

CHAPTER 1

INTRODUCTION

1.1 Statement and significance of problem

Persistent organic pollutants (POPs) are chemicals that are toxic, persist in the environments for long periods, bio-accumulate through food chain, and pose a risk of causing harmful effects on wildlife and humans. POPs can be classified in endocrine disrupting chemicals (EDCs), acting as hormone receptor agonists and antagonists (Colborn et al., 1993; Kelce et al., 1997; Longnecker et al., 1997; Turusov et al., 2002). Because of their long-term adverse effects on living organisms, POPs have been banned in most developed countries. However, it is still used in some developing countries for essential public health purposes (Curtis and Lines, 2000; Turusov et al., 2002).

Several studies showed that exposure to POPs, and dichlorodiphenyltrichloroethane (DDT) in particular, was linked to adverse reproductive effects in mammals, birds, amphibians, and fish (Colborn et al., 1993; Guillette, Jr. et al., 1994, 1996; Vos et al., 2000). In addition, POPs have been mentioned as goitrogenic chemicals that disrupt thyroid hormone system (Capen, 1994). However, it was rather difficult to conclude which particular causal agents produced the hormonal effects.

Epidemiological human data regarding health effects of POPs are generally lacking. Most studies in men used end points of adverse reproductive effects such as decreased sperm counts, reproductive tract abnormalities, infertilities, and reproductive cancers (Ayotte et al., 2001; Longnecker et al., 2002; Skakkebaek, 2002; Dalvie et al., 2004a). Few previous studies investigated the effects of POPs on hormonal status in adult men, and available data were inconclusive. In fact, the hormonal actions of POPs could be expected to have some impact on secretion of endocrine releasing factors, resulting in varying effects on reproductive hormonal metabolism. Further hormonal disturbance or imbalance could potentially occur in organs of the endocrine system (Jaga, 2000). Most studies in mother-infant pairs have considered prenatal and postnatal exposure to POPs, however, the studies regarding their effects on reproductive and thyroid hormone levels are lacking. Since exposure to POPs during fetus development may result in a permanent change of endocrine system and neurodevelopment in infants, adverse health effects on infants are of concern (Dorea et al., 2001; Sala et al., 2001a).

In northern Thailand, POPs were widely used for malaria vector control programs and farming purposes. The predominant chemicals detected in humans were DDT and its metabolites. Stuetz et al. (2001) reported that total DDT levels in human milk of women from Mae Sa Mai Village were still high with a median and a maximum level of 209 and 2,012 ng/ml, respectively. DDT was first introduced in 1949 and finally stopped altogether for public health in 1999. DDT was extensively used for farm activities until it was banned in 1983, but most farmers illegally continued using in spite of its ban (Chareonviriyaphap et al., 2000; Stuetz et al., 2001).

The present study aimed to investigate POPs contaminated in the environment and humans, and their effects on the human endocrine system. Twelve pig fats from the pigs fed in Mae Rim District of Chiang Mai province were randomized as environmental samples. Ninety-seven adult men from Mae Rim District and 127 mother-infant pairs from Mae Rim and Chiang Dao Districts, Chiang Mai Province, who eligible for the inclusion criteria, willing to participate the study, and signed written consents, were enrolled as studied population. The research study was divided into 3 sections:

Research section 1: Distribution of POPs in the environment.

Research section 2: Effect of POPs on reproductive hormone levels in adult men.

Research section 3: Effect of POPs on reproductive and thyroid hormone levels in infants.

1.2 Literature reviews

1.2.1 Categories and uses of POPs

POPs are chemicals that are toxic, persist in the environments for long periods, bio-accumulate through food chain, and pose a risk of causing harmful effects on wildlife and humans. The initial group of POPs includes 12 POPs in three major categories:

- 1) Organochlorine pesticides (OCPs) include aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex and toxaphene.
- 2) Polychlorinated biphenyls (PCBs), which are used in electrical transformers, paint additives, and plastics

- 3) Chemical by-products from combustion, such as dioxins and furans, which are produced unintentionally from most forms of combustion.

OCPs were widely used for insect control and malaria vector control programs. The banned years of individual OCPs in Thailand are presented in Table 1.1. Uses of OCPs, and DDT in particular, enormously increased after the second World War because of their effectiveness against mosquitoes and other insects. However, problem related to extensive use of DDT began to appear in the year after the 1940s. Consequently, DDT has been banned since the 1970s in most developed countries although it is still used in some developing countries for essential public health purposes (Turusov et al., 2002). In Thailand, DDT for farming purposes has been banned since 1983; however, its use for public health was completely banned in 1999 (Chareonviriyaphap et al., 2000; Stuetz et al., 2001).

Table 1.1 The banned years of individual OCPs in Thailand

OCPs	The banned year
aldrin	1988
chlordane	2000
DDT	1983 (Farming purposes) 1999 (malaria control programs)
dieldrin	1988
HCB	1980
Heptachlor	1988
Toxaphene	1983

Source: www.itdoa.com/crop_itda/toxic.htm, 2002.

Although POPs have been banned for long periods, they are still found in ecosystem. The current intakes of OCPs in Asia are up to 100 times greater than those in developed countries, and the estimated intake of DDT by children is reported to be 100 times greater than the acceptable daily intake (ADI) established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (Turosov et al., 2002).

1.2.2 Exposure to POPs and their environmental and health effects

POPs can be found in every level of food chain. Humans are placed at the top of food chain, therefore high levels of POPs have been found in the human body (Ataniyozawa et al., 2001; Ntow et al., 2001). Once inside the body, they can be readily stored in fatty tissue where they don't exert any effects. However, when fat is mobilized for energy, these chemicals are consequently released into bloodstream and caused adverse health effects. In pregnant women, these contaminants are also released from fatty tissue into the bloodstream during pregnancy and breastfeeding and subsequently transferred to their offspring. Since fetuses and infants may not have adequate defense mechanisms to combat exposure to toxicants, they are therefore vulnerable group to their toxic actions (You et al., 1999; Miller et al., 2002). Some studies have mentioned that exposure during fetus development may result in a permanent change of immune and endocrine system, and neurodevelopment in infants even at low levels of prenatal exposure (Dorea et al., 2001; Sala et al., 2001a).

A number of the reports have indicated that many synthetic chemicals, and POPs in particular, act as endocrine disrupting chemicals (EDCs) (Table 1.2). These chemicals can exert their effects in many ways. They can bind to hormone's receptor and mimic the hormone action. Alternatively, they can stimulate or inhibit enzymes responsible for synthesis or clearance of the hormone, and thereby give rise to an increase or decrease action of the hormone. Studies in field and laboratory animals have found that exposure to POPs was linked with abnormal hormonal status, reduced fertility, undescended testicle, altered sexual behavior, and feminization of male (Colborn et al., 1993; Guillette, Jr. et al., 1994, 1996; You et al., 1998; Mills et al., 2001). Human epidemiological studies have concerned the association between exposure to these contaminants and endpoints of adverse health outcomes. It has been linked with an increase incidence of reproductive tract abnormalities, decreased sperm quantities and qualities, and reproductive cancers (Ekbom et al., 1996; Hoyer et al., 1998; Ayotte et al., 2001; Skakkebaek, 2002; Hauser et al., 2003; Saiyed et al., 2003). The potential human effects of individual POPs are presented in Table 1.3. However, few previous studies investigated their effects on hormonal status in humans and available data are inconclusive.

Few studies investigated the adverse health effects of POPs on adult men. DDT in North Carolina farmers, Latvian and Swedish men, and Italian men found no effect on reproductive hormonal status (Hagmar et al., 2001a; Martin, Jr. et al., 2002; Cocco et al., 2004). On the other hand, the studies in Mexican men and South African male workers found an effect on hormonal status. The study in Mexican men found a

negative association of plasma *p,p'*-DDE levels with the ratio of bio-available to total testosterone, semen volumes, and sperm counts (Ayotte et al., 2001). The study in South African male workers found positive associations of *p,p'*-DDT and *p,p'*-DDD with E₂ and testosterone; however, they suggested that these associations might be due to chance, since *p,p'*-DDT and *p,p'*-DDD were not known to be a strongly anti-androgenic or estrogenic (Dalvie et al., 2004a).

Adverse health effects on pregnant women and infants: POPs have an ability to transfer from mothers to fetuses through placenta barrier (Kanja et al., 1992; Bjerregard and Hansen, 2000; Waliszewski et al., 2000; Sala et al., 2001a; Butler et al., 2003). Some epidemiological studies have related prenatal exposure to these contaminants with an increase risk of preterm birth, low birth weight, birth defect, acute infection, cryptorchidism, and hypospadias (Longnecker et al., 2001, 2002, Ribas-fito et al., 2002; Dallaire et al., 2004; Salazar-Garcia et al., 2004).

Field and laboratory animal studies have reported that exposure to OCPs has an effect on thyroid hormone levels (Scollon et al., 2004; Verreault et al., 2004; Sormo et al., 2005). The study in rats has found increased thyroid weight, reduced iodine transport and increased hepatic metabolism of thyroxine as a result of chronic ingestion of DDT (Fregly et al., 1968).

Table 1.2 Synthetic chemicals known as or suspected to be endocrine disrupting chemicals (EDCs)

HERBICIDES AND FUNGICIDES	
<ul style="list-style-type: none"> • Dichlorophenoxyacetic acid • Trichlorophenoxyacetic acid • Alachlor • Amitrole • Atrazine • Metribuzin • Nitrofen • Trifluralin 	<ul style="list-style-type: none"> • Benomyl • Mancozeb • Zineb • Metriam complex • Maneb • Ziram • Tributyltin • Hexachlorobenzene
INSECTICIDES	
<ul style="list-style-type: none"> • Benzenehexachlorocyclohexane (β-HCH) • Lindane (γ-HCH) • Methoxychlor • Toxaphene • DDT and its metabolites • Carbaryl • Endosulfan • Mirex • Transnonochlor 	<ul style="list-style-type: none"> • Chlordane • Oxychlordane • Dicofol • Heptachlor • Heptachlor epoxide • Dieldrin • Parathion • Methomyl • Synthetic pyrethroids • Chlodecone (kepone)
NEMATOCIDES	
<ul style="list-style-type: none"> • Aldicarb 	<ul style="list-style-type: none"> • 1,2-dibromo-3-chloropropane
INDUSTRIAL CHEMICALS	
<ul style="list-style-type: none"> • Dioxins • Polychlorinated biphenyls (PCBs) • Polybrominate biphenyls (PBBs) • Pentachlorophenol (PCP) 	<ul style="list-style-type: none"> • Phthalates • Styrenes

Source: Pocar et al., 2003.

Table 1.3 Potential human effects of individual POPs

Health Effects	Aldrin, Dieldrin	Chordane	DDT	Toxaphene	Mirex	HCH	PCBs, Dioxin, Furans
Reproduction and development	+	+	+	+	+	+	+
Cytochrome P450 system	+	+		+	+	+	+
Porphyria						+	+
Immune system	+	+	+	+	+	+	+
Adrenal effects			+	+		+	+
Thyroid and retinal effects			+	+		+	+
Mutagenic effects							
Carcinogenic effects	+	+		+	+	+	+
Skeletal changes				+			+

Source: Papiya, 2004.

1.2.3 Measurement of POPs

Measurement of exposure to POPs or EDCs has two methods, measurement of specific chemicals and biologically based methods. Measurement of specific chemicals utilizes special instruments, including gas chromatograph-electron capture detector (GC-ECD) and gas chromatograph-mass spectrometer (GC-MS). The biologically based methods are in vitro assay for assessing estrogenic and anti-androgenic substances, which are as follows: receptor binding assays, cell proliferation assays, receptor-dependent gene expression assays, and immunoassays (Baker, 2001). Receptor binding assays have been widely used because they are easy to perform, rapid, and relatively cheap. Although these assays cannot distinguish between estrogen agonistic and antagonistic effects, they are suited to indicate estrogenic activities of chemicals for a first and/or a large-scale screenings (Baker, 2001; Li et al., 2004). Enzyme-linked receptor assay (ELRA) is a newly developed receptor binding assay for xeno-estrogens (Seifert et al., 1998).

1.3 Objectives

The objectives of this research were divided into 3 sections.

(1) **Objectives of research section 1:**

Distribution of POPs in the environment

It was to investigate types and levels of POPs in pig fats.

(2) **Objectives of research section 2:**

Effect of POPs on reproductive hormone levels in adult men

The objectives of this section were as follows:

(2.1) to investigate types and levels of POPs in adult male plasma.

(2.2) to investigate the association of POP levels with reproductive hormone levels in adult male plasma.

(2.3) to investigate estrogenic activities of chemicals in adult male plasma.

(2.4) to investigate the association of POP levels and estrogenic activities of chemicals in adult male plasma.

(3) **Objectives of research section 3:**

Effect of POPs on reproductive and thyroid hormone levels in infants

The objectives of this section were as follows:

(3.1) to investigate types and levels of POPs in maternal and cord sera.

- (3.2) to compare mean levels of POPs in maternal and cord sera.
- (3.3) to compare mean levels of POPs in mother-infant pairs from Mae Rim and Chiang Dao Districts.
- (3.4) to investigate the association between POP levels in maternal and cord sera.
- (3.5) to investigate the association of POP levels with reproductive and thyroid hormone levels in cord serum.
- (3.6) to investigate estrogenic activities of chemicals in maternal and cord sera.
- (3.7) to investigate the association of POP levels with estrogenic activities of chemicals.

1.4 Scope of the study

1.4.1 Scope of research section 1:

Distribution of POPs in the environment

In January 2003, twelve pig fat samples from Mae Sa Mai Village, Mae Rim District, Chiang Mai Province, were randomized as environmental samples for analysing types and levels of POPs.

1.4.2 Scope of research section 2:

Effect of POPs on reproductive hormone levels in adult men

In May 2003, adult men, aged from 18 to 45 years old and resided in Mae Sa Mai Village, Mae Rim District, for at least 10 years, were invited to participate in the study. Ninety-seven adult men who eligible for the inclusion criteria, willing to

participate the study, and signed written consents were studied population. Fasting venous plasma was collected for analysing POPs, total lipids, reproductive hormones, and estrogenic activities of chemical. POP levels were expressed on a lipid basis.

1.4.3 Scope of research section 3:

Effect of POPs on reproductive and thyroid hormone levels in infants

During March 2003 to June 2004, the pregnant women who had lived in three subdistricts of Mae Rim District (Pong Yang, Mae Ram, and Sun Kha Yom) and eight subdistricts of Chiang Dao District (Maung Na, Maung Ngai, Mae Na, Tung Khoa Paung, Aru No tai, Pang Feung, Pang Ma Yao, and Bann Na Wai) of Chiang Mai Province for at least 5 years were invited to participate in the study. Because iodine deficiency during pregnancy has a strong impact on thyroid status in infants, therefore, the pregnant women who had urinary iodine levels lower than 100 $\mu\text{g/l}$ or who were diagnosed as hypothyroidism were excluded. The pregnant women who eligible for the inclusion criteria, willing to participate the study, and signed written consents were enrolled and interviewed for socio-demographic data. Data on questionnaire consisted of personal data, lifestyle, pesticide use and exposure, and delivery history.

During delivery, one hundred and twenty-seven pregnant women, 39 women from Mae Rim District and 88 women from Chiang Dao District, who were enrolled during pregnancy, had normal delivery and full term gestation were studied population. Maternal serum was collected for analysing POPs, total lipids, and

estrogenic activities of chemicals. Cord serum was analysed for measuring POPs, total lipids, reproductive and thyroid hormones, and estrogenic activities of chemicals. POP levels in maternal and cord serum were expressed on a lipid basis.

1.5 Definitions

- 1.5.1 POPs are organochlorine pesticides, including 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (*p,p'*-DDT), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (*p,p'*-DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (*p,p'*-DDD), 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (*o,p'*-DDT), 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene (*o,p'*-DDE), dieldrin, heptachlor, heptachlor epoxide, beta-hexachlorocyclohexane (β -HCH), gamma-hexachlorocyclohexane (γ -HCH), and hexachlorobenzene (HCB).
- 1.5.2 Estrogen hormone is 17β -estradiol (E_2).
- 1.5.3 Androgen hormone is testosterone.
- 1.5.4 Thyroid hormones are total thyroxine (TT_4), free thyroxine (FT_4), thyroid stimulating hormone (TSH).
- 1.5.5 Adult men are the men aged from 18-45 years old and resided in Mae Sa Mai, Mae Rim District for at least 10 years.
- 1.5.6 Full term gestation is gestation between 36 and 42 weeks.
- 1.5.7 Mother-infant pairs are the mother-infant pairs which the mothers had lived in Mae Rim and Chiang Dao Districts for at least 5 years, and had normal delivery and full term gestation.
- 1.5.8 Low birth weight is weight at birth lower than 2,500 g.

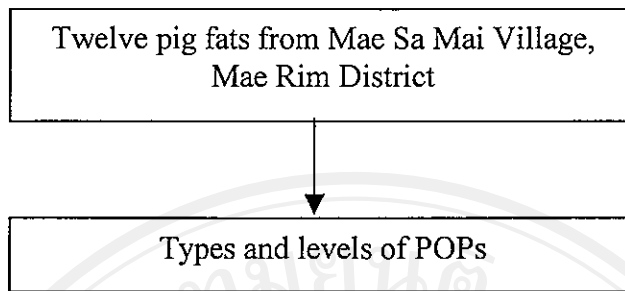


Figure 1.1 Conceptual framework of research section 1:

Distribution of POPs in the environment

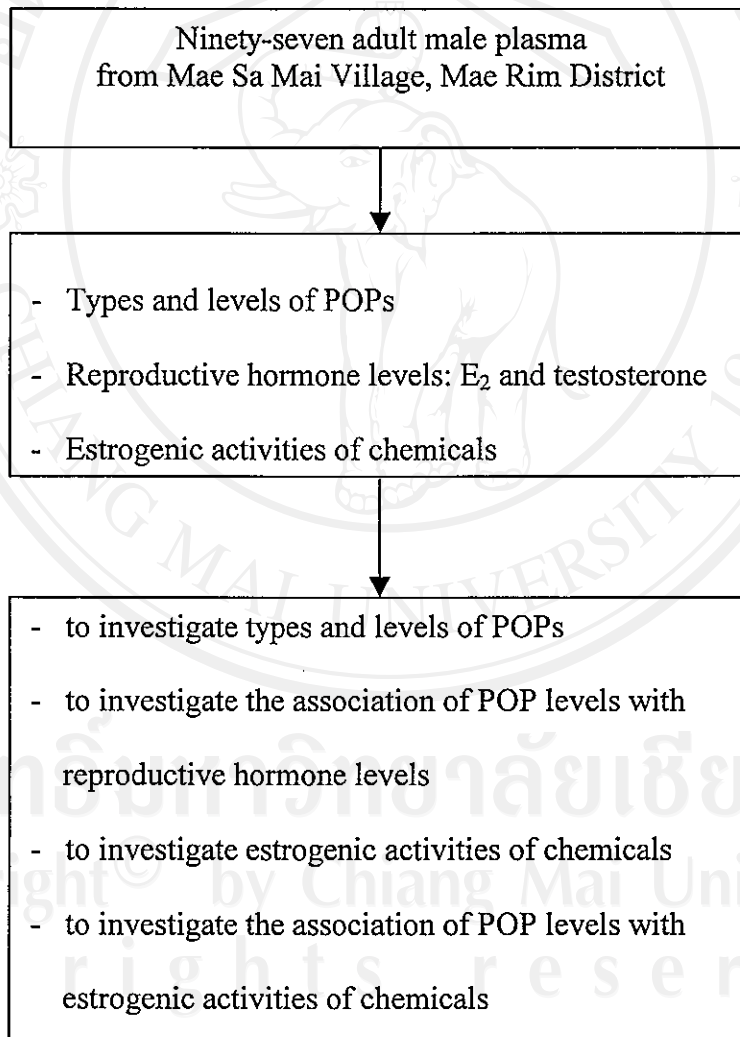


Figure 1.2 Conceptual framework of research section 2:

Effect of POPs on reproductive hormone levels in adult men

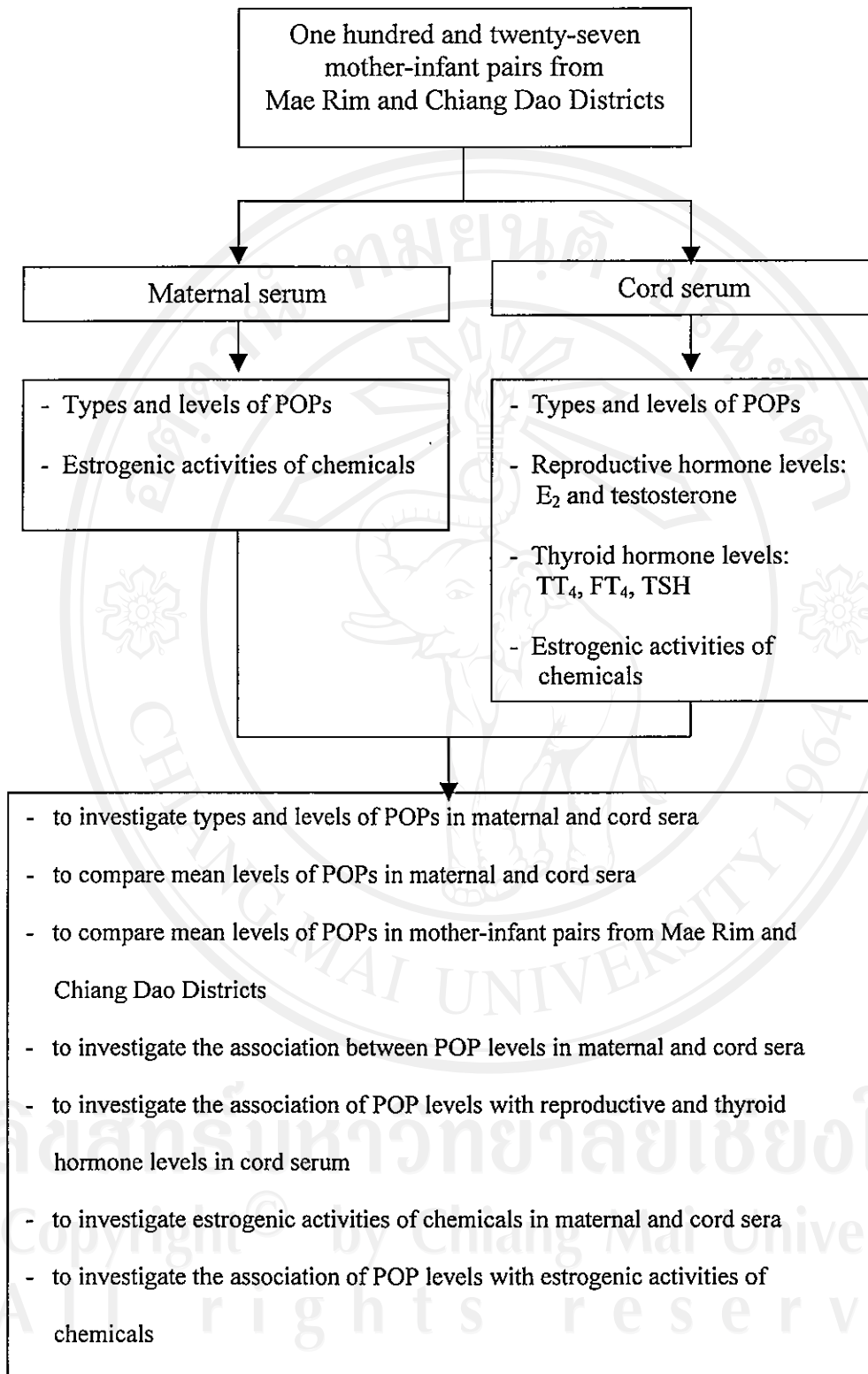


Figure 1.3 Conceptual framework of research section 3:

Effect of POPs on reproductive and thyroid hormone levels in infants