ASSOCIATION BETWEEN ARSENIC EXPOSURE AND DIABETES IN RONPIBOON DISTRICT NAKHON SI THAMMARAT THAILAND: CASE CONTROL RESEARCH

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Public Health
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การศึกษาความสัมพันธ์ระหว่างการได้รับสัมผัสสารหนูกับการเกิดโรคเบาหวาน ในอำเภอร่อนพิบูลย์ จังหวัดนครศรีธรรมราช ประเทศไทย: การศึกษาแบบเปรียบเทียบระหว่างกลุ่มศึกษาและกลุ่มควบคุม

นางสาวขวัญยืน ศรีเปารยะ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาสาธารณสุขศาสตรดุษฎีบัณฑิต
สาขาวิชาสาธารณสุขศาสตร์
วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559

Thesis Title	ASSOCIATION BETWEEN ARSENIC EXPOSURE AND DIABETES IN RONPIBOON DISTRICT NAKHON SI THAMMARAT THAILAND: CASE CONTROL RESEARCH
By	Miss Kwanyuen Sripaoraya
Field of Study	Public Health
Thesis Advisor	Robert S. chapman, M.D., M.P.H.

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

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ขวัญยืน ศรีเปารยะ : การศึกษาความสัมพันธ์ระหว่างการได้รับสัมผัสสารหนูกับการเกิดโรคเบาหวานในอำเภอร่อนพิบูลย์ จังหวัดนครศรีธรรมราช ประเทศไทย: การศึกษาแบบเปรียบเทียบระหว่างกลุ่มศึกษาและกลุ่มควบคุม (ASSOCIATION BETWEEN ARSENIC EXPOSURE AND DIABETES IN RONPIBOON DISTRICT NAKHON SI THAMMARAT THAILAND: CASE CONTROL RESEARCH) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: นพ. โรเบิร์ท เอส. แชบแม็น, 102 หน้า.

ความเป็นมา: เบาหวานเป็นโรคเรื้อรังที่เกิดจากตับอ่อนผลิตอินซูลินลดลง (เบาหวานชนิดที่๑) หรือเกิดจากร่างกายใช้อินซูลินได้ไม่เต็มที่ (เบาหวานชนิดที่ ๒) ปัจจัยเสี่ยงการเกิดเบาหวาน โดยเฉพาะอย่างยิ่งชนิดที่ ๒ ที่รู้จักกันดีได้แก่ การมีอายุมาก เป็นโรคอ้วน ลงพุง รับประทานอาหารไม่สมดุลย์ ไม่ออกกำลังกาย มีความเครียด บุคคลในครอบครัวมีประวัติเป็นโรคนี้ และลักษณะทางพันธุกรรม การได้รับสัมผัสสารหนูในปริมาณสูงถูกจัดเพิ่มให้เป็นปัจจัยเสี่ยงเบาหวานชนิดที่สอง แต่ผลการศึกษาทางระบาดวิทยากรณีความสัมพันธ์การได้รับสารหนูระดับปานกลางหรือต่ำกับการเกิดเบาหวานยังไม่สามารถสรุปได้เนื่องจากยังมีผลขัดแย้งกันอยู่ พบปัญหาการปนเปื้อนสารหนูที่อำเภอร่อนพิบูลย์ จังหวัดนครศรีธรรมราช ประเทศไทยมาตั้งแต่ปี พ.ศ. ๒๕๓๐ อัตราการเกิดเบาหวานของประชากรที่อาศัยในพื้นที่นี้ที่สูงขึ้นกว่าเดิม ทำให้คณะผู้วิจัยสนใจศึกษาหาปัจจัยเสี่ยงการเกิดโรคนี้ เพื่อให้ได้ข้อมูลมาใช้ลดความเสี่ยงต่อไป โดยการศึกษานี้เน้นหาความสัมพันธ์ระหว่างปัจจัยเสี่ยงต่างๆ รวมถึงการได้รับสัมผัสสารหนูกับการเกิดเบาหวานในกลุ่มประชากรที่อาศัยในพื้นที่ 3 หมู่บ้านคือ หมู่ ๒ หมู่ ๑๒ และหมู่ ๑๓ ของตำบลร่อนพิบูลย์ จังหวัดนครศรีธรรมราช ประเทศไทย

วิธีการวิจัย: เป็นการออกแบบการศึกษาเพื่อเปรียบเทียบปัจจัยทางสังคมและเศรษฐกิจ ตลอดจนการได้รับสัมผัสสารหนูในระดับต่ำระหว่างกลุ่มศึกษาที่ได้รับการวินิจฉัยว่าเป็นเบาหวานจำนวน ๑๘๕ ราย และกลุ่มควบคุมที่ไม่ได้เป็นเบาหวานชนิดนี้ จำนวน ๒๐๐ ราย (กรณีจับคู่แบบไม่ควบคุม) และจำนวน ๑๘๕ ราย (กรณีจับคู่แบบควบคุม ใช้ข้อมูลทุติยภูมิจากการศึกษาที่ได้ดำเนินการก่อนหน้านั้นเมื่อ พ.ศ. ๒๕๔๓ และ ๒๕๕๑ และเนื่องจากตัวแปรอิสระที่จำเป็นต้องใช้วิเคราะห์มีข้อมูลไม่สมบูรณ์ มีบางส่วนขาดหายไป รวมทั้งลักษณะการกระจายตัวของข้อมูลจากการตรวจวัด ไม่ได้มีการกระจายแบบปกติ จึงใช้เทคนิคทางสถิติในโปรแกรม เอส พี เอส เอส คือ มัลติเพิล อิมพิวเตชั่น และเลือกใช้วิธีการที่เรียกว่า พรีดิกทีพ มีน แม็ทชิ่งเพื่อจัดการกับค่าที่เป็นลบหลังการคำนวณ ได้มีการสร้างโมเดลการคำนวณเพื่อศึกษาอิทธิพลของตัวแปรอิสระได้แก่ ปัจจัยทางสังคมและเศรษฐกิจรวมทั้งปัจจัยการที่เกี่ยวกับสารหนู กับความเสี่ยงการเกิดเบาหวาน อย่างเป็นลำดับขั้นตอน สำหรับข้อมูลชุดการจับคู่แบบไม่ควบคุมระหว่างกลุ่มศึกษาและกลุ่มควบคุมนั้นวิเคราะห์ความสัมพันธ์โดยใช้วิธีวิเคราะห์การถดถอยแบบโลจิสติคแบบไม่มีเงื่อนไข ส่วนกรณีที่มีการจับคู่ เพศ อายุ ระหว่างกลุ่มศึกษาและควบคุมใช้วิธีวิเคราะห์การถดถอยแบบโลจิสติคแบบมีเงื่อนไข (รุ่นค็อกซ์) เพื่อหาความสัมพันธ์ระหว่างตัวแปรที่สนใจ

ผลการวิจัย ในทั้งสองกลุ่มศึกษา คือ แบบไม่จับคู่และแบบจับคู่ระหว่างกลุ่มศึกษาและกลุ่มควบคุมนั้น พบว่า ดัชนีมวลกาย (p=<0.001, 0.007) อายุ ((p=0.003 เฉพาะกลุ่มไม่จับคู่) ประวัติการเป็นเบาหวานของพี่น้องพ่อแม่เดียวกัน (p=0.021, 0.031) การดื่มแอลกอฮอล์ (p=0.002,เฉพาะกลุ่มจับคู่) สัมพันธ์กับการเพิ่มความเสี่ยงการเกิดเบาหวานชนิดที่ ๒ และยังพบอีกว่า การมีรถขับขี่ที่รวมถึงมอเตอร์ไซค์ อันเป็นตัวบ่งชี้ฐานะทางเศรษฐกิจประการหนึ่ง (p= 0.020, 0.010) การออกกำลังกาย (p=0.051, 0.027) สัมพันธ์กับการลดความเสี่ยงการเกิดเบาหวาน และสังเกตไม่พบความสัมพันธ์ใดๆ ระหว่างระดับสารหนูในน้ำกับการเกิดเบาหวานในพื้นที่นี้จากผลการศึกษาในทั้งสองกลุ่มข้างต้น

สรุปผลการวิจัย ผลจากการศึกษาในทั้งสองกลุ่ม คือ กลุ่มการศึกษาจับคู่แบบไม่ควบคุม และกลุ่มจับคู่แบบควบคุมในครั้งนี้ ยืนยันว่า การมีอายุมาก มีดัชนีมวลกายสูง มีประวัติการเป็นเบาหวานของพี่น้องพ่อแม่เดียวกัน เป็นปัจจัยสำคัญที่เพิ่มความเสี่ยงการเกิดเบาหวานชนิดที่สอง ในขณะที่การมีรถขับขี่ที่รวมถึงมอเตอร์ไซค์ อันเป็นตัวบ่งชี้ฐานะทางเศรษฐกิจ การออกกำลังกาย สัมพันธ์กับการลดความเสี่ยงการเกิดเบาหวานในพื้นที่การศึกษานี้ แม้พบว่าการใช้น้ำฝนในปี ๒๕๕๑ มีความสัมพันธ์อย่างจำกัดกับการลดความเสี่ยงเบาหวาน แต่ไม่พบความสัมพันธ์ระหว่างระดับความเข้มข้นสารหนูในน้ำกับการเกิดเบาหวานชนิดที่สองในพื้นที่การศึกษาที่อำเภอร่อนพิบูลย์ในครั้งนี้ การศึกษาในบริบทที่เกี่ยวเนื่องกับเรื่องนี้ยังคงมีความสำคัญ

คำสำคัญ การได้รับสัมผัสสารหนู เบาหวาน ประเทศไทย กลุ่มศึกษาและกลุ่มควบคุม

สาขาวิชา สาธารณสุขศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORDS: ARSENIC EXPOSURE / DIABETES / THAILAND / CASE-CONTROL

KWANYUEN SRIPAORAYA: ASSOCIATION BETWEEN ARSENIC EXPOSURE AND DIABETES IN RONPIBOON DISTRICT NAKHON SI THAMMARAT THAILAND: CASE CONTROL RESEARCH. ADVISOR: ROBERT S. CHAPMAN, M.D., M.P.H., 102 pp.

Background: Diabetes mellitus (DM) is a chronic metabolic disease resulting from diminished insulin production by pancreas (type 1) or the ineffective use of insulin by the body (type 2). Known risk factors for DM, especially DM type2, include older age, obesity; unbalanced diet, physical inactivity, stress, family history, and genetic polymorphisms. Chronic arsenic exposure at high level was considered as additional DM risk factors, but inconclusive epidemiological results still exist. Contamination of arsenic in environment had been found since 1987 in Ronphiboon district, Nakhon Si Thammarat Province, Thailand. The increased rates of DM patients in that area also led us to the investigation of DM risk factors, to add more information for DM risk mitigation. Thus, this study focused on investigation of determinants of DM type2 risk among residents of 3 Moo Ban (villages) of Ronphiboon sub-district, Nakhon Si Thammarat Province, Thailand.

Methods: This unmatched and matched case-control studies aimed to compare the socioeconomic as well as low dose arsenic exposure patterns between villagers with DM Type 2 (Cases, N=185) and those who had not been diagnosed with DM (controls, N=200 for unmatched; N=185 for matched). The data used were based on previous community-based studies in 2000 and 2008. The technique of Multiple Imputation (MI), with the Predictive Mean Matching (PMM, an imputation method used to prevent negative value after MI) was used to impute missing values for independent variables. The stepwise modelling was constructed to investigate the influence of socio-economic background and arsenic-related independent variables on DM risk. For fully imputed two data set of cases-unmatched controls and cases-matched controls, multiple logistic regression and conditional logistic (cox model) were respectively used to assess associations.

Results: BMI (p=<0.001, 0.007), age (p=0.003, unmatched), and history of sibling illness (p=0.021, 0.031), drinking (p=0.002, matched) were statistically significantly associated with increased risk of DM type 2, whereas having motorcar (representing better economic status, p= 0.020, 0.010), exercise (p=0.051, 0.027) were associated with lower DM type2 risk in the unmatched and matched case-control, respectively. We did not observe convincing association of water arsenic concentration with diabetes risk in both unmatched and matched controls studies.

Conclusions: Our findings on sociodemographic information of both unmatched and matched case control studies, confirm that older age, BMI, having history of illness in siblings were the determinants for increased DM type2, whereas having better economic status, exercise were associated with lower DM type2 risk in this area. Our analysis suggested no association between water arsenic concentration and DM type2 risk, though a limit inconsistent association was identified for the use evidence of rain water year 2008. Further research is needed on this topic.

Field of Study: Public Health Student's Signature .....

Academic Year: 2016 Advisor's Signature .....

ACKNOWLEDGEMENTS

This research was supported by the Royal Thai Government under the Department of Medical Sciences (for year 2000 and 2008 studies), and the EU funds on the project ‘‘The Mechanistic Basis for Providing a Realistic Cancer Risk Assessment for Exposure to Inorganic Arsenic within the EU Community’(during year 2000 study). For her initiative research collaboration and supportive, I am grateful for Dr. Sumol Pavittranon, my mentor and my boss. Without her persistence and understanding, I would not be able to complete this work.

I am grateful for my supervisor, Dr. Robert S. Chapman, for pushing me through all obstacles with the data complexity. I have learned a lot from his statistical expertise in the field of epidemiology.

We would like to thank all the health volunteers and the Ron Phibul people for their cooperation. We appreciate the cooperative affort of staff members of Ron Phibun hospital for allowing us to use their network and supporting facilities to collect all necessary information. I am grateful for all villagers in Moo 2 12 and 13 of Ron Phibun Sub-district for allowing me and the team to work in their villages and allowed us to walk around their houses and kitchens to collect water samples as well as answering our questions during those years.

Special thanks to Dr. Siriluck Thaichareun, for her identification of arsenicosis and skin cancer of all patients at Ronphibun hospital. I am also appreciating Khun Wilaiwan Putapreuk, Dr. Peera Kongthong and staffs, for their supportive works in Ronphibun hospital and in the field that allow me to fulfil all the required avtivities.

It is also impossible not to mention the laboratory support team (toxicology lab) from Trang and Surat Thanee Regional Medical Sciences Centers, Department of Medical Sciences. I am grateful for allowing me to work with them and thus, could have valuable data regarding arsenic concentration in water.

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CHAPTER I

INTRODUCTION

1.1 Background and Significance

1.1.1 Diabetes

Diabetes mellitus (DM) is a chronic metabolic disease resulting from impaired insulin production by pancreas (type 1) or the ineffective use of insulin by the body (type 2). It is incurable once it happens. If elevated blood sugar, a common effect of uncontrolled diabetes is persistent, it might lead to many severe complications of the body's systems such as blood vessels, eyes, kidneys, nerves or amputation of organs. Diabetes is one of major health problems among Thai population. Known risk factors for diabetes, especially type2 diabetes, are older age, obesity, eating habit, physical inactivity, stress, family history, and genetic polymorphisms. Since 1994, a number of epidemiological studies' results have suggested that environmental toxicants such as chronic arsenic exposure could be one of the DM risk factors (C.-J. Chen et al., 2007).

There are two types of diabetes were mainly recognized worldwide. DM type 1 results from the body react against pancreatic β cells (or autoimmune process) that produces insulin, the hormone that helps bringing sugar into the cells. Since the body could not produce insulin, the DM type 1 patients usually need insulin injection for treatment. The onset of DM type 1 is normally before adulthood and ketoacidosis is generally found. DM type 2 is due to resistance to insulin action and/or insulin deficiency. It does not need insulin in treatment and it is normally found in adults aged over 30 years. DM type 2 is the most common form of diabetes. Its risk factors include age, family history, genetic, high blood pressure, central adiposity, lack of exercise, obesity and diet (Longnecker & Daniels, 2001). The onset of DM type2 is a slow process and the symptoms develop slowly; thus rapid diagnosis is difficult. Apart from genetics, life style and occupation, some toxicants in environment including arsenic might be responsible for increased prevalence of DM type 2 worldwide (Longnecker & Daniels, 2001).

1.1.2 Diabetes situation in Thailand

According to survey of Thai population on burden of diseases and its risk factors of the year 1999 and 2004 by the working group on burden of disease and injuries among Thais, the Thai Ministry of Public Health (information from published announcement), the DALYs (Disability Adjusted Life Years) caused by DM of the year 2004 was 1.7 years per 100,000 persons among male and 2.7 years per 100,000 persons among female. It was reported that the estimated national prevalence of diabetes in Thai adults (aged ≥35 years) was 9.6 % (2.4 million people), which included 4.8% previously diagnosed and 4.8 % newly diagnosed (Aekplakorn et al., 2003). In the year 2007, diabetes was ranked second among top five leading diseases in Thais, as reported and published online on February 1, 2009 by the permanent secretary of the ministry of public health. There were 757,031 people have been diagnosed and treated for diabetes.

The following tables show amount and rate per 100,000 people of DM patients during 2004 – 2007 as reported from the chronic disease surveillance system of the

epidemiological division, Department of Disease Control, Ministry of public health, Thailand.

Table 1 Amount of DM patients and rate of DM per 100,000 people in Thailand from 2004 – 2007

year	Sex		Total Patients Being reported	Rate/100,000 people
	Male	Female		
2004*	NA	NA	228,309	382.46 – 2282.15
2005**	96,788	213,613	310,401	218.58 – 2834.57
2006***	201,699	407,955	609,654	159.45 – 4772.14
2007****	229,715	485,177	714,892	391.13 – 6719.62

* Report from 26 provinces, ** report from 28 provinces, *** report from 45 provinces,**** Report from 46 provinces, NA = not available
These reports from the chronic diseases surveillance system account only for DM patients that came to government's hospitals and health service facilities.

Table 2 Number of DM patients and rate of DM per 100,000 people in Nakhon Si Thammarat Province from 2003 – 2008(Data from provincial health office)

year	Patients categorized by Sex		Total Patients Being reported	Nakhon Si Thammarat population	Rate/100,000 people
	Male	Female			
1987-2003	657	1567	2224	1,531,072*	145.3
2004	896	2101	2997	1,500,343	199.8
2005	1286	3035	4321	1,502,382**	287.6
2006	1773	4157	5930	1,510,460**	392.6
2007	3237	7513	10758	1,506,997	713.9
2008	5335	12077	17412	1,513,163	1150.7

* Data as of 2003, ** use Nakhon Si Thammarat provincial data which is different from the annual epidemiological surveillance report of the epidemiological division.

Table 3 Number of DM patients and rate of DM per 100,000 people in Ron Phibun sub-district, Nakhon Si Thammarat Province from 2003 – 2008
(Source; Diabetes clinic of Ron Phibun hospital, Provincial Health Office, Department of local administration, Ministry of public health)

year	**Ron Phibun District** (report of Provincial Health Office)			**Ron Phibun Sub-District** (report of Ron Phibun hospital)		
	Pop	**No. case**	**Rate/10^6**	**Pop**	**No. case**	**Rate/10^6**
1987-2003	NA	38	NA	23000	166	721.7
2004	82915	116	139.9	24477*	238	972.3
2005	82754	161	194.6	25000*	304	1216.0
2006	82832	196	236.6	25000*	373	1492.0
2007	80729	275	340.7	25500*	421	1651.0
2008	NA (~80750)	419	~518.9	26000*	453	1742.0

Note: * = estimate number (source: Ron Phibun hospital)
Abbreviation: Pop = population, No. = Number, NA = not available

Figure 1 Number of DM patients of Ron Phibun hospital from 1988-2008 (Source: OPD card)

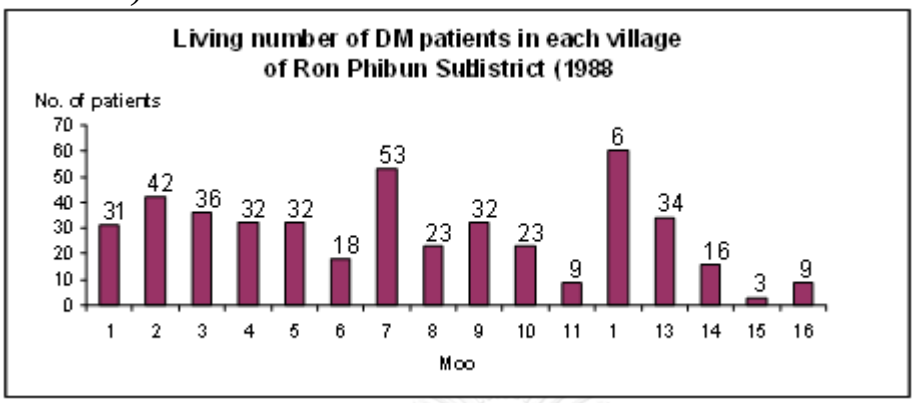
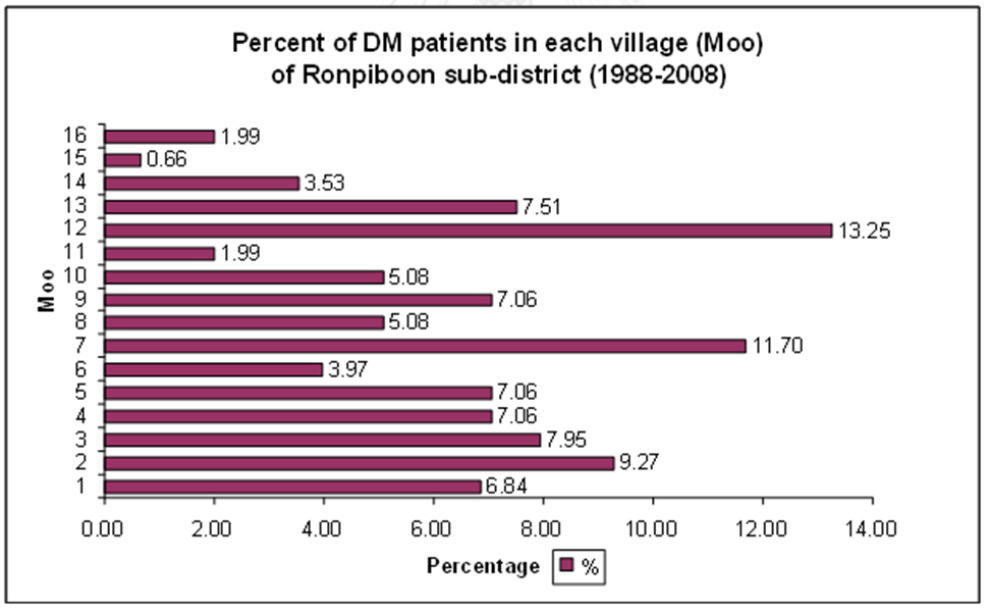


Figure 2 Percentages of DM patients in each of 16 Moo Ban of Ron Phibun sub-district from 1988-2008



As shown in figure1 and 2, the number and percentage of DM patients in Moo 12, Moo 2 and Moo 13 rank first, third, and fifth respectively, among the one of 16 Moo of Ron Phibun sub-district. The DM patients of these three villages contribute 30% of the whole sub-district. Table 4 shows number of DM cases in Moo 2, 12 and 13 in the year 2000 and 2008.

Table 4 Number of DM patients in Moo 2, 12, and 13 in 2000 and 2008 (Ron Phibun hospital report)

Moo	No. DM reported in 2000	No.DM reported in 2008 at Ron Phibun hospital
2	16	40
12	13	58
13	7	36

1.1.3 Arsenic exposure and its health effect

Arsenic is a toxic metalloid element occurring naturally and manmade. It can be found in soil, water and air and low level of 1 – 5 mg/L was found in rock in the form of amorphous or various forms of arsenopyrites. One third of arsenic compounds (both organic and inorganic forms) in the air come from volcano eruption, geothermal waters and wild fire, whereas mining is the major source of arsenic in soil and water. Arsenic compounds, especially Orpiment (As_2O_3) and Realgar (AsS) were used by men as coloring agents and alloys for arsenic bronzes, ormental/painting, cosmetics and copper arsenic alloys since old aged. During the 19^{th} to 20^{th} century, arsenic were used in pharmaceutical and medicinal areas, used as coloring agents in toys, wallpapers and wrapping papers, pesticides/insecticides, cotton defoliant, growth promoter in pigs/cattle/sheep dips, copper-chrome-arsenate wood preservative, wire alloys, electronics and ceramic/glassware (IPCS/WHO, 2001).

There were many reports of arsenic toxicity from Taiwan, Germany, Western U.S.A., Mexico, Chile, Argentina, India, Bangladesh, Mongolia, Thailand, China, Japan and some others.

Arsenic was categorized into three groups; organic arsenic forms (oAs), inorganic arsenic forms (iAs), and arsine gas. Arsenic compounds exist in one of these valency states; 0, +3, +5, and -3. Trivalent iAs includes arsenic (III) oxide, arsenic (III) chloride, and arsenenous acid. Pentavalent iAs includes arsenic (V) oxide, arsenic acid, arsenates whereas arsenosugars, arsenilic acid, dimethylarsinic acid or cacodylic acid (DMA), arsenobetaine were considered as organic arsenic (oAs). Inorganic arsenic (iAs) is more toxic than organic arsenic (oAs), and trivalent form of oAs is more toxic than the pentavalent one (ATSDR, 2007).

Sakurai et al. (2004) gave purified arsenobetaine to mice and found some changes in organs related to immunity such as thymus gland and gallbladder, but it exerts no toxicity (Sakurai, Kojima, Ochiai, Ohta, & Fujiwara, 2004). This evidence implies that chronically exposed to arsenobetaine via seafood consumption is not toxic to the human body.

Arsenic compounds, once exist in environment, it cannot be destroyed but it can be transformed. For physical mobilization, it can be attached to small particles and float along with the wind, and when it rains, arsenic-attached particles can be flushed onto the ground. Some microbial in soil can use arsenic and change it to arsine form, a garlic-like smell toxic gas. Arsenic attached to particles can be flushed or leached into surface water, sediment transport, aquifer sediments, and finally, sea water causing high accumulation of organic arsenic in seafood and seaweed as well as fresh water animals. Arsenic-bearing rock/minerals leached arsenic into water (surface and groundwater) by either reducing or oxidizing condition. With the slow groundwater flow and continuous leaching, the accumulations of arsenic in

groundwater found were usually high compared to the surface water that having a faster flow. Reports from many countries around the world showed over WHO standard level (10 ppb) of arsenic contamination in groundwater. Natural cause of contamination in groundwater was identified in Bangladesh, Argentina, Vietnam, Cambodia, Chile, China, Ghana, Hungary, Mongolia, Nepal, New Zeeland, Taiwan and England, whereas Australia, Canada, Japan, Mexico, Thailand, USA and England have arsenic problems related to mining operations.

There are arsenopyrite related mineral lines in many provinces in Thailand that could leach arsenic to environment if geographical conditions are suitable or improper mining operation occurs in that area. Those provinces include Saraburee, Supan Buri, Rayong, Srakaeo, Kanchanaburi, Ratchaburi, Prachuap Khiri Khan, Loei, Nong Bua Lam Phu, Nong Khai, Tak, Phetchabun, Nan, Uttaradit, Chiang Mai, Chiang Rai, Mae Hong Son, Lampang, Lamphun, Nakhon Si Thammarat, Trang, Yala, Songkhla, Phatthalung, and Satun (Sripuang, N, Meeting report).

1.1.4 Arsenic exposure and Diabetes: a review of epidemiological and experimental studies

Navas-Acien et al. (2006) did a systematic review of the experimental and epidemiological reports on arsenic exposure and diabetes type 2 (Navas-Acien et al., 2006). Among 19 epidemiologic studies, the researchers categorized it into three groups according to exposure levels. Firstly, they identified 9 studies focusing on population living in high arsenic contaminated areas (Taiwan, Bangladesh) that reported the relative risk estimate of 2.52 (95%CI, 1.69-3.75), though methodological problems hinder the interpretation of the casual association. Secondly, as a moderate exposure, they grouped 9 reports from occupational populations, and thirdly, they identified 4 studies in other populations (as a low exposure). It was concluded that (1) there were inconsistent evidence from occupational studies and from general population studies; and (2) the available evidence until the year 2006 was not sufficient to establish causal relationship between DM type2 and arsenic exposure. These conclusions were supported by Chien et al. (2007), after conducting similar reviews.

Chen et al. (2007) reviewed previously published epidemiological studies designed to investigate association between chronic arsenic exposure and diabetes from different research groups around the world and found that 6 of 7 reports were cross-sectional designed, including the one from Bangladesh (C.-J. Chen et al., 2007). There is only one study by Tseng et al. (2000) that had a cohort design (Tseng et al., 2000). This research group biannually followed 446 no diabetic participating residents living 5 days per week in three villages in Taiwan which have arsenic contamination level in drinking water range between 0.70 – 0.93 mg/L. Fasting plasma glucose and oral glucose tolerance test were used to identify diabetic etiology of every participating individual. They used criteria set by the World Health Organization (WHO) to define DM, and selected two townships where there were none arsenic problems with the availability of the DM type 2 data as control/reference data. The reference odd ratio was one when accumulative arsenic exposure was < 17 mg/L_year and the DM incidence was 1.9 %. They reported the relative risks of 1.6, 2.3 and 2.1 for adjustment of age (55 years), body mass index (25 kg/m^2) and cumulative arsenic exposure (17 mg/L_year) respectively, after the follow-up period of 1499.5 person-years and found that age and body mass index were significantly associated with

diabetes incidence. These authors suggested that to study the association between DM type 2 and low to moderate arsenic exposure, a well-designed prospective cohort with accurate diagnosis of DM, a precise estimation of individual arsenic exposure with intensive use of appropriate biomarker, large sample size plus longer studied period, a good control of identified confounding variables and an intensive analysis of interaction between important variables and exposure are needed.

After reviewing all available related information, Tseng (2004) proposed that there are 4 potential mechanisms involved in the DM induction by arsenic exposure (Tseng, 2004). Those four are phosphorus substitution, high affinity of sulfhydryl groups, increased oxidative stress and interference with gene expression whereby arsenic interferes expression of genes related to the maintenance of glucose homeostasis in peripheral tissue (adipocyte, muscle), pancreatic ß-cells and liver. Arsenic is characterized as both carcinogenic and non-carcinogenic agent. The available information from many research groups implied that there are some common mechanisms and pathways such as oxidative stress and MAPK or ERK (Mitogen-Activated Protein Kinase, Extracellular signal Regulated Kinase) that involved in both carcinogenic and non-carcinogenic mode of actions after arsenic exposure. Oxidative stress is a result of imbalance between antioxidants and oxidants. It can stimulate production of reactive oxygen species (ROS), which play a role in insulin resistance (the condition implicated DM type2) as well as it can act as signal molecules that promote cell cycle progression by affecting growth factor receptors (eg.AP-1, NF-kB) and induce oxidative DNA damage. Shi et al. (2004) as cited in Kligerman and Tennant (2007) suggested that methylated trivalent arsenicals induced oxidative DNA damage (most likely by generating superoxide and hydrogen peroxide which generate hydroxyl radical, an important inducer of DNA damage and possibly skin cancer if the damage was beyond being repaired, leading to the formation of stable chromosome-type aberration which play an important role in induction of cancer (Kligerman & Tennant, 2007). To date, little has been known about mechanism of arsenic-induced DM type2 in human.

Meliker et al. (2007) did an ecologic study to investigate relationship between moderate arsenic exposure and selected disease outcomes including diabetes among resident of six county of southeastern Michigan(Meliker, Wahl, Cameron, & Nriagu, 2007). A standardized mortality ratio (SMR) analysis was conducted with direct adjustment for age and race. With a population-weighted mean arsenic concentration of 11 ppb, elevated mortality rates were observed for both male and female for DM (Male SMR, 1.28; CI_99%, 1.18-1.37; Female SMR, 1.27; CI_99%, 1.19-1.35).

There were not sufficient information from previous laboratory studies both in vivo and in vitro to draw a conclusive mechanism networks for DM type 2 caused by arsenic exposure, especially at the low dose one (Navas-Acien et al., 2006).

1.1.5 Arsenic exposure population in Ron Phibun District

The residence of Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat province, Thailand had exposed to arsenic contaminated in their living environment for three generations. Since the problems were first observed in 1987, there were some efforts to reduce arsenic exposure such as continuous campaign aiming to replace usage of well water with tap and rain water for households' consumption and the closure of contaminated wells. At the present, the situations have been contained at some degree. However, there are still low to moderate contaminated

levels of arsenic in consumption waters of the residents living in risk area. An epidemiological survey in the year 2000 by the epidemiological division, Department of Disease Control, found that the prevalence of skin lesions caused by arsenic exposure was 0.63% (156 persons out of 24477 population of the whole sub-district). Sripaoraya et al. (2001) did a similar survey focusing on 3 villages that have history of high arsenic contamination in environment, and identified 49 people had skin lesions, skin cancer or bladder cancer(Sripaoraya, Jankong, Puttaprug, & Pavittranon, 2001).

At the time when the arsenic problems occurred in 1987, information or evidence suggesting that there might be association between arsenic exposure and diabetes was not available. Among the residents of the Ron Phibun sub-district, it is impossible to get information on who had or had not DM at that time. During October 2000 to March 2001, Pavittranon et al. (2003) did a cross-sectional survey on arsenic exposure by measuring arsenic level in consumption water and inorganic arsenic (iAs) in morning urine of individuals in each households, and measured glucosuria level of 783 people living in village number 13 and 2 of Ron Phibun sub-district where it was reported by other research groups that it have high level of arsenic in water and in soil(Pavittranon et al., 2003). Apart from using strip test for glucosuria measurement, the outpatient cards (OPD) at the Ron Phibun hospital were reviewed to identify the DM patients. Constructed questionnaires were used to get all needed information such as personal information (e.g. house number, age, sex, length of staying in the area), occupation, food (seafood and most frequent intake) and water consumption, alcohol, smoking, pattern of water use, herbal drug consumption, self-identified health data (including DM), history of treatment for arsenic poisoning by health authorities in the past. It was found that 2.94% of participated population or 0.58% of total population of those two villages had sugar level in urine ≥100 mg/dl. Until the year 2004, according to Ron Phibun hospital's record; there were 177 DM patients out of 24477 populations of the Ron Phibun sub-district (or 0.723%, or 723 people per 100,000 people). When compared to the Nakhon Si Thammarat DM-provincial rate of 146.59 people per 100,000 people, as reported in the year 1999, the DM rate of Ron Phibun sub-district was 4.9 times higher. Moreover, the 1996-1997 second national health survey report indicated that the DM rate for Thais was 0.147%, whereas the rate of Ron Phibun sub-district was 0.723%.

1.1.6 Knowledge gap

Though there were many epidemiological studies' results indicated association between high dose of chronic arsenic exposure and DM type 2, the association at low to moderate exposure dose is still questionable. Some limitation from previous studies such as study design, exposure assessment in epidemiological study, and application of suitable biomarkers both marker of exposure and effect lead to the need for more field studies that cover the limitation of the previous one. Navas-Acien A. et al. (2006), after reviewing a number of epidemiological studies, found that the majority of the researchers used cause of death in the death certificate, history of drug use, measurement of fasting blood sugar for DM type2 identification and used arsenic level in water and in working environment, which are the estimated values, to assess the exposure(Navas-Acien et al., 2006). Arsenic contamination levels in Ron Phibun sub-district was vary from low to moderate (<10 ppb up to 3 mg/L) compared to the level reported from Taiwan's studies. Study to investigate the association between

low to moderate arsenic exposure and DM type 2 in Thailand has never been done and published before.

Though it is recognized that cohort design is the most desirable epidemiological study, it could not fit in this study due to following reasons: (1) the data on DM type2 occurrence among residents of Ron Phibun sub-district in 1987 was not available, (2) there were a few number of DM type2 patients available to be followed up, and (3) the available budget is not sufficient for follow up. Thus, this study was designed as case-control with a retrospective enquiry in populations with previously low to moderate level of arsenic exposure.

1.2 Research Question

- Was there an association of low and moderate arsenic exposure with type 2 diabetes risk in Moo 2, 12, and 13 of Ron Phibun sub-district?

1.3 Objectives

To investigate the association between diabetes mellitus type 2 and different levels of arsenic exposure in Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat province, Thailand.

1.4 Research Hypothesis

Null hypothesis: **DM type 2 is not associated with arsenic exposure.**

1.5 Operational definition

DM; diabetic mellitus is a group of metabolic diseases characterized by hyperglycemia (abnormally high levels of sugar in the blood) resulting from impairment of glucose and insulin metabolism or more specifically, resulting from defects in insulin secretion by pancreatic β-cells, impairment of insulin action on peripheral tissue (muscle, adipocytes) and/or increase endogenous glucose production by liver.

DM type1 means individual diagnosed of DM before age of 30 and prescribed insulin treatment after 6 months of diagnosis.

DM type2 means the majority of DM with insulin resistance as main figure (high sugar level in blood).

Diabetes: the WHO definition;

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

DM type 2
definition

(http://www.nlm.nih.gov/medlineplus/ency/article/000313.htm#Definition)

DM Type 2 is diabetes mellitus Type 2. It is a chronic (lifelong) disease marked by high levels of sugar in the blood. It begins when the body does not

respond correctly to insulin which is a hormone released by the pancreas. It is the most common form of diabetes, usually occurs with obesity and insulin resistance.

Insulin resistance

It means that fat, liver and muscle cells do not respond normally to insulin. As a result they do not store sugar for energy. Since the tissues do not respond well to insulin, the pancreas produces more and more insulin. Because sugar is not getting into the tissues, abnormally high levels of sugar build up in the blood. (called hyperglycemia). Many people with insulin resistance have hyperglycemia and high blood insulin levels at the same time. Overweight People have a higher risk of insulin resistance, because fat interferes with the body's ability to use insulin.

Diabetes type 2 diagnosis

DM patient will be identified when (1) fasting plasma glucose value ≥126 mg/dl, or (2) has a record of previous diagnosis of diabetes by doctors (known diabetes), or (3) being newly diagnose diabetes through DM screening program and further confirmation step at hospital. Impair fasting glucose means fasting plasma glucose is equal to 110-125 mg/dl in the absence of previous diagnosis of diabetes.

Term and definition for arsenic exposure

- Exposure means contact.
- Exposure assessment is an estimate exposure.
- Arsenic exposure means inorganic arsenic exposure. Exposure agent is inorganic arsenic (As^{3+}, As^{5+})
- Water consumption is water used for drinking, cooking, bathing where inorganic arsenic is the majority.
- Exposed population referred to people living more than 1 year in high contaminated area as identified by high arsenic level in water (chronic, intermittent). They are villagers in Moo 2, 12, 13 of Ron Phibun sub-district.
- For Each individual who actively stays in specified area > 1 year :
    - expose group is those with intake dose >3.0 µg As/Kg body weight per day
    - non-expose group is those with intake dose ≤3.0 µg As/Kg body weight per day
- Exposure route is ingestion. It is the main route of exposure in this study. Arsenic exposure via soil and air is negligible.
- In this Ron Phibun sub-district area, we called arsenic levels contaminated in its environment (including in water) “low to moderate” because, while they are not negligible, they are lower than the high levels that have been observed in other locations such as India, Bangladesh, and Taiwan(Navas-Acien et al., 2006). There is no cutoff point between low and moderate. Rather, this is a general descriptive term.

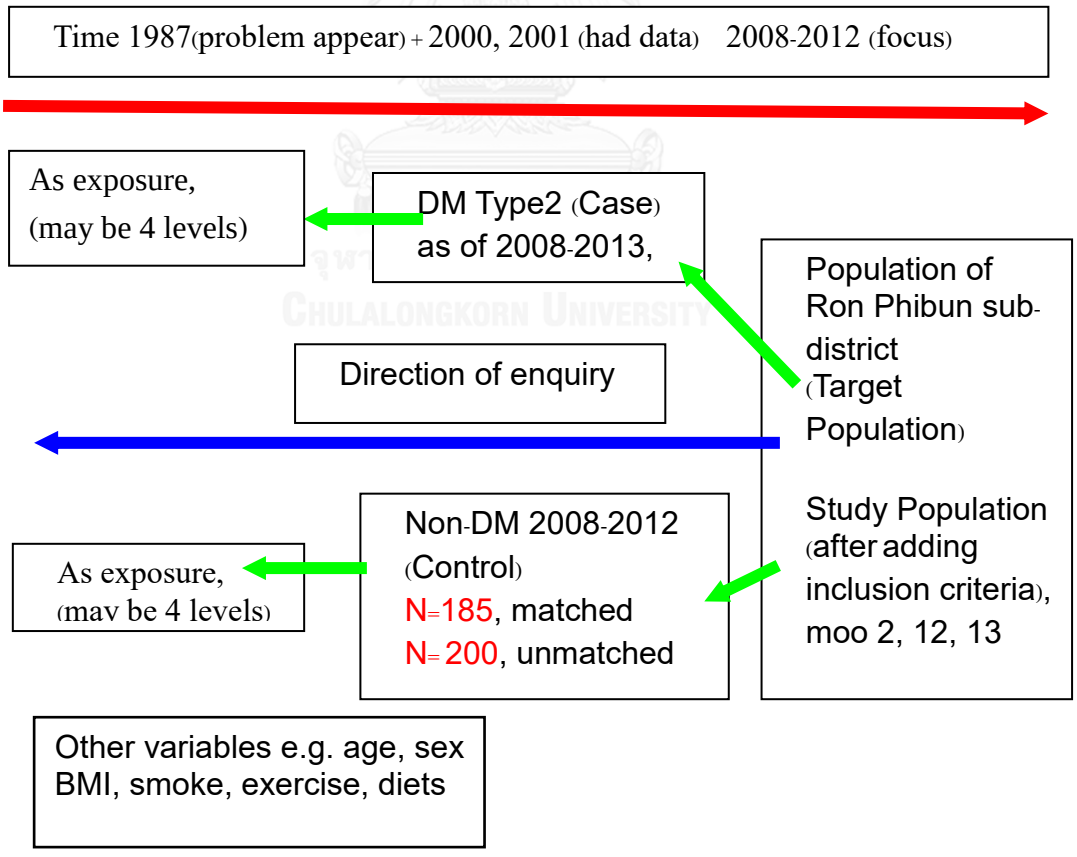
1.6 Scope and work plan

This study was a case control design including 2 sets of controls, one unmatched and one matched, where selection of case and control is population-based and based on status of DM type2. A multivariable analysis model was used with unconditional logistic for unmatched control and conditional logistic for matched control.

1.7 Expected Benefits and Public Health Significance

- Out put
  - Know association between DM type2 occurrence and As exposure in Ron Phibun district
  - Ron Phibun hospital has information to follow DM type2 risk group
- Benefits
  - Lead to initiative of early DM type2 identification scheme and extra DM type2 monitoring in risk area (e.g. having arsenic contaminated in environment such as in Supanburee province)
  - Add up to new knowledge in the area of chronic arsenic toxicity (As, as a new risk factor of DM type 2)
  - Villagers and local authorities have supporting information to raise awareness of arsenic contamination problems

1.8 Conceptual framework



CHAPTER II

LITERATURE REVIEW

2.1 Arsenic: Introduction

Arsenic is a toxic metalloid element occurring naturally and manmade. It can be found in soil, water and air and low level of 1 – 5 mg/L was found in rock in the form of amorphous or various forms of arsenopyrites. One third of arsenic compounds (both organic and inorganic forms) in the air come from volcano eruption, geothermal waters and wild fire, whereas mining is the major source of arsenic in soil and water. Arsenic compounds, especially Orpiment (As_2O_3) and Realgar (AsS) were used by men as coloring agents and alloys for arsenic bronzes, ormental/painting, cosmetics and copper arsenic alloys since old aged. During the 19^{th} to 20^{th} century, arsenic were used in pharmaceutical and medicinal areas, used as coloring agents in toys, wallpapers and wrapping papers, pesticides/insecticides, cotton defoliant, growth promoter in pigs/cattle/sheep dips, copper-chrome-arsenate wood preservative, wire alloys, electronics and ceramic/glassware (IPCS/WHO, 2001).

There were many reports of arsenic toxicity from Taiwan, Germany, Western U.S.A., Mexico, Chile, Argentina, India, Bangladesh, Mongolia, Thailand, China, Japan and some others.

Arsenic (As) has atomic number of 33, atomic weight of 74.91. It was categorized into three groups; organic arsenic forms (oAs), inorganic arsenic forms (iAs), and arsine gas. Arsenic compounds exist in one of these valency states; 0, +3, +5, and -3. Trivalent iAs includes arsenic (III) oxide, arsenic (III) chloride, and arsenenous acid. Pentavalent iAs includes arsenic (V) oxide, arsenic acid, arsenates whereas arsenosugars, arsenilic acid, dimethylarsinic acid or cacodylic acid (DMA), arsenobetaine were considered as organic arsenic (oAs). Inorganic arsenic (iAs) is more toxic than organic arsenic (oAs), and trivalent form of oAs is more toxic than the pentavalent one (ATSDR, 2007).

Organic arsenics found in nature are normally in the form of arsenobetaine, arsenocholine, where dimethylarsinate and arsenosugar forms are usually found in seafood (Word Health Organization [WHO] & International Agency For Cancer Registry [IARC], 2004). It was reported that there was organic arsenic in bird's nest used as healthy soup among Asians (Luong & Nguyen, 1999). However, this form is not considered toxic to the body and it is rapidly eliminated via urine without changing the form within 4 days. Moreover, Sakurai et al. (2004) gave purified arsenobetaine to mice and found some changes in organs related to immunity such as thymus gland and gallbladder, but it exerts no toxicity(Sakurai et al., 2004). This evidence implies that chronically exposed to arsenobetaine via seafood consumption is not toxic to the human body.

Arsenic compounds, once exist in environment, it cannot be destroyed but it can be transformed. For physical mobilization, it can be attached to small particles and float along with the wind, and when it rains, arsenic-attached particles can be flushed onto the ground. Some microbial in soil can use arsenic and change it to

arsine form, a garlic-like smell toxic gas. Arsenic attached to particles can be flushed or leached into surface water, sediment transport, aquifer sediments, and finally, sea water causing high accumulation of organic arsenic in seafood and seaweed as well as fresh water animals. Arsenic-bearing rock/minerals leached arsenic into water (surface and groundwater) by either reducing or oxidizing condition. With the slow groundwater flow and continuous leaching, the accumulations of arsenic in groundwater found were usually high compared to the surface water that having a faster flow.

Reports from many countries around the world showed over WHO standard level (10 ppb) of arsenic contamination in groundwater. Natural cause of contamination in groundwater was identified in Bangladesh, Argentina, Vietnam, Cambodia, Chile, China, Ghana, Hungary, Mongolia, Nepal, New Zealand, Taiwan and England, whereas Australia, Canada, Japan, Mexico, Thailand, USA and England have arsenic problems related to mining operations. There are arsenopyrite related mineral lines in many provinces in Thailand that could leach arsenic to environment if geographical conditions are suitable or improper mining operation occurs in that area. Those provinces include Saraburi, Supan Buri, Rayong, Srakaeo, Kanchanaburi, Ratchaburi, Prachuap Khiri Khan, Loei, Nong Bua Lam Phu, Nong Khai, Tak, Phetchabun, Nan, Uttaradit, Chiang Mai, Chiang Rai, Mae Hong Son, Lampang, Lamphun, Nakhon Si Thammarat, Trang, Yala, Songkhla, Phatthalung, and Satun (Sripuang, N, Internal Meeting report).

2.2 Arsenic Exposure

The main routes of Arsenic exposure in general population are inhalation, oral and dermal. People can expose to arsenic contaminated in environment by eating food, drinking water or breathing air. Small children can expose to arsenic by accidently eating soil.

2.2.1 Inhalation exposure

Small amount of Arsenic in the air people breathe can go to the lung and dissolve in blood stream. Normally, Arsenic exists in atmosphere as As_2O_3 particles or bound to particulate matter (Panel, 2009). However, some still accumulate in the lung. Many reports showed that people who work in smelters and chemical plants, where airborne arsenic such as arsenic trioxide dust is the predominate form, have high level of arsenic in their lungs (ATSDR, 2007). The Occupational Safety and Health Administration (OSHA) issued exposure limit in workplace of 10 $\mu g/m^3$ per 8-hours per day or 40 hours per week. The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) is 2 $\mu g/m^3$.

2.2.2 Oral exposure

The main dietary sources of arsenic are drinking water (iAs form), seafood (oAs; Arsenobetaine) such as fish and shellfish, rice/rice cereal, mushrooms and poultry. Some seaweed may contain arsenic in inorganic forms that may be more harmful than the organic one. Children could expose to arsenic by eating small amount of arsenic contaminated soil or dust while playing.

oAs forms are mainly found in seafood as Arsenobetaine (in sea animals) and Dimethylarsinylriboside derivatives or Arsenosugars (in seaweeds). So far, there is no report on arsenic toxicity from eating seafood, though high arsenic level was found. This is because the organic forms (such as Arsenosugars) are the majority compared to inorganic one and the low bioavailability of the oAs form. At the present,

WHO, US EPA, and Codex recommended level of iAs in drinking water is 0.01 mg/L or 10 ppb whereas the Thai-recommended level is 50 ppb. Though it was not mentioned separately how much iAs or how much oAs, it is well recognized that this recommended value means only for iAs because oAs had never been found and reported in drinking water(ATSDR, 2007).

After modeling the dose-response data from key epidemiological studies and selecting a benchmark response of 1% extra risk, the EFSA (European Food Safety Authority) Panel on Contaminants in the food chain (CONTAM proposed a range of benchmark dose lower confidence limit ($BMDL_{.01}$) of values between 0.3 to 8 μg/Kg b.w. per day based upon cancers of lung, skin, bladder and skin lesions (Panel, 2009).

Codex recommended Tolerable Daily Intake (TDI) values for arsenic in foods (not seafood) is as total arsenic. It ranges from 10 ppb to 2 mg/L, depending on type of foods. According to the Notification No. 273, 2003 of the Ministry of Public Health, Thailand, the maximum limit of iAs level in freshwater food and seafood as well as other food was 2 mg/L. Lately, according to the seventy-second meeting report of the JECFA (Joint FAO/WHO Expert Committee on Food Additives) released issued 16th March 2010, the inorganic arsenic lower limit on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL0.5) was 3.0 μg/Kg b.w. per day (2-7 μg/Kg b.w. per day based on the range of estimated total dietary exposure). A range of assumptions have been used to estimate total dietary exposure to inorganic from drinking-water and food(World Health Organization & International Agency For Research on Cancer [IARC], 2010).

In Thailand, the maximum limit of arsenic concentration (total arsenic) in surface water was 10 ppb (environmental standard) whereas the maximum value for drinking water both from groundwater and processed water (bottle water) was 50 ppb. Average concentration of arsenic in river water in the 100 km^2 area of Ron Phibun district, Nakhon Si Thammarat Province, Thailand, was found to be 218 ppb (4.8 – 583 ppb), with the exposed population of 15,000. (Williams, Fordyce, Paijitprapapon, & Charoenchaisri, 1996)

2.2.3 Dermal exposure

Dermal contact with contaminated soil or water is another route of arsenic exposure. Though very little information is available, several studies indicate that local irritation and dermatitis is the main effect of this contact(ATSDR, 2007). (Wester et al., 2004) reported that absorption rate of iAs in soluble form on the skin of monkeys (closest to that in human) was average 2.8±1.9% , range 0.6 - 4.4 %, which was much higher rate than that of iAs (average 0.04±0.07%, range 0.00 - 0.12%), from CCA-treated wood residue (arsenic residues collected from surface of wood preserved with chromate copper arsenate; CCA), as measured by urinary excretion of arsenic above background level from dietary exposure).

2.3 Arsenic Toxicokinetic

(ATSDR, 2007)

2.3.1 Arsenic Absorption

iAs in both forms (Arsenate and Arsenite) is well absorbed by both oral and inhalation routes whereby absorption by the dermal route is low compared to the others. For inhalation exposure, iAs is absorbed across the lung by deposition of the arsenic particles onto the lung surface and absorption of arsenic from the deposition material. After breathing arsenic contaminated dusts, the dust particles will be settled onto the

lining of the lungs before arsenic is taken up from the lungs into the body. Dermal absorption of arsenic begins with arsenic binding to skin, and that bound arsenic may be slowly taken up into the blood.

Oral exposure is the major route of arsenic exposure. Inorganic arsenic (iAs) could be absorbed better than the organic one. Once enter the body via oral route, arsenic will be absorbed at the gastrointestinal tract where high solubility form like As_2O_3 could be absorbed more than 90%, whereas AsS_3, GaAs and PbAs, the low solubility forms, will be absorbed only about 30% (ATSDR, 2007).

2.3.2 Arsenic Distribution

Once in blood stream, arsenic bounds to protein on red blood cells and distribute throughout the body to different organs within 24 hours.

2.3.3 Arsenic Metabolisms (ATSDR, 2007) and (IPCS/WHO, 2001)

Once absorbed on red blood cells, arsenic will circulate in blood stream and goes to the liver where there are some preliminary changes by methylation process, before the majority of it leave the body in the urine. Normally, the absorption and secretion of Arsenic occurs very quick in the body. About 50 – 80% of absorbed inorganic arsenic will be changed to the metabolite forms such as Monomethylarsonic Acid (MMA) and Dimethylarsinic Acid (DMA). These forms can later be changed back to trivalent and pentavalent iAs, the higher toxic forms that can accumulate in nail and hair, as well as secrete through urine within about 2 days.

Once absorbed, arsenites (As^{3+}) are finally oxidized to arsenates (As^{5+}) and methylated forms (MMA, DMA). The process may then be repeated to result in DMA. These processes take place in the liver by enzymic methylation. The rate and proportion of methylation varies among arsenic species. Organic arsenic (oAs) compounds are less absorbed at the gastrointestinal tract than the inorganic form. oAs, a less toxic form will be absorbed and eliminated from the body quicker than the iAs.

When arsenic is absorbed in digestive lining, pentavalent arsenic will be reduced (reduction reaction) by reductase enzymes to trivalent arsenic, which is further methylated to MMA, DMA and TMA (Trimethylarsenate) by methyltransferase enzyme. In methylation reaction, it has S-adenosyl-methionine (SAM) as methyl donor group and glutathione (GSH) as co-factor. Moreover, trivalent forms of arsenic (As_2O_3 and Arsenite) could accumulate in body tissues more than pentavalent forms, even though it can be methylated more. oAs such as MMA, DMA, arsenobetaine go to enzymic changes only a little, and thus 80 – 90 % of it will be eliminated in urine. For Arsenosugar, another form of oAs, it will be changed to DMA and pentavalent form (WHO).

It is previously believed that methylation process that changes iAs to oAs is the detoxification process. However, recent finding indicated that MMA^{3+}, the metabolism product that line between iAs (As^3) and DMA exert more toxicity than iAs. This MMA^{3+} is not stable in solution such as urine; as a result, it will be rapidly changed further to DMA^v (Hopenhayn-Rich et al., 2000)

Arsenic metabolisms of the ethnic Andeans living in North Argentina who exposed to approximately 200 ppb of arsenic from drinking water were studied (Vahter & Concha, 2001). The research team reported that this people had little percentage of MMA in urine; the only group compared to other population from other studies, and proposed that arsenic metabolism is vary depending on species, ethnic groups and individual differences. It was also reported that average percentage

proportion of metabolites of arsenic in human urine as iAs : MMA : DMA are 20 : 15 : 65, though individual variation was noted(Vahter, Concha, & Nermell, 2000). They suggested that this variation might result from the function of genes that control arsenic methyltransferase, an enzyme involved in methylation process. Moreover, DMA found in children's urine was lower than that of adults, indicating that arsenic could accumulate and show its toxicity more in children. High accumulation of arsenic in the body, if it occurs, high amount of DMA in children urine is normally found. An increase of arsenic intake in adults could result in decreased level of DMA, and in increased level of iAs and MMA. In pregnant women, arsenic could transport pass umbilical cords to fetus in the uterus where induction of methylation process taking place (ATSDR, 2007). Christian et al. (2006) found an elevated DMA (79-85%) level, iAs (8-16%), and MMA (5-6%) in the urine of pregnant women exposed to iAs in drinking water (Christian, Hopenhayn, Centeno, & Todorov, 2006).

2.3.4 Arsenic Elimination/Excretion

For oral exposure, most arsenic is immediately excreted in the urine as a mixture of As^{3+}, As^{5+}, MMA and DMA, where DMA is the dominant form (ATSDR, 2007). The relative proportions of these mixtures are vary depending on the arsenic forms administered, time after exposure, route of exposure, dose level, and exposed species. Small amounts are excreted in feces and even smaller amounts are in breast milk and sweat. Some remain bound to tissues, where the amounts depending inversely on the rate and extent of methylation. Half life ($t_{1/2}$) of arsenic in human blood and in urine is approximately 1 and 4 hours respectively. Within 2-3 days, the body can excrete arsenic more than 50% of the original total intake, then 38% in the next 48 hours or 58% in 5 days (IPCS/WHO, 2001). The range of percentages of metabolites of arsenic in urine found in many studies are 10 -30 % of iAs, 10-20% of MMA, and 55 – 75% of DMA (IPCS, 2001). The range of arsenic found in feces is 4 – 8 %. Approximately 75% of oAs will be eliminated from the body within 4 days whereas it takes 7 days for 62% of iAs to be excreted (IPCS, 2001). However, the rates vary by forms of arsenic and types of food. When the metabolite products are not eliminated completely from the body, accumulation of arsenic in many target organs such as skin, nail, bone and gastrointestinal lining occur. Chronicly expose to arsenic above reference level might lead to accumulation of arsenic in nail, hair and skin.

2.4 Arsenic Toxicity

Arsenic is toxic element that can enter the body by breathing, skin absorption and digestion. Arsenic compounds are considered both carcinogen and non-carcinogen. Most cases of arsenic toxicity in human have been associated with iAs exposure. Toxic effects of arsenic are complicated due to its existing in different oxidation states and many differences in inorganic and organic compounds. Trivalent arsenic (arsenite) shows higher toxic than the pentavalent arsenic (arsenate). After exposure, Arsenic-bounded red blood cells could be circulated to organs such as liver, kidneys, pancreases, lungs, heart, intestine and skin within 24 hours and slowly perfused into tissues. Arsenic could inhibit enzymic reaction of succinic dehydrogenase by binding to Sulfhydryl (-SH), thus, affects oxidation phosphorylation reaction which could cause disruption of production of ATP in the oxidation phosphorylation chain. When ATP production is disrupted, it will affect many body systems such as brain (headache, confuse, emotionally unstable,

forgetful), heart system, inflammatory of the liver, nervous system, destruction of red blood cells, inhibition of activities of bone marrows.

2.4.1 Effects of Arsenic to human organs (ATSDR, 2007)

2.4.1.1 Effect on gastrointestinal tract, livers and kidney

Acute exposure of arsenic compounds by oral route could cause damage to mucus membranes, damage in intestinal membranes, bloody diarrhea, jaundice (liver), glomerular damage, tubular necrosis and finally, kidney failure. For chronic exposure, it can cause fatty necrosis (jaundice, cirrhosis) in liver as well as improper works of the kidney.

2.4.1.2 Effect on cardiovascular system

Acute effects of arsenic in this system are cardiac arrhythmias, shock, vasodilation, failure of blood circulatory system, high blood pressure, where capillary vasodilation, gangrene of extremities, and heart diseases are considered the effects of chronic exposure.

2.4.1.3 Effect on nervous system

Acute effects found are sensory loss (peripheral), cerebral edema (central nervous system). For chronic effects of arsenic, hearing loss, peripheral neuropathy such as stocking and glove distribution and muscle weakness, and cerebral lesions, hemorrhagic necrosis are identified.

2.4.1.4 Effect on the skin

Skin is considered the most sensitive no cancer target after chronic arsenic exposure where typical dermal effects are hyperkeratosis of the skin (on the palms and soles), formation of multiple hyper keratinized corns or warts, and hyperpigmentation of the skin with interspersed spots of hypopigmentation.

2.4.1.5 Effect on respiratory system

Breathing of arsenic dusts such as arsenic trioxide dust could cause irritation of mucus membrane in nasal cavity, or damage in nasal cavity. Apart from that, arsenic could be accumulated in the lung as found in miners and farmers who used pesticides containing arsenic.

2.4.1.6 Effect on hematologic system

Chronic effect of arsenic to bloods is inhibition of production of blood cells by bone marrows, which lead to anemia and leucopenia.

2.4.1.7 Effect on reproductive system

Prenatal exposure of arsenic in a human population results in gene expression changes in newborns (Fry et al. 2007). Prenatal and early childhood exposures could lead to increased mortality rates and abortion.

2.4.1.8 Carcinogenic effects of arsenic

Arsenic was classified by IARC (International Agency for Research on Cancer) in 1987 as a group I carcinogen. Concrete evidence supporting this conclusion was from Taiwan's studies where increased deaths from liver cancer among people living in arsenic endemic areas were reported. Epidemiological studies indicate that chronically expose to arsenic in drinking water increased risk of skin, lung, liver, bladder, kidney and intestine, as well as chromosomal damage.

It was found that children having high arsenic levels in hairs showed less IQ than those with lower levels, though inconclusive evidences on association between arsenic exposure and learning abilities, growth development of the children were reported (Siripitayakunkit, Lue, & Choprapawan, 2001).

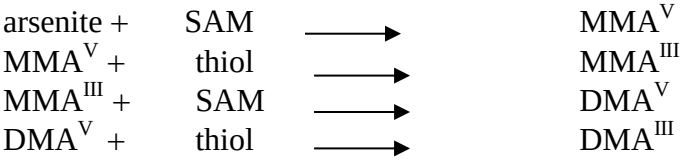
Colon was found to be the first target organ when applying MMA to rats and mices (Lora L Arnold, Eldan, Van Gemert, Capen, & Cohen, 2003). The research team also reported that at colon, MMA exerts more toxic effect in rat than in mice, and more toxic in male than in female, where the maximum tolerance dose was 400 mg/L, the exposure levels below which no adverse effects (NOAELs) have been observed in female and male rat and mice were 50 and 200 mg/L subsequently.

2.4.2 Carcinogenicity and genotoxicity of arsenic species

The different abilities of entering inside the cells dictate arsenite and arsenate toxicities where arsenite (As^3) has more toxic effect than arsenate (As^5). Many different cell lines studies found that, for the same exposed levels, arsenite could accumulate more inside the cells than arsenate [(Lerman, Clarkson, & Gerson, 1983);(Bertolero, Pozzi, Sabbioni, & Saffiotti, 1987); (Delnomdedieu, Styblo, & Thomas, 1995); (Styblo et al., 2000); (Vega et al., 2001)]. It was explained that at normal pH in the body, arsenite is in the uncharged form that could enter pass cell surface better than arsenate that is in negative charge. At the present, it is well recognized that arsenite and arsenate enter the cells by active transport process where arsenite is carried inside by aqua glycoprotein 7 and 9 which normally transporting water and glycerol across cells (Liu, 2002); and arsenate is carried inside by phosphate transporter (R. N. Huang & Lee, 1996). The mechanisms at which organic arsenic enter the cells is still unclear though it might relate to organic ion transporter.

Arsenic compounds in the body are metabolized by methylation process and excrete in urine where excretion rate of metabolized forms of arsenic is higher than that of inorganic arsenic forms (Erminio Marafante et al., 1987). Methylation process occurs mainly in liver and some occur in kidney and lung. Reduction reaction of arsenate to arsenite always occurs before methylation process. Enzyme used to reduce arsenate to arsenite in vitro is purine nucleoside phosphorylase, where di thiol, not glutathione (GSH), is used as reductant [(Gregus & Németi, 2002); (Radabaugh, Sampayo-Reyes, Zakharyan, & Aposhian, 2002)]. GSH could reduce arsenate to MMA and DMA without using any enzyme (Scott, Hatlelid, MacKenzie, & Carter, 1993).

Arsenite is methylated to monomethyl arsenic acid (MMA^V) by addition of methyl group from S-adenosylmethionine (SAM) , and cyt 19 gene was found to encode production of enzyme relating to this process, glutathione-S-transferase omega class 1-1 (GSTO1-1) (Lin et al., 2002). After that, MMA^V is further reduced to MMA^{III} by the same enzyme (Zakharyan et al., 2001). Reductant for Cyt 19 is thioredoxin and NADPH while GSTO1-1 uses GSH as reductant. Next, MMA^{III} is methylated to DMA^V that could be further reduced to DMA^{III} which prone to be changed by cyt 19 (Thomas, Styblo, & Lin, 2001). A simplified reaction involved in iAs biotransformation pathway is as follow.



The proportions of excreted arsenic in urine normally found in population are 10-30% iAs, 10-20% $MMA^{(V+III)}$ and 60-80% $DMA^{(V+III)}$, though exemption had been reported in the Andeans in North Argentina (Vahter & Concha, 2001). Human body excretes arsenic as MMA more than other animal species (Vahter, 2002). Pentavalent

metabolites (MMA^V, DMA^V) are less toxic than arsenite (As^3) and arsenate (As^V) (Erminio Marafante et al., 1987).

Oral LD_{50} of arsenate, arsenite, $MMA^V$and DMA^V are approximately 100, 41, 961, and 644 mg/KgBw subsequently (Brown, Kitchin, & George, 1997). Thus, it is believed that bio methylation of arsenic is the detoxifying process. However, recent findings indicated that trivalent methylated metabolites (MMA^{III}, DMA^{III}) are more toxic than arsenite both in vivo and in vitro [(Styblo et al., 2000); (Styblo, Serves, Cullen, & Thomas, 1997); (Petrick, Jagadish, Mash, & Aposhian, 2001)]. These methylated trivalent metabolites are very reactive and strong inhibitor of GSH reductase (Styblo et al., 1997) as well as thioredoxin reductase (Lin, Cullen, & Thomas, 1999), compared to arsenite or pentavalent metabolites. Thioredoxin reductase enzyme catalyzes NADPH-dependent reduction of disulfide bond of oxidized thioredoxin, an oxidoreductase having a lot of biological activities (Powis & Montfort, 2001).

2.4.2.1 Carcinogenicity of methylated arsenic species

DMA^V (cacodylic acid) is normally used as ingredient of herbicides where people can expose it while producing or using herbicides in the fields. Moreover, it can enter the body via intake of contaminated food and seaweeds. DMA^V is the main metabolite products of intake of iAs and arsenogars found naturally in seaweeds (Francesconi, Tanggaar, McKenzie, & Goessler, 2002). Once enter the body, the majority of it is rapidly excreted in an unchanged form in urine, and only 5% of it is changed to trimethylarsine oxide (TMAO) [(E. Marafante et al., 1987); (Buchet, Lauwerys, & Roels, 1981). Very little amount of TMAO was found in urine after high iAs intake (ATSDR, 2007).

DMA^V given to rat was considered an important promoter of malignant tumor of bladder (the highest response), kidney, liver and thyroid gland (Yamamoto et al., 1995). It was found to increase amount of premalignant renal and hepatic foci; and increase ODC activity as well. At the bladder, administration levels of DMA^V in drinking water related to its promoter activities whereby at 10 mg/L, it acts as promoter for papilloma; at 25 mg/L, it acts as promoter for carcinoma (Wanibuchi et al., 1996). Yamanaka et al. (1989) found urothelial hyperplasia in rats orally exposed to DMA^V 40 mg/L from food, and found necrosis even at its lower intake levels(Yamanaka, Hasegawa, Sawamura, & Okada, 1989). The authors proposed that cytotoxicity effects, followed by regenerative hyperplasia are the related mechanisms. Wanibuchi et al. (1996) examined promotional effects of N-butyl-N-(4-hydroxybutyl) nitrosamine-induced arsenic compounds to rats' bladders and found that arsenite 17.3 mg/L did not act as a promoter while DMA^V (184 mg/L) is the strongest one, and MMA^V (187 mg/L) ,TMAO (182 mg/L) are the promoter as well(Wanibuchi et al., 1996). DMA^V given to mouse caused single DNA strand breaks in their lung tissues, but liver and kidney [(Brown et al., 1997); (L. L. Arnold et al., 1999); (Yamanaka, Hasegawa, Sawamura, & Okada, 1991)]. DMA^V acts as complete carcinogen, as transitional cell carcinomas in rat's bladders was identified after chronically exposed them to $\geq$ 50 mg/L of DMA^V in drinking water for 2 years (Wei, Wanibuchi, Yamamoto, Li, & Fukushima, 1999). The lowest concentration of DMA^V that promotes bladder papilloma and complete bladder carcinogen are 10 and 50 mg/L accordingly [(Wanibuchi et al., 1996); (Wei et al., 1999)].

2.4.2.2 Genotoxicity of methylated metabolites of arsenic

Metabolic pathways that explain the toxic effects of DMA^V include (1) reduction reaction that changes DMA^V to DMA^{III}, the more genotoxic form; (2) production of TMAO; (3) reduction reaction that changes DMA^V to dimethyl arsine, that could lead to occurrence of peroxyradical, hydroxy radical and superoxide. Moore et al. (1997) reported that DMA^V at high dose (> 10,000 μg/ml) could induce mutation in mouse lymphoma L5 178Y/TK+/- cells(Moore, Harrington-Brock, & Doerr, 1997). Studies of clastogenic effects of arsenic compounds to human fibroblasts showed that order of the potency of clastogenic activities (based on concentration needed) is arsenite (0.8 μM) > arsenate > DMA^V (> 7mM) > MMA^V > TMAO, where DMA^V at the level of more than 7 mM is strong inducer of chromosome pulverization in nearly all metaphase (Oya-Ohta, Kaise, & Ochi, 1996). DMA^V (10mM) could induce DNA strand breaks and DNA-protein crosslinks in human alveolar type II cells. This might result from production of DMA peroxy radical (Yamanaka et al., 1990). DMA peroxy radical also causes DNA strand break in in-vitro study (Yamanaka et al., 2001). It was proposed that occurrence of DMA peroxy radical is important pathway for carcinogenicity of DMA^V.

Fenton reactions (reaction relating to iron) could stimulate ROS (reactive oxygen species) production that lead to genotoxic effects of methylated metabolites of arsenic compounds (Ahmad, K. T., & Cullen, 2002). Both DMA^V and DMA^{III} (10 mM) cause the release of iron (Fe) from ferritin, and iron chelator could inhibit the nicking of plasmid DNA by DMA^{III}. Excess free iron could induce heme oxygenase [(Stocker, Yamamoto, McDonagh, Glazer, & Ames, 1987); (Ryter & Choi, 2002)]. Arsenite is a good heme oxygenase inducer while DMA^V and MMA^V are not) (Brown et al., 1997). The activities of arsenic and its metabolites in inducing DNA strand break in lymphocyte cells (as analyzed by comet assay) is as following : DMA^{III} > MMA^{III} >> arsenite = arsenate > MMA^V > DMA^V (Peng, Sharma, Mass, & Kligerman, 2002).

Cohen et.al. (2013) reviewed related papers to evaluate the carcinogenicity of iAs and made some remarked conclusions on mode of action (MOA) of carcinogenicity of iAs(Cohen, Arnold, B. D., & Eldan, 2013). They proposed that MOA of arsenic toxicity involved formation of trivalent metabolites interacting with cellular sulphydryl groups, which lead to cytotoxicity and regenerative cell proliferation. They also concluded that the cytotoxicity induced by iAs results in non-cancer toxicities, whereby regenerative cell proliferation enhances development of epithelial cell. This proposed MOA suggested that there is a non-linear, threshold dose-responses relationship for both non-cancer and cancer end points.

2.5 Mechanisms of actions of DM type 2

Diabetes Mellitus Type 2 (DM Type2) is a metabolic disease caused by defects in insulin secretion by pancreatic β-cells, by impairment of insulin action on peripheral tissues (adipocytes and muscle), and/or by increased endogenous glucose production by liver (Diaz-Villasenor, Burns, Hiriart, Cebrian, & Ostrosky-Wegman, 2007). Generally, insulin resistance and insulin deficiency co-exist among DM type2 patients. Increased production of sugar by liver and decreased usage of sugar by muscles lead to insulin resistance, whereas decreased secretion of insulin by pancreatic β-cells causes insulin deficiency. Type2 DM represent 90-95% of the total DM. DM is considered a metabolic disease resulting from (1) defects in insulin secretion by pancreatic ß-cells, (2) impairment of insulin action on peripheral tissue namely adipocytes and muscle, and/or (3) an increase

in endogenous glucose production by liver. Known risk factors for DM are age, sex, family history, obesity, physical inactivity, diet, stress and genetic. However, a number of published studied reports mainly performed in animal and cell line models indicated that chronic arsenic exposure related to increased risk of DM occurrence, especially DM type 2.

For the patients with insulin resistance, an increased blood sugar level due to increased glucose production by liver and decreased usage of glucose in skeletal muscles and adipose tissue is an important figure. The mechanism explaining improper function of skeletal muscles in eliminating sugar from the blood is poorly understood. However, it was observed that the function of insulin to post receptor is abnormal, the capillary density of the muscles and white muscle fibers decreases. An increased free fatty acid levels inhibit sugar to enter the cells, and inhibit phosphorylation process in the muscles. In liver, free fatty acid will activate fatty acid oxidation, increase production of sugar from liver and decrease secretion of insulin from pancreatic β-cells. An increased gluconeogenesis and decreased glycolysis in the liver caused by improper function of insulin lead to high level of blood sugar while fasting. Identified main related factors for insulin resistance are age, sex, central adiposity, genetic, circulating insulin antagonist (hormone, free fatty acid, TNF-α), obesity, lack of physical activity, glucose toxicity and others such as pregnancy, ageing, and drug use (Longnecker & Daniels, 2001). For insulin deficiency, it is the result of abnormality of pancreatic β-cells that lead to decreased insulin secretion. The lower amount of insulin secreted is not sufficient for up taking increased blood sugar.

Diabetes Mellitus (DM) is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (WHO) .It is characterized by hyperglycemia, or raised blood sugar, a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

2.6 Related reports explaining mechanisms involved in arsenic toxicity and DM type2 occurrence

Diaz-Villasenor et al., (2007) did a reviewed studies focused on mechanism by which arsenic induces impairment of expression of genes related to type 2 diabetes and suggested that arsenic might increase DM type 2 risk via multiple mechanisms and pathways (Diaz-Villasenor et al., 2007). The researchers proposed that after arsenic exposure, expression of genes related to type 2 diabetes were altered and results in (1) alteration of gluconeogenesis in the liver, (2) the synergistic effect of insulin synthesis and secretion reduction as well as induction of oxidative stress in pancreatic ß-cells, (3) an abnormal proliferation and differentiation pattern of peripheral tissue and (4) insulin resistance in peripheral tissue. Arsenic might affect protein tyrosine kinases (PTK) function, tyrosine phosphorylation (the induction of hypo phosphorylation of insulin receptor tyrosine kinase: IRK), interfere translocation process of insulin, interfere transportation of insulin to cell surface (via cytoskeleton), affect phosphorylation of insulin receptor and translocation of glucose transporter (Diaz-Villasenor et al., 2007). Expressions of 16 genes, namely Insulin, PDX-1, catalase, PPARɣ, AP2, c/EBPα, NF-κB, $p21^{Cip1/waf1}$, $p27^{Kip1}$, Cyclin D1, AP-1(c-fos and c-jun), TNF- α, IL-6, AkT, mSOS-Ras-MAPK, PEPCK were reported to be affected by different concentration of arsenic with different exposure time and studied models.

Reports from laboratories studies in the past could not give conclusive evidences explaining complete mechanisms involved in arsenic causing DM type2 (Díaz-Villaseñor, Sánchez-Soto, Cebrián, Ostrosky-Wegman, & Hiriart, 2006). There were three main types of studies: (1) studies on signal transduction (using pancreatic β-cells) and gene expression (using pre-adipocytes tissues) that mainly treated the cells with high concentration of arsenite (37 – 75 mg/L), (2) studies on glucose uptake mechanisms using animal-origin cell lines and pre-adipocytes tissue treated with arsenite and its metabolite products concentration range from ppb to 750 mg/L, (3) other related studies.

2.6.1 Studies related to pancreas

It is known that pancreatic β-cells in pancreas produce insulin that maintains glucose homeostasis in the body. Villasenor et al. (2006) reported that inorganic arsenic interfered production and secretion of insulin by pancreatic β cell, thus, the body fail to maintain glucose homeostasis. They found that after treating pancreatic β cells with inorganic arsenic (iAs^{III}) 5 μM)374.5 ppb) for 72 hours, insulin production was decreased as measured by decreased expression of insulin mRNA. Moreover, the treated cells cannot differentiate the difference in glucose concentration. It was also proposed that arsenite (1 μM) modify transcription of genes involved in glucose sensing process, the necessary signal transduction to couple the stimulus with secretion, and/or the secretion machinery itself without directly interfering with insulin genes. Arsenic may increase insulin transcription acting through PDX-1 (pancreatic duodenal homeobox-1) activation (Macfarlane et al., 1997). PDX-1 is a transcription factor required for development of the pancreas and has been reported to influence expression of many β-cells genes including gene coding for insulin, glucokinase, islet amyloid polypeptide and glucose transporter GLUT2 (Johnson et al., 2003) The dose-dependent-decreased in expression and activity as well as increased production of reactive oxygen species (ROS) were found after in vitro treatment of keratinocyte cell line HaCaT with 10 -20 μM of Sodium arsenite for 24 hours (Sun et al., 2006). After arsenic exposure, decrease in pancreatic β-cells catalase expression and activity values was also observed (Sun et al., 2006). ROS production plays a key role in insulin resistance, a prevalent condition implicated in the development of DM type2. It is stimulated by oxidative stress resulting from imbalance between antioxidants and oxidants during arsenic metabolism [(Goering et al., 1999); (Sun et al., 2006)]. Antioxidants include superoxide dismutase; H_2O_2-inactivating enzyme, catalase and glutathione peroxidase. In vivo study's results by Izquierdo-Vega et al. confirmed that stress and oxidative damage really occur(Izquierdo-Vega, Soto, Sanchez-Peña, De Vizcaya-Ruiz, & Del Razo, 2006). In this study, rats were exposed to 1.7 mg/Kg every 12 hours for 90 days and the activity of pancreatic thioredoxin reductase (an enzyme involved in regulatory system to maintain intracellular redox status by scavenging ROS), the levels of total glutathione, lipoperoxidation in pancreas significantly increased.

2.6.2 Studies related to liver

There are few studies of effect of arsenic in liver. Hamilton et al. (1998) studied the induction of phosphoenolpyruvate carboxykinase (PEPCK) mRNA in liver by giving a single dose of 100 μM/kg of sodium arsenite to 14-days chick embryos and found significantly increase in basal expression of PEPCK overtime and found altered response of PEPCK gene to glucocorticoid induction after first 2-4

hours of treatment, before its response was back to normal(Hamilton et al., 1998). Arsenic can alter PEPCK expression by interacting directly with glucocorticoid receptor (GR) complexes, whereby it inhibits GR-mediated transcription without interfering with hormone induced nuclear translocation or activation of GR complexes (Kaltreider, Davis, Lariviere, & & Hamilton, 2001). It was found that the activity of hepatic glucose -6– phosphate dehydrogenase (G6PDH) significantly reduced in a time-related manner in mice treated with 3.2 mg/L for 6-15 months (Santra, Maiti, Chowdhury, & Mazumder, 2000). G6PDH is an enzyme in pentose phosphate pathway. A decrease in blood activity of G6PDH related to an increased risk of oxidative-stress-induced DM (Wan, Tsai, & Chiu, 2002), and to a decrease in generation of nitric oxide (Gaskin, Estwick, & Peddi, 2000).

2.6.3 Studies related to peripheral tissue (adipocytes and muscle cells)

It is known that insulin involves in regulation of glucose, lipid and protein metabolisms. Insulin-binded receptor will cause increased glucose uptake in muscle and fat and trigger a network of signaling pathways that could lead to translocation of glucose transporter (GLUT4) from intracellular sites to cell membrane (Saltiel & Kahn, 2001). Arsenic could alter expression and/or activity of different genes/proteins expressed in peripheral tissue where those genes involved in (1) adipocyte differentiation, (2) cell cycle, (3) pro-inflammatory response-transcription factor, (4) two signalling pathways (Ras-MAPKinase-AP-1 cascade, PI(3) K-Akt), and (5) protein-induced insulin resistance (Diaz-Villasenor et al., 2007). Differentiation of C3H 10T1/2 pre-adipocytes treated with sodium arsenite (6 μM) for 2 months to adipocytes was inhibited (Trouba, Wauson, & Vorce, 2000). The authors proposed that this was the results of disrupting the expression of genes involved in adipogenesis and down-regulation of fat-cell-specific genes. Moreover, expression of genes (PPARγ: peroxisome proliferative-activated receptor gamma; AP1, adipocyte selective fatty acid binding protein; C/EBPα, transcription factor CCAAT-enhance binding protein; $p21^{Cip1/waf1}$ and $p27^{Kip1}$, gene involved in cell cycle regulation) involved in adipogenesis (a cellular process) measured as mRNAs was found to decrease significantly.

Arsenic was found to involve in regulation of pro-inflammatory response-transcription factor (Kapahi et al., 2000). Nuclear factor- kappaB (NF-kB) is a transcription factor relating to chronic disease like DM where it play a central role in regulating transcription of cytokines involved in insulin resistance such as tumour necrosis factor-α (TNF-α) and interleukin-1 (IL1) (Kumar, Takada, Boriek, & Aggarwal, 2004). Arsenite (12.5 μM) inhibits activations of NF-kB, I-κB (Inhibitory-kappaB protein) degradation, IKK (I-κB kinase protein) activity (Kapahi et al., 2000).

Arsenic affects Ras-MAP kinase-AP-1 cascade by preventing activation of Ras (by preventing the guanine nucleotide exchanger factor SOS from converting RAs to active GTP-bound state) by insulin (Doza, Hall-Jackson, & Cohen, 1998). Arsenite also affect PI(3)K-AKt signalling pathway where there was a report by Sandoval et al. (2007) that arsenite treatment through this pathway is able to induce either cell differentiation or proliferation depending on cell type and p53 status(Sandoval et al., 2007). Paul et al. (2007) proposed that inhibition of the PDK-1/PKB/Akt-mediated transduction step is the key mechanism for the inhibition of ISGU in adipocytes exposed to iAs^{III} or MAs^{III} (Paul, Harmon, Devesa, Thomas, & & Styblo, 2007).

Wu et al. (2003) collected lymphocytes of 24 subjects exposing low to high levels of arsenic from drinking contaminated well water as indicated by arsenic level in blood and measured gene expression by microarray with 708 human cDNAs, and confirmed the most significantly altered genes by RT-PCR(Wu, Chiou, Ho, Chen, & Lee, 2003). Nearly threefold increase in IL-6 expression was found, compared between the low and the high exposed groups. In the study by Walton et al. (2004), measuring basal and insulin-stimulated glucose uptake in 3T3-L1 adipocytes cells exposed to arsenate and to methylated arsenic compound, reported that methylarsine oxide ($MAs^{III}O$) inhibited insulin-stimulated glucose uptake at the concentration of 0.4 and 0.04 mg/L after 4 and 24-hr exposure consequently(Walton et al., 2004). Paul et al. (2007), upon their study on molecular mechanism that cause inhibition of ATP production of PKB/Akt (phosphorylation of protein kinase B) using 3T3Li adipose tissue treated with $iAs^{(III)}$ (50 μM) and MAs^{III} (2 μM), proposed that inhibition of PDK-1/PKB/Alk-mediated transduction step was the main mechanism that inhibit insulin-stimulated glucose uptake (ISGU) process(Paul et al., 2007).

2.7 Reviews of Epidemiological studies of arsenic and DM type2

Published epidemiological studies of association of DM type2 and arsenic exposure from environmental and occupational sources around the world since are minimal. The only study that claimed a cohort design is the one of Tseng et al. (2000). The majority is cross-sectional studies, and a few is the case-control. Navas-Acein et al. (2006) identified 19 epidemiological studies (4 in general population representing low exposure, 9 in occupational population representing moderate exposure, and 6 in Taiwan and Bangladesh where the exposure is high) published from 1980 to 2004(Navas-Acien et al., 2006). They reported pooled odds ratio of studies' results in high exposure area (Taiwan, Bangladesh) of 2.5 (95% confidence interval, 1.69-3.75), though methodology problems were being identified. Inconsistent evidence of association between low (study in general population other than in Taiwan and Bangladesh) and moderate (study in occupational population) arsenic exposure and DM type2 was identified (Navas-Acien et al., 2006).

It was also observed that cross-sectional and case-control study designs are the most used in occupational and general population studies subsequently [(Navas-Acien et al., 2006); (C.-J. Chen et al., 2007)]. The diabetes diagnosis tools mostly used by those researchers are death certificate, self-reported DM type2, OGTT (Oral Glucose Tolerance test) or self-report, OGTT and currently treated diabetes, only OGTT, currently treated diabetes reimbursed by the National Health Insurance, glycosylated hemoglobin (HbA1c), self-reported + glucosuria test + OGTT, only glucosuria test (Navas-Acien et al., 2006). Almost all occupational studies used job title (7/9) in assessing arsenic exposure, whereas in general population studies, plasma arsenic levels, urinary arsenic levels, cumulative exposure index (CEI) of village or community drinking water, history of living in HAA (high arsenic exposure) area, living in HAA + keratosis, years of residency and subject drinking water were used [(Navas-Acien et al., 2006); (C.-J. Chen et al., 2007)]. Among those studies, at least age and sex were adjusted for odds ratio of diabetes.

Rahman and Axelson (1995) extended the analysis of a previous case-control study of DM and arsenic exposure from 1978 (Rahman & Axelson, 1995). Death certificates of employed Swedish copper smelter were used for re-analysis. Cases (12 people) were selected based on death certificate and clinical information on

the DM disease. Controls (31 people) were those free from cancer, cardiovascular, and cerebrovascular diseases. Objective information provided and categorized (3 groups; 1 = <0.5 mg/m^3, 2 = ~0.5 mg/m^3, 3 = >0.5 mg/m^3) by experience safety engineers were used in arsenic exposure assessment. The odds ratio found for DM with increasing arsenic exposure categories were 2.0 (95%CI, 0.1-27), 4.2 (95%CI, 0.3-54), 7.0 (0.7-79). Based on the Mantel-Haenszel procedure and its extension for trend testing, the total odds ratio found were 3.3 (95%CI, 0.5-30).

Coronado-Gonzalez et al. (2007) did a community-based case-control study to evaluate relationship between arsenic exposure and DM type2 in Mexico, and a dose-response relationship between arsenic concentration in urine (applied as marker of exposure) and the occurrence of DM type2 was observed(Coronado-González, Del Razo, García-Vargas, Sanmiguel-Salazar, & Escobedo-de la Peña, 2007). It was reported that subjects with intermediate (63.5-104 µg/g creatinine) and high (>104 µg/g creatinine) total arsenic in urine had two and three times higher risk of having DM type2 accordingly (odds ratio, 95%CI for intermediate exposure = 2.16, 1.23-3.79; odds ratio, 95%CI for high exposure = 2.84, 1.64-4.92). The cases and controls in this study were obtained from previous cross-sectional study. Cases (200 people) are those with DM type2 as diagnosed by having glucose fasting blood sugar levels ≥ 126 mg/100ml, or a history of diabetes treated with insulin, or oral hypoglycemic agents. Controls (200 people) were the persons taken from the immediate order of the identification of the cases in a cross-sectional study (the next subject studied). Individual spot-urine of all cases and controls were collected in the morning (after everyone was requested not to eat seafood diet for 5 days before collection). The collected urine samples were acid-digested and measured for total arsenic concentration by HG-AAS. Total urine arsenic levels (iAs) of individuals were used as direct marker for exposure assessment. Adjusted odds ratios for potential confounding such as sex, age, triglycerides, body mass index, hypertension, family history of DM were reported (using multivariate analysis model with un-conditional logistic regression).

Navas-Acein et al. (2008) did a cross-sectional study in representative of 788 US adults aged ≥ 20 years who participated in the 2003-2004 National Health and Nutrition Examination survey (NHANRS), and reported that total urine arsenic was associated with increased prevalence of DM type2 and with levels of glycated hemoglobin after adjustment for diabetes risk factors and markers of seafood intake(Navas-Acien, Silbergeld, Pastor-Barriuso, Clark, & Guallar, 2008). It was also found that low to moderate inorganic arsenic exposure, not organic form that associated with increased risk of DM type2. Participants with DM type2 had a 26% higher level of total arsenic (CI = 2% to 56%), and a non-significant higher (10%) level of dimethyl arsenate (CI = -8 to 33%) than participants without DM type2, and level of arsenobetaine were similar to those without DM type2.

Wang et al. (2009) observed that blood glucose levels of DM individuals (mean ±SD; 8.1± 2.2 mM) living in the arsenic-endemic areas in Xinjiang Autonomous Region, PR China were lower than those (mean ±SD; 9.7± 3.4 mM) from the nearby control site(Wang et al., 2009). Elevated levels of urinary NAG (N-acetyl-β-glucosaminidase), a lysosomal enzyme involved in the breakdown metabolism of glycoproteins, was used as indicative of kidney dysfunction in both human and rat studies (Wang et al., 2009). It was found that (1) urinary NAG levels

of the DM type2 and non DM individuals in the endemic areas were higher than the corresponding group from the control area, and the urinary NAG levels found were significantly higher in villagers with the DM than those without DM. Higher urinary arsenic (total arsenic, analysed with ICP-MS) concentrations were found in villagers from endemic areas than in those from the control site. These observations were confirmed in rat model and corresponding results were found. It was suggested that arsenic affect the kidney function significantly in individuals with diabetic conditions. It was also proposed that arsenic alters glucose metabolism significantly in individuals with diabetes, and chronic arsenic exposure has an inhibitory effect on glucose metabolism both in human and rat (Wang et al., 2009).

Pattern of arsenic metabolism in different ethnic group of general population is different (Brima et al., 2006). Normally, the half-life of iAs in the body is 2 days, thus measuring arsenic in urine can reflect an individual's recent exposure (Watanabe et al., 2001). It is also known that sequestering of arsenic in hair, fingernail and toenail occurs over 2 – 18 months, thus evaluation of arsenic concentration in these tissues reflect chronic exposure (Brima et al., 2006). The proportions of arsenic species in urine of Asians healthy volunteers residing in Leicester, UK are: Arsenobetaine (AB) = 83%; Dimethylarsinate (DMA) = 16%; As^{3} = 0%; Methylarsonate (MA) = 1%; As^{V} = 0%. The concentrations (µg/g creatinine) of arsenic species (mean±SD) in urine of Asians healthy volunteers are: AB =15.2±20.2, DMA = 2.9±2.9, As^{3} = 0.0±0.0, MA = 0.1±0.2, As^{V} = 0.0±0.0. Levels of arsenic concentration in different tissues of healthy Asians in the UK study are 20.6 µg/g creatinin for urine samples; 117 µg/kg for hair samples, and 154 µg/kg for fingernail samples (Brima et al., 2006). The levels of arsenic in toenails among 32 pregnant women in Ron Phibun sub-district ranged from 0.1 to 68.63 µg/g (Fry, 2007).

Pavittranon et al. (2003) reported that off 568 arsenic exposed individuals of Moo 2, 12, 13 of Ron Phibun sub-district, Nakhon Si Thammarat province, 47 (2.11%) were found having glucosuria >100 mg/dl(Pavittranon et al., 2003). Correlation between urinary arsenic level (total arsenic) and glucosuria level (using cut off point 50µg/g creatinine) among exposed group was not found in this study, and the higher prevalence of DM occurrence in those three villages which have high arsenic level in environment, compared to the nearby villages, were observed (unpublished data).

CHAPTER III

RESEARCH METHODOLOGY

3.1 Study Setting

It was an analytical epidemiologic study to prove the association between diabetes (DM Type 2 in particular) with arsenic exposure in the population of Ron Phibun sub-district's three villages where arsenic contamination has been found at its highest level. Two case-control studies, one unmatched and one matched was designed to compare the arsenic exposure patterns between villagers with DM Type 2 and those who had not been affected.

3.2 Study Population

3.2.1 Target group (case) was the population with diabetes, both male and female, age 35 years old up. The target group had been residents of Moo 2, 12 and 13 of Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat Province for more than one year.

3.2.2 Reference (Control) group was the diabetes free population, both male and female, age 35 years old up. The control groups had been residents of Moo 2, 12 and 13 of Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat Province for more than one year as well.

3.2.3 Sample size

According to national census in January 2008, the number of population age 35 years old up in Moo 2, 12 and 13 of Ron Phibun sub-district was 1956, 2554 and 1321 respectively. The STATCALC, an expansion of EpiInfo version 6 programs is used to calculate sample size. Assuming an exposure rate in the cases of 20.7% and the unmatched controls of 10%, the required sample size, for 95% confidence level of data ($\alpha = 0.05$), 80% power (1-β), a ratio of case and control of 1:2, expected odds ratio of exposure for cases compared to the odds ratio of exposure for control of 2.35 the estimated sample size of case and control (matched and unmatched) is 141 and 282 respectively.

In this study, we actually collected data from 185 cases, 185 matched controls and 200 unmatched controls. In the matched, cases and controls were matched on gender and age (within 2 years). It was a community based case-control, not hospital.

Calculation details of sample size are as follows:

Table 5Calculation details of sample size

Unmatched Case-Control Study (Comparison of ILL and NOT ILL) Sample Sizes for 10.00 % Exposure in NOT ILL Group							
	NOT ILL		Exposure In ILL	Odds Ratio	Sample Size		
Conf.	Power	: ILL			NOT ILL	ILL	Total
95.00 %	80.00 %	2:1	20.70 %	2.35	282	141	423
90.00 %	"	"			228	114	342
95.00 %	"	"			282	141	423
99.00 %	"	"			402	201	603
99.90 %	"	"			572	286	858
95.00 %	**80.00 %**	"			**282**	**141**	**423**
"	90.00 %	"			374	187	561
"	95.00 %	"			460	230	690
"	99.00 %	"			648	324	972
"	80.00 %	1:1			195	195	390
"	"	2:1			282	141	423
"	"	3:1			366	122	488
"	"	4:1			452	113	565
"	"	5:1			535	107	642
Reference: Fleiss, "Statistical Methods for Rates and Proportions", 2nd Ed., Wiley, 1981, pp. 38-45(Fleiss, 1981).							

Note: Conf., means confidence level; ILL, means illness.

3.3 Criteria for Volunteer Recruiting

3.3.1 Inclusion criteria of target group (diabetes patients)

Cases were those who were diagnosed with diabetes (new patients diagnosed according to specified criteria of the Ron Phibun hospital, WHO and old patients from OPD card), had lived in Moo 2, 12 and 13 of Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat province for more than one year, could be male or female, at least 35 years old, and had no occupations related to arsenic exposure as being assessed by questionnaire response on their present and past occupations.

3.3.2 Inclusion criteria of reference or control group

There were two control groups, one unmatched and the other matched. Selection of controls was as followings:

For unmatched control group;

They were those who did not have DM type2 (using the same diagnosed criteria as the target group, at time of occurrence of the case). They were male or female, at least 35 years old, and had no occupations related to arsenic exposure as being assessed by questionnaire response on their present and past occupations. They were selected randomly as a subsample of combined population of the 3 Moo Ban

(Moo 2, 12 and 13) of Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat province for more than one year. Thus, the controls were intended to be representative of the overall study

For matched control group;

They were selected after the cases were identified and characterized. Since the total number of cases was 185, the matched control was the same figure. They were pair-matched on age and sex. Apart from that, the matched controls were selected in the same manner as the unmatched one. Thus, it is possible that one person could be selected as unmatched and matched control. Among all of this study population, 27 persons were selected as both matched and unmatched control (14.6%of matched: N185, or 13.5% of unmatched: N200).

3.3.3 Steps for control selection (Both unmatched and matched);

1. Opened the excel file of screening results according to "active community-based screening of chronic diseases in the country in 2008 by Sor Por Sor Chor / National Health Security Office" which was received from Ron Pi Boon hospital after the permission from Nakhon Si Thammarat Provincial Health Office (Appendix B; data from risk assessment forms).
2. Selected all identification number (a unique number as identified on questionnaire) and its accompanying information of those who live in Moo 2, 12, 13 and combined all three Moo ban in one sheet.
3. Excluded those who aged less than 35 years old.
4. Wrote each identification number on small piece of blank paper that had been equally cut beforehand, and rolled it properly. Put it all together in a big box. Mixed it thoroughly before picking it up one by one and checked again (whether it is a case or not) for an unmatched control.
5. Put back all the slots of selected unmatched controls in the same box after finishing the unmatched control selection and confirmation process.
6. Mixed all the slots in the box thoroughly.
7. Picked up one slot; look at the unique identification number, then searched for gender and age information in the sheet mentioned in steps 3. At this step, we knew gender and age of that individual.
8. Opened the sheet containing confirmed cases (185 cases), searched for match of gender, then age (fit criteria). The slot that was selected was kept separately and not put back in the box.
9. Always confirmed immediately after matching, to make sure that the selected matched control is not the case.
10. Filled in the information of matched control in the same cell as case in the spread sheet constructed periodically.
11. Followed the same from steps 7to 10 until finishing all the 185 matches.

3.4 Data Collection: Procedures and Instruments

A retrospective enquiry case-control was used to study the association between diabetes and arsenic level in water used. The data used for exposure assessment of arsenic and diabetes identification among cases and controls is based on the studies in the year 2000 and 2008 performed by researcher, with permission to use the data from the Department of Medical Sciences. Data were taken from these two

studies (year 2000 and 2008) because these were simply years for which funding was obtained, without any particular scientific reason. These years were scientifically appropriate for the research conducted for this thesis. All the identified DM type 2 cases were based on the database of DM clinic at Ron Phibun hospital. The identification and confirmation of new cases during 2009-2014 was made via reviewing the Ron Phibun hospital's OPD cards.

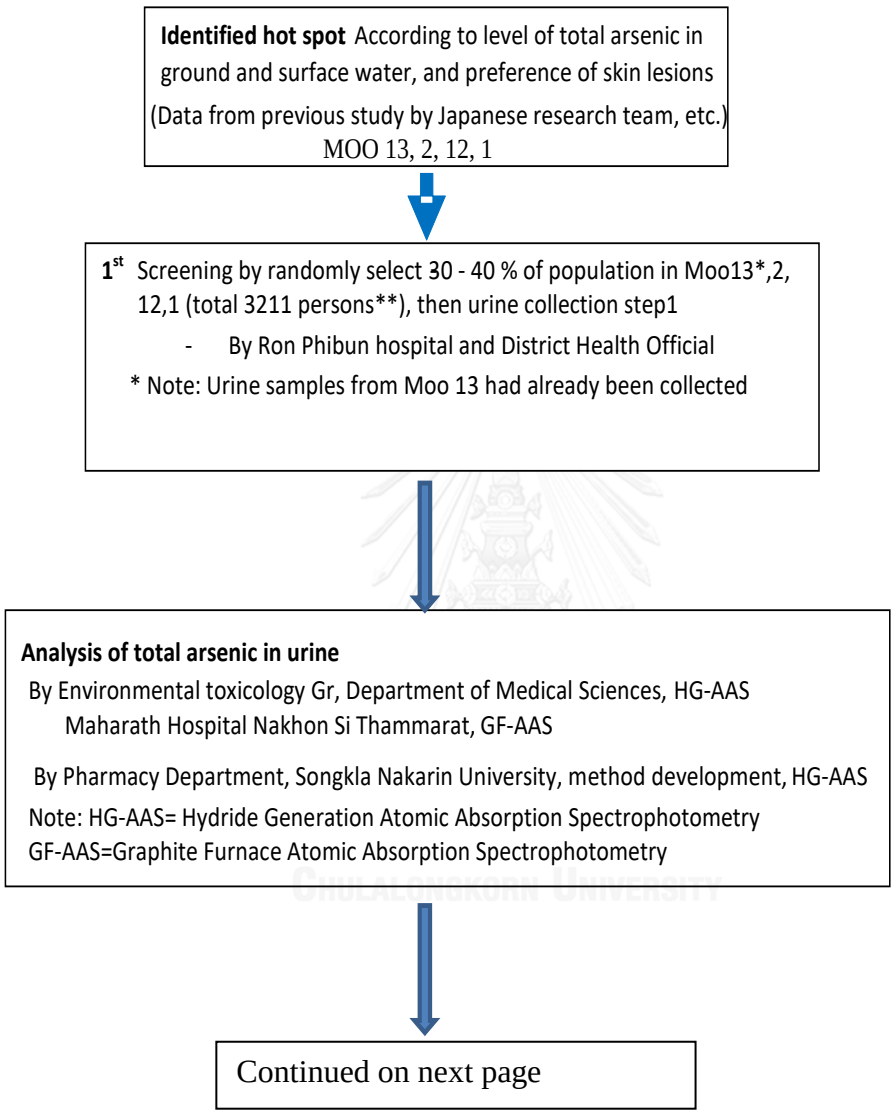
Individual-level data for cases and controls, including water arsenic levels, sources of water used, and other potentially relevant characteristics, had been collected in the community studies in 2000 and 2008.

3.4.1 Related details of the research methodology of the year 2000 study are as follows:

- This study was performed under the project named "Selection of arsenical exposed populations and individuals in Ron Pi Boon district, Thailand for the mechanistic study of arsenic cancer and a low dose risk assessment" by the Department of Medical Sciences whereby the researcher is working. The project was part of the European Union funded project called "the mechanistic basis for providing a realistic cancer risk assessment for exposure to inorganic arsenic within the European community".

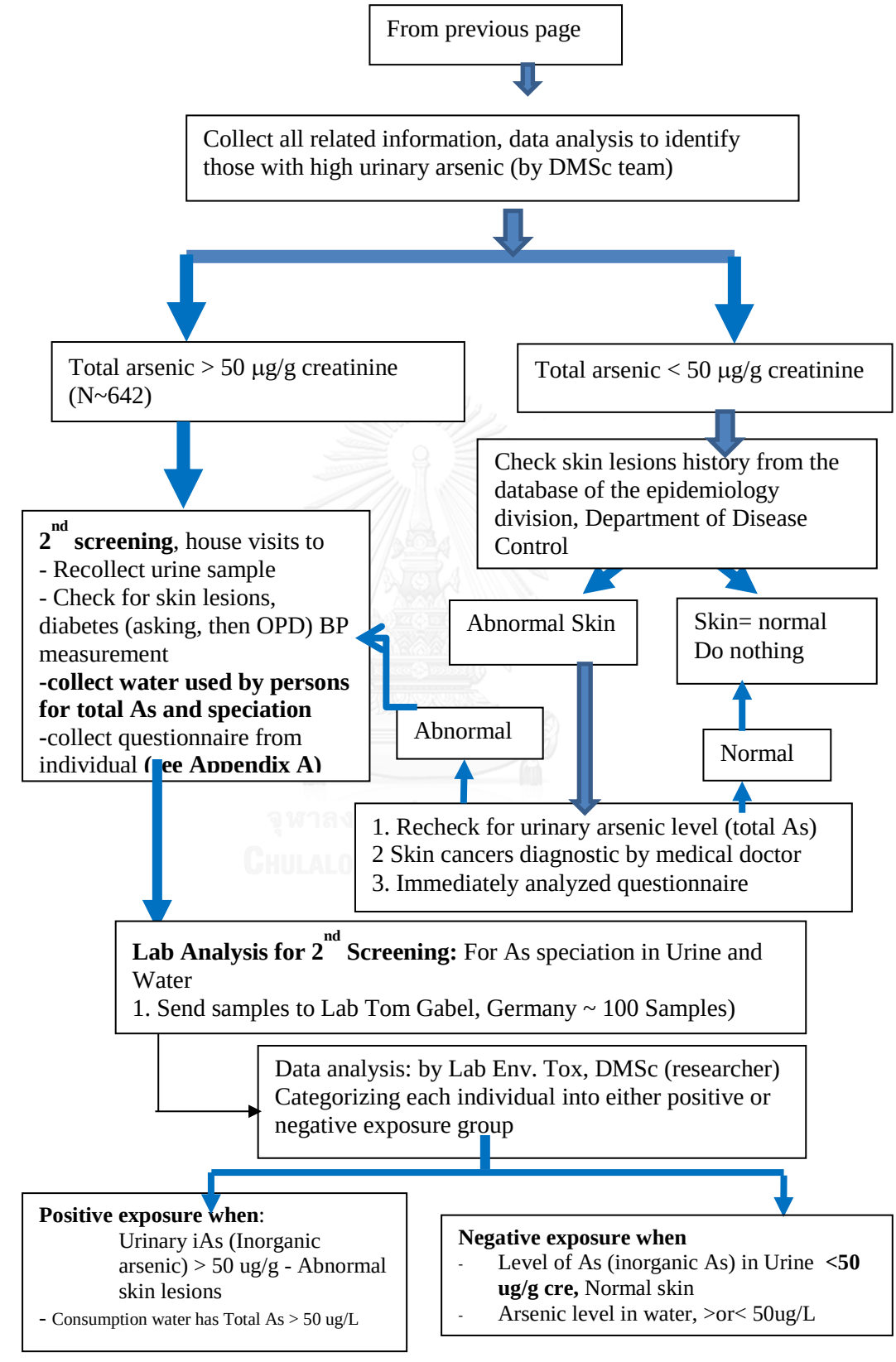
- Following is a flow chart showing methodology of the abovementioned project:

Figure 3Flow chart of research methodology of the year 2000 study



Continued on next page

Figure3 (continue)



3.4.2 Details of the year 2008 study are as follows:

- We did literature reviewed, both locally and internationally, and started contacting local authorities, developed questionnaires to assess arsenic exposure, personal data, including lifestyles. Local health authorities were to conduct the interview.
- The protocol was developed and approved by the ethical research committee of the Medical Sciences Department.
- Then, we had the first field trip aimed to meet community leaders, learning about their issues and announcing the project, soliciting cooperation for the project and assessing the qualified population from the database of the Ron Phibun Hospital.
- Second field trip aimed to train village health volunteers to interview population (using the designed questionnaires), to acquire water consumption data, and to collect water samples (using short questionnaire and short check list, see **Appendix C**.
- The 3rd-5th field trips aimed to collect the data on diabetes risk of the community by using risk assessment forms of specified chronic diseases including DM to select the potential target group and control group (see **Appendix B**). Data collection was done in cooperation with the hospital and the trained village health volunteers.
- Data analysing (from the risk assessment forms of specified chronic disease collected from the 3rd-5th field trips), to separate the diabetes prone group and have them taken further tests for confirmation of DM at the Ron Phibun hospital. This risk assessment form was developed and used for active community-based screening of chronic diseases like diabetes in the country in 2008 by the Sor Por Sor Chor (National Health Security Office).
- Searching for the diabetes patients by checking with the OPD card of the hospital. This work was supported by the hospital staffs that were in the research team.
- Researchers and village health volunteers collected water samples from participating households, if they allowed (using form as in Appendix C). For those who used village tap water, the water was sampling from the tap after opening it for some time, the storage tanks in the community and from every households' storage containers. For rain water consumption, it was not collected from every household.
- Water samples were sent for analysis of arsenic concentration with GF-AAS (with detection limit of 1 µg/Liter or 1 ppb) at reference laboratory of the Trang Medical Sciences Center, Department of Medical Sciences in Trang Province.
- Researcher analysed the data on the arsenic level in consumption water of households in the three villages. The data then was used to calculate and compare intake levels of arsenic form water consumption, as average individual intake concentration.
- The reports (results of arsenic concentration in consumption water and the DM risk individuals) were delivered to the households through co-researchers from the Ron Phibun hospital and village health volunteers. Attached to the report

was the short questionnaire asking whether the DM risk person had come to any hospital or clinic for the DM confirmation.

- Assigned village health volunteers compiled the questionnaires to deliver to the hospital. Researchers followed up those who were the risk group and reported back that they did not visit any health facility for confirmation, to make appointment to the hospital's DM clinic later.
- The 5^{th}-7^{th} field trips aimed to collect the blood samples of diabetes volunteers (both the existing patients and the newly diagnosed one) for further testing. The details were as follows:
    - Researchers and the Diabetes clinic of the hospital plan to schedule diabetes patients from the three villages for blood collecting without disrupting their regular hospital visits. The hospital's diabetes clinic operates only on Wednesday and Friday. The frequency of clinic visit by each patient also depends on their symptoms, thus it this study, the duration for blood collecting and blood analysing took 3-4 months.
    - However, the special initiative of organized blood collecting in the village was set up. Researchers visited each of those 3 villages on Saturday and Sunday to collect blood of the DM patients and the specified DM risk individual who never visit hospital, to confirm for the DM. Following steps were employed for every blood collecting activities:
        - The researchers and nurses explained in details the information from the volunteer manual to the patients before having them signed their consent forms. The researchers explained clearly to the volunteers of their risk and benefit from the project activities.
            - Nurses collected blood samples in the morning as patients were asked to fast at least 8 hours before. The nurses also always present while collecting 10 mL of venous blood. Sterile technique was strictly used in this step and the 2-4 ml of blood was drawn in tube for determination of fasting plasma glucose by the hospital's clinic laboratory.

3.5 Measurements

3.5.1 Identifying DM type 2 subjects:

Steps/ criteria for DM identification

The hospital's criteria complying with the one of WHO was used as following:

First step: Screening process and criteria for identification of DM high risk individual in the community.

There were two categories to be considered before data analysis for identification of the DM risk individual.

Second step: Confirmation process at hospital

Table 6 Steps and Criteria for identification and confirmation of DM high risk individual

First step: Screening process and criteria for identification of DM high risk individual in the community		
Category 1: Collect blood (DTX; capillary blood testing for sugar level) after meal 2 hours		
Result of Blood sugar level (mg/dl) or mg%	**Conclusion**	**Further actions to be taken**
<140	normal	no
140-199	Risk	Asked to recheck 3 months later at home, lifestyle change recommended
≥200	High risk	record result in given form and send the case to hospital for confirmation step
Category 2: Collect blood (DTX; capillary blood testing for sugar level) before meal (fasting) 8 hours		
120-125	Risk	Asked to recheck 3 months later at home, lifestyle change recommended
≥126	High risk	record result in given form and send the case to hospital for confirmation step
Second step: Confirmation process at hospital		
<100		Recheck every year
101-125	Risk	Doctor asks to recheck 3 months later at hospital, lifestyle change recommended
≥126+ DM symptom	DM patient	DM clinic, report to the system

All identified cases identification was included in the hospital's OPD card and computer system for DM clinic. Researcher had a permission to obtain the case information from Ron Phibun hospital.

3.5.2 Estimation of arsenic concentration

This data was obtained by a retrospective enquiry, after case and control allocation. Since arsenic exposure appeared to public in 1987 (2530 BC) to year 2008 (2551 BC), the most polluted area identified were Moo 2, 12 and 13 at Ron Phibun sub-district. Thus, exposed study population referred to villagers living in Moo 2, 12, and 13 of Ron Phibun sub-district more than 1 year. As mentioned in data collection, for both years 2000 and 2008 studies, the information of different water types (e.g. municipal tap water, village tap water, well water, bottle water, rain water) individual used for drinking, cooking, bathing/teeth brushing came from questionnaire, whereas information on arsenic concentration in each type of water collected from individual's house was measured with GF-AAS (Graphite Furnace Atomic Absorption Spectrophotometry) and HG-AAS (Hydride Generation Atomic Absorption Spectrophotometry) at reference laboratory of the Department of Medical Sciences. The American Public Health Association (APHA): Standard methods for the examination of water and wastewater; Method 3030E and 3113B:21st edition (21sted.), was utilized. The detection limit of 0.001 mg/L or 1 µg/L was obtained.

Though inorganic arsenic is considered the most toxic form, according to a toxicological point of view, its concentration in water was not measured in the study, in fact, we measured total arsenic. Since rain water was not collected in the year 2008 study, the results of arsenic concentration of the year 2000 study were used in 2008 study. Only a few bottled water samples were available for arsenic level analysis and the concentration we detected was less than the detection limit of 0.001 mg/L; whereas there were a number of individual said that they used this water type for either drinking or cooking. Thus, it was assumed that arsenic concentration in bottled water was zero in both years. It was probably very slightly higher than zero, but below detection limits.

According to toxicological profile of arsenic (ATSDR, 2007), little information are available for adverse effects from dermal exposure of inorganic arsenic. Villagers did not use bathtub when taking a bath, according to results of field observation during house visits of the year 2000 study. They used cement or plastic tanks or earth jars to collect water for bathing and teeth brushing and used a bowl to shower. They also brushed their teeth while bathing in the morning and evening. By this practice, the amount of water is too little and the contact time is too short to cause any effective absorption. However, teeth brushing water was considered as oral-incidental exposure source instead. Arsenic concentration in those water types used for teeth brushing were measures. Concentration of arsenic in each purpose of water use (drinking, cooking, and teeth brushing) was expressed as Mean ± S.E (Standard Error).

3.6 Data Analysis

Secondary data from previous two community studies in 2000 and 2008 were used. For all needed socioeconomic information and sources of water that individuals used for consumption, questionnaire (year 2000) and worksheet for health risk screening with additional worksheet for water collection (year 2008) with face to face interviews were used in data collection. Information on melanosis or hyperkeratosis (as a potential marker of water arsenic exposure) was obtained after skin examination

of individuals by specially trained nurse at the time of interviewing. Information from those two years' studies was combined into a single data file for further analysis.

Among others, data of independent variables such as gender, age, BMI, having exercise, residency in different Moo Ban (Moo2, 12, 13) and length of residence (years), history of illness of parents and siblings, smoking, drinking, observation of melanosis or hyperkeratosis, married status, education, occupation (being farmer, being government officer or own business), and having a motorcar (representing economic status) were scrutinized for association with DM type2 in the area. Having exercise in this study means those who have exercise at least 30 minutes per time and 3 times per week. We classified having either diabetes (DM), hypertension (HT), gout, chronic renal failure (CRF), myocardial Infarction (MI), stroke, chronic obstruction pulmonary disease (COPD) and paralysis or myocardial ischemia as having history of illness of both parent and sibling categories, though some of this disease are not quite relate to DM. This is because of limitation of data availability since the questionnaire that we used as source document was aimed to screen health risks among population for national survey purposes, not specifically for screening of DM frequency.

Variables indicating evidence of use of 5 types of water (municipal tap water, village tap water, bottled water, well water and rain water were constructed by combining evidence of use of that type of water either from questionnaire responses regarding water source or from the availability of arsenic concentration in that type of water for individual subjects. For each individual, arsenic concentration (mg/L) in water types both years 2000 and 2008 were imputed and expressed as mean and S.E.

For average arsenic levels in drinking water, cooking water and teeth brushing water variables, a number of models were constructed in such a way that it combines the measured concentration of arsenic in each water type and questionnaire response regarding water source into one variable for individual subjects.

Data set of case and unmatched control and data set of case and matched control were separately fully imputed, using Multiple Imputation (MI) method, before association analysis. We imputed for missing data for all analysed variables, both sociodemographic and arsenic relating metrics relating variables.

MI is a computational statistical method used to impute missing value for independent variables. There are 3 steps for MI analysis; first, formulation of imputation model and a series of imputed dataset are then created. Second, each imputed dataset is analyzed separately. And third, a single set of estimates are generated from the pooled imputed datasets. The MI estimate of the standard error (S.E) of a parameter is square root of within imputation variance plus between imputation variance. Within imputation variance is the average of variances across imputations and between imputations variance is function of variance of parameters estimated across the imputed datasets and number of imputation. Thus, uncertainty in the imputed values is accounted for by combining the results across imputations.

To prevent negative values after MI of quantitative variables that is not normally distributed, the Predictive Mean Matching (PMM) (as imputation method in method subcommand of MI, SPSS version 22.0) was used (Allison, 1999). To reduce uncertainty, variables that have percentage of missing value more than 80% were not imputed or included in the model.

An imputation model was constructed (based on variables of interested when some of those variables have missing value) to investigate the influence of socio-economic background , usage of consumption water and average concentration of arsenic in drinking, cooking, teeth brushing water on DM type2 occurrence.

A multivariable analysis model (a tool for determination of relative contribution of different causes or variables of interested to DM type2 occurrence) with unconditional logistic regression analysis for unmatched control and conditional logistic for matched control were employed for association analysis whereby a significant association is identified when p-value is <0.05. In the conditional logistic regression, we used cox models whereby the strata were the separate case-control pairs. Matching factors like age and gender were not considered as independent variables in the conditional logistic regression models.

For each of fully imputed data set of unmatched and matched control groups, we first settled on sociodemographic variables to be used. Those variables include being male (only unmatched control), age as of year 2008 (only unmatched control), BMI year 2008, exercise, living in different Moo Ban (Moo 2 12 13; Moo2 as reference), having history of illness of parents as well as of siblings, smoking, drinking, having symptom of either melanosis or hyperkeratosis, married status, education level, year of residency in Ron Phi Boon, being farmer and being Government official or having own business (being labours and others as reference), and having motorcar (represent a better economic status).

For association analysis, 3 modelling steps were made as following:

1 To bring forward sociodemographic independent variables to subsequent models, we made 3 consecutive intermediate models for unmatched and 2 models for matched control, whereby a cut-off point for p-value of 0.200 was used for selection of input variables to the next model We finally selected 8 and 6 variables for unmatched and matched control groups, respectively.

2 Two consecutive intermediate models comprising of 8 selected variables and variables for evidence of use of water types in 2000 and 2008 were then made in the unmatched control group. In the final model, a cut-off point for p-value of 0.200 was also used for selection of input variables. Only 1 model comprising of 6 selected sociodemographic variables and evidence of use of different water types was made in the matched control group.

3 One model was constructed; both in unmatched and matched controls, to combine selected sociodemographic variables (8 for unmatched, 6 for matched control groups) with average arsenic concentrations in different water types, and to combine with average arsenic concentrations in drinking water, cooking water, and teeth brushing/bathing water variables in 2000 and 2008.

For case and unmatched control groups, a model comprising of adjusted sociodemographic variables including being male, age as of year 2008, BMI year 2008, exercise, history of illness of parents, history of illness of siblings, ever drink, having motorcar (including motorcycle)), and arsenic relating metric variables (average arsenic concentration in water used for drinking, for cooking, for teeth brushing/bathing in 2000 and 2008) was constructed for association analysis. The constructed final model for association analysis in the matched control group comprised of BMI year 2008, exercise, history of illness of siblings, ever drink, being government official or owning business, having motorcar (including motorcycle), and

average arsenic concentration in water used for drinking, for cooking, for teeth brushing/bathing in 2000 and 2008.

3.7 Strengths and Limitations

The strong points of this study are: (1) The researcher was the local people, speaking the same dialect, and had good relationship with local authorities, leader communities and local people; and (2) the availability of good exposure data via questionnaire response on types of water used for consumption, and arsenic level in those types of water at household of individual. The water Arsenic measurement data available was unusual in their completeness, the strongest point of the exposure assessment in this study.

DM is not considered a rare disease but it can be a relatively infrequent. The case-control designed for this study might be problematic if special attention was not paid to the selection of control group and confounding factors.

3.8 Actions to Ensure Validity of Data

Following activities were implemented to ensure the validity of study data:

(1) Random sampling of the whole qualified population (those fitting criteria) for selection of control was applied (dealing with selection bias);

(2) To deal with confounding error, the use of accredited laboratory analysis results, validated questionnaires and carefully considering contribution of other existing DM type2 risk factors (by: matching, direct &indirect adjustment, multivariate analysis etc.) were explored;

(3) To deal with information bias, following measures were taken:

- Make sure to achieve participation and acknowledgement of the project activities by target population,
- Make sure to get participation of well-trained village health volunteers, and actively involvement of medical officers at the Ron Phibun hospital and at the Provincial Health Office.
- Cross checking of the available data (DM and arsenic exposure) separately before merging it at the end.

3.9 Ethical Consideration

The secondary data from two previous studies (Year 2000, 2008) were used with the permission of principle investigators. Since researcher was the main investigator in that team, the protocols for those studies were developed and received approval from ethical committees of the Department of Medical Sciences (according to Memorandum No. 0625/0387; Appendix D). When collecting the biological samples and handling of personal data of the participating individual, following points were taken into account:

- Obtain approval from the Department of Medical Sciences' ethic committee before sample collections
- Groups inform consent was performed and participating individual was asked to sign consent form
- Not individual reporting
- Only specified researchers handling raw data after getting permission from volunteers
- Blind coding was generally applied
- Transparent approach via two way communication
- Use familiar dialog / environment to explain the activities

- Provide understandably & clear message to participant (the right to know) and give a fair chance for making decision.
- No monetary compensation to be allocated for volunteers. Volunteers' blood and nails were collected by trained nurses of the Ron Phi boon hospital. In case of emergency, volunteers would be admitted to the Ron Phibun Hospital. Complication cases would be referred to Nakhon Si Thammarat Regional Hospital.
- Volunteers can exit the program at anytime.

3.10 Work Places

All field works were taken place in Moo 2, 12 and 13 of Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat Province. For laboratory analysis and measurement of arsenic concentrations, it was done in toxicology and biochemistry laboratory of the National Institute of Health, as well as Surat Thani and Trang Regional Medical Sciences Center, the Department of Medical Sciences. The College of Public Health Sciences, Chulalongkorn University was the venue for statistical analysis, report writing, consulting with supervisor. Ron Phibun Hospital, Nakhon Si Thammarat, played a role of co-operation center for field works.

CHAPTER IV

RESULTS

All cases were patients of DM clinic at Ron Phibun Hospital who had residency in Moo2, Moo12, and Moo13. The numbers of diagnosed cases each year by Moo Ban updated as of year 2013 are shown in table7. Total number of cases (both sex) was diagnosed as of year 2013 in Moo 2, Moo 12, and Moo 13 are 58, 73, and 54, respectively. The highest number was found in Moo 12.

Table 7 Numbers of diagnosed cases each year by Moo Ban (since ≤2008- 2013)

Moo	**Total Number of DM Type 2 cases (age ≥35 y) in each diagnosed year**						
	Gender	**≤2008**	**2009**	**2010**	**2011**	**2013**	**Total/Sex**
2	Male	9	3	1	6	-	19
	Female	27	2	1	7	2	39
12	Male	11	4	6	1	-	22
	Female	39	5	4	2	1	51
13	Male	7	3	2	5	3	20
	Female	25	3	2	2	2	34
Total		118	20	16	23	8	185
Accumulated Number		118	138	154	177	185	

Selected characteristics of case and unmatched control groups are presented in table 8, whereby mean and standard error (SE) of age as year 2008, Body Mass Index (BMI) as of the year 2008, Length of residence (years) in Ron Phibun sub-district were 58.5 (.828), 25.3 (.32), 37.3 (13.69) in case group, and 52.8 (.932), 22.4 (.26), 38.4 (14.85) in control group, respectively. The number (%) of individual who: Being male; Doing exercise; Having history of illness of parents; Having history of illness of siblings; Ever smoking; Ever drinking; Melanosis or hyperkeratosis (as a metric of potential water arsenic exposure) is observed; Actively married; Have basic and higher education; Being farmer; Being government employer; Having and driving a motorcar are 61(33), 81 (43.7), 83 (44.9), 88 (47.8), 74 (39.9), 81 (43.8), 106 (57.3), 96 (51.8), 67 (36.4), 19 (10.3), 18 (9.7), 116 (62.7) in case group and 78(39), 51 (25.4), 42 (21), 29 (14.5), 39 (19.7), 37 (18.6), 115 (57.5), 93 (46.7), 86 (43.2), 15 (7.5), 12 (6), 170 (85) in unmatched control group, respectively.

Table 8 Selected characteristics among case (n: 185) and unmatched control (n: 200) groups

Variables	Case group, n=185	Unmatched Control group, n=200	Total n=385
Male; Count (%)	61 (33)	78 (39)	139
Age as of year 2008; Mean (SE)	58.5 (0.83)	52.8 (0.93)	55.5 (0.64)
BMI year 2008; Mean (SE)	25.3 (0.32)	22.4 (0.26)	23.8 (0.29)
Exercise at least 30 minute/time and > 3 times/week; count (%)	81 (43.7)	51 (25.4)	132
Having history of illness of parents; count (%)	83 (44.9)	42 (21)	125
Having history of illness of siblings; count (%)	88 (47.8)	29 (14.5)	117
Ever smoking; count (%)	74 (39.9)	39 (19.7)	113
Ever drinking; count (%)	81 (43.8)	37 (18.6)	118
Melanosis or hyperkeratosis is observed; count (%)	106 (57.3)	115 (57.5)	221
Actively married; count (%)	96 (51.8)	93 (46.7)	189
Have basic and higher education; count (%)	67 (36.4)	86 (43.2)	153
Length of residence (years) in Ron Phibun sub-district; Mean (SE)	37.4 (13.69)	38.4 (14.85)	37.9 (14.24)
Being farmer; count (%)	19 (10.3)	15 (7.5)	34
Being government employer; count (%)	18 (9.7)	12 (6)	30
Having and driving motorcar; count (%)	116 (62.7)	170 (85)	286

Note: SE = Standard Error (For continuous variable). For continuous variables, when performing the independent t-test (in SPSS Version 22.0) from imputed data set, it does not give the Standard Deviation (SD), instead, it gives Standard Error (SE).

Table 9 presents frequencies of use of different water types (municipal tap water, village tap water, bottled water, well water and rain water) used for consumption in year 2000 and year 2008 in case and unmatched control groups. For the year 2000, those usage percentages of municipal tap water, village tap water, bottled water, well water, rain water are 55.4, 42.8, 36.9, 52.4, 68.4 in case group and 47.5, 44.4, 40.4, 61.1, 68.2 in unmatched control group respectively. In the year 2008, percentage of use of municipal tap water, village tap water, bottled water, well water, rain water are 50.5, 53.4, 34.2, 39.8, 42.3 in case group and 46.8, 64.5, 38.5, 40.3, 58.3 in unmatched control groups. Rain, well and municipal tap water are top three ranking in year 2000, whereas village tap water, rain water , and municipal tap water are the top three ranking in the year 2008.

Table 9 Descriptive information on evidence of types of water used for consumption in case and unmatched control groups

Variables	Case group, N=185	Unmatched Control group N=200	Total N=385
Use of municipal tap waterY2000; count (%)	102 (55.4)	95 (47.5)	197 (51.2)
Use of village tap water Y2000; count (%)	79 (42.8)	89 (44.4)	168 (43.6)
Use of bottle water Y2000; count (%)	68 (36.9)	81 (40.4)	149 (38.7)
Use of well water Y2000; count (%)	97 (52.4)	122 (61.1)	219 (56.9)
Use of rain water Y2000; count (%)	127 (68.4)	136 (68.2)	263 (68.3)
Use of municipal tap waterY2008; count (%)	93 (50.5)	94 (46.8)	187 (48.6)
Use of village tap water Y2008; count (%)	99 (53.4)	129 (64.5)	228 (59.2)
Use of bottled water Y2008; count (%)	63 (34.2)	77 (38.5)	140 (36.4)
Use of well water Y2008; count (%)	74 (39.8)	81 (40.3)	155 (40.3)
Use of rain water Y2008; count (%)	78 (42.3)	117 (58.3)	195 (50.6)

For unmatched control groups, descriptive results of arsenic concentration (mg/L or mg/L) in different water types and in water used for drinking, cooking, and teeth brushing of both years 2000 and 2008 are present in Table10. In both years 2000 and 2008, the highest concentration level were found in well water whereby in year 2000; mean arsenic concentration was 0.5383 mg/L (S.D=0.7636 mg/L), in year 2008; mean arsenic concentration was 0.1214 mg/L (S.D=0.1350 mg/L).

Table 10 Descriptive information on Arsenic concentration (mg/L) in different water types and water used for consumption in 2000 and 2008 of case and unmatched control groups (n=385)

Variables	**Mean (mg/L)**	**S.D. (mg/L)**	**Minimum (mg/L)**	**Maximum (mg/L)**
Concentration of As Municipal Tap Water 2000	0.0058	0.0049	0.0009	0.0110
Concentration of As Well Water 2000	0.5383	0.7636	0.0009	8.5830
Concentration of As Village Tap Water 2000	0.0402	0.0413	0.0009	0.0920
Concentration of As Rain Water 2000	0.0217	0.0304	0.0009	0.0730
Concentration of As Bottled water 2000*	0.0000	0.0000	0.0000	0.0000

Table 10 (continue)

Variables	**Mean (mg/L)**	**S.D. (mg/L)**	**Minimum (mg/L)**	**Maximum (mg/L)**
Concentration of As Municipal Tap Water 2008	0.0281	0.0426	0.0009	0.1120
Concentration of As Well Water 2008	0.1214	0.1350	0.0009	0.3290
Concentration of As Village Tap Water 2008	0.0605	0.0588	0.0009	0.1500
Concentration of As Rain Water 2008	0.0215	0.0304	0.0009	0.0730
Concentration of As Bottle water 2008	0.0000	0.0000	0.0000	0.0000
Arsenic Concentration of DW 2000	0.0125	0.0208	0.0000	0.0670
Arsenic Concentration of DW 2008	0.0495	0.0551	0.0000	0.1410
Arsenic Concentration of CW 2000	0.1532	0.3055	0.0000	1.0700
Arsenic Concentration of CW 2008	0.0364	0.0433	0.0000	0.1410
Arsenic Concentration of TW 2000	0.2772	0.3613	0.0000	1.3510
Arsenic Concentration of TW 2008	0.0964	0.0951	0.0009	0.3020

Note: S.D means Standard Deviation; mg/L, means milligram per liter; DW, means drinking water; CW, means cooking water; TW, means teeth brushing/bathing water.

Table 11 presents average concentration of arsenic in mg/L in different types of water and in water that individual said they used for drinking, cooking, teeth brushing/bathing in case and unmatched control groups of both years 2000 and 2008. In year 2000, average (S.E.) concentration in mg/L of arsenic in municipal tap water, well water, village tap water, rain water, bottled water, were 0.0059 (0.0005), 0.5505(0.0850), 0.0397(0.0055), 0.0201(0.0029), 0.0000 in case group, and 0.0058(0.0004), 0.5270(0.0645), 0.0407(0.0047), 0.0231(0.0031), 0.0000 in unmatched control group. In year 2008, average (S.E.) concentration in mg/L of arsenic in municipal tap water, well water, village tap water, rain water, bottle water, were 0.0252(0.0043), 0.1209(0.0111), 0.0628(0.0088), 0.0200(0.0032), 0.0000 in case group, and 0.0308(0.0050), 0.1219(0.0138), 0.0584(0.0081), 0.0229(0.0026), 0.0000 in unmatched control group. For continuous variables, when performing the independent t-test (in SPSS Version 22.0) from imputed data set, it does not give the Standard Deviation (SD), instead, it gives Standard Error (SE).

In year 2000 and 2008, arsenic concentration in mg/L (S.E.) in water that individual said they used for drinking, cooking, teeth brushing/bathing are 0.0106(0.0060), 0.0461(0.0100), 0.1420(0.0759), 0.0352(0.0145), 0.2824(0.0321), 0.0943(0.0221) in case group, and 0.0143(0.0073), 0.0526(0.0116), 0.1635(0.1181), 0.0374(0.0134), 0.2724(0.0319), 0.0984(0.0228) in unmatched control group respectively.

Table 11 Comparison of Arsenic concentrations (mg/L) in water types and water used in case and unmatched control groups.

Variables	Case Group N=185		Unmatched control group N=200		Total N=385	
	Mean (mg/L)	**S.E. (mg/L)**	**Mean (mg/L)**	**S.E. (mg/L)**	**Mean (mg/L)**	**S.E. (mg/L)**
Concentration of As Municipal Tap Water 2000	0.0059	0.0005	0.0058	0.0004	0.0058	0.0003
Concentration of As Well Water 2000	0.5505	0.0850	0.5270	0.0645	0.5383	0.0599
Concentration of As Village Tap Water 2000	0.0397	0.0055	0.0407	0.0047	0.0402	0.0029
Concentration of As Rain Water 2000	0.0201	0.0029	0.0231	0.0031	0.0217	0.0022
Concentration of As Bottled water 2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Concentration of As Municipal Tap Water 2008	0.0252	0.0043	0.0308	0.0050	0.0281	0.0035
Concentration of As Well Water 2008	0.1209	0.0111	0.1219	0.0138	0.1214	0.0096
Concentration of As Village Tap Water 2008	0.0628	0.0088	0.0584	0.0081	0.0605	0.0063
Concentration of As Rain Water 2008	0.0200	0.0032	0.0229	0.0026	0.0215	0.0022
Concentration of As Bottle water 2008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Arsenic Concentration of Drinking Water 2000	0.0106	0.0060	0.0143	0.0073	0.0125	0.0065
Arsenic Concentration of Drinking Water 2008	0.0461	0.0100	0.0526	0.0116	0.0495	0.0101
Arsenic Concentration of Cooking Water 2000	0.1420	0.0759	0.1635	0.1181	0.1532	0.0959
Arsenic Concentration of Cooking Water 2008	0.0352	0.0145	0.0374	0.0134	0.0364	0.0136
Arsenic Concentration of Teeth brushing/bathing Water 2000	0.2824	0.0321	0.2724	0.0319	0.2772	0.0204
Arsenic Concentration of Teeth brushing/ bathing Water 2008	0.0943	0.0221	0.0984	0.0228	0.0964	0.0219

Note: S.E, Standard Error (For continuous variable). For continuous variables, when performing the independent t-test (in SPSS Version 22.0) from imputed data set, it does not give the Standard Deviation (SD), instead, it gives Standard Error (SE).

Comparison results of association analysis after MI of 3 models comprise of selected input variables are shown in table12. Those variables are grouped into 2, a group of established risk factors and a group of potential risk factors for DM type2. A cutoff point for p-value of 0.200 was used for selection of 17 input demographic variables in consecutively constructed model 2 and 3. In the final model (model 3), the p-values (OR) of those adjusted variables of age as of year 2008, BMI year 2008, exercise, having history of illness of parent, history of illness of sibling, drinking, having motorcar (represent a better economic status) are 0.003 (1.034), <0.001 (1.195), 0.079 (2.143), 0.125 (2.314), 0.034 (3.529), 0.064 (3.016), and 0.016 (0.377) respectively. Thus, all blank cells in table 12 represent excluded variables' results due to their p-values being above the cutoff point of 0.200.

Table 12 Results after Multiple Imputation of 3 consecutively constructed models analysis for different input variables for DM type2 risk association in case and unmatched control groups.

Input variables	**Model1**		**Model2**		**Model3**	
	Odds Ratio	**p-value**	**Odds Ratio**	**p-value**	**Odds Ratio**	**p-value**
1 Established risk factors for DM type 2						
Male	0.620	0.159	0.684	0.220		
Age As of year 2008	1.034	0.006	1.034	0.004	1.034	0.003
BMI year 2008	1.201	<0.001	1.189	0.000	1.195	<0.001
Exercise	2.154	0.086	2.171	0.070	2.143	0.079
2. Potential risk factors for DM type2						
Moo12 (Reference; Moo2)	0.847	0.655				
Moo13 (Reference;Moo2)	1.024	0.950				
History of illness in parents	2.251	0.168	2.252	0.147	2.314	0.125
History of illness in siblings	3.688	0.026	3.579	0.033	3.529	0.034
Ever smoke?	1.814	0.303				
Ever drink?	2.956	0.176	3.446	0.049	3.016	0.064
Having either melanosis or hyperkeratosis?	0.916	0.896				
Married, living with spouse	1.105	0.781				
Higher education?	0.874	0.790				
Length of residence in Ron Phibun	1.002	0.846				

Table12 (continue)

Input variables	**Model1**		**Model2**		**Model3**	
	Odds Ratio	**p-value**	**Odds Ratio**	**p-value**	**Odds Ratio**	**p-value**
Being farmer (Reference; Labor and others)	0.560	0.279				
Being Government official or owning business (Reference; Labor and others)	0.815	0.753				
Having motorcar (including motorcycle)	0.399	0.014	0.397	0.020	0.377	0.016

Table 13 presents results of association analysis for DM type2 risk in case and unmatched control groups after Multiple Imputation (MI) of 2 consecutively constructed models. Those variables in the model are grouped into 2, a group of adjusted socioeconomic variables (Age As of year 2008, BMI year 2008, Exercise, History of illness of parents, History of illness of siblings, Ever drink?, Having motorcar (including motorcycle)), and a group of evidence of types of water used (use of municipal tap, village tap, bottled, well, rain water in year 2000 and 2008).

In the final model (model 2), the p-values (OR) of those adjusted variables of age as of year 2008, BMI year 2008, exercise, having history of illness of parents, history of illness of siblings, drinking, having motorcar (represent a better economic status), and use of rain water in 2008 are 0.006 (1.035), <0.001 (1.194), 0.095 (2.089), 0.132 (2.348), 0.024 (3.668), 0.073 (3.085), 0.029 (0.388), and 0.099 (0.478) respectively. A cutoff point for p-value of 0.200 was used for selection of input variables in consecutively constructed model 2. We found indirect association, though not significant, between use evidence of rain water in year 2008 and diabetes risk in model 2.

Table 13Results after Multiple Imputation of 2 consecutively constructed models analysis of selected socioeconomic and evidence of used of water types for DM type2 risk association in case and unmatched control groups

Input variables	**Model1**		**Model2**	
	Odds Ratio	**p-value**	**Odds Ratio**	**p-value**
Age As of year 2008	1.037	0.006	1.035	0.006
BMI year 2008	1.202	0.001	1.194	<0.001
Exercise	2.215	0.084	2.089	0.095
History of illness in parents	2.615	0.093	2.348	0.132
History of illness in siblings	3.889	0.040	3.668	0.024
Ever drink?	3.244	0.058	3.085	0.073
Having motorcar (including motorcycle)	0.371	0.030	0.388	0.029
Use of municipal tap waterYear2000	1.428	0.512		
Use of village tap water Year2000	0.973	0.951		
Use of bottled water Year2000	1.090	0.842		
Use of well water Year2000	0.828	0.708		
Use of rain water Year2000	0.892	0.832		
Use of municipal tap waterYear2008	0.800	0.586		
Use of village tap water Year2008	0.911	0.873		
Use of bottled water Y2008	0.579	0.277		
Use of well water Year2008	0.907	0.830		
Use of rain water Year2008	0.463	0.150	0.478	0.099

Table 14 shows association analysis results of DM type2 and selected socioeconomic variables as well as arsenic concentration variables in case and unmatched control groups. The p-value (OR) for being male, age as of year 2008, BMI year 2008, exercise, history of illness of parents, history of illness of siblings, ever drink, having motorcar (including motorcycle), arsenic concentration of municipal tap water, well water, village tap water, rain water in year 2000 and in year 2008 are 0.004 (1.034), <0.001 (1.202), 0.095 (2.161), 0.169 (2.260), 0.047 (3.678), 0.049 (3.053), 0.013 (0.360), 0.712 (1.84×10^{6}), 0.969 (1.009), 0.943 (0.674), 0.876 (322), 0.828 (0.354), 0.821 (0.759), 0.741 (4.963), and 0.860 (0.002) respectively. Significant association between arsenic concentration in different types of water used for consumption of both years 2000 and 2008 and DM type 2 risk were not identified.

Table 14 Results after Multiple Imputation of constructed model analysis of selected socioeconomic and arsenic concentration in different water types variables for DM type2 risk association in case and unmatched control groups

Input variables	**Case and unmatched control groups**	
	Odds Ratio	**p-value**
Age As of year 2008	1.034	0.004
BMI year 2008	1.202	<0.001
Exercise	2.161	0.095
History of illness in parents	2.260	0.169
History of illness in siblings	3.678	0.047
Ever drink?	3.053	0.049
Having motorcar (including motorcycle)	0.360	0.013
Concentration of As Municipal Tap Water 2000	1840245	0.712
Concentration of As Well Water 2000	1.009	0.969
Concentration of As Village Tap Water 2000	0.674	0.943
Concentration of As Rain Water 2000	322	0.876
Concentration of As Municipal Tap Water 2008	0.354	0.828
Concentration of As Well Water 2008	0.759	0.821
Concentration of As Village Tap Water 2008	4.963	0.741
Concentration of As Rain Water 2008	0.002	0.860

Table 15 shows association analysis results of DM type2 and adjusted demographic variables as well as arsenic relating metrics variables in case and unmatched control groups. The p-value (OR) for being male, age as of year 2008, BMI year 2008, exercise, history of illness of parents, history of illness of siblings, ever drink, having motorcar(including motorcycle), arsenic concentration of water individual said they used for drinking, for cooking, for teeth brushing/bathing in year 2000 and 2008 are 0.270(0.706), 0.003(1.036), <0.001(1.200), 0.051(2.255), 0.101(2.322), 0.021(3.858), 0.076(3.301), 0.020(0.400), 0.430(0.000), 0.587(1.830), 0.655(1.271), 0.536(0.074), 0.742(20.969), and 0.470(0.148) respectively. Meaningful associations of estimated arsenic concentration of water used for drinking, cooking, teeth brushing/bathing by individual and diabetes risk was not observed in this group.

Table 15 Results after Multiple Imputation of constructed models analysis for adjusted input variables of sociodemographic and arsenic relating metrics for DM type2 risk association in case and unmatched control groups

Input variables	**Case and unmatched control Group**	
	Odds Ratio	**p-value**
1 Demographic variables		
Male	0.706	0.270
Age As of year 2008	1.036	0.003
BMI year 2008	1.200	<0.001
Exercise	2.255	0.051
History of illness in parents	2.322	0.101
History of illness in siblings	3.858	0.021
Ever drink?	3.301	0.076
Having motorcar (including motorcycle)	0.400	0.020
2 Arsenic relating metrics variables		
Arsenic Concentration of Drinking Water 2000	0.000	0.430
Arsenic Concentration of Cooking Water 2000	1.830	0.587
Arsenic Concentration of Teeth brushing/bathing Water 2000	1.271	0.655
Arsenic Concentration of Drinking Water 2008	0.074	0.536
Arsenic Concentration of Cooking Water 2008	20.969	0.742
Arsenic Concentration of Teeth brushing/bathing Water 2008	0.148	0.470

Selected characteristics of case and matched control groups are presented in table 16. The mean and standard error (SE) of Body Mass Index (BMI) as of the year 2008, Length of residence (years) in Ron Phibun sub-district were 25.3 (.34), 45.2 (11.21) in case group, and 22.4 (.28), 48.8 (10.78) in matched control group, respectively. The number (%) of individual who: Doing exercise; Having history of illness of parents; Having history of illness of siblings; Ever smoking; Ever drinking; Melanosis or hyperkeratosis (as a metric of potential water arsenic exposure) is observed; Actively married; Have basic and higher education; Being farmer; Being government employer; Having and driving motorcar were 76 (41.1), 84 (45.4), 78 (42.2), 81 (43.8), 94 (50.8), 100 (54.1), 120 (64.9), 103 (55.1), 19 (10.3), 18 (9.7), 110 (59.5) in case group and 48 (25.9), 28 (15.1), 18 (9.7), 30 (16.2), 28 (15.1), 111 (60.0), 117 (63.2), 106 (57.3), 20 (10.8), 11 (5.9), 154 (83.2) in matched control group, respectively.

Table 16 Selected characteristics among case and matched control groups

Variables	**Case group,** n=185	**Matched Control group,** n=185	**Total** n=370
BMI year 2008; Mean (SE)	25.3 (0.34)	22.4 (0.28)	23.9 (0.23)
Doing exercise at least 30 minute/time and > 3 times/week; count (%)	76 (41.1)	48 (25.9)	124
Having history of illness in parents; count (%)	84 (45.4)	28 (15.1)	112
Having history of illness in siblings; count (%)	78 (42.2)	18 (9.7)	96
Ever smoking; count (%)	81(43.8)	30 (16.2)	111
Ever drinking; count (%)	94 (50.8)	28 (15.1)	122
Melanosis or hyperkeratosis is observed; count (%)	100 (54.1)	111(60.0)	211
Actively married; count (%)	120 (64.9)	117 (63.2)	237
Have basic and higher education; count (%)	102 (55.1)	106 (57.3)	208
Length of residence (years) in Ron Phibun sub-district; Mean (SE)	45.2 (11.2)	43.9 (10.78)	44.5 (10.8)
Being farmer; count (%)	19 (10.3)	20 (10.8)	39
Being government employer; count (%)	18 (9.7)	11(5.9)	29
Having and driving motorcar; count (%)	110 (59.5)	154(83.2)	264

Note: SE = Standard Error (For continuous variable). For continuous variables, when performing the independent t-test (in SPSS Version 22.0) from imputed data set, it does not give the Standard Deviation (SD), instead, it gives Standard Error (SE).

Table17 shows frequencies of use of different water types (municipal tap water, village tap water, bottled water, well water and rain water) used for consumption in year 2008 and year 2000 in case and matched control groups. For the year 2000, those usage percentages of municipal tap water, village tap water, bottled water, well water, rain water are 53.0, 30.8, 34.1, 49.7, 67.6 in case group and 40.5, 38.4, 31.4, 53.5, 68.1 in matched control group respectively. In the year 2008, percentage of use of municipal tap water, village tap water, bottle water, well water, rain water are 50.3, 48.6, 42.7, 37.8, 43.2 in case group and 44.9, 58.4, 44.9, 41.1, 58.9 in matched control groups.

Table 17Descriptive information on evidence of types of water used for consumption in case and matched control groups

Variables	Case group, n=185	Matched Control group n=185	Total n=370
Use of municipal tap waterY2000; count (%)	98(53.0)	75(40.5)	173 (46.8)
Use of village tap water Y2000; count (%)	57(30.8)	71(38.4)	128 (34.6)
Use of bottle water Y2000; count (%)	63.(34.1)	58(31.4)	121 (32.7)
Use of well water Y2000; count (%)	92(49.7)	99(53.5)	191 (51.6)
Use of rain water Y2000; count (%)	125(67.6)	126(68.1)	251 (67.8)
Use of municipal tap waterY2008; count (%)	93(50.3)	83(44.9)	176 (47.6)
Use of village tap water Y2008; count (%)	90(48.6)	108(58.4)	198 (53.5)
Use of bottled water Y2008; count (%)	79(42.7)	83(44.9)	162 (43.8)
Use of well water Y2008; count (%)	70(37.8)	76(41.1)	146 (39.5)
Use of rain water Y2008; count (%)	80(43.2)	109(58.9)	189 (51.1)

For matched control groups, descriptive results of arsenic concentrations (mg/L or mg/L) in different water types and in water used for drinking, cooking, and teeth brushing of both years 2000 and 2008 are present in Table18. In both years 2000 and 2008, the highest concentration level were found in well water whereby in year 2000; mean arsenic concentration was 0.5176 mg/L (SD=0.7619 mg/L), in year 2008; mean arsenic concentration was 0.2264 mg/L (SD=0.2923 mg/L).

Table 18 Descriptive information of Arsenic concentration (mg/L.) in different water types and water used for consumption in 2000 and 2008 of case and matched control groups (n=370)

Variables	**Mean (mg/L)**	**S.D. (mg/L)**	**Minimum (mg/L)**	**Maximum (mg/L)**
Concentration of As Municipal Tap Water 2000	0.0057	0.0049	0.0009	0.0110
Concentration of As Well Water 2000	0.5176	0.7619	0.0009	8.5830
Concentration of As Village Tap Water 2000	0.0421	0.0439	0.0009	0.0980
Concentration of As Rain Water 2000	0.0173	0.0277	0.0009	0.0730
Concentration of As Bottle water 2000	0.0000	0.0000	0.0000	0.0000
Concentration of As Municipal Tap Water 2008	0.0307	0.0429	0.0009	0.1050
Concentration of As Well Water 2008	0.2264	0.2923	0.0009	1.1070
Concentration of As Village Tap Water 2008	0.0808	0.0915	0.0009	0.2470
Concentration of As Rain Water 2008	0.0175	0.0279	0.0009	0.0730
Concentration of As Bottle water 2008	0.0000	0.0000	0.0000	0.0000
Arsenic Concentration of DW 2000	0.0143	0.0225	0.0000	0.0670
Arsenic Concentration of DW 2008	0.0433	0.0509	0.0000	0.1410
Arsenic Concentration of CW 2000	0.1010	0.1954	0.0000	1.0700
Arsenic Concentration of CW 2008	0.0395	0.0497	0.0000	0.1410
Arsenic Concentration of TW 2000	0.3685	0.4646	0.0000	1.3510
Arsenic Concentration of TW 2008	0.1873	0.2224	0.0009	0.7270

Note: S.D means Standard Deviation; mg/L, means milligram per liter; DW, means drinking water; CW, means cooking water; TW, means teeth brushing/bathing water.

Table 19 compares results of average concentration of arsenic in mg/L in different types of water and in water that individual said they used for drinking, cooking, teeth brushing/bathing in case and matched control groups in both year 2000 and 2008. In year 2000, average (S.E.) concentration in mg/L of arsenic in municipal tap water, well water, village tap water, rain water, bottled water, were 0.0054(0.0005), 0.5452(0.0722), 0.0408(0.0061), 0.0180(0.0029), 0.0000 in case group, and 0.0060(0.0006), 0.4900(0.0759), 0.0433(0.0065), 0.0165(0.0030), 0.0000 in matched control group. In year 2008, average (S.E.) concentration in mg/L of arsenic in municipal tap water, well water, village tap water, rain water, bottled water, were 0.0265(0.0045), 0.2054(0.0273), 0.0807(0.0157), 0.0181(0.0025), 0.0000 in case group, and 0.0349(0.0073), 0.2474(0.0347), 0.0809(0.0183), 0.0170(0.0028), 0.0000 in matched control group. In year 2000 and 2008, arsenic concentration in mg/L (S.E.) in water that individual said they used for drinking, cooking, teeth brushing/bathing were 0.0144(0.0025), 0.0412(0.0177), 0.0970(0.0752), 0.0371(0.0127), 0.3555(0.2721), 0.1690(0.0979) in case group, and 0.0142(0.0035), 0.0454(0.0212), 0.1049(0.1030), 0.0418(0.0130), 0.3815(0.3041), 0.2057(0.1593) in matched control group respectively.

Table 19 Comparison of Arsenic concentrations (mg/L) in water types and water uses in case and matched control groups

Variables	Case Group N=185		Matched control group N=185		Total N=370	
	Mean (mg/L)	**S.E. (mg/L)**	**Mean (mg/L)**	**S.E. (mg/L)**	**Mean (mg/L)**	**S.E. (mg/L)**
Concentration of As Municipal Tap Water 2000	0.0054	0.0005	0.0060	0.0006	0.0057	0.0004
Concentration of As Well Water 2000	0.5452	0.0722	0.4900	0.0759	0.5176	0.0540
Concentration of As Village Tap Water 2000	0.0408	0.0061	0.0433	0.0065	0.0421	0.0038
Concentration of As Rain Water 2000	0.0180	0.0029	0.0165	0.0030	0.0173	0.0015
Concentration of As Bottled water 2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Concentration of As Municipal Tap Water 2008	0.0265	0.0045	0.0349	0.0073	0.0307	0.0050
Concentration of As Well Water 2008	0.2054	0.0273	0.2474	0.0347	0.2264	0.0167
Concentration of As Village Tap Water 2008	0.0807	0.0157	0.0809	0.0183	0.0808	0.0161
Concentration of As Rain Water 2008	0.0181	0.0025	0.0170	0.0028	0.0175	0.0016
Concentration of As Bottled water 2008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Arsenic Concentration of Drinking Water 2000	0.0144	0.0025	0.0142	0.0035	0.0143	0.0027
Arsenic Concentration of Drinking Water 2008	0.0412	0.0177	0.0454	0.0212	0.0433	0.0192
Arsenic Concentration of Cooking Water 2000	0.0970	0.0752	0.1049	0.1030	0.1010	0.0881
Arsenic Concentration of Cooking Water 2008	0.0371	0.0127	0.0418	0.0130	0.0395	0.0124
Arsenic Concentration of Teeth brushing/ bathing Water 2000	0.3555	0.2721	0.3815	0.3041	0.3685	0.2866
Arsenic Concentration of Teeth brushing/ bathing Water 2008	0.1690	0.0979	0.2057	0.1593	0.1873	0.1278

Note: S.E, Standard Error (For continuous variable). For continuous variables, when performing the independent t-test (in SPSS Version 22.0) from imputed data set, it does not give the Standard Deviation (SD), instead, it gives Standard Error (SE).

Table 20 shows comparison results of association analysis after MI of 2 models comprise of selected input variables. Those variables are grouped into 2, a group of established risk factors and a group of potential risk factors for DM type2. In the first model (model 1), the p-values (OR) of those input variables of BMI year 2008, exercise, Moo12, Moo13, having history of illness of parents, history of illness of siblings, smoking, drinking, Having either melanosis or hyperkeratosis , Married (living with spouse), Higher education, Length of residence in Ron Phibun, Being farmer, Being Government official or having own business, having motorcar (represent a better economic status) are 0.002 (1.304), 0.014 (4.701), 0.74 (0.810), 0.925 (1.058), 0.355 (1.741), 0.018 (8.755), 0.215 (3.013), 0.027 (7.843), 0.671 (0.779), 0.606 (1.373), 0.762 (1.230), 0.995 (1.000), 0.516 (0.580), 0.187 (0.289) and 0.057 (0.205) respectively. A cutoff point for p-value of 0.200 was also used for selection of input variables in consecutively constructed model 2. Thus, all blank cells in table 20 represent excluded variables' results due to their p-values being above cutoff point. In model2, the p-values (OR) of those adjusted variables of BMI year 2008, exercise, history of illness of sibling, drinking, Being Government official or having own business, having motorcar (represent a better economic status) are 0.002 (1.271), 0.028 (4.354), 0.001 (8.505), 0.001 (11.26), 0.155 (0.309), and 0.003 (0.199) respectively.

Table 20 Results after MI of 2 consecutively constructed models analysis for different input variables for DM type2 risk association in case and matched control groups

Input variables	**Model1**		**Model2**	
	Odds Ratio	**p-value**	**Odds Ratio**	**p-value**
1 Established risk factors for DM type2				
BMI year 2008	1.304	0.002	1.271	0.002
Exercise	4.701	0.014	4.354	0.028
2 Potential risk factors for DM type2				
Moo12 (Reference;Moo2)	0.810	0.740		
Moo13 (Reference;Moo2)	1.058	0.925		
History of illness in parents	1.741	0.355		
History of illness in siblings	8.755	0.018	8.505	0.001
Ever smoke?	3.013	0.215		
Ever drink?	7.843	0.027	11.260	0.001
Having either melanosis or hyperkeratosis?	0.779	0.671		
Married, living with spouse	1.373	0.606		
Higher education?	1.230	0.762		
Length of residence in Ron Phibun	1.000	0.995		
Being farmer (Reference; Labor and others)	0.58	0.516		
Being Government official or having own business (Reference; Labor and others)	0.289	0.187	0.309	0.155
Having motorcar (including motorcycle)	0.205	0.057	0.199	0.003

Results of association analysis for DM type2 and adjusted socioeconomic and water types that had used evidence by individual variables in case and matched control groups are shown in table 19. The p-value (OR) for BMI year 2008, exercise, history of illness of siblings, ever drink, being government official or owning business, having motorcar (including motorcycle), evidence of use of municipal tap water, village tap water, bottled water, well water, rain water, in year 2000 and in year 2008 are 0.032(1.319), 0.071(5.706), 0.002(20.299), 0.005(17.613), 0.212(0.227), 0.041(0.198), 0.647(1.587), 0.375(0.427), 0.602(1.610), 0.524(0.665), 0.817(1.175), 0.660(0.675), 0.990(1.015), 0.484(0.638), 0.738(0.821), 0.299(0.356), respectively. We did not find meaningful association between evidence of use of different water types and diabetes risk in this setting model.

Table 21 Results after Multiple Imputation of constructed model analysis for adjusted input socioeconomic and evidence of used of water types variables for DM type2 risk association in case and matched control groups

Variables	**Case and Matched Control Group**	
	Odds Ratio	**P-value**
BMI Y2008	1.319	0.032
Exercise	5.706	0.071
History of illness in siblings	20.299	0.002
Drinking (Ever drink?)	17.613	0.005
Government & Own Business	0.227	0.212
Having motorcar?	0.198	0.041
Evidence of use of municipal tap water 2000	1.587	0.647
Evidence of use of village tap water 2000	0.427	0.375
Evidence of use of bottled water 2000	1.610	0.602
Evidence of use of well water 2000	0.665	0.524
Evidence of use of rain water 2000	1.175	0.817
Evidence of use of municipal tap water 2008	0.675	0.660
Evidence of use of village tap water 2008	1.015	0.990
Evidence of use of bottled water 2008	0.638	0.484
Evidence of use of well water 2008	0.821	0.738
Evidence of use of rain water 2008	0.356	0.299

Table 22 shows association analysis results of DM type2 and selected socioeconomic variables as well as arsenic concentration variables in case and matched control groups. The p-value (OR) for BMI year 2008, exercise, history of illness of siblings, ever drink, being government official or owning business, having motorcar (including motorcycle), arsenic concentration of municipal tap water, well water, village tap water, rain water in year 2000 and in year 2008 are 0.001 (1.316), 0.024 (5.400), 0.004 (10.525), 0.004 (11.993), 1.136 (0.258), 0.002 (0.167), 0.707 (0.000), 0.963 (0.975), 0.835 (0.206), 0.726 (42059), 0.426 (0.011, (0.589 (0.498), 0.747 (0.301), and 0.701 (0.000), respectively. Significant association between arsenic concentration in different types of water used for consumption of both years 2000 and 2008 and DM type 2 risk were not identified in this matched control group.

Table 22 Results after Multiple Imputation of constructed model analysis of selected socioeconomic and arsenic concentration in different water types variables for DM type2 risk association in case and matched control groups

Input variables	**Case and matched control groups**	
	Odds Ratios	**p-value**
BMI year 2008	1.316	0.001
Exercise	5.400	0.024
History of illness in siblings	10.525	0.004
Ever drink?	11.993	0.004
Government & Own Business	0.258	0.136
Having motorcar (including motorcycle)	0.167	0.002
Concentration of As Municipal Tap Water 2000	0.000	0.707
Concentration of As Well Water 2000	0.975	0.963
Concentration of As Village Tap Water 2000	0.206	0.835
Concentration of As Rain Water 2000	42059	0.726
Concentration of As Municipal Tap Water 2008	0.011	0.426
Concentration of As Well Water 2008	0.498	0.589
Concentration of As Village Tap Water 2008	0.301	0.747
Concentration of As Rain Water 2008	0.000	0.701

Results of association analysis for DM type2 and adjusted socioeconomic and arsenic relating metrics variables in case and matched control are shown in table 23. The p-value (OR) for BMI year 2008, exercise, history of illness of sibling, ever drink, being government official or owning business, having motorcar (including motorcycle), arsenic concentration of water individual said they used for drinking, for cooking, for teeth brushing/bathing in year 2000 and in year 2008 are 0.007(1.311), 0.027(6.099), 0.031(10.131), 0.002(15.410), 0.245(0.254), 0.010(0.164), 0.902(0.141), 0.614(2.488), 0.687(0.647), 0.716(36.345), 0.723(0.042), and 0.886(0.586) respectively. Again, association between arsenic concentration in water used for drinking, cooking, teeth brushing/bathing by individual in year 2000 and 2008 was not observed.

Table 23 Results after Multiple Imputation of constructed model analysis for adjusted socioeconomic and arsenic relating metrics variables for DM type2 risk association in case and matched control groups

Variables	**Case and Matched Control Group**	
	Odds Ratios	**P-value**
BMI Y2008	1.311	0.007
Exercise	6.099	0.027
History of illness in siblings	10.131	0.031
Drinking (Ever drink?)	15.410	0.002
Government & Own Business	0.254	0.245
Have motorcar?	0.164	0.010
Arsenic Concentration of Drinking Water 2000	0.141	0.902
Arsenic Concentration of Cooking Water 2000	2.488	0.614
Arsenic Concentration of Teeth brushing/bathing Water 2000	0.647	0.687
Arsenic Concentration of Drinking Water 2008	36.345	0.716
Arsenic Concentration of Cooking Water 2008	0.042	0.723
Arsenic Concentration of Teeth brushing/bathing Water 2008	0.586	0.886

Table 24 shows tabulated results after MI for DM type 2 risk associations for socioeconomic variables in unmatched and matched control groups. In the unmatched control group, the p-values (OR) of those adjusted variables (final model) of age as of year 2008, BMI year 2008, exercise, having history of illness of parents, history of illness of siblings, drinking, having motorcar (represent a better economic status) are 0.003 (1.034), <0.001 (1.195), 0.079 (2.143), 0.125 (2.314), 0.034 (3.529), 0.064 (3.016), and 0.016 (0.377) respectively. For the matched control group, the p-values (OR) of those adjusted variables (final model) of BMI year 2008, exercise, history of illness of siblings, drinking, Being Government official or having own business, having motorcar (represent a better economic status) are 0.002 (1.271), 0.028 (4.354), 0.001 (8.505), 0.001 (11.26), 0.155 (0.309), and 0.003 (0.199) respectively.

Table 24 Tabulated results after Multiple Imputation for DM type 2 risk associations of socioeconomic variables in unmatched and matched control groups.

Variables	**Unmatched control**		**Matched control**	
	Odds Ratio	**P-value**	**Odds Ratio**	**P-value**
Full selected variables, first model results				
1 Established risk factors for DM type2				
Male	0.620	0.159		
Age as of year 2008	1.034	0.006		
BMI year 2008	1.201	<0.001	1.304	0.002
Exercise	2.154	0.086	4.701	0.014
2 Potential risk factors for DM type2				
Moo12 (Reference;Moo2)	0.847	0.655	0.810	0.740
Moo13 (Reference;Moo2)	1.024	0.950	1.058	0.925
History of illness in parents	2.251	0.168	1.741	0.355
History of illness in siblings	3.688	0.026	8.755	0.018
Ever smoke?	1.814	0.303	3.013	0.215
Drinking (Ever drink?)	2.956	0.176	7.843	0.027
Having either melanosis or hyperkeratosis?	0.916	0.896	0.779	0.671
Married, living with spouse	1.105	0.781	1.373	0.606
Higher education?	0.874	0.790	1.230	0.762
Length of residence in Ron Phibun	1.002	0.846	1.000	0.995
Farmer (Ref.; Labor &others)	0.560	0.279	0.58	0.516
Government official or own business (Ref; Labor & others)	0.815	0.753	0.289	0.187
Having motorcar (including motorcycle)	0.399	0.014	0.205	0.057
Adjusted selected variables, final model results				
1 Established risk factors for DM type2				
Age as of year 2008	1.034	0.003	-	-
BMI year 2008	1.195	<0.001	1.271	0.002
Exercise	2.143	0.079	4.354	0.028
2 Potential risk factors for DM type2				
History of illness of parents	2.314	0.125	-	-
History of illness of siblings	3.529	0.034	8.505	0.001
Drinking (Ever drink?)	3.016	0.064	11.258	0.001
Government official or own business (Ref; Labor & others)	-	-	0.309	0.155
Having motorcar (including motorcycle)	0.377	0.016	0.199	0.003

Table 25 gives comparison results after MI of selected demographic variables, concentration of arsenic (mg/L) in water that individual said they used for drinking, cooking, and teeth brushing/bathing in year 2000 and 2008, in unmatched and matched control.

Table 25 Tabulated results after Multiple Imputation for DM type 2 risk associations of socioeconomic variables, and arsenic relating metrics in unmatched and matched control groups.

Variables	**Unmatched control**		**Matched control**	
	Odds Ratio	**P-value**	**Odds Ratio**	**P-value**
1 Demographic variables				
Male	0.706	0.270		
Age as of Y2008	1.036	0.003		
BMI Y2008	1.200	<0.001	1.311	0.007
Exercise	2.255	0.051	6.099	0.027
History of illness in parents	2.322	0.101		
History of illness in siblings	3.858	0.021	10.131	0.031
Drinking (Ever drink?)	3.301	0.076	15.410	0.002
Being Government official or having own business (Reference; Labor and others)			0.254	0.245
Having motorcar (including motorcycle)	0.400	0.020	0.164	0.010
2 Arsenic relating metrics variables				
Arsenic Concentration of Drinking Water 2000	0.000	0.430	0.141	0.902
Arsenic Concentration of Cooking Water 2000	1.830	0.587	2.488	0.614
Arsenic Concentration of Teeth brushing/bathing Water 2000	1.271	0.655	0.647	0.687
Arsenic Concentration of Drinking Water 2008	0.074	0.536	36.345	0.716
Arsenic Concentration of Cooking Water 2008	20.969	0.742	0.042	0.723
Arsenic Concentration of Teeth brushing/bathing Water 2008	0.148	0.470	0.586	0.886

In the unmatched control group, the p-value (OR) for being male, age as of year 2008, BMI year 2008, exercise, history of illness of parent, history of illness of sibling, ever drink, having motorcar (including motorcycle), arsenic concentration of water individual said they used for drinking, for cooking, for teeth brushing/bathing in year 2000 and in year 2008 are 0.270(0.706), 0.003(1.036), <0.001(1.200), 0.051(2.255), 0.101(2.322), 0.021(3.858), 0.076(3.301), 0.020(0.400), 0.430(0.000),

0.587(1.830), 0.655(1.271), 0.536(0.074), 0.742(20.969), and 0.470(0.148) respectively. In the matched control group, the p-value (OR) for BMI year 2008, exercise, history of illness of siblings, ever drink, being government official or owning business, having motorcar (including motorcycle), arsenic concentration of water individual said they used for drinking, for cooking, for teeth brushing/bathing in year 2000 and in year 2008 are 0.007(1.311), 0.027(6.099), 0.031(10.131), 0.002(15.410), 0.245(0.254), 0.010(0.164), 0.902(0.141), 0.614(2.488), 0.687(0.647), 0.716(36.345), 0.723(0.042), and 0.886(0.586) respectively.

CHAPTER V

DISCUSSION

DM is considered a metabolic disease resulting from (1) defects in insulin secretion by pancreatic ß-cells, (2) impairment of insulin action on peripheral tissue namely adipocytes and muscle, and/or (3) an increase in endogenous glucose production by liver (Diaz-Villasenor et al., 2007). Identified main related factors for insulin resistance are age, sex, central adiposity, genetic, circulating insulin antagonist (hormone, free fatty acid, TNF-α), obesity, lack of physical activity, glucose toxicity and others such as pregnancy, ageing, and drug use (Longnecker & Daniels, 2001). According to a meta-analysis by Navas-Acien et al. (2006), potential mechanism of actions of arsenic on DM type2 was not conclusive; among identified 10 in *vivo* studies in animals, inconsistent effects of arsenic on glucose metabolism were found; and among 19 in vitro studies, 5 studies reported that arsenic interfered with transcription factors involved in insulin-related gene expression (Navas-Acien et al., 2006).

We observed no convincing association between arsenic exposure and DM type2 risk in this study area, when combining selected socioeconomic and arsenic related metrics. However, our results do not rule out higher-level arsenic exposure than we observed could actually be a risk factor for diabetes.

We combined selected socioeconomic variables (being male, age year 2008, BMI year 2008, exercise, having history of illness in parents, history of illness in siblings, drinking, having motorcar for the unmatched control; and BMI year 2008, exercise, history of illness in siblings, drinking, being government official or having own business, having motorcar for the matched control group) with different set of arsenic relating metrics variables in the constructed model for association analysis. Those arsenic relating metrics include (1) set of evidence of use of different water types (municipal tap, village tap, bottled, well, rain water) in both years 2000 and 2008, (2) set of arsenic concentrations in each water types both years 2000 and 2008, and (3) set of arsenic concentrations in water used for drinking, cooking, teeth brushing/bathing in years 2000 and 2008.

Limited negative associations with rain water use suggest that there may actually be some limited association of arsenic exposure with diabetes risk in the study area. Further, more extensive study of this issue would be desirable if feasible. Research on association of arsenic exposure with other diseases would also be desirable. For a set of variables indicating evidence of use of 5 types of water (municipal tap water, village tap water, bottled water, well water and rain water), it was constructed by combining evidence of use of that type of water from either they said they use it in questionnaire or from the availability of arsenic concentration of that type of water.

Average concentrations of Arsenic in different water types and water used for DW, CW, and TW in year 2000 and 2008 are consistency not significantly associated with risk of DM type2, both in unmatched and matched control groups. To obtain average arsenic levels in drinking water, cooking water and teeth brushing/bathing

water variables, a number of models were constructed in such a way that it combines the measured concentration of arsenic in each water type and questionnaire response regarding water source into one variable for individual subjects.

Chen et al. (2010) reported no association of arsenic exposure and glucosuria and they found no association between arsenic in well water, total arsenic in urine and HbA1c (blood glycosylated hemoglobin) levels in a population based cross-sectional study in Bangladesh (Y. Chen et al., 2010). In the subsequently prospective cohort study to evaluate association between arsenic exposure and cardiovascular diseases, adversely association between arsenic exposure via drinking water and mortality from heart disease, especially among smokers was observed (Y. Chen et al., 2011). Off 568 arsenic exposed individuals of Moo 2, 12, 13 of Ron Phibun sub-district, Nakhon Si Thammarat province, 47 (2.11%) were found having glucosuria >100 mg/dl and correlation between urinary total arsenic level and glucosuria level among exposed group (using cut off point 50 μgAs/g creatinine) was not found in this study (Pavittranon et al., 2003).

Our findings is different from that of a case cohort study on the association between DM risk and lifetime exposure to low levels of inorganic arsenic in drinking water in the US where they found that for every 15 mg/L increase in arsenic concentration in drinking water, risk for DM increased by 27 percent (95% CI =1% to 59%) after adjusting for ethnicity, and time varying measures of BMI and physical activity (James et al., 2013). In a community-based case-control study in Mexico, after adjusting for potential confounding such as sex, age, triglycerides, body mass index, hypertension, family history of DM, and using tertiles distribution of arsenic in urine as cut-off point in the model, Coronado-Gonzalez et al. (2007) reported that the higher risk of DM type2 were related to age, being female, and the presence of high blood pressure (Coronado-González et al., 2007). They also provided evidence that the higher risk of DM was related to the higher urinary total arsenic concentration.

In both case and unmatched control groups, the highest average concentration of arsenic is found in well water, followed by village tap water. A similar pattern is observed in the case and matched control groups. The average concentration of arsenic in well water in year 2000 is higher than that of year 2008, whereas the one in village tap water in year 2008 is higher than that of year 2000 in both groups. It was assumed that arsenic concentration in bottled water was zero in both years. This assumption might have introduced a little error into the modeled estimates for arsenic, but not enough to change the interpretation of findings.

The highest average concentration of arsenic is found in water that those individuals said they used for teeth brushing/bathing, in both years and both groups. We did not directly measure consumption rate (gram of water/person/day) of drinking, cooking and oral-accidental source of teeth brushing water and arsenic concentration in food consumed by each individual in both year 2000 and 2008 studies. To calculate arsenic intake dose (μg As /Kg body weight /day) of individual, a number of assumption and scenarios had to be made, and large uncertainty was expected. Realizing this, we only estimated concentration of arsenic that individual had exposed, though it might not reflex estimated intake concentration. For example, if individual used only well water that had high arsenic concentration for teeth brushing, the intake dose could be very low because the amount of water used for teeth brushing is very little.

In this study, BMI is significantly associated with increased DM type2 risk in both unmatched and matched control groups, whereas older age (as of year 2008) was associated with increased DM type2 risk in the unmatched control group. Like others previous epidemiology report worldwide ((Longnecker & Daniels, 2001), (Navas-Acien et al., 2006), (Meliker et al., 2007)), we consistency found that BMI always associated with increased DM type2 risk, even when arsenic concentration relating metrics (evidence of use of different water types, arsenic concentration in different water types, arsenic concentration in water used for drinking(DW), cooking(CW), teeth brushing(TW)) were combined with the selected socioeconomic variables in the analysis models. After the follow-up period of 1499.5 person-years and bi-annually followed 446 non-diabetic residents living 5 days/ week in three villages in Taiwan, age and body mass index were found to be significantly associated with diabetes incidence (Tseng et al., 2000).

Physical inactivity is considered as known risk factor for DM type2 ((Longnecker & Daniels, 2001), (Meliker et al., 2007)). We found that less exercise was associated with increased DM type2 risk, though not significant in the unmatched control group, when only socioeconomic variables were included in analysis models. When we combined selected variables involving being male, age as of year 2008, BMI, exercise having history of illness in parents or siblings, drinking, having motorcar (including motorcycle) and arsenic concentration in water used for DW, CW, TW, less exercise was found to be marginally associated with increased DM type2 risk. To evaluate this unexpected finding in the unmatched control group, we looked at the association of exercise with age, BMI, history of illness in parents and siblings and having a motorcar. We found little or no association between exercise and age, BMI, and motorcar. We did see strong positive association between exercise and history of illness in parents or siblings, whereby those who have reported that having parents or siblings illness did exercise more than those who had not. In view of this, it is conceivable that our observed positive association of exercise and DM type2 risk (data not shown) could actually be a reflection of reverse causality, whereby the knowledge that DM type2 or other chronic illness had occurred in their family induced participants to increase their exercise levels.

In the matched control group whereby cases and controls were matched on age and gender, we found that less exercise is significantly associated with increased DM type2 risk in almost all categories of modeling both with and without arsenic relating metrics variables, except when the evidence of use of different water types were combined in the model analysis. When only group of selected socioeconomic variables (BMI, exercise, having history of illness in siblings, drinking, being government official or having own business, having motorcar (including motorcycle) were added in the model, the p-value (OR) of exercise is 0.028 (4.354), but when arsenic concentration in water used for DW, CW, TW and evidence of use of different water types variables were combined with those previously mentioned 6 variables, the p-value (OR) are 0.027 (6.099) and 0.071(5.706), respectively.

Since it was reported that lack of exercise and other risk factors including age, family history, genetic, high blood pressure, central adiposity, obesity, diet, life style and occupation, some toxicants in environment including arsenic might be responsible for increased prevalence of DM type 2 worldwide (Longnecker & Daniels, 2001), we proved, in the our matched control study, that less exercise is associated with increased DM type2 risk. Further research is still required to ascertain the true

relationship between exercises, the combination of exercise and evidence of use of different water types and DM type2 risk in this study area, especially in the unmatched control settling.

History of illness in siblings was found to be associated with higher DM type2 risks in both unmatched and matched control groups. Our finding is similar to those reported previously. ((Longnecker & Daniels, 2001), (C.-J. Chen et al., 2007)) Though we found that history of illness of siblings is potential risk factor of DM type2, the knowledge on linkage between genetic effects and DM type2 risk is limited. After evaluating effects of gene and socioeconomic characteristics on concentration of toxic metals such as arsenic (As), cadmium (Cd), mercury (Hg) and of essential elements such as Selenium (Se), Zinc (Zn) in blood samples of Australian twin pairs, it was reported that variation in concentration of arsenic and mercury were due mainly to common genetic effect (Whitfield et al., 2010). This group reported that alcohol consumption is significantly associate with increased As, Hg, Pb and Se concentrations, whereas increased years of education associate with decreased As, Hg, Pb concentrations. They also found that genetic effect on essential elements such as Cu, Se, Zn could modulate concentration of toxic effects of other elements. This finding could explain why epidemiological studies of association between heavy metals exposure at low to moderate dose and DM type2 risk have yielded inconclusive results.

We did not find significant association between history of illness in parents and DM type2 risk in both unmatched and matched control studies in every analysis modeling, though the association had been previously reported (Longnecker & Daniels, 2001). This could be due to the limitation of data availability since the questionnaire that we used as source document was aimed to screen health risks among population for national survey purposes, not specifically for screening of DM frequency. Thus, we classified having either diabetes (DM), hypertension (HT), gout, chronic renal failure (CRF), myocardial Infarction (MI), stroke, chronic obstruction pulmonary disease (COPD) and paralysis or myocardial ischemia as having history of illness of both parents and siblings categories, though some of this disease are not quite relate to DM. Moreover, we suspected that the older age participants could not be able to classify or remember that what diseases their parents had. The mean age in case group was 58.5 years old, whereas in unmatched control group was 52.8 years old.

For smoking history, we classified never and ever smoking by face to face interview using constructed questionnaire. We did not find association of smoking with DM type2 risk in both unmatched and matched control groups. Thus, this variable was not further included in the models that combine selected socioeconomic variables with arsenic relating metrics variables. A meta-analysis result by J.S. Tsuji et al. that aimed to assess the risk of arsenic at low dose exposure on bladder cancer showed that the summary relative risk estimate (SRRE) of 9 related studies in never smokers were inconsistent with predicted values extrapolated from the high dose exposure from Taiwan's studies and they proposed to examine the risk of arsenic exposure among smokers (Tsuji, Alexander, Perez, & Mink, 2014). In our unmatched control study, after pursuing different model analysis, we could identify the interaction of drinking and being male, as well as smoking (data not shown). We found that drinking is marginally associated with increased DM type2 risk ($p = 0.055$) when keeping male and exclude smoking in analysis model. However, when excluding both male and smoking variables, the association was less strong ($p = 0.073$). It

would be useful to find out whether different diet habits among smokers and never smokers could contribute to this outcome result or not. Chen et al.(2011) observed synergetic effect on ischemic heart disease of individual after exposing both cigarette smoking, and arsenic at concentrations as low as 0.0253- 0.1140 mg/L (Y. Chen et al., 2011). Pan et al. (2013) did a case control study in Bangladesh to investigate association of DM type2 and moderate dose of arsenic exposure, and their results suggested that being overweight, smoking, and arsenic exposure increased risk of DM type2 (Pan et al., 2013).

In our unmatched control study, after pursuing different model analysis for only socioeconomic variables, we could identify the interaction of drinking and being male, as well as smoking. We found that drinking is associated with increased DM type2 risk (p= 0.049, OR= 3.446) when keeping male and excluding smoking in analysis model consisting of being male, age year 2008, BMI year 2008, exercise, having history of illness in parents, history of illness in siblings, drinking, having motorcar (represent a better economic status). However, when both male and smoking variables were excluded in the model, the association is less strong (p= 0.064, OR=3.016).

In the matched control group, we found that drinking consistency associated with higher DM type2 risk, even when we combined selected socioeconomic variables (BMI year 2008, exercise, history of illness in siblings, drinking, being government official or having own business , having motorcar (represent a better economic status) with arsenic relating metrics in the analysis model. The association of DM type2 and drinking is even stronger (p= 0.001, OR= 11.260) when smoking was further excluded from the model. For the difference between the estimates for drinking in the unmatched and matched case-control groups, we have no convincing explanation for this. Our findings were generally similar for the other sociodemographic variables. Study on effect of synergistic interaction between arsenic exposure at low to moderate dose and smoking, drinking on DM type2 is needed.

Having motorcar, representing better economic status is found to be consistency strongly associated with lower DM type2 risk in both unmatched and matched control groups. One possible explanation is that those who have enough income to buy and drive motorcar (including motorcycle) could access more choices of water source that usually had low arsenic contamination such as rain water, or bottled water (product from outside study area). However, cautious interpretation is required here; the finding might be related to other factors such as gender, lifestyles.

We did not find association between residency in different Moo Ban (Moo 2: reference, Moo 12, Moo13), length of resident in this area and DM type2 risk in both unmatched and matched control groups. Our study population spent 38 years, on average, staying in these 3 Moo Ban. We selected Moo 2 as reference group just because its location is not in the municipal area as Moo 12 and 13. Skin lesions (having symptom of melanosis or hyperkeratosis), married status (actively married), higher educational, occupation (Labors and other: reference, being farmer, government official/having own business) were not found to be associated with DM type2 risk either. There is different level of arsenic contamination in environment in the study area, especially in water that the residence of those 3 Moo Ban used for their consumption. Its range was between less than detection limit of 0.001 mg/L to 8.583 mg/L.

So far, though many research groups had tried to establish prospective case cohort study, a causal relationship between low dose arsenic exposure and DM type2 could not be established and limited sample size was found to be the major limitation (Kuo, Moon, Thayer, & Navas-Acien, 2013).

Similarities of significantly association results between independent variables like BMI, history of illness in siblings, having motorcar and DM type2 risk were identified in both set of controls; unmatched and matched. Comparing similar constructed models, we found that exercise, and drinking was associated with DM type2 risk only in the matched control setting whereby age and sex were matched. Results regarding no meaningful associations between arsenic-related independent variables and DM type2 risk in every related constructed model were identified in both unmatched and matched control studies. The consistency findings in both unmatched and matched control groups allowed us to be more confident in our association analysis results in general. Future study in prospective cohort design could yield fruitful results to contribute to the inconsistence epidemiological findings on association of low to moderate arsenic exposure and DM type2 risk worldwide ((C. F. Huang et al., 2011), (James et al., 2013)).

Strength of this study

This study has the strongest point of the arsenic exposure estimation via the availability of good arsenic concentration data and the questionnaire response regarding purpose of use of each water type for both year 2000 and 2008. This allowed us to specify exposed arsenic concentration at individual level according to purpose of use for consumption. Water samples were analysed in accredited laboratory (certified ISO17025, ISO15189) of the Department of Medical Sciences, using established and validated methods, thus, the quality of arsenic concentration results was ensured. All related data was collected by trained personals according to developed guidelines.

Limitations and uncertainties

In this study, some limitations with missing data exist. Inappropriate handling of missing data or missing data itself may lead to bias and loss of information (Sterne et al., 2009). Multiple Imputation (MI) technique with Predictive Mean Matching (PMM) method attached in SPSS v22.0 (IBM) was used to reduce risk of bias. To prevent negative value after MI of quantitative variables that is not normally distributed like arsenic concentration in different water types, the Predictive Mean Matching (PMM) (as imputation method in method subcommand of MI, SPSS version 22.0) was used (Allison, 1999). To reduce uncertainty, variables that have percentage of missing value more than 80% were not imputed or included in the model.

Unavoidable uncertainty could occur due to: (1) fluctuation in exposed dose of arsenic and exposed time; (2) time for collection of water samples to measure arsenic concentration might or might not correspond with actual exposure point; and (3) indirect measurement of exposure level (measure arsenic level in water in each household), since confirmation of exposure via biological samples in each individual was not available due to high cost of analysis of arsenic in nails samples, thus the data was not available.

Uncertainty could also occur from a number of following assumptions:

- Only consumption of arsenic contaminated water is considered as the main route of exposure in this study.
- Inorganic arsenic is considered the most toxic form, according to a toxicological point of view, its concentration in water was not measured in this study. Thus, it is assumed that all "total arsenic" in water that was measured is inorganic arsenic form.
- It was also assumed that arsenic concentration in rain water collected from each household in the study area in the year 2000's study should not be significantly different from that of the year 2008. Since rain water was not collected in the year 2008's study, the results of arsenic concentration of the year 2000's study were used in 2008 study as well.
- The intake of arsenic from food sources is neglected, and water used for cooking (CW) is accounted instead. We did not investigate consumption of seafood of individual, even though it could be considered as another source of arsenic exposure.
- Teeth brushing water was considered as oral-accidental exposure source in this study. Villagers did not use bathtub when taking a bath, according to results of field observation during house visits of the year 2000's study. They used cement or plastic tanks or earth jars to collect water for bathing and teeth brushing and used a bowl to shower. They also brush their teeth while bathing in the morning and evening.

Conclusions and recommendations

To conclude, our analysis suggested no convincing association of water arsenic concentration with diabetes risk in both unmatched and matched controls studies. Further research is needed on this topic. The findings regarding sociodemographic information of the unmatched case control study confirm that older age, BMI, having history of illness in siblings were associated with higher DM type2 risk, whereas having motorcar, representing better economic status, is associated with lower DM type2 risk in the study area. In the matched case-control study, BMI, exercise, having history of illness in siblings, drinking were associated with higher DM type2 risk, whereas having motorcar, representing better economic status, was associated with lower DM type2 risk. The findings for sociodemographic variables tend to confirm the overall validity of modelling strategy.

We proposed to establish a case-cohort study in this area to estimate lifetime arsenic exposure of individual, to inspect causal relationship between chronic arsenic exposure and other diseases. Multidisciplinary team of family doctors should be equipped with the message of DM risk determinants when they plan for household visit. Arsenic level in water used for cooking, especially village tap water, should be closely monitored.

APPENDIXES

APPENDIX A

Questionnaire to study arsenic exposure among villagers of Moo 2, 12, 13
, Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat Province
Date/Month/year (of interview and collection of water samples)…….. For coding

Please mark "/" in the small box in front of chosen information or fill in the blank

I Personal Information

1. Name-Surname……
   ID……
2. Age……year……Month; Date of birth (dd/mm/yy)…. ……..□□□□
3. Sex □ 1. Male □ 2. Female □
4. Marriage status □ 1.single □ 2. Married □ 3. Divorce □
   □ 4. Widow □ 5. Separate
5. Highest educational level □
   □ 1. Not study □ 2. Elementary school
   □ 3. secondary school □ 4. High school / Vocational certificate
   □ 5. Higher vocational certificate /Diploma □ 6. Bachelor degree
   □ 7. Higher than Bachelor degree
6. Present address: House No…….Moo……..Sub-district…….. □□□□
   District……Province…… □□□□
7. How long have you been in this house/or this village? □□□□
   ……year…….Month
8. Previous occupation □
   □ 1. Agriculturalist □ 2. Government employee □ 3. Merchandise
   □ 4. Labors □ 5. Others…….
9. Do you ever work or stay in other sub-district more than 6 months?
   □ 1. Yes □ 2. Never □
   <u>If yes:</u> please identity the period of that staying
   From; month……year……To; Month…….year…….
   Total period…….year……month
10. Present occupation
    □ 1. Agriculturalist □ 2. Government employee □ 3. Merchandise □
    □ 4. Labors □ 5. Others…….
11. How long have you worked for this job? ……year □□
12. Present working address: Moo……Sub-district…… □□□□
    District……..Province……. □□□□

2. Exposure Information

1. Are you smoking?

□ 1. Smoke □ 2. Not smoke □

If smoke: How long have you been smoking? …….year □□

Type of cigarette - Tobacco leaves)ใบจาก: Baijark(

- Cigarette (มวนขาว: Moun Kao(

Amount smoke per day ……..piece/day □□

2. If do not smoke now, do you ever smoke in the past? □ 1 yes □ 2. Never □

If yes, for how long: ……year □□

3. How often do you drink alcohol? □

□ 1. Every day □ 2. 4 days/week □ 3. Once a week □ 4. Twice a month

□ 5. Once a month □ 6. Only in ceremonial events, but less than 10 times a year □ 7. Never drink

4. How much do you drink each time? □

Rice whisky……glass Beers……glass

Liquor/whisky ……glass others ……..

(Ranking criteria; answer 1-2 = minor drink, 3-4 = moderate drink, 5-6 = heavy drink)

5. How long have you been drinking? ……year □□

6. Are you still drinking alcohol? □ 1. Drink □ 2. Not drink □

7. How many glasses of water do you drink per day? ……..glass (=……CC) □□

8. What type of water does you most frequent drink? □

□ 1. Rain water □ 2. Tap water □ 3. Well water

□ 4. Bottle water □ 5. Mineral water

□ 6. Others……..

9. Specify action normally taken before you drink water: □

□ 1. Do nothing □ 2. Boiling

□ 3. Filtering □ 4. Leave it to precipitate □ 5. Others……

10. Do you ever change source of drinking water? □

□ 1. Change □ 2. Never change

If change, when? ……../……../……..

Please identify old source: (specify old location)……

(Identify used period)……year □

Please identify new source: (specify new location)……

(Identify past to present used period)……year □

If you used to drink shallow well water, please specify the period

Start from: mm …. / yy….. To: mm……/ yy……Total … ....year □

Location of shallow well water: House No. ……Moo……. □□

11. Specify type of container used to collect drinking water: □

□ 1. Not collect □ 2. Metal tank □ 3. Cement tank

□ 4. Water jar □ 5. Glass bottle □ 6. Plastic bottle

□ 7. Others ……..

12. Specify type of water used for everyday-cooking: □

□ 1. Rain water □ 2. Tap water □ 3. Well water

□ 4. Bottle water □ 5. Mineral water

□ 6. Others……..

13. Specify action normally taken before you use water for cooking: □
□ 1. Do nothing □ 2. Boiling
□ 3. Filtering □ 4. Leave it to precipitate □ 5. Others……

14. Specify type of container used to collect cooking water: □
□ 1. Not collect □ 2. Metal tank □ 3. Cement tank
□ 4. Water jar □ 5. Glass bottle □ 6. Plastic bottle
□ 7. Others ……..

15. Specify bathing water: □
□ 1. Rain water □ 2. Tap water
□ 3. Well water □ 4. Others ……..

16. Specify type of container used to collect cooking water: □
□ 1. Not collect □ 2. Metal tank □ 3. Cement tank
□ 4. Water jar □ 5. Glass bottle □ 6. Plastic bottle
□ 7. Others ……..

17. What type of food source did you eat yesterday? (Can choose >1 answer) □
□ 1. Fresh water food (fish, Shrimp, mussel) □ 2. Seafood
□ 3. chicken, pork, beef □ 4. Fruits, vegetables
□ 5. Others……

18. Identify types of food you eat most frequently (answers can be more than 1): □
□1 Fresh water food (fish Shrimp, mussel) □ 2 seafood
□ 3 Chicken, pork, beef □ 4 Fruits, vegetables

19. How many times a week does you eat seafood? □
□ 1. Not eat □ 2. 1-4 times □ 3. 5-8 times
□ 4. 9-12 times □ 5. Others……..

20. How many times did you eat seafood in the last 7 days? □
□ 1. Not eat □ 2. 1-4 times □ 3. 5-8 times
□ 4. 9-12 times □ 5. Others……..

21. Identify types of drug you ate yesterday: (answers can be more than1) □
□ 1. Not eat □ 2. Bolus □ 3.Herbal medicine
□ 4. Prescript drug…….

22. Identify the most frequent take drugs: □
□ 1. Not eat □ 2. Bolus □ 3.Herbal medicine
□ 4. Prescript drug…….

III. Health Information

1. Have you ever been sick from following list of diseases since childhood?

- Goiter	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- Toxic thyroid gland	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- Asthma	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- High Blood Pressure	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- Diabetes	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- Jaundice	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- convulse	□ 1 Yes	□ 2. No	□ 3. Don't know	□
- Lung tuberculosis	□ 1 Yes	□ 2. No	□ 3. Don't know	□

- Kidney disease □ 1 Yes □ 2. No □ 3. Don't know □
- Cancer □ 1 Yes □ 2. No □ 3. Don't know □
- Accidents □ 1 Yes □ 2. No □

If yes; please give more details on that diseases such as when it happen, for how long, any type of treatment (please mention every one of them):

……
……

2. Have you ever been in operation by any mean? □

□ 1. Yes □ 2. No

If yes, Please specify (every time)…….

When it happen? ……..…/……..…/……..

3. Have you ever been admitted in hospital?

□ 1. Yes □ 2. No □

If yes; Please give diseases' name, type of treatment and period you stayed in hospital:

……
……

4. Did you ever have your hair and nails cut by medical doctor or nurse for arsenic analysis?

□ 1. Yes □ 2. No □

If yes, When? ……../……../…….

Do you know the result? It yes, please specify: ……..

5. Have you ever been told by health official that you are sick because of arsenic poisoning?

□ 1. Yes □ 2. No □

If yes, specify when you were firstly notified: year……

6. Have you ever been treated for arsenic poisoning?

□ 1. Yes □ 2. No □

If yes, where did you go for treatment? For how long did you have been treated?

……
……

If you have been treated, please specify type of treatment: □

□ 1. Drug prescription □ 2. Drug injection

□ 3. vanishing cream □ 4. Others……..…

Identify drugs' name, if possible:

……

7. Did your closed relative have diabetes? □

□ 1. Not have □ 2. Have, identified……. □ 3. Don't know

8. Do you have any skin disease? □ 1. Have □ 2. Not have □

In case of abnormal skin

9. When the symptoms begin, and for how long? ……year □□

10. What did you do with this skin problem, have you been treated? □□

□ 1. Not treated □ 2. Treated by medical doctor

□ 3. Treated by someone else Identify…….

11. Where did you go for treatment of your skin problem? □

□ 1. At home □ 2. Go to hospital, Name: ……

□ 3. Go to primary health care unit, Name: …….

□ 4. Others……..

12. Identify the most frequent skin symptoms you encountered:

……
……

13. What do you think about your health, in general, when compared to the others at your age? □

□ 1. Excellent □ 2.Good □ 3. Sufficient

□ 4. Not good □ 5. Don't know □ 6. Not answer

Interviewers' name……..

Date……Month……..Year……..

Note: Sample collection (Please check):

Water collection

…Rain water ……2. Tap water …….3. Well water ……4. Bottle water

Urine collection: ……..Yes …….No

External Body examination: screening (by nurse)

Blood pressure_____/_____pulse_____/minute

General characterization:_____

List	Normal	Abnormal	Opinion
Sentiment			
Skin			
Eyes			
Ear neck, nose			
Mouth, tongue, teeth			
Neck, Thyroid gland			
Chest			
Heart			
Abdominal pain			
Nervous system			
Others			
Conclusion	1 = Normal…	2 = abnormal…… 3. = not sure…….	

Inspector's name: ……

Skin examination for those suspected of having chronic arsenic poisoning (By specially trained nurse only)

Skin color or skin pigmentation (melanosis)

_____ 1. **White spot** scattering on the body 10 -50 spots _____ 2. Rain drop

_____ .3 Black skin color especially on palms and soles

1. **Hyperkeratosis**

_____ Level 1 small lump < 5 _____ Level 2 small lump > 5

_____ Level 3 _____ Level 4

Inspector's name: ……

APPENDIX B

Worksheet for Health risk screening program (2008 study)

☐ Access to social security Government employee Access to health security

No. ID Address: House No. …… Moo …….. Tambon……..
□ - □□□□ - □□□□□ - □□ - □
Interviewee: Name……. Surname…….. Age……y Date of Birth……..
Social security number (For social security system)
□□ - □□□□□□□ - □ Branch □□□□□□
Name of health office …….
District …….Province……..
Screening Date…….

1. Family information
   1.1 Does your parent have history of illness from any of following diseases?

   ∗□ Diabetes (DM) □ Hypertension (HT) □ Gout
   □ Chronic renal failure (CRF) □ Myocardial Infarction (MI) □ Stroke
   □ Chronic obstruction pulmonary disease (COPD) □ don't know
   □ Others (e.g. blind, amputation of leg)……
   ♥□ Paralysis, myocardial ischemia □ don't have

   1.2 Do any of your brothers and sisters have history of illness from any of following diseases?

   ∗□ Diabetes (DM) □ Hypertension (HT) □ Gout
   □ Chronic renal failure (CRF) □ Myocardial Infarction (MI) □ Stroke
   □ Chronic obstruction pulmonary disease (COPD) □ don't know
   □ Others (e.g. blind, amputation of leg)……
   ♥□ Paralysis, myocardial ischemia □ don't have

2. Have you ever been ill or went to see doctors due to any of following diseases or symptoms?

2.1	Diabetes (DM)	∗□ have	□ not have	□ never check
2.2	Hypertension (HT)	□ have	□ not have	□ never check
2.3	Liver disease	□ have	□ not have	□ never check
2.4	Paralysis	♥□ have	□ not have	□ never check
2.5	Heart disease	♥□ have	□ not have	□ never check
2.6	Abnormality of cholesterol in blood	♥∗□ have	□ not have	□ never check
2.7	Amputation (arms, legs)	□ have	□ not have	
2.8	Give birth & child wt. > 4 Kg	∗□ have	□ not have	
2.9	Drinking water more than usual, and more frequently	∗□ have	□ not have	
2.10	Night urinate more than 3 times	∗□ have	□ not have	
2.11	Getting slimmer, instead of eating more	∗□ have	□ not have	
2.12	Weight Lost /be fatigued	∗□ have	□ not have	
2.13	Having lib blemish often, & slowly heal	∗□ have	□ not have	
2.14	Itchy of skin, reproductive organ	∗□ have	□ not have	
2.15	Eyes is blurred, change eyeglass often	∗□ have	□ not have	
2.16	Beriberi, Without cause	∗□ have	□ not have	

3. If you had history of illness as marked in question no2, what did you do about it?
   □ Still treating/ follow doctor's instruction □ treat, but infrequent
   □ Used to treat but not now/ buy and take drug myself

4. Do you smoke? (Risk = smoke >1 piece/day)
   □ Smoking……piece/day; Type of cigarette ……Period……year (since start to present)
   □ Not smoke

□ Used to smoke but quit, Type of cigarette ……Period……year (since start to quitting)

□ Amount smoke, pack-year……..……..

5. Do you drink alcohol? (Risk = drink >2 times/week)

□ Drink…….times/week (liquor >45 cc/day/ beer > 240 cc/day/ wine > 120 cc/day)

□ Not drink □ Used to drink, but quit

6. Do you exercise or play any sport? (Risk = exercise < 3 times/week)

□ Not at all

□ Yes, less than 3 time per week

□ Yes, 3 times a week, 30 minutes each time, very often

□ Yes, >3 times a week, 30 minutes each time, very often

□ Yes, every day, 30 minutes each time

7. What type of food/taste do you like when eating food?(answer can be >1)(Risk =select any one, or more)

□ Sweet □ Salty □ oily □ don't like any of these

8. Do you drive motorbike or car? (Risk = not ware helmet, not tie seatbelt)

□ Not drive motorbike / do not use car

□ Drive motorbike/ car, and always use helmet or tie seatbelt

□ Drive motorbike/ car and, sometime, use helmet or tie seatbelt

□ Drive motorbike/ car and use helmet or tie seatbelt only when seeing police at check point

9. Do you use condom when having sexual relationship with either your partner or someone else that is not your husband or wife?

□ Use every time □ use only when requested

□ not use

□ Never have sex with someone else □ not answer

.....

Body examination

Name-Surname.....Gender.....

1. Weight.....Kg Height.....cm BMI……Risk = >23 for female,>25 male)

□ 1. Less than normal (<18.5) □ 2. Normal(22.9-18.5) ∗◷ □ 3. Plump(24.9-23)

∗◷♥□ 4. Fat (30-25) ∗◷♥□ 5. Very fat)>30)

2. Waistline…….inch/cm, waist hip…….Inch/cm □ 1. Normal ◷□ 2. Risk

3. Blood Pressure; 1st measurement……mm.Hg, 2nd measurement……mm.Hg
Average blood pressure……mmHg

□ 1. Normal (<129/79 mmHg) ◷□ 2. Risk (≥130/80 – 139/89)

∗◷♥□ 3. Suspect of high blood pressure (>140 mmHg)

4.1 Blood glucose……mg/dl; finger puncture (use DTX, a machine), before eating breakfast

□ 1. Normal (<100 mg/dl) ∗◷□ 2. Risk (100 – 125 mg/dl)

∗◷♥□ 3. Suspect of DM (>126 mg/dl)

4.2 Finger puncture after breakfast……hr. Blood glucose……..mg/dl (ref. value for blood glucose after breakfast 2 hr.

□ 1. Normal (<140 mg/dl) ∗◷□ 2. Risk (140 – 199 mg/dl)

∗◷♥□ 3. Suspect of DM (≥200 mg/dl)

5. Anemic looking □ 1. Pale □ 2. Not pale

6. Examination, in general……

Risk Conclusion (by doctor/nurse/health official)

( ) 1. Not found ( ) ◷2. Risk found (1 in 7 or more than 1 of symbols◷)

( ) 3. Chronically ill from disease…… ( ) ∗ 4. DM Risk

( ) ♥ 5. Risk of paralysis □ extremely high (>5) □ very high (3-5)□ high (2)

Recommendation

( ) Frequently exercise, 30-45 minutes each day, at least 3 times a week

( )Control eating oily food, reduce salty food

( )Control food with high carbohydrate (starch, sugar, sweet), increase eating vegetables

( )Reducing, stopping, quitting of smoking, caffeine drinking, alcohol drinking

( )Make appointment to measure blood pressure again.

At.....Date.....

( )Make appointment to recheck DM (Fasting blood sugar)

at.....Date.....

( )Recommend for further examination ……..

( )Sent for further treatment at

Examined by

(name).....Position.....

APPENDIX C

Worksheet for water collection2008

This form is used by only trained village health volunteer, to collect all types of water from each household and to interview representative person in that house

.1Name..... Surname.....

.2Date of birth.....Present age .....year

.3House No .....Moo.....

4. Please specify type of water used in your household

4.1 Drinking water	□ municipal tap	□ village tap	□ bottle water
	□ Well water	□ rain water	□ filtered water
4.2 Cooking water	□ municipal tap	□ village tap	□ bottle water
	□ Well water	□ rain water	□ filtered water
4.3 Bathing water	□ municipal tap	□ village tap	□ bottle water
	□ Well water	□ rain water	□ filtered water

Water sample collection

(Please make "x" in front of collected type of water)

□ Municipal tap water
□ Village tap water
□ Well water
□ Filtered water

Note: Do not collect rain and bottle water

APPENDIX D

Approval letter from ehhical committees

บันทึกข้อความ

ส่วนราชการ สำนักวิชาการวิทยาศาสตร์การแพทย์ โทร. 99360

ที่ สธ 0625/ 0387 **วันที่** 30 เมษายน 2552

เรื่อง ส่งรายงานการประชุมคณะกรรมการพิจารณาการศึกษาวิจัยในคนกรมวิทยาศาสตร์การแพทย์

เรียน นางสาวขวัญยืน ศรีเปารยะ

ตามที่ได้มีการประชุมคณะกรรมการพิจารณาการศึกษาวิจัยในคน เมื่อวันศุกร์ที่ 24 เมษายน 2552 ณ ห้องประชุม 216 อาคาร 3 ชั้น 2 กรมวิทยาศาสตร์การแพทย์ นนทบุรี สำนักวิชาการวิทยาศาสตร์การแพทย์ จึงขอส่งรายงานการประชุมดังกล่าวรายละเอียดตามเอกสารแนบท้าย หากมีข้อแก้ไขประการใดโปรดแจ้งให้สำนักวิชาการฯ ทราบภายในวันพุธที่ 6 พฤษภาคม 2552 หากพ้นกำหนดถือว่ารับรองรายงานการประชุมดังกล่าว

จึงเรียนมาเพื่อทราบ

(นางสาวมานัญยา เอี่ยมเล็ก)
กรรมการและผู้ช่วยเลขานุการ
คณะกรรมการพิจารณาการศึกษาวิจัยในคน
กรมวิทยาศาสตร์การแพทย์

ทั้งนี้ นายแพทย์อาชวินทร์ โรจนวิวัฒน์ ได้มีการประสานกับทางสำนักงานคณะกรรมการอาหารและยา และทั้งนี้ได้มีหนังสือผ่อนผันจากทางสำนักงานคณะกรรมการอาหารและยาแล้ว

ที่ประชุมรับทราบ และมีมติอนุมัติให้ดำเนินโครงการได้

3.2 โครงการเดิมเรื่อง "การศึกษาความสัมพันธ์ระหว่างการเกิดโรคเบาหวานกับการได้รับสัมผัสสารหนูในระดับกลางและต่ำในประชากร ตำบลร่อนพิบูลย์ จังหวัดนครศรีธรรมราช ประเทศไทย" โดยมี นางสาวขวัญยืน ศรีเปารยะ สถาบันวิจัยวิทยาศาสตร์สาธารณสุข เป็นหัวหน้าโครงการวิจัย

ที่ประชุมรับทราบ และมีมติอนุมัติให้ดำเนินโครงการได้

3.3 โครงการเดิมเรื่อง "การศึกษาวิวัฒนาการของเชื้อไข้หวัดใหญ่ทัยป์เอ (H1 และ H3) และทัยป์บีที่แยกได้ในประเทศไทย" ของสถาบันวิจัยวิทยาศาสตร์สาธารณสุข โดยมี นางสาวมาลินี จิตตกานต์พิชย์ สถาบันวิจัยวิทยาศาสตร์สาธารณสุข เป็นหัวหน้าโครงการวิจัย

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APPENDIX

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