentre Medical Advisory Board of Health on the Net Foundation (HON). *Fetal Development - 33 Weeks Pregnant*. 2015 August 2009 [cited 2016 Feb, 20]; Available from: http://www.babycentre.co.uk/33-weeks-pregnant.

63. Opydo-Szymaczek, J. and M. Borysewicz-Lewicka, *Urinary Fluoride Levels For Assessment Of Fluoride Exposure Of Pregnant Women In Poznan, Poland.* Research report Fluoride 2005. **Vol. 38**(4): p. 312-317.

64. Bashash, M., et al., *Prenatal Fluoride Exposure and Cognitive Outcomes in Children at 4 and 6-12 Years of Age in Mexico.* Environ Health Perspect, 2017. **125**(9): p. 097017.

65. Deena B.Thomas, et al., *Urinary and plasma fluoride levels in pregnant women from Mexico City.* Environmental Research, 2016. **Volume 150, October 2016**: p. 489-495.

66. Gurumurthy Sastry M, et al., *Transplacental Transport of Fluoride, Calcium and Magnesium.* National Journal of Integrated Research in Medicine, 2011. **Vol. 2(3). July- September**: p. 51-55.

67. Caldera, R., et al., *Maternal-Fetal Transfer Of Fluoride In Pregnant Women.* Biol Neonate, 1988. **Vol. 54**(5): p. 263-9.

68. Gurumurthy Sastry. M, Shruti Mohanty, and Pragna Rao, *Role Of Placenta To Combat Fluorosis (In Fetus) In Endemic Fluorosis Area.* NJIRM, 2010. **Vol. 1(4)**(Role Of Placenta To Combat Fluorosis): p. 16-19.

69. J Opydo-Szymaczek and M Borysewicz-Lewicka, *Transplacental Passage Of Fluoride In Pregnant Polish Women Assessed On The Basis Of Fluoride Concentrations In Maternal And Cord Blood Plasma.* Research report Fluoride, 2007. **Vol. 40(1)**: p. 46-50.

70. John D. MacArthur. *Placental Fluorosis : Fluoride And Preeclampsia*. 2015 May 2015 [cited 2016 May 12]; Available from: http://townsendletter.com/May2015/placental0515_2.html.

71. Maduska, A.L., et al., *Placental Transfer Of Intravenous Fluoride In The Pregnant Ewe.* Am J Obstet Gynecol, 1980. **Vol. 136**(1): p. 84-6.

72. Malhotra, A., et al., *Placental Transfer Of Fluoride In Pregnant Women Consuming Optimum Fluoride In Drinking Water.* J Indian Soc Pedod Prev Dent, 1993. **Vol. 11**(1): p. 1-3.

73. Moshe Ron, et al., *Fluoride Concentration In Amniotic Fluid And Fetal Cord Ad Maternal Plasma.* 1985.

74. Michael Connett and Hardy Limeback, *Fluoride And Its Effect On Human Intelligence - A Systematic Review.* IADR 83rd General Session and Exhibition (International Association for Dental Research), 2008.

75. Hart, R., et al., *Relationship Between Municipal Water Fluoridation And Preterm Birth In Upstate New York.* American Public Health Association (APHA), 2009(water public health).

76. A. K. Susheela, *Fluoride toxicity manifestations in pregnant women and children in India and the way forward*, in *3rd Indo-Global Summit & Expo on Healthcare*. 2015: New Delhi,India. p. October 05-07, 2015

77. A.K. Susheela, N.K. Monda, and Rashmi Gupta, *Fluoride Ingestion, The Primary Cause for High Prevalence of Anemia in Pregnant Women and Low Birth Weight Babies :Simple and Easy to Practice Interventions for Improving Maternal and Infant Health* in *XXXIInd Conference Of The International Society For Fluoride,Fluorosis Prevention: From Research At The Cellular Level To Mitigation November 25–28, 2014* 2014: Chiangmai, Thailand.

78. Sirikul Isaranurug, *Maternal and Child Health Handbook in Thailand.* Journal of International Health, 2009. Vol.24 No.2.

79. WHO, *WHO Antenatal Care Randomized Trial: Manual For The Implementation Of The New Model*. 2002.

80. Liammongkolkul, S., et al., *AB133. Expanded Newborn Screening Program In Thailand* Annals of Translational Medicine, 2017. **Vol 5, Suppl 2 September**.

81. S Pangkanon, et al., *Detection Of Phenylketonuria By The Newborn Screening Program In Thailand.* SOUTHEAST ASIAN J TROP MED PUBLIC HEALTH, 2009. **Vol 40 No. 3 May**: p. 525 - 529.

82. Thiboonboon, K., et al., *An Economic Evaluation of Neonatal Screening for Inborn Errors of Metabolism Using Tandem Mass Spectrometry in Thailand.* PLoS One, 2015. **10**(8): p. e0134782.

83. International Atomic Energy Agenc VIENNA *Screening Of Newborns For Congenital Hypothyroidism: Guidance For Developing Programmes*. 2005.

84. WHO (South-East Asia Region), *Neonatal - Perinatal Database: Working Definitions.* 2006: p. 7.

85. Woramongkol, N., et al., *Mother And Child Health Handbook (2012)*. 2012, The Veteran Press, Bangkok: Bureau of Health Promotion, Department of Health, Ministry of Pubic Health, Thailand.

86. Department of Health, *Mother And Child Health Handbook (2015)*. 2015, The Veteran Press, Bangkok: Bureau of Health Promotion, Department of Health, Ministry of Pubic Health, Thailand.

87. Chumnijarakij, T., et al., *Maternal risk factors for low birth weight newborn in Thailand*. Vol. 75. 1992. 445-52.

88. Ajchara Kasunan. *Preterm & Low Birth Weight Risks & Prevention*. 2015 [cited 2016 Mar, 22]; Available from: http://www.mhso.moph.go.th/mhs/attachments/article/446/2.Preterm%20%20Low%20birth%20weight%20risks%20%20prevention.pptx.

89. Khunpradit, S., *Fetal Origins Of Adult Disease.* Chulalongkorn Medical Journal, 2004. **Vol. 48 (5)**: p. 309-322.

90. Health Promotion Center Region1 Chiang Mai, D.O.H., *Strategic Plan 2016 :Mother and Child Health Dataset 2015*. 2016, Health Promotion Center Region1 Chiang Ma: Chaing Mai. p. 45-49.

91. UNICEF Office For Thailand, *Multiple Indicator Cluster Survey Of Children And Women In Thailand 2006*. 2007.

92. Kongpun, M., W. Choompolkul, and J. PromsakhaNaSakolnakorn, *Fluorotoxicosis Management In Chiang Mai And Lumphoon Territory.* 2007.

93. Chaiwong, S., et al., *Blood And Urine Fluoride And Iodine, And Blood Thyroid Functions Tests In 1st Trimester Pregnant Women In Phayao Province, Thailand*, in *The XXXIInd Conference of the International Society for Fluoride Research (ISFR), 2014*. 2014: Chiang Mai, Thailand.

94. Namkaew, M. and P. Wiwatanadate, *Association Of Fluoride In Water For Consumption And Chronic Pain Of Body Parts In Residents Of San Kamphaeng District, Chiang Mai, Thailand.* Trop Med Int Health, 2012. **17**(9): p. 1171-6.

95. Leatherwood, E.C., et al., *Dental Caries And Dental Fluorosis In Thailand.* American Journal of Public Health, 1965. **Vol. 55 No.11**(11): p. 1792-1799.

96. Chuah Chong Joon, *The Assessment Of The Quality Of Water Resources In Northern Thailand: Towards Safe Drinking-Water Access For Vulnerable Populations*. 2016, National University of Singapore. p. 144.

97. Min Qi Wang, et al., *Use of GIS in Maryland Environmental Public Health.* Eectronic Journal of Health Imformatics, 2011. **Vol. 6(2): e15**: p. 8.

98. Marilyn F. Vine, Darrah Degnan, and Carol Hanchette, *Geographic Information Systems: Their Use in Environmental Epidemiologic Research.* Environmental Health Perspectives, 1997. **Vol. 105, No.6**: p. 598-605.

99. Kasemsuppakorn, P., *GIS for Collaborative Research.* Information Technology Journal, 2015. **Vol. 11, No. 1,**(Academic Paper): p. 102-109.

100. Laosuwan, T., *Design and Development GIS Web Services for Epidemiology Data Distribution: The Pilot Project for Dengue Fever in Maha Sarakham Province Thailand.* International Journal of Advances in Engineering, Science and Technology (IJAEST), 2012. **Vol. 2 No. 3**: p. 193-201.

101. Blossom, J.C., et al., *Applying GIS Methods to Public Health Research at Harvard University.* Journal of Map & Geography Libraries, 2011. **Vol. 7**(3): p. 349-376.

102. Laosuwan, T., *Online web GIS- based services for spatial data and sharing of leptospirosis Epidemiology information; Development of pilot projct in Mahasarakham province Thailand.* INTERNATIONAL JOURNAL OF GEOMATICS ANDGEOSCIENCES, 2012. **Vol. 3**: p. 121-133.

103. Soytong, P. and R. Perera, *Use of GIS Tools for Environmental Conflict Resolution at Map Ta Phut Industrial Zone in Thailand.* Sustainability, 2014. **Vol. 6**(5): p. 2435-2458.

104. Google Inc. *Google Earth,*. 2016; 7.1.8.3036 (32-bit):[Available from: https://www.google.com/earth/.

105. Wikipedia.org. *Google Maps*. 2017 31 May 2017 [cited 2017 May]; Available from: https://en.wikipedia.org/wiki/Google_Maps.

106. Google Inc. *Google Maps*. 2017 [cited 2016 January 21]; Available from: https://www.google.com/maps.

107. Kaewpitoon, S.J., et al., *GIS Database and Google Map of the Population at Risk of Cholangiocarcinoma in Mueang Yang District, Nakhon Ratchasima Province of Thailand.* Asian Pacific Journal of Cancer Prevention, 2016. **17**(3): p. 1293-1297.

108. GADM.org, *Global Administrative Areas: Thailand Boundaries*. 2014.

109. Wikipedia.org. *Lamphun Province*. 2016 20 May 2015 [cited 2016 Feb 20]; Available from: https://en.wikipedia.org/wiki/Lamphun_Province.

110. Chow S, S.J., Wang H., *Sample Size Calculations In Clinical Research. 2nd Ed. Chapman & Hall/Crc Biostatistics Series*. 2008. 89.

111. Powerandsamplesize.com. *Power and Sample size Calculators*. 2016 [cited 2016 Mar, 20]; 2016:[Available from: http://powerandsamplesize.com/Calculators/.

112. Lamphun Provincial Health Office, *Health Data Center: Mather and Child Health 2014-2016.* 2015.

113. Mary S Fewtrell, et al., *How much loss to follow-up is acceptable in long-term randomised trials and prospective studies?* British Medical Journal, 2008. **93 No 6**: p. 458-461.

114. Auyporn Reungtrakul, *Technical Creating Research Tools*. 2008.

115. Ecler Studios. *Smart System Info*. 2013 [cited 2016 January 21]; 1.3:[Available from: https://www.appsapk.com/smart-system-info/.

116. WHO. *Water-Related Diseases : Fluoride*. n.d; Available from: http://www.who.int/water_sanitation_health/diseases/fluorosis/en/.

117. Amador, D.O.R., et al., *Evaluation Of Thyroid Hormones (TSH and T4) In Pregnant Women Exposed To Fluoride (F-) In Drinking Water.* 27th Conference of the International Society for Environmental Epidemiology (ISEE), , 2015.

118. Sananpanichkul, P. and S. Rujirabanjerd, *Association Between Maternal Body Mass Index And Weight Gain With Low Birth Weight In Eastern Thailand.* Southeast Asian J Trop Med Public Health, 2015. **Vol 46 No. 6 November 2015**: p. 1085-1091.

119. Traisathit, P., et al., *The Association Between Fluorides From Drinking Water And Other Factors Involved In Iodine Deficiency In Pregnant Women In Thailand.* 2014.

120. Locator, C.M. *Drinking water quality in Thailand*. 2017 June 23, 2017 [cited 2017 June 19]; Available from: http://www.chiangmailocator.com/wiki-drinking-water-quality-in-thailand-p152#sources.

121. Organization, W.H. *Water safety and quality*. 2017 March 2018 [cited 2017 22 Dec]; Available from: http://www.who.int/water_sanitation_health/publications/wsp-roadmap.pdf?ua=1.

APPENDICS

Appendix A:

Research Ethicals Approval

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 154/2016

Certificate of Approval

Study Title No. 129.1/59 : ASSOCIATION BETWEEN MATERNAL FLUORIDE LEVEL AND PRETERM DELIVERY AND LOW BIRTH WEIGHT AMONG PREGNANCY AT ENDEMIC FLUORIDE AREAS IN LAMPHUN THAILAND: A PREGNANCY-BIRTH COHORT STUDY

Principal Investigator : MR.NONTHAPHAT THEERAWASTTANASIRI

Place of Proposed Study/Institution : College of Public Health Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature: ..... Signature: .....

(Associate Professor Prida Tasanapradit, M.D.) Chairman

(Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.) Secretary

Date of Approval : 12 September 2016 Approval Expire date : 11 September 2017

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire

Protocol No. 129.1/59
Date of Approval 12 SEP 2016
Approval Expire Date 11 SEP 2017

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์/โทรสาร: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 154/2559

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 129.1/59 : ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน ประเทศไทย : การศึกษาไปข้างหน้า

ผู้วิจัยหลัก : นายณรภัทร ธีระวรรธนะสิริ

หน่วยงาน : วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม.....
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)
ประธาน

ลงนาม.....
(ผู้ช่วยศาสตราจารย์ ดร.นันทริ ชัยชนะวงศาโรจน์)
กรรมการและเลขานุการ

วันที่รับรอง : 12 กันยายน 2559 **วันหมดอายุ** : 11 กันยายน 2560

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัยและใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย
3) ผู้วิจัย
4) แบบสอบถาม

เลขที่โครงการวิจัย 129.1/59
วันที่รับรอง 12 ก.ย. 2559
วันหมดอายุ 11 ก.ย. 2560

เงื่อนไข

1. ข้าพเจ้ารับทราบว่าหากมีการเปลี่ยนแปลงโครงการวิจัย ข้าพเจ้าต้องดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ
2. หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องยุติ เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย
3. ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด
4. ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารชี้ชวนเข้าร่วมวิจัย (ถ้ามี) เฉพาะที่ประทับตราคณะกรรมการเท่านั้น
5. หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในการเก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ
6. หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ
7. โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น

เอกสารรับรองจริยธรรมการวิจัยในมนุษย์ โรงพยาบาลลำพูน

Research ID :	Ethic LPN ๔๐/๒๕๕๙
ชื่อผู้วิจัย :	นายนณธภัทร ธีระวรรธนะสิริ
หน่วยงาน :	นิสิตหลักสูตรสาธารณสุขศาสตรดุษฎีบัณฑิต(หลักสูตรนานาชาติ) วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
ชื่อโครงการ(ภาษาไทย) :	ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน ประเทศไทย : การศึกษาไปข้างหน้า
ชื่อโครงการ(ภาษาอังกฤษ) :	Association Between Maternal Fluoride Level and Preterm Delivery and Low Birth Weight Among Pregnancy At Endemic Fluoride Areas In Lamphun, Thailand: A Pregnancy-Birth Cohort Study

ที่	รายการเอกสาร	การอ้างอิง
๑	โครงร่างการวิจัย	ฉบับวันที่ ๑๑ พฤษภาคม ๒๕๕๙
๒	หนังสือแสดงความยินยอมเข้าร่วมการวิจัย	ฉบับวันที่ ๑๑ พฤษภาคม ๒๕๕๙
๓	ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย	ฉบับวันที่ ๑๓ พฤษภาคม ๒๕๕๙
๔		
๕		

เสนอรายงานความก้าวหน้า :ทุกๆ ☐ ๓ เดือน ☐ ๖ เดือน ☑ ๑ ปี ☐ อื่นๆ.....

วันที่เริ่มอนุมัติ	25 ก.ค. 2559	วันหมดอายุ	25 ก.ค. 2560

ได้ผ่านการพิจารณาด้านจริยธรรมการวิจัยในมนุษย์จากคณะกรรมการจริยธรรมการวิจัยโรงพยาบาลลำพูน และเห็นว่าผู้วิจัยต้องดำเนินการตามโครงการวิจัยที่ได้กำหนดไว้ หากจะมีการปรับเปลี่ยนหรือแก้ไขใดๆ ควรผ่านความเห็นชอบหรือแจ้งต่อคณะกรรมการจริยธรรมการวิจัยโรงพยาบาลลำพูนก่อน

.....

(นพ.กวินทร์ ภักดี)

ประธานคณะกรรมการจริยธรรมการวิจัย

โรงพยาบาลลำพูน

หลังการรับรอง

- กรณีขยายเวลาดำเนินการวิจัย ให้ขอความเห็นชอบก่อนหนังสือรับรองหมดอายุ ๒ เดือน
- หากมีเหตุการณ์ที่อาจมีผลต่อสวัสดิภาพ ความปลอดภัยของอาสาสมัคร ให้รายงานต่อคณะกรรมการฯโดยด่วน

แบบการขออนุมัติขยายเวลาการทำวิจัย

เรียน ประธานคณะกรรมการวิจัยในมนุษย์โรงพยาบาลลำพูน
เรื่อง ขออนุมัติขยายเวลาในการดำเนินงานวิจัย

ตามที่ข้าพเจ้า นายนณธภัทร ธีระวรรธนะสิริ สังกัด วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย ได้รับอนุมัติโครงการวิจัย เรื่อง ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในสังหวัดลำพูน, ประเทศไทย : การศึกษาไปข้างหน้า (Association between Maternal fluoride level and Preterm delivery and Low Birthweight among Pregnancy at Endemic fluoride areas in Lamphun Thailand: A Pregnancy-Birth Cohort Study) มีระยะเวลาดำเนินการ 1 ปี ตั้งแต่วันที่ 25 กรกฎาคม 2559 ถึงวันที่ 25 กรกฎาคม 2560 นั้น

มีความประสงค์

☑ ขอขยายระยะเวลาการดำเนินการวิจัย ครั้งที่ ...1.....
ตั้งแต่วันที่ 25 เดือน กรกฎาคม พ.ศ. 2560 ถึง วันที่ 30 เดือน พฤศจิกายน พ.ศ. 2560
(ระบุสาเหตุที่ไม่สามารถทำการวิจัยให้แล้วเสร็จตามโครงการ และงานวิจัยที่ได้ทำไปแล้วโดยสังเขป)

เนื่องจาก การเก็บข้อมูลหญิงตั้งครรภ์ระหว่างตั้งครรภ์ไม่ครบถ้วน และยังไม่ได้เก็บข้อมูลการคลอดมารดาและทารกหลังคลอด เนื่องจากยังไม่ครบกำหนดคลอด

ดังนั้น เพื่อให้การดำเนินงานโครงการเป็นไปด้วยความเรียบร้อยดีและบรรลุตามวัตถุประสงค์ ข้าพเจ้าขอขยายระยะเวลาดำเนินการวิจัย เป็นระยะเวลา 4 เดือน โดยจะดำเนินการให้แล้วเสร็จภายใน วันที่ 30 พฤศจิกายน 2560

จึงเรียนมาเพื่อโปรดพิจารณาอนุมัติ

(นายนณธภัทร ธีระวรรธนะสิริ)
วันที่ 15 เดือน พฤษภาคม พ.ศ. 2560

เรียน ประธาน ฯ
ลงชื่อ .....
(.....)
ผู้ประสานงานวิจัยคณะกรรมการวิจัยในมนุษย์ รพ.ลำพูน
(วันที่ 24 ก.ค. 2560)

ความเห็น
( ) เห็นควรอนุมัติ ( ) ไม่เห็นควรอนุมัติ
ลงชื่อ .....
(.....)
ประธานคณะกรรมการวิจัยในมนุษย์ รพ.ลำพูน
(วันที่ 24 ก.ค. 2560)

Appendix B

Test validity of the Questionnaires

Questionnaires	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	IOC
Part 1 Resident areas						
1. **Now, you live in District** ☐ 1. Mueang Lamphun ☐ 2. Pasang ☐ 3 Ban Thi	+1	+1	+1	+1	+1	1
2. Name of village _____ No. of village _____ Name of sub district _____	+1	+1	+1	+1	+1	1
3. How many years do you live in this village? ☐ (1) more than 5 years ☐ (2) 1-5 years (specify) _____ year	+1	0	0	+1	+1	0.6
Part 2 Socio-demographic and Characteristics						
4. Age _____ years	+1	+1	+1	+1	+1	1
5. Family status ☐ (1) Single mom ☐ (2) live together ☐ (3) separated ☐ (4) other (specify) _____	+1	+1	+1	+1	+1	1
6. Education of pregnant woman ☐ (1) non-education ☐ (2) primary school ☐ (3) secondary school ☐ (4) high school ☐ (5) Diploma ☐ (6) Bachelor or higher	+1	+1	+1	+1	+1	1

Questionnaires	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	IOC
7. Education of spouse / partner ☐ (1) non-education ☐ (2) primary school ☐ (3) secondary school ☐ (4) high school ☐ (5) Diploma ☐ (6) Bachelor or higher	+1	+1	+1	+1	+1	1
8. Family income ☐ (1) less than 5,000 baht ☐ (2) 5,001 -10,000 baht ☐ (3) 10,001 -15,000 baht ☐ (4) 15,001 -20,000 baht ☐ (5) more than 20,001 baht	+1	+1	+1	+1	+1	1
3. Risk Factor related to Premature delivery & LBW						
9. Source of drinking water ☐ (1) village tap ☐ (2) Shallow well ☐ (3) bottled water ☐ (4) other (specify) _____	+1	0	0	+1	+1	0.6
10. Source of cooking water ☐ (1) village tap ☐ (2) Shallow well ☐ (3) bottled water ☐ (4) other (specify) _____	+1	0	0	+1	+1	0.6
11. Are your spouse / partner smoke? ☐ (0) No ☐ (1) Yes	+1	+1	+1	+1	+1	1
12. Do you standing work continuing more than 1 hour per day? ☐ (0) No (Move to No 13) ☐ (1) Yes (Answer No 12.1)	+1	+1	+1	+1	+1	1

Questionnaires	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	IOC
12.1 How about your standing work Amount of hour of standing work per day _____ hours Amount of day of standing work per week _____ days	+1	+1	+1	+1	+1	1
13. Do you drink coffee? ☐ (0) No (Move to No 13) ☐ (1) Yes (Answer No 13.1)	+1	+1	+1	+1	+1	1
13.1 How often do you drink coffee per week? ☐ (1) 1-2 days ☐ (2) 3-4 days ☐ (3) 5-6 วัน ☐ (4) every day	+1	+1	+1	+1	+1	1
14. Do you drink/eat tea or food make from tea? ☐ (0) No (Move to No 13) ☐ (1) Yes (Answer No 14.1)	+1	+1	+1	+1	+1	1
14.1 How often do you drink/eat tea food make from tea per week? ☐ (1) 1-2 days ☐ (2) 3-4 days ☐ (3) 5-6 วัน ☐ (4) every day	+1	+1	+1	+1	+1	1
15. Do you eat letpet ? ☐ (0) No ☐ (1) Yes (Answer No 15.1)	+1	+1	+1	+1	+1	1
15.1 How often do you eat letpet per week ☐ (1) 1-2 days ☐ (2) 3-4 days ☐ (3) 5-6 วัน ☐ (4) every day	+1	+1	+1	+1	+1	1

Appendix C:

Questionnaires (English)

Title: Association between Maternal fluoride level and Preterm delivery and Low Birthweight among Pregnancy at Endemic fluoride areas in Lamphun Thailand: A Pregnancy-Birth Cohort Study

Researcher: Mr. Nonthaphat Theerawasttanasiri,

College of Public Health Sciences, Chulalongkorn University, Bangkok,

Thailand, e-mail: p.theerawattanasiri@gmail.com Mobile: 66 9 7969 6283

Explanation

This questionnaire is prepared for Association between Maternal fluoride level and Preterm delivery and Low Birthweight among Pregnancy at Endemic fluoride areas in Lamphun Thailand: A Pregnancy-Birth Cohort Study.

The questionnaires are composed of 3 parts.

Part 1 Residence Area

Part 2 Sociodemographic and Characteristic

Part 3 Risk Factor related to Premature delivery & LBW

**** Please fill in .....and check ✓ the true answer in ☐ ****

For the benefit of research. Please answer the questions fully and please return to the staff. Your information will be kept strictly confidential.

Questionnaires

Title: Association between Maternal fluoride level and Preterm delivery and Low Birthweight among Pregnancy at Endemic fluoride areas in Lamphun Thailand: A Pregnancy-Birth Cohort Study

Areas Code_____ **Participant ID**_____ **(HN)** _____

Questionnaires	For researcher
Part 1 Resident areas	
1. **Now, you live in District** ☐ 1. Mueang Lamphun ☐ 2. Pasang ☐ 3 Ban Thi	
2. Name of village _____ No. of village _____ Name of sub district _____	
3. How many years do you live in this village? ☐ (1) more than 5 years ☐ (2) 1-5 years (specify) _____ year	
4. Source of drinking water ☐ (1) village tap ☐ (2) Shallow well ☐ (3) bottled water ☐ (4) other (specify) _____	
5. Source of cooking water ☐ (1) village tap ☐ (2) Shallow well ☐ (3) bottled water ☐ (4) other (specify) _____	
Part 2 Socio-demographic and Characteristics	
6. Age _____ years	
7. Family status ☐ (1) Single mom ☐ (2) live together ☐ (3) separated ☐ (4) other (specify) _____	
8. Education of pregnant woman ☐ (1) non-education ☐ (2) primary school ☐ (3) secondary school ☐ (4) high school ☐ (5) Diploma ☐ (6) Bachelor or higher	
9. Education of spouse / partner ☐ (1) non-education ☐ (2) primary school ☐ (3) secondary school ☐ (4) high school ☐ (5) Diploma ☐ (6) Bachelor or higher	

Questionnaires	For researcher
10. Family income ☐ (1) less than 5,000 บาท ☐ (2) 5,001 -10,000 ☐ (3) 10,001 -15,000 ☐ (4) 15,001 -20,000 ☐ (5) 20,001 -25,000 ☐ (6) more than 25,000	
3. Risk Factor related to Premature delivery & LBW	
11. Are your spouse / partner smoke? ☐ (0) No ☐ (1) Yes	
12. Do you standing work more than 1 hour per day? ☐ (0) No (Move to No 13) ☐ (1) Yes (Answer No 12.1)	
12.1 How about your standing work Amount of hour of standing work per day _____ hours Amount of day of standing work per week _____ days	
13. Do you drink coffee? ☐ (0) No (Move to No 13) ☐ (1) Yes (Answer No 13.1)	
13.1 How often do you drink coffee per week? ☐ (1) 1-2 days ☐ (2) 3-4 days ☐ (3) 5-6 วัน ☐ (4) every day	
14. Do you drink/eat tea or food make from tea? ☐ (0) No (Move to No 13) ☐ (1) Yes (Answer No 14.1)	
14.1 How often do you drink/eat tea food make from per week? ☐ (1) 1-2 days ☐ (2) 3-4 days ☐ (3) 5-6 วัน ☐ (4) every day	
15. Do you eat letpet? ☐ (0) No ☐ (1) Yes (Answer No 15.1)	
15 How often do you eat letpet per week ☐ (1) 1-2 days ☐ (2) 3-4 days ☐ (3) 5-6 วัน ☐ (4) every day	

Appendix D:

Medical Record information (English)

Postpartum Page 1

Areas Code_____

Participant ID_____ **(HN)** _____

Place of ANC ( ) Lamphun Hospital ( ) Pasang Hospital ( ) Ban Thi Hospital

Place of Delivery ( ) Lamphun Hospital ( ) Pasang Hospital ( ) Ban Thi Hospital

Date of Delivery _____

Information	For researcher
History & medical record 1. Gravida ____ Para ____	
2. LMP _____	
3. EDC _____	
4. High _____ centimeter	
5. Body weight before pregnant _____ Kg	
6. Body weight before delivery _____ Kg	
7. Gestational age of 1-st ANC _____ weeks	
8. Complete 5 times of ANC visit ☐ (0) No (specify the last visit) _____ ☐ (1) Yes	
9. History of cesarean delivery. ☐ (0) No ☐ (1) Yes	
10. Clinical assessment 10.1. Infection during pregnant ☐ (0) No ☐ (1) Yes (specify) _____	
10.2 Hematocrit _____ %	
10.3 Serum fluoride _____ mg/L	
10.4 Urine fluoride _____ mg/L	

Appendix D

Medical Record information (English)

Newborn Page 2

Newborn ID_____ **(HN)** _____

Information	For researcher
1. Gestational age at birth _____ weeks ☐ (1) Premature delivery (less than 37 weeks) ☐ (2).Term (37 – less than 42 weeks) ☐ (3) Post term (42 week or over)	
2. Sex ☐ (1) boy ☐ (2) girl	
3. Body weight _____ gram. ☐ (0). Less than 2500 gram ☐ (1) equal 2500 gram or over	
4 Height _____ centimeter Head circumference _____ centimeter	
5. APGAR Score 5.1 1 minute _____ 5.2 5 minutes _____	
6.type of delivery ☐ (1) Normal delivery ☐ (2) other (specify) _____	
7. Congenital abnormalities ☐ (0) Yes ☐ (1) No (specify) _____	
8. Baby Health ☐ (0) Yes ☐ (1) No (specify) _____	
9. Clinical Assessment 9.1 TSH level ☐ (0) No ☐ (1).Yes	
9.2 PKU level ☐ (0) No ☐ (1).Yes	

Appendix E:

Questionnaires (Thai)

แบบสอบถาม

หัวข้อวิจัย: ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน, ประเทศไทย : การศึกษาไปข้างหน้า

ชื่อผู้วิจัย: นายนณธภัทร ธีระวรรธนะสิริ นักศึกษาปริญญาเอก วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย หมายเลขโทรศัพท์ 097 9696 283 หรือ ที่อยู่อีเมล์ p.theerawattanasiri@gmail.com

โปรดอ่านต่อไปนี้ก่อนที่จะเริ่มทำแบบสอบถาม

ผู้วิจัยขอขอบคุณท่านที่ยินดีเข้าร่วมโครงการวิจัยครั้งนี้ แบบสอบถามนี้จะใช้สำหรับเก็บข้อมูลครั้งแรก โดยจะใช้เวลาในการตอบคำถาม 10 - 15 นาที แบบสอบถามประกอบด้วย 3 ส่วน ดังนี้

ส่วนที่ 1 ปัจจัยที่เกี่ยวกับการได้รับฟลูออไรด์

ส่วนที่ 2 ลักษณะทางสังคมและประชากร

ส่วนที่ 3 พฤติกรรมที่เสี่ยงต่อการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย

คำแนะนำทั่วไป:

กรุณากรอกคำตอบลงในช่องว่าง ..... หรือ ทำเครื่องหมาย ✓ ลงใน ☐ โดยเลือกคำตอบที่ตรงกับความเป็นจริงของท่านมากที่สุด

เพื่อประโยชน์ที่ได้จากการวิจัย ขอความกรุณาท่านตอบคำถามให้ครบถ้วนและโปรดส่งคืนกับเจ้าหน้าที่ ข้อมูลของคุณจะถูกเก็บเป็นความลับอย่างเคร่งครัด

แบบสอบถาม

หัวข้อวิจัย: ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน, ประเทศไทย : การศึกษาไปข้างหน้า

รหัสพื้นที่_____ รหัสหญิงตั้งครรภ์ (HN) _____

คำถาม	สำหรับผู้วิจัย
ส่วนที่ 1 เขตที่อยู่อาศัย	
1. **<u>ปัจจุบันท่านอาศัยอยู่จริง</u>**ในเขตอำเภอ ใด ☐ 1. อ.เมืองลำพูน ☐ 2. อ.ป่าซาง ☐ 3 อ.บ้านธิ	
2. ท่านอาศัยอยู่จริง หมู่บ้าน _____ หมู่ที่ ____ ตำบล _____	
3. ระยะเวลาที่ท่านอาศัยจริงในหมู่บ้านนี้ ถึงปัจจุบัน นานเท่าไร ☐ (1) มากกว่า 5ปี ☐ (2) 1-5 ปี ให้ระบุจำนวน _____ ปี	
4. แหล่งน้ำดื่มที่ท่านใช้เป็นประจำ คือ ☐ (1) น้ำประปา ☐ (2) น้ำบ่อ ☐ (3) น้ำบรรจุขวดหรือถัง ☐ (4) อื่น ๆ ระบุ _____	
5. แหล่งน้ำที่ท่านใช้ในการปรุง ประกอบอาหาร เป็นประจำ คือ ☐ (1) น้ำประปา ☐ (2) น้ำบ่อ ☐ (3) น้ำบรรจุขวดหรือถัง ☐ (4) อื่น ๆ ระบุ _____	
ส่วนที่ 2 ลักษณะทางสังคมและประชากร	
6. อายุหญิงตั้งครรภ์ _____ ปี	
7. สถานะภาพครอบครัว ☐ (1) โสด (คุณแม่เลี้ยงเดี่ยว) ☐ (2) อยู่ด้วยกัน ☐ (3) ไม่ได้อยู่ด้วยกัน ☐ (4) อื่น ๆ ระบุ_____	
8. ระดับการศึกษาสูงสุดของหญิงตั้งครรภ์ ☐ (1) ไม่ได้ศึกษา ☐ (2) ประถมศึกษา ☐ (3) มัธยมศึกษาตอนต้น ☐ (4) มัธยมศึกษาตอนปลาย/ปวช. ☐ (5) ปวส./อนุปริญญา ☐ (6) ปริญญาตรี ☐ (7) ปริญญาโท หรือ สูงกว่า	
9. ระดับการศึกษาสูงสุดของสามี/คู่ครอง ☐ (1) ไม่ได้ศึกษา ☐ (2) ประถมศึกษา ☐ (3) มัธยมศึกษาตอนต้น ☐ (4) มัธยมศึกษาตอนปลาย/ปวช. ☐ (5) ปวส./อนุปริญญา ☐ (6) ปริญญาตรี ☐ (7) ปริญญาโท หรือสูงกว่า	

คำถาม	สำหรับผู้วิจัย
10. รายได้ต่อเดือนของครอบครัว (หญิงตั้งครรภ และสามี/คู่ครอง) ☐ (1) น้อยกว่า 5,000 บาท ☐ (2) 5,001 -10,000 ☐ (3) 10,001 -15,000 ☐ (4) 15,001 -20,000 ☐ (5) 20,001 -25,000 ☐ (6) มากกว่า 25,000	
ส่วนที่ 3 ปัจจัยที่เสี่ยงต่อการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย	
11. ปัจจุบัน คู่สมรส / สามี ของท่านสูบบุหรี่ หรือไม่ ☐ (0) ไม่สูบ ☐ (1) สูบ	
12. อาชีพของท่านมีลักษณะการทำงานที่ต้องยืนเป็นเวลานานมากกว่า1ชั่วโมงหรือไม่ ☐ (0) ไม่ใช่ (ข้ามไป ข้อ13) ☐ (1) ใช่ ให้ตอบข้อ 12.1	
12.1 ลักษณะการยืนทำงานในแต่ละวัน และในสัปดาห์ (1) จำนวนชั่วโมง ที่ยืนทำงานในแต่ละวัน _____ ชั่วโมง (2) จำนวนวัน ที่ยืนทำงานในแต่ละสัปดาห์ _____ วัน	
13. ปัจจุบันท่านดื่มกาแฟหรือไม่ ☐ (0) ไม่ดื่ม ☐ (1) ดื่ม ให้ตอบข้อ 13.1	
13.1 ในแต่ละสัปดาห์ ท่านกาแฟ บ่อยครั้งแค่ไหน ☐ (1) 1-2 วัน ☐ (2) 4-3 วัน ☐ (3) 5-6 วัน ☐ (4) ทุกวัน	
14. ท่านดื่มน้ำชา หรือรับประทานอาหารที่ทำจากใบชาหรือไม่ เช่น น้ำชา ☐ (0) ไม่ดื่ม /ไม่รับประทาน ☐ (1) ดื่ม/รับประทาน ให้ตอบข้อ 14.1	
14.1 ในแต่ละสัปดาห์ ท่านนดื่มน้ำชา หรือรับประทานเมี่ยง บ่อยครั้งแค่ไหน ☐ (1) 1-2 วัน ☐ (2) 4-3 วัน ☐ (3) 5-6 วัน ☐ (4) ทุกวัน	
15. ท่านรับประทานเมี่ยหรือไม่ ☐ (0) ไม่รับประทาน ☐ (1) รับประทาน ให้ตอบข้อ 15.1	
15 ในแต่ละสัปดาห์ ท่านรับประทานเมี่ยง บ่อยครั้งแค่ไหน ☐ (1) 1-2 วัน ☐ (2) 4-3 วัน ☐ (3) 5-6 วัน ☐ (4) ทุกวัน	

Appendix F:

Medical Record information (Thai)

แบบบันทึกข้อมูลทางการแพทย์ : หญิงหลังคลอด หน้า 1

รหัสพื้นที่ _____

รหัสผู้เข้าร่วมวิจัย ID_____ **(HN)** _____

สถานที่ฝากครรภ์ ( ) รพ.ลำพูน ( ) รพ.ป่าซาง ( ) รพ.บ้านธิ

สถานที่คลอด ( ) รพ.ลำพูน ( ) รพ.ป่าซาง ( ) รพ.บ้านธิ

วัน เดือน ปี ที่คลอด _____

ข้อมูลหญิงคลอด	**สำหรับนักวิจัย**
ประวัติข้อมูลทางการแพทย์ 1. ครรภ์ที่ ____ จำนวนบุตรคลอดมีชีวิต ____	
2. ว ด ป. ประจำเดือนครั้งสุดท้าย (LMP) _____	
3. EDC _____	
3. วันกำหนดคลอด (EDC) _____	
4. ส่วนสูง _____ เซนติเมตร	
5. น้ำหนักก่อนตั้งครรภ์ _____ กิโลกรัม	
6. น้ำหนักก่อนคลอด _____ กิโลกรัม	
7. ฝากครรภ์ครั้งแรกเมื่ออายุครรภ์ได้ _____ สัปดาห์	
8. ฝากครรภ์ครบ 5 ครั้ง ตามเกณฑ์ฝากครรภ์คุณภาพ กรมอนามัย ☐ (0) ไม่ใช่ ☐ (1) มี ระบุ _____	
9. ประวัติการผ่าคลอดหรือไม่ ☐ (0) ไม่มี ☐ (1) มี	
<u>10. ผลตรวจทางห้องปฏิบัติการ</u> 10.1. ประวัติการติดเชื้อขณะตั้งครรภ์ปัจจุบัน ☐ (0) ไม่มี ☐ (1) มี ระบุ _____	
10.2 ความเข้มข้นของเม็ดเลือดแดง (Hematocrit) _____	
10.3 ความเข้มข้นของฟลูออไรด์ในเลือด _____	
10.4 ความเข้มข้นของฟลูออไรด์ในปัสสาวะ _____	

Appendix G:

Medical Record information (Thai)

แบบบันทึกข้อมูลทางการแพทย์ : ทารกแรกคลอด หน้า 2

รหัสทารก ID_____ **(HN)** _____

Information	For researcher
1. อายุครรภ์ขณะคลอด _____ สัปดาห์ ☐ (1) คลอดก่อนกำหนด (น้อยกว่า 37 สัปดาห์) ☐ (2). ครบกำหนดคลอด (37- น้อยกว่า 42 สัปดาห์) ☐ (3) เกินกำหนดคลอด (42 สัปดาห์ หรือมากกว่า)	
2. เพศทารก ☐ (1) ชาย ☐ (2) หญิง	
3. น้ำหนักทารกแรกคลอด ระบุ _____ กรัม ☐ (0). น้อยกว่า 2500 กรัม ☐ (1) เท่ากับ 2500 กรัม หรือมากกว่า	
4 ความยาว _____ เซนติเมตร เส้นรอบศีรษะ _____ เซนติเมตร	
5. APGAR Score 5.1 1 นาที _____ 5.2 5 นาที _____	
6. วิธีการคลอด ☐ (1) คลอดเอง ☐ (2) ใช้เครื่องมือ ระบุ _____	
7. ความผิดปกติแต่กำเนิด ☐ (0) ไม่มี ☐ (1) มี ระบุ	
8. ภาวะสุขภาพแรกเกิด ☐ (0) แข็งแรง ☐ (1) ผิดปกติ ระบุ _____	
8. Baby Healthภาวะสุขภาพแรกเกิด ☐ (0) Yes ☐ (1) No (specify) _____	
9. ผลตรวจทางห้องปฏิบัติการ 9.1 การตรวจความบกพร่องไทรอยด์ฮอร์โมน ☐ (0) ผิดปก ☐ (1).ปกติ	
9.2 การตรวจ PKU ☐ (0) ผิดปก ☐ (1).ปกติ	
9.3 ระดับความเข้มข้นของฟลูออไรด์ในสายสะดือทารก _____ mg/L	

Appendix H:

Information Sheet for Research Participant (Thai)

ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย

ชื่อโครงการวิจัย

ภาษาไทย: ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน, ประเทศไทย : การศึกษาไปข้างหน้า

ภาษาอังกฤษ: Association between Maternal fluoride level and Preterm delivery and Low Birthweight among Pregnancy at Endemic fluoride areas in Lamphun Thailand : A Pregnancy-Birth Cohort Study

ชื่อผู้วิจัย นายนณธภัทร ธีระวรรธนะสิริ นิสิตระดับ ปริญญาเอก หลักสูตรสาธารณสุขศาสตรดุษฎีบัณฑิต (หลักสูตรนานาชาติ) วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย

สถานที่ติดต่อ วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย อาคารสถาบัน 3 ชั้น 10 ซอยจุฬา 62 ปทุมวัน กรุงเทพ 10330 โทรศัพท์ 02-2188194 โทรสาร 02-2556046 (ที่ทำงาน) ศูนย์อนามัยที่ 1 เชียงใหม่ 51 ถ.ประชาสัมพันธ์ ต.ช้างคลาน อ.เมือง จ.เชียงใหม่ 50100 โทรศัพท์ 053 272 256 ต่อ 616 โทรศัพท์มือถือ 097 9822 296 E-mail: p.theerawattansiri@gmail.com

1. เรียน ผู้เข้าร่วมโครงการวิจัยทุกท่าน

ท่านได้รับเชิญให้เข้าร่วมโครงการวิจัยนี้เนื่องจากท่านเป็นกลุ่มประชากรเป้าหมายของการศึกษา ก่อนที่ท่านจะตัดสินใจเข้าร่วมการวิจัยนี้ ขอให้ท่านอ่านเอกสารฉบับนี้อย่างถี่ถ้วน เพื่อจะได้ทราบถึงเหตุผลและรายละเอียดของการวิจัย หากท่านมีข้อสงสัยใด ๆ กรุณาสอบถามเพิ่มเติมจากผู้วิจัยได้ตลอดเวลา เพื่อให้งานวิจัยนี้ประสบความสำเร็จ ผู้วิจัยใคร่ขอความร่วมมือจากท่านโปรดตอบข้อมูลแบบสอบถามให้ตรงกับความเป็นจริงมากที่สุด เพื่อประโยชน์ที่ได้จาการวิจัยต่อไป

2. เหตุผลความเป็นมา

ฟลูออไรด์เป็นแร่ธาตุตามธรรมชาติที่พบได้ในทุกภูมิภาค แต่จะพบมากที่สุดในภาคเหนือของประเทศไทยถึงร้อยละ 61 นอกจากนี้ยังพบว่าระดับความเข้มข้นของฟลูออไรด์ในภาคเหนือมีค่า สูงกว่าภูมิภาคอื่น ๆ

จากการสำรวจข้อมูลของกลุ่มพัฒนาทันตสาธารณสุขระหว่างประเทศและกรมทรัพยากรน้ำในปี 2553 พบว่า ลำพูนเป็น 1 ใน 6 จังหวัดทั่วประเทศ ที่แหล่งน้ำดื่มสาธารณะของหมู่บ้านมีปริมาณฟลูออไรด์สูงกว่าค่ามาตรฐาน (0.70 มิลลิกรัมต่อลิตร) มากกว่าร้อยละ 50 ของจำนวนหมู่บ้านที่สำรวจ โดยมีค่าตั้งแต่มากกว่า 0.7 – 9.80 มิลลิกรัมต่อลิตร และ จากการสำรวจฟลูออไรด์ในบ่อน้ำตื้นและบ่อน้ำลึกของ Joon Chuah และคณะ ในปี 2557 ในพื้นที่จังหวัดลำพูน พบว่าบ่อน้ำลึกร้อยละ 35 มีฟลูออไรด์ที่ระดับความเข้มข้น 1.5 มิลลิกรัมต่อลิตร ในขณะบ่อน้ำตื้นพบร้อยละ 7 ข้อมูลจากองค์การอนามัยโลกและการศึกษาวิจัยในต่างประเทศพบว่า ฟลูออไรด์สามารถผ่านจากรกมารดาไปยังตัวอ่อนที่อยู่ในครรภ์ได้ *การได้รับ*

ฟลูออไรด์ในปริมาณสูงในระหว่างตั้งครรภ์จะส่งผลต่อการเจริญเติบโตของทารกในครรภ์ และเสี่ยงต่อการคลอดก่อนกำหนดและทารกรกแรกคลอดน้ำหนักน้อยได้

การคลอดก่อนกำหนดหนด หมายถึง ทารกคลอดก่อนอายุครรภ์ครบ 37 สัปดาห์ และทารกแรกเกิดน้ำหนักน้อย หมายถึง ทารกมีน้ำหนักแรกคลอดน้อยกว่า 2500 กรัม การคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยเป็นปัญหาสำคัญด้านอนามัยแม่และเด็กของประเทศทั่วโลก รวมทั้งประเทศไทย สาเหตุหลักของทารกแรกเกิดน้ำหนักน้อยมาจากการคลอดก่อนกำหนด องค์การอนามัยโลกประมาณการว่า มีการคลอดก่อนกำหนดหนดถึงร้อยละ 10 ของการเกิดทั่วโลก และ 15-20% เป็นทารกแรกเกิดน้ำหนักน้อยและอัตรานี้เพิ่มสูงขึ้น ซึ่งเป็นสาเหตุการตายคลอดและการตายของเด็กอายุต่ำกว่า 5 ทั่วโลก ในประเทศไทยพบว่า มีอัตราทารกแรกเกิดน้ำหนักร้อยละ 8-9 % ส่วนจังหวัดลำพูนนั้น ในปี2557-2558 มีทารกแรกเกิดน้ำหนักน้อยร้อยละ 10-11% ซึ่งสูงกว่าค่ามาตรฐานของประเทศไทยที่กำหนดไว้คือ 7 %

การศึกษาผลกระทบต่อสุขภาพของฟลูออไรด์ในประเทศไทย ส่วนใหญ่ที่ศึกษาได้แก่ โรคฟันตกกระ โรคกระดูกโก่งงอผิดรูป เป็นต้น นอกจากนี้ยังมีการศึกษาโรคฟันตกกระในหญิงตั้งครรภ์ ซึ่งพบว่าอุบัติการณ์การโรคฟันตกกระ มีอัตราสูงในพื้นที่ภาคเหนือรวมทั้งพื้นที่จังหวัดลำพูน สาเหตุส่วนใหญ่เกิดจากการบริโภคน้ำดื่มที่มีปริมาณฟลูออไรด์สูง อย่างไรก็ตาม ยังไม่มีข้อมูลการศึกษาระดับความเข้มข้นของฟลูออไรด์ในเลือดหญิงตั้งครรภ์และในสายสะดือทารกแรกคลอด นอกจากนี้การคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยเป็นปัญหาที่ได้รับความสนใจในช่วงที่ผ่านมา แต่ยังไม่ได้มีการศึกษาเกี่ยวกับผลกระทบที่ไม่พึงประสงค์ของการได้รับฟลูออไรด์สูงกับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย

3. วัตถุประสงค์ของการศึกษา

1) เพื่อตรวจวัดฟลูออไรด์ในเลือดหญิงตั้งครรภ์และเลือดจากสายสะดือทารกแรกคลอด

2) เพื่อเปรียบเทียบความเข้มข้นของฟลูออไรด์ในเลือดหญิงตั้งครรภ์และเลือดจากสายสะดือทารกแรกคลอด ระหว่างพื้นที่ปกติและพื้นที่มีปริมาณฟลูออไรด์สูง

3) เพื่อประเมินความสัมพันธ์ระหว่างระดับความเข้มข้นของฟลูออไรด์ในเลือดมารดากับการคลอดก่อนกำหนด

4) เพื่อประเมินความสัมพันธ์ระหว่างระดับความเข้มข้นของฟลูออไรด์ในเลือดมารดาและทารกแรกเกิดน้ำหนักน้อย

5) เพื่อเปรียบเทียบอุบัติการณ์การคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยระหว่างพื้นที่ปกติและพื้นที่มีฟลูออไรด์สูง

4. วิธีการที่เกี่ยวข้องกับการวิจัย

4.1 กลุ่มตัวอย่างในการศึกษาวิจัย คือ หญิงตั้งครรภ์ในเขตอำเภอเมืองจังหวัดลำพูน โดยมีเกณฑ์การคัดเข้า และเกณฑ์การคัดออก ดังนี้

4. 1.1 เกณฑ์การคัดเข้า

1) หญิงตั้งครรภ์ชาวไทยที่มีอายุ ระหว่าง 20 – 35 ปี

2) อาศัยอยู่ในเขตอำเภอเมืองลำพูนมาเป็นระยะเวลา 5 ปีหรือมากกว่า

3) ได้รับการประเมินความเสี่ยงตามเกณฑ์ประเมินความเสี่ยงหญิงตั้งครรภ์ของกรมอนามัย และไม่พบว่ามีประวัติเสี่ยงจากการตั้งครรภ์ก่อนหรือการตั้งครรภ์ครภ์ปัจจุบัน

4) อายุครรภ์ 28 – 30 สัปดาห์ ขณะมารับบริการที่คลินิกฝากครรภ์ โรงพยาบาลลำพูน

5) วางแผนทีการคลอดที่โรงพยาบาลลำพูน

6) ยินดีเข้าร่วมโครงการวิจัยและลงลายมือชื่อในใบยินยอมด้วยความสมัครใจ

4.1.2 เกณฑ์การคัดออก

1) หญิงตั้งครรภ์ทีมีทารกในครรภ์มากกว่า 1 คน

2) เก็บปัสสาวะได้เพียงครั้งเดียว หรือ เข้าร่วมโครงการวิจัยไม่ครบ

4.1.3 การแบ่งกลุ่มผู้มีส่วนร่วมในการวิจัย

1) ผู้เข้าร่วมวิจัย จะถูกแบ่งกลุ่มเป็น 2 กลุ่ม ดังนี้

1.1) หญิงตั้งครรภ์ที่อาศัยอยู่ในพื้นที่ปกติ (Normal areas) หมายถึง หมู่บ้านที่มีปริมาณฟลูออไรด์ในน้ำประปาหรือแหล่งสาธารณะหลักของหมู่บ้านไม่เกิน 0.70 มิลลิกรัม/ลิตร

1.2) หญิงตั้งครรภ์ที่อาศัยอยู่ในพื้นที่มีปริมาณฟลูออไรด์สูง (Endemic areas) หมายถึง หมู่บ้านที่มีปริมาณฟลูออไรด์ในน้ำประปาหรือแหล่งสาธารณะหลัก เกิน 0.70 มิลลิกรัม/ลิตร

2) จำนวนผู้เข้าร่วมวิจัยทั้งหมด 304 คน เป็นหญิงตั้งครรภ์ที่อาศัยอยู่ในพื้นที่ปกติ จำนวน 152 คน และหญิงตั้งครรภ์ที่อาศัยอยู่ในพื้นที่มีปริมาณฟลูออไรด์สูง จำนวน 152 คน

4.2 การให้คำแนะนำและการสาธิตวิธีเก็บตัวอย่างน้ำดื่มและน้ำใช้ปรุงประกอบอาหาร

ภายหลังจากลงลายมือชื่อยินยอมเข้าร่วมโครงการ ผู้วิจัยและ/หรือผู้ช่วยผู้วิจัยจะอธิบายและสาธิตวิธีเก็บตัวอย่างน้ำดื่มและน้ำที่ใช้ปรุงประกอบอาหาร และสนับสนุนอุปกรณ์สำหรับเก็บตัวอย่างน้ำ พร้อมป้ายฉลากระบุ “น้ำดื่ม” และ “น้ำใช้ปรุงประกอบอาหาร” อย่างละ 1 ขวด จำนวน 2 ขวด

4.3 การเก็บข้อมูล เมื่ออายุครรภ์ 32 – 33 สัปดาห์

4.3.1 การเก็บตัวอย่างน้ำดื่มและน้ำใช้ปรุงประกอบอาหาร โดยเก็บตัวอย่างน้ำจากแหล่งน้ำที่หญิงตั้งครรภ์ในเป็นประจำมากที่สุด ตั้งแต่เริ่มรู้ว่าตั้งครรภ์ จนกระทั่งอายุครรภ์ 32-33 สัปดาห์ อย่างละขวด 1 ขวด ๆ ละ 20 มิลิลิตร เช่น น้ำบ่อ น้ำประปา น้ำบาดาล น้ำบรรจุขวด หรือแหล่งอื่น ๆ โดยหญิงตั้งครรภ์นำมาส่งวันมารับบริการฝากครรภ์ขณะอายุครรภ์ได้ 32-33 สัปดาห์ ที่คลินิกฝากครรภ์ โรงพยาบาลำพู

4.3.2 การเก็บข้อมูลแบบสัมภาษณ์โดยผู้วิจัยหรือผู้ช่วยผู้วิจัย 1 ครั้ง ใช้เวลาประมาณ 15 -20 นาที

4.3.3 การเก็บตัวอย่างเลือดเพื่อตรวจวิเคราะห์หาระดับฟลูออไรด์ในขณะอายุครรภ์ 32-33 สัปดาห์ โดยเจาะเลือดที่ข้อพับแขน 1 ครั้ง ปริมาณไม่เกิน 1 ช้อนชา การเจาะเลือดนี้จะดำเนินการร่วมกับการเจาะเลือดปกติตามโปรแกรมการให้บริการของคลีนิคฝากครรภ์ โดยโครงการวิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการตรวจวิเคราะห์ระดับฟลูออไรด์

4.3.4 การเก็บตัวอย่างเลือดจากสายสะดือทารกแรกคลอดเพื่อตรวจวิเคราะห์หาระดับฟลูออไรด์ จำนวน 1 ครั้งๆ ละไม่เกิน 1 ช้อนชา การเก็บเลือดนี้จะดำเนินการร่วมกับการเก็บเลือดจากสายสะดือปกติ

ตามโปรแกรมให้บริการทารกแรกคลอด ห้องคลอดด โดยโครงการวิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการตรวจวิเคราะห์ระดับฟลูออไรด์

5. การเข้าร่วมและการสิ้นสุดการเข้าร่วมโครงการวิจัย

ระยะเวลาเข้าร่วมโครงการตั้งแต่อายุครรภ์ 28 – 30 สัปดาห์ และสิ้นสุดการเมื่อคลอด

การเข้าร่วมในโครงการวิจัยครั้งนี้เป็นไปโดยความสมัครใจ หากท่านไม่สมัครใจจะเข้าร่วมการศึกษา ท่านสามารถถอนตัวได้ตลอดเวลา การขอถอนตัวออกจากโครงการวิจัยจะไม่มีผลต่อการฝากครรภ์แต่อย่างใด

6. ความเสี่ยงที่อาจได้รับจากการเจาะเลือด

ท่านมีโอกาสที่จะเกิดอาการเจ็บ เลือดออก ช้ำจากการเจาะเลือด อาการบวมบริเวณที่เจาะเลือดหรือหน้ามืด และโอกาสที่จะเกิดการติดเชื้อบริเวณที่เจาะเลือด แต่พบได้น้อยมาก

7. การปกป้องรักษาข้อมูลความลับของอาสาสมัคร

ข้อมูลที่อาจนำไปสู่การเปิดเผยตัวท่าน จะได้รับการปกปิดอยู่เสมอและจะไม่เปิดเผยแก่สาธารณชน โดยจะใช้เฉพาะรหัสประจำโครงการวิจัยของท่าน ในการใช้ข้อมูลเวชระเบียน โดยจะเก็บข้อมูลประวัติการฝากครรภ์ การตรวจรักษา ขณะตั้งครรภ์จนกระทั่งคลอดที่ การตรวจร่างกาย และผลตรวจทางห้องปฏิบัติการ ภาวะแทรกซ้อนขณะตั้งครรภ์ การคลอด และข้อมูลทารกหลังคลอด โดยผู้วิจัยจัดทำหนังสือขออนุญาตศึกษาข้อมูลดังกล่าวจากผู้อำนวยการโรงพยาบาลลำพูน

8. ประโยชน์ที่อาจได้รับ

เมื่อสิ้นสุดโครงการวิจัย ผลสรุปของโครงการวิจัยจะทำให้ทราบระดับความเข้มข้นของฟลูออไรด์ในน้ำประปาหรือแหล่งน้ำดื่มสาธารณะของหมู่บ้าน ภาพรวมระดับความเข้มข้นของฟลูออไรด์ระดับนตำบลและอำเภอ ระดับฟลู่ออไรด์ในเลือดหญิงตั้งครรภ์และในสายสะดือทารก ความสัมพันธ์ระหว่างระดับความเข้มข้นของฟลูออไรด์ในเลือดมารดาและการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ความแตกต่างของอุบัติการณ์ของการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อยระหว่างพื้นที่ปกติและพื้นที่มีฟลูออไรด์สูง ผลสรุปของโครงการวิจัยจะเป็นประโยชน์ต่อการสร้างความตระหนักต่อปัญหาฟลูออไรด์ ตลอดจนการป้องกันและลดการสัมผัสฟลูออไรด์ในกลุ่มหญิงตั้งครรภ์เพื่อการป้องกันผลกระทบต่อสุขภาพต่อไป

9. ค่าตอบแทนสำหรับผู้เข้าร่วมวิจัย (ถ้ามี)

ผู้ร่วมวิจัยจะได้รับค่าสมนาคุณเมื่อร่วมโครงการครั้งละ 100 บาท รวม3ครั้ง เป็นเงิน 300 บาท

หากท่านไม่ได้รับการปฏิบัติตามที่ปรากฏในเอกสาร **“ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย”** ท่านสามารถร้องเรียนได้ที่

1) คณะกรรมการจริยธรรมการวิจัยในคน โรงพยาบาลลำพูน 177 ถนน สายริมปิง ต.ต้นธง อ.เมือง จ.ลำพูน 51000 โทรศัพท์ 0-5356-9100

2) คณะกรรมการจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย 254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 0-2218-3202 E-mail: eccu@chula.ac.th

Appendix I

Informed Consent (Thai)

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย

ทำที่ โรงพาบาล ..... จ.ลำพูน

วันที่.....เดือน.....พ.ศ. .....

เลขที่ ..... (รหัสประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย)

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย ความสัมพันธ์ระหว่างระดับฟลูออไรด์ในมารดากับการคลอดก่อนกำหนดและทารกแรกเกิดน้ำหนักน้อย ในกลุ่มหญิงตั้งครรภ์พื้นที่มีฟลูออไรด์ในจังหวัดลำพูน ประเทศไทย: การศึกษาไปข้างหน้า

ชื่อผู้วิจัย นายนณธภัทร ธีระวรรธนะสิริ นักศึกษาปริญญาเอก หลักสูตรสาธารณสุขศาสตรดุษฎีบัณฑิต (หลักสูตรนานาชาติ) วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย

ที่อยู่ที่ติดต่อ วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย อาคารสถาบัน 3 ชั้น 10 ซอย จุฬา 62 ปทุมวัน กรุงเทพ 10330 โทรศัพท์ 02-2188194 โทรสาร 02-2556046 โทรศัพท์มือถือ 097 982 2296 ที่อยู่อีเมล์ p.theerawattanasiri@gmail.com

ข้าพเจ้า **ได้รับทราบ**รายละเอียด **ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย** เกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่าง ๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด และ**ได้รับคำอธิบาย**จากผู้วิจัย **จนเข้าใจเป็นอย่างดี**แล้ว

ข้าพเจ้าจึง**สมัครใจ**เข้าร่วมโครงการวิจัย โดยข้าพเจ้าเข้าร่วมกิจกรรมและยินยอมให้เปิดเผยข้อมูลเวชระเบียนโดยจะเก็บข้อมูลประวัติการฝากครรภ์ การตรวจรักษาขณะตั้งครรภ์จนกระทั่งคลอดที่ การตรวจร่างกายและผลตรวจทางห้องปฏิบัติการ ภาวะแทรกซ้อนขณะตั้งครรภ์ การคลอดและข้อมูลทารกแรกคลอด ข้อมูลมารดาหลังคลอด ตลอดระยะเวลา 1 ปี ตั้งแต่เริ่มต้นจนกระทั่งสิ้นสุดโครงการ ดังต่อไปนี้

1) ข้าพเจ้าจะให้ข้อมูลและตอบแบบสอบถามตามความเป็นจริง

2) ข้าพเจ้ายินยอมให้เก็บตัวอย่างเลือดเพื่อตรวจวิเคราะห์หาระดับฟลูออไรด์ในขณะอายุครรภ์ 24-30 สัปดาห์ โดยเจาะเลือดที่ข้อพับแขน 1 ครั้ง ปริมาณไม่เกิน 1ช้อนชา (5ซีซี) ซึ่งจะดำเนินการร่วมกับแผนงานปกติตามโปรแกรมการให้บริการฝากครรภ์ของคลีนิคฝากครรภ์โรงพยาบาลลำพูน โดยโครงการวิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการตรวจหาระดับฟลูออไรด์

3) ข้าพเจ้ายินยอมให้เก็บตัวอย่างปัสสาวะ ปริมาณครั้งละไม่เกิน 2ช้อนชา (10ซีซี), 3 ครั้ง ขณะอายุครรภ์ 24-30, 31-33 และ 34-36 สัปดาห์ เพื่อวิเคราะห์หาระดับฟลูออไรด์ ซึ่งจะดำเนินการร่วมกับ

แผนงานปกติตามโปรแกรมการให้บริการฝากครรภ์ของคลีนิคฝากครรภ์โรงพยาบาลลำพูน โดยโครงการวิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการตรวจหาระดับฟลูออไรด์

4) ข้าพเจ้ายินยอมให้เก็บตัวอย่างเลือดจากสายสะดือทารกแรกคลอด ปริมาณไม่เกิน 1 ช้อนชา (5ซีซี), 1ครั้งขณะแรกคลอด เพื่อตรวจวิเคราะห์หาระดับฟลูออไรด์ ซึ่งจะดำเนินการร่วมกับแผนงานปกติตามโปรแกรมการให้บริการทารกแรกคลอดของห้องคลอดโรงพยาบาลลำพูน โดยโครงการวิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการตรวจหาระดับฟลูออไรด์

5) ข้าพเจ้าจะได้รับค่าสมนาคุณเมื่อร่วมโครงการครั้งละ 100 บาท รวม3ครั้ง เป็นเงิน 300 บาท

6) ข้าพเจ้ามีสิทธิ**ถอนตัว**ออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ **โดยไม่ต้องแจ้งเหตุผล** ซึ่งการถอนตัวออกจากการวิจัยนี้ จะไม่ส่งผลกระทบใด ๆ ต่อการดูแล การรักษา การให้บริการทางการแพทย์ขณะตั้งครรภ์และการคลอดของข้าพเจ้าทั้งสิ้น

7) ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใด ๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะ**เก็บรักษาเป็นความลับ** โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่

1) คณะกรรมการจริยธรรมการวิจัยในคน โรงพยาบาลลำพูน 177 ถนน สายริมปิง ต.ต้นธง อ.เมืองลำพูน จ.ลำพูน 51000 โทรศัพท์ 0-5356-9100

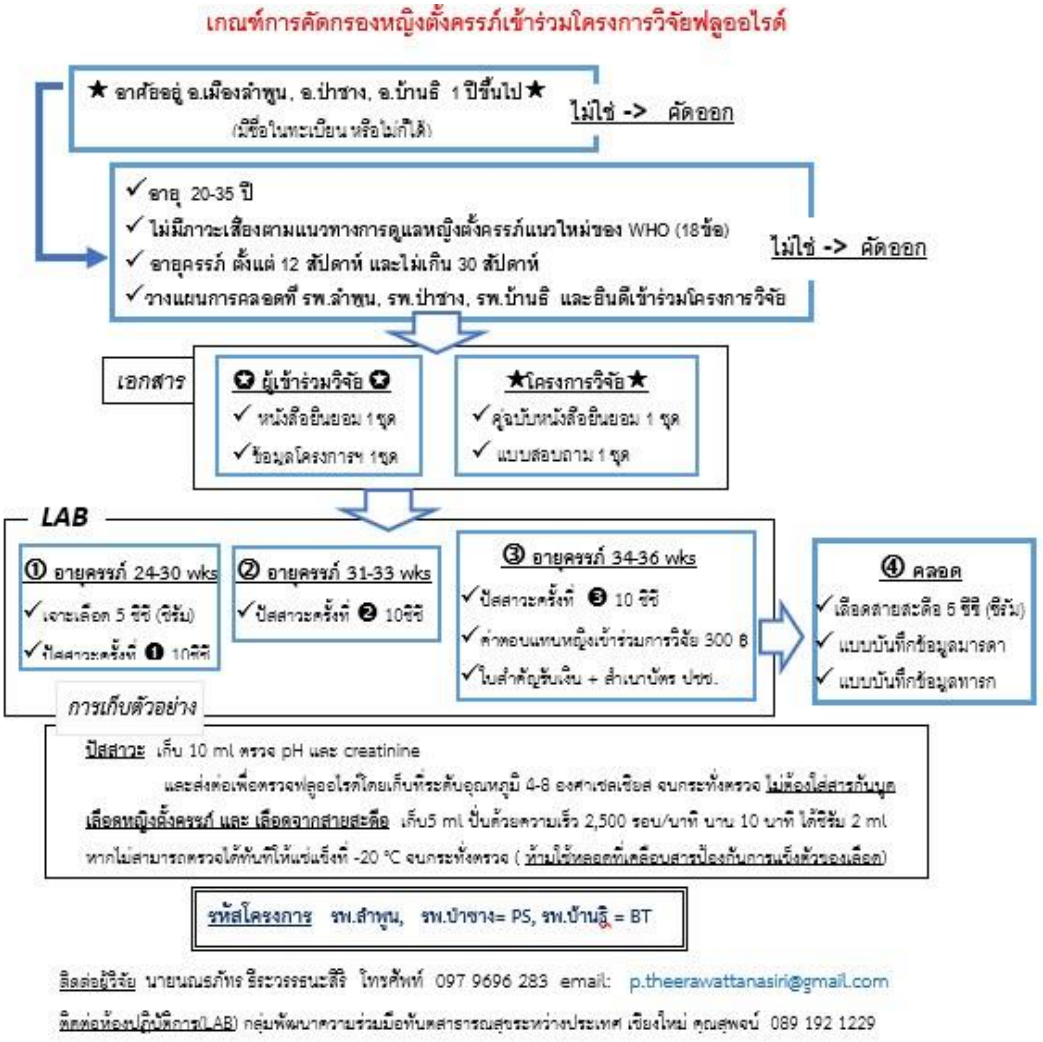
2) คณะกรรมการพิจารณาจริยธรรมการวิจัยในคนกลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย 254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 0-2218-3202 **E-mail: eccu@chula.ac.th**

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นหลักฐานต่อหน้าพยาน และข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัยและสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ.....	ลงชื่อ.....
(นายนณธภัทร ธีระวรรธนะสิริ)	(.....)
ผู้วิจัยหลัก	ผู้เข้าร่วมในการวิจัย
	ลงชื่อ.....
	(.....)
	พยาน

Appendix J

Screening protocol and Follow up Checklist



วิจัยฟลูออไรด์ HN _____ ID No _____		
อายุครรภ์	**กิจกรรม**	**วันที่**
1. 12-30 wks	1.1 แบบสอบถาม	
	1.2 เจาะเลือด 5ml	
	1.3 เก็บปัสสาวะครั้งที่ ❶ 10 ml	
2. 31-33 wks	2.1 เก็บปัสสาวะครั้งที่ ❷ 10 ml	
3. 34-36 wks	3.1 เก็บปัสสาวะครั้งที่ ❸ 10 ml	
4. คลอด	4.1 เลือดสายสะดือ 5ml	
	4.2 ข้อมูลมารดา	
	4.3 ข้อมูลทารก	

Appendix K

Maternal Serum & Laboratory Record Form(Thai)

แบบนำส่งส่งตรวจทางห้องปฏิบัติการ

***ตัวอย่างซีรัมจากเลือดหญิงตั้งครรภ์*เพื่อตรวจระดับฟลูออไรด์**

รหัสพื้นที่ ( ) รพ.ลำพูน MA ( ) รพ.ป่าซาง PS ( ) รพ. บ้านธิ BT

วันที่ส่งตัวอย่าง _____

วันที่เก็บตัวอย่าง	**รหัสผู้เข้าร่วมวิจัย**	**HN**	**อายุครรภ์ (สัปดาห์)**	**ระดับ F Mg/L**	**หมายเหตุ**

หมายเหตุ *รหัสผู้เข้าร่วมวิจัย* ให้ใส่ตัวอักษรภาษาอังกฤษ ตามด้วยลำดับผู้เข้าร่วมวิจัยของแต่ละโรงพยาบาล เช่น รพ.ลำพูน = MA001, รพ.ป่าซาง = PS001, รพ. บ้านธิ = BT001

ผู้ส่ง _____ โทรศัพท์ติดต่อ _____

ติดต่อผู้วิจัย นายนณธภัทร ธีระวรรธนะสิริ โทรศัพท์ 097 9696 283

ติดต่อห้องปฏิบัติการ(LAB)กลุ่มพัฒนาความร่วมมือทันตสาธารณสุขระหว่างประเทศ เชียงใหม่ คุณสุพจน์ 089 192 1229

Appendix L

Information of Public Water Supply (Thai)

แบบนำส่งและบันทึกการตรวจระดับฟลูออไรด์ในน้ำประปาหรือแหล่งน้ำสาธารณะหมู่บ้าน

ลำดับ	รหัส	ตำบล	หมู่บ้าน	ละติจูด	ลองจิจูด	จำนวนผู้ใช้ (ครัวเรือน)	ระดับความเข้มข้นฟลูออไรด์ .mg / L	หมายเหตุ
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

หมายเหตุ หมู่บ้านที่ไม่มีระบบประปา กรุณาระบุแหล่งประปาที่ใช้ร่วม เช่น ใช้ร่วมกับหมู่ .... (ระบุ)

Appendix M

Maternal Serum & Laboratory Record Form(Thai)

แบบนำส่งส่งตรวจทางห้องปฏิบัติการ

การตรวจระดับฟลูออไรด์ **ในปัสสาวะหญิงตั้งครรภ์**

รหัสพื้นที่ ( ) รพ.ลำพูน MA ( ) รพ.ป่าซาง PS ( ) รพ. บ้านธิ BT

วันที่ส่งตัวอย่าง _____

รหัส	วันที่เก็บ	รหัส	HN	อายุครรภ์	ครั้งที่ตรวจ			น้ำหนัก	pH	creatinine	ระดับ	หมายเหตุ
ตัวอย่าง	ตัวอย่าง	โครงการ		(สัปดาห์)	1	2	3	Kg.		mg/L	F mg/L	
					<30	31-33	34--36					
01												
02												
03												
04												
05												
06												
07												
08												
09												
10												
11												
12												
13												
14												

หมายเหตุ *รหัสผู้เข้าร่วมวิจัย* ให้ใส่ตัวอักษรภาษาอังกฤษ ตามด้วยลำดับผู้เข้าร่วมวิจัยของแต่ละโรงพยาบาล เช่น รพ.ลำพูน = MA001, รพ.ป่าซาง = PS001, รพ. บ้านธิ = BT001

ผู้ส่ง _____ โทรศัพท์ติดต่อ _____

ติดต่อผู้วิจัย นายนณธภัทร ธีระวรรธนะสิริ โทรศัพท์ 097 9696 283

ติดต่อห้องปฏิบัติการ(LAB)กลุ่มพัฒนาความร่วมมือทันตสาธารณสุขระหว่างประเทศ เชียงใหม่ คุณสุพจน์ 089 192 1229

Appendix N

Umbilical Serum Fluoride & Laboratory Record Form(Thai)

แบบนำส่งส่งตรวจทางห้องปฏิบัติการ

***ตัวอย่างซีรัมจากสายสะดือทารกภ์*เพื่อตรวจระดับฟลูออไรด์**

รหัสพื้นที่ ( ) รพ.ลำพูน MA ( ) รพ.ป่าซาง PS ( ) รพ. บ้านธิ BT

วันที่ส่งตัวอย่าง _____

วันที่เก็บตัวอย่าง	**รหัสผู้เข้าร่วมวิจัย**	**HN มารดา**	**HN ทารก**	**ระดับ F Mg/L**	**หมายเหตุ**

หมายเหตุ *รหัสผู้เข้าร่วมวิจัย* ให้ใส่ตัวอักษรภาษาอังกฤษ ตามด้วยลำดับผู้เข้าร่วมวิจัยของแต่ละโรงพยาบาล เช่น รพ.ลำพูน = MA001, รพ.ป่าซาง = PS001, รพ. บ้านธิ = BT001

ผู้ส่ง _____ โทรศัพท์ติดต่อ _____

ติดต่อผู้วิจัย นายนณธภัทร ธีระวรรธนะสิริ โทรศัพท์ 097 9696 283

ติดต่อห้องปฏิบัติการ(LAB)กลุ่มพัฒนาความร่วมมือทันตสาธารณสุขระหว่างประเทศ เชียงใหม่ คุณสุพจน์ 089 192 1229

Appendix O

Maternal Serum & Laboratory Record Form(Thai)

แบบนำส่งส่งตรวจทางห้องปฏิบัติการ

***ตัวอย่างซีรัมจากเลือดหญิงตั้งครรภ์*เพื่อตรวจระดับฟลูออไรด์**

รหัสพื้นที่ ( ) รพ.ลำพูน MA ( ) รพ.ป่าซาง PS ( ) รพ. บ้านธิ BT

วันที่ส่งตัวอย่าง _____

วันที่เก็บตัวอย่าง	รหัสผู้เข้าร่วมวิจัย	HN	อายุครรภ์ (สัปดาห์)	ระดับ F Mg/L	หมายเหตุ

หมายเหตุ *รหัสผู้เข้าร่วมวิจัย* ให้ใส่ตัวอักษรภาษาอังกฤษ ตามด้วยลำดับผู้เข้าร่วมวิจัยของแต่ละโรงพยาบาล เช่น รพ.ลำพูน = MA001, รพ.ป่าซาง = PS001, รพ. บ้านธิ = BT001

ผู้ส่ง _____ โทรศัพท์ติดต่อ _____

ติดต่อผู้วิจัย นายนณธภัทร ธีระวรรธนะสิริ โทรศัพท์ 097 9696 283

ติดต่อห้องปฏิบัติการ(LAB)กลุ่มพัฒนาความร่วมมือทันตสาธารณสุขระหว่างประเทศ เชียงใหม่ คุณสุพจน์ 089 192 1229

VITA

Nonthaphat Theerawasttanasiri graduated Diploma in Public Health from Sirindhorn College of Public Health, Phitsanulok. He earned his Bachelors' in Community Health from Uttaradit Rajabhat University, Public Administration from Sukhothai Thammathirat Open University, and Masters' in Political Science from Ramkhamhaeng University, Man & Environment Management from ChiangMai University. Also, now he is a Ph.D. candidate on Doctor of Philosophy in Public Health program (Environmental & Occupational Health) at College of Public Health Sciences, Chulalongkorn University, Thailand.

He is currently working as a Professional public health technical officer at Health Promotion Center Region1 Chiang Mai, Department of Health, Ministry of Public Health. His research focuses on Public Health, Environmental & Occupational Health, and Occupational Health & Safety.