The organochlorine pesticides, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) is a persistent organic pollutant (POP), which historically was used widely in agricultural pest control and for control of vector-borne diseases. DDT is converted in the environment to other more stable forms, including 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (p,p´-DDD) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p´-DDE). Similar to the mother compound these degradation products are highly persistent in the environment, they bioaccumulate, and may undergo biomagnification food chains (Laden et al., 1999; Perry et al., 2005; Chevrier et al., 2008; Thomas et al., 2008). Hence, global contamination by DDTs has caused serious concerns about possible adverse health effects to humans and animals (UNEP, 2001). Several previous studies have used DDT and its degradation products in human blood serum as biomarkers for health effects (Laden et al., 1999; Perry et al., 2005; Tiido et al., 2006; Qin et al., 2011), including possible negative effects of DDT on thyroid hormone function during pregnancy (Asawasinsopon et al., 2006; Chevrier et al., 2008; Langer et al., 2009), the effect of serum concentration of POPs in female during gestation (Meeker et al., 2009; Wang et al., 2009) as well as effect to their childhood (Verhulst et al., 2009). Other studies have suggested associations between organochlorine pesticides and type 2 diabetes as well as cardiovascular diseases (Codru et al., 2007; Rignell-Hydbom et al., 2009; Lee et al., 2011).

Thailand is a tropical country of 500,000 square kilometers with population of approximately 60 million. Agricultural occupation is the foundation of the economy. During the last 2 decades agricultural production and industrialization have steadily increased concomitantly with and increased, import of pesticides. In 1949, DDT was first introduced for a malaria vector control trials in Northern Thailand and was simultaneously used for agricultural pest control. In 1983, the use of DDT in agriculture was banned, except in emergencies. However, as malaria was epidemic and widespread, DDT was used for...
vector control until it was phased out between 1995 and 1999 (Malikul, 1988; Chareonviriyapap et al., 1999). In this study we investigate serum concentrations of \( p,p'\)-DDE and \( p,p'\)-DDT as exposure biomarkers for potential adverse health effect in adult Thai residents and compare the pollutant concentrations with body mass index (BMI), thyroid hormones, cholesterol, triglycerides and fasting blood sugar levels in human serum. We chose to study adult subjects as adults historically have had a higher exposure to DDT than younger individuals.

**MATERIALS AND METHODS**

**Subjects**

We recruited subjects in the sixteen participating regions in Thailand (Fig. 1) during May to August 2011. A total of 1,137 adults (484 males and 653 females; age mean ± S.D. 48 ± 10 and 46 ± 10, respectively) were examined. The study was approved by the Ethical Review Committee for Research in Human Subjects at Department of Medical Sciences, Ministry of Public Health (Nonthaburi, Thailand). All participants signed a written informed consent form with a short questionnaire recording details on age, weight and height.

**Measurements in blood serum**

For each subject 12 ml of blood samples were obtained by venipuncture between 08.00 and 10.00 hr after an overnight fast and allowed to clot at room temperature as well as centrifuged in a refrigerated centrifuge. The blood sample of 10 ml was collected in a Vacuette® silicone tube without an anticoagulant agent for analyses of cholesterol, \( p,p'\)-DDE, \( p,p'\)-DDT, triglycerides and thyroid hormones. A remaining sample of 2 ml was collected in Vacuette® sodium fluoride tube with K3EDTA for analysis of fasting blood sugar. The aliquots of serum were stored at -80°C until being transported to the laboratories analysis. BMI was measured weight/height\(^2\) (kg/m\(^2\)). Serum total cholesterol and triglycerides as well as fasting plasma glucose concentrations were determined using enzymatically methods by Professional Laboratory Management Corp. Co., Ltd. (Bangkok, Thailand), which is accredited for those analyses. The serum total lipids concentrations for cholesterol and triglycerides were calculated by Phillips formula (Phillips et al., 1989; Bernert et al., 2007).

Serum total \( p,p'\)-DDE and \( p,p'\)-DDT concentrations were measured using gas chromatography with tandem mass spectrometry (Agilent 7890 series GC and Agilent 7000 series GC/MS Triple Quadrupole system, Santa Clara, CA, USA) as described by Zhao and Meng (2011). Those standards were purchased from Chem Service (West Chester, PA, USA). Isodrin was used as internal standard. A number of blank and control samples were analyzed together with the sample to verify the accuracy and precision of the measurements. Quantification was achieved using National Institute of Standards & Technology’s certified reference material (Gaitersburg, MD, USA). The limit of quantification (LOQ) was set to ten times the average noise for each compound. None of the compounds were detected in blanks. The LOQ was determined by the lowest calibration level. Quantitative limit was 0.12 \( \mu g/l \) with the correlation coefficients value of 0.9995. The coefficient of variation for the analyzed compound was less than 10%. Recovery varied from 78% to 110% for these compounds. Serum thyroid hormones levels were performed using radioimmunoassay in the Neonatal Screening Operation Center at Department of Medical Sciences (Ministry of Public Health, Nonthaburi, Thailand).

Fig. 1. Map of the study area in Thailand, defined as dense color.
Data and statistical analysis

Data was presented as mean with 95% confidence interval. The statistical analyses were performed using Sigma Plot for Windows (version 11.0, Systat Software, USA). The values below the LOQ were treated as half of this limit. Correlation analysis of serum total \( p,p' \)-DDE and \( p,p' \)-DDT concentrations and body mass index, thyroid hormones levels, serum total cholesterol and triglycerides levels as well as fasting blood sugar levels were conducted using spearman’s correlations \((r_s)\) method. Kruskal-Wallis One Way Analysis of Variance on Ranks was performed to test significance level of serum total \( p,p' \)-DDE and \( p,p' \)-DDT concentrations in each region compare against total amount of serum \( p,p' \)-DDE and \( p,p' \)-DDT concentrations in all regions.

RESULTS

Characteristics of the study subjects for the entire are presented in Table 1. In a total of 1,137 adults (484 males and 653 females) obtained from 16 regions in Thailand. Region P defined as control site that expected to be low background level of exposure to DDT (Fig. 1). We chose the subjects average age of 46-48 (± 10) years old because they were alive longer during the period when DDT was used in Thailand, which had a greater opportunity for high background levels of exposure to this chemical.

Total amount of serum \( p,p' \)-DDT concentration was less than the first quartile of total amount of serum \( p,p' \)-DDE concentration in both subjects (Fig. 2). We assessed the serum total \( p,p' \)-DDE concentration in each region compare with total amount of serum \( p,p' \)-DDE and \( p,p' \)-DDT concentrations of all regions (Fig. 2). The result findings that there was a statistically significant difference between serum total \( p,p' \)-DDE concentration in each region (males: Kruskal-Wallis \( H = 236.368, p \leq 0.001 \) and females: Kruskal-Wallis \( H = 253.231, p \leq 0.001 \)). At least seven regions showed a higher serum concentration of \( p,p' \)-DDE than or equal to the interquartile ranges of total amount of serum \( p,p' \)-DDE concentration from all regions (Fig. 2). There were regions C, I, K, L, M, N & O in both male and female subjects. While the regions I and N showed the highest of serum \( p,p' \)-DDE concentration in both populations. Regions A, B, D, E, F, G, H, J and P were less than the interquartile ranges of total amount of serum \( p,p' \)-DDE concentration in both subjects. The lowest or equal to median of total amount of serum \( p,p' \)-DDE concentration were regions B, E, F, H & J in male subjects and regions A, B, D, E, F & H in female subjects.

The significant positive correlation was found between total amount of serum \( p,p' \)-DDE and \( p,p' \)-DDT concentra-
levels (Table 2). There was no statistically significant relationship between total amount of serum \(p,p'\)-DDT and \(p,p'\)-DDE concentrations with any of serum thyroid hormones (Table 2).

**DISCUSSION**

Pesticides DDT can persist in the environmental for long periods of time tend to their highly resistant to biodegradation and long-range dispersion to regions far from their original such as the arctic regions (Oehme, 1991; Bidleman et al., 1992; Iwata et al., 1993, 1994; El-Shahawi et al., 2010). Due to their lipophilic property, they have an ability to deposit in adipose tissue, serum and breast milk in human body as well as in animals (Laden et al., 1999; Hauser et al., 2003; Jaga and Dharmani, 2003; Kannan et al., 2004; Tiido et al., 2006; Miranda-Fiho et al., 2007; Meeker et al., 2009; Wang et al., 2009; Alava et al., 2011; Bergkvist et al., 2012). As the reason above, in 2001, all of the organochlorine insecticides classified under Stockholm Convention as the persistent organic pollutants were prohibited or banned from use, import, export and produce in the country (Pollution Control Department, 2013).

Although the use of DDT was banned in the late 1999s in Thailand, however, as a consequence of our study showed a rather high level of total amount of serum \(p,p'\)-DDE concentration than previous study by Asawasinsopon et al. (2006). They have investigated serum concentration of \(p,p'\)-DDE in 39 mother-infants from northern Thailand, which had 1,191 ng/g lipid. Serum concentrations of \(p,p'\)-DDE in this present studies were 1,539 ng/g lipid in male subjects and 1,547 ng/g lipid in female subjects, respectively. Generally, \(p,p'\)-DDT is a lipophilic compound which tends to persist much longer periods in the body, especially in adipose tissue (Laden et al., 1999; Wang et al., 2009). Therefore, the ratio of \(p,p'\)-DDE/DDT is an indicator of whether the DDT observes was recently released or had been emitted to the environment (Pinkney and McGowan, 2006; Mishra et al., 2011; Bergkvist et al., 2012). A relatively low \(p,p'\)-DDE/DDT ratio indicates a recent exposure. Conversely, a relatively high \(p,p'\)-DDE/DDT ratio implies a past usage of DDT. The mean ratio of \(p,p'\)-DDE/DDT in present study was 11.4 and 11.6 in males and females, respectively. There was a high \(p,p'\)-DDE/DDT ratio above 5, which indicated a past usage of DDT. We have evaluated serum total \(p,p'\)-DDE concentration within each region in Thailand.

**Table 1.** Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (n)</td>
<td>484</td>
<td>653</td>
</tr>
<tr>
<td>Age ± S.D. (years)</td>
<td>48 ± 10</td>
<td>46 ± 10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.44</td>
<td>24.26</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(22.14-22.74)</td>
<td>(23.97-24.57)</td>
</tr>
<tr>
<td>(p,p')-DDE (ng/g lipid)</td>
<td>1,539</td>
<td>1,547</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(1,242-1,837)</td>
<td>(1,293-1,806)</td>
</tr>
<tr>
<td>(p,p')-DDT (ng/g lipid)</td>
<td>135</td>
<td>133</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(116-164)</td>
<td>(112-147)</td>
</tr>
<tr>
<td>(p,p')-DDE/(p,p')-DDT ratio</td>
<td>11.4</td>
<td>11.6</td>
</tr>
<tr>
<td>Thyroid–stimulating hormone (mIU/l)</td>
<td>1.48</td>
<td>2.16</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(1.22-1.74)</td>
<td>(1.80-2.52)</td>
</tr>
<tr>
<td>Thyroxine (T4) (μg/dl)</td>
<td>8.12</td>
<td>8.43</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(7.71-8.53)</td>
<td>(8.27-8.59)</td>
</tr>
<tr>
<td>Sugar (mg/dl)</td>
<td>94.84</td>
<td>95.99</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(92.53-97.15)</td>
<td>(93.41-98.57)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>203.80</td>
<td>216.32</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(199.93-207.67)</td>
<td>(212.47-220.16)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>155.72</td>
<td>130.22</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(146.78-164.66)</td>
<td>(123.32-137.12)</td>
</tr>
</tbody>
</table>
At least eight regions showed serum total p,p'-DDE concentration less than interquartile ranges of total amount of serum p,p'-DDE concentration. The highest of serum total p,p'-DDE concentration found in region I and N which located in southern and northern part of Thailand. Those areas have been used this pesticide to control insects in agriculture and insects that carry diseases such as malaria (Malikul, 1988; Chareonviriyaphap et al., 1999).

We have compared mean concentration of serum total p,p'-DDE among countries. The highest proportion of serum total p,p'-DDE concentrations were found in Mexico with mean of 2,770 ng/g lipid (Waliszewski et al., 2002), while the least concentrations were observed in Japan with mean of 93 ng/g lipid (Fukata et al., 2005).

Furthermore, recent studies revealed that there was also high level of p,p'-DDE concentration in human breast milk such as Hong Kong (1,380 ng/g lipid) (Hui et al., 2008), Bangladesh (1,645 ng/g lipid) (Bergkvist et al., 2012), Dalian (2,000 ng/g lipid) (Kunisue et al., 2004) and South Africa (4,600 ng/g lipid) (Sereda et al., 2009). This was raising serious concerns about infant health.

For the correlation coefficients analysis, a number of studies reported both positive and negative as well as zero correlations between BMI and DDT (Laden et al., 1999; Schildkraut et al., 1999; Chevrier et al., 2000; Perry et al., 2005). The apparent inconsistencies in the observed cross-sectional association of BMI with DDT may explain by pharmacokinetics model (Wolff et al., 2007). This model has explained that low BMI subjects should have greater DDT concentrations in adipose tissue than high BMI subjects because of elimination rate of DDT/DDE in persons with low BMI was faster than high BMI persons. However, it depends on how recent exposure has occurred and the distribution of BMI in the population. Likewise, the statistically significant inconsistency between serum DDT concentration and serum lipids were observed in our study. The significant inverse correlation was found between total amount of serum p,p'-DDT concentration and serum cholesterol in both subjects, except the significant inverse correlation between this pesticide and serum triglycerides levels was only observed in female subjects. This was explained by high lipid solubility of DDT was associated with triglycerides in the interior of fat globule (Hugunin and Bradley, 1971). One prospective study revealed that serum concentrations of organochlorine compounds tended to change with serum concentrations of cholesterol and triglycerides in patients with exocrine pancreatic cancer (Porta et al., 2007). Moreover, the different association between organochlorine compounds and serum lipids may depend on a body burden, exposure timing, elimination rate and a various aspects of metabolic dysregulation (Perry et al., 2005; Rignell-Hydbom et al., 2007).

Table 2. Spearman rank correlation coefficients analysis between serum total p,p'-DDE and p,p'-DDT concentrations with BMI, plasma glucose, serum thyroid hormones and serum lipid profile

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>BMI (kg/m²)</th>
<th>p,p'-DDE (ng/g lipid)</th>
<th>p,p'-DDT (ng/g lipid)</th>
<th>TSH (mIU/l)</th>
<th>T4 (μg/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>-</td>
<td>0.056</td>
<td>0.021</td>
<td>0.052</td>
<td>-0.018</td>
<td>0.181(2)</td>
<td>0.176(2)</td>
<td>0.241(2)</td>
</tr>
<tr>
<td>p,p'-DDE (ng/g lipid)</td>
<td>0.056</td>
<td>-</td>
<td>0.757(2)</td>
<td>0.038</td>
<td>0.010</td>
<td>0.240(2)</td>
<td>0.007</td>
<td>0.061</td>
</tr>
<tr>
<td>p,p'-DDT (ng/g lipid)</td>
<td>-0.113</td>
<td>0.764(2)</td>
<td>-</td>
<td>0.020</td>
<td>0.019</td>
<td>0.085</td>
<td>-0.192(2)</td>
<td>-0.117(1)</td>
</tr>
<tr>
<td>TSH (mIU/l)</td>
<td>0.083</td>
<td>-0.044</td>
<td>0.074</td>
<td>-</td>
<td>-0.096(1)</td>
<td>-0.018</td>
<td>0.015</td>
<td>0.099(1)</td>
</tr>
<tr>
<td>T4 (μg/dl)</td>
<td>-0.060</td>
<td>-0.049</td>
<td>-0.023</td>
<td>-0.091(1)</td>
<td>-</td>
<td>0.057</td>
<td>-0.029</td>
<td>-0.049</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.145(2)</td>
<td>0.162(2)</td>
<td>0.116</td>
<td>0.015</td>
<td>0.043</td>
<td>-</td>
<td>0.256(2)</td>
<td>0.204(2)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.348(2)</td>
<td>-0.038</td>
<td>-0.158(1)</td>
<td>0.073</td>
<td>-0.020</td>
<td>0.136(2)</td>
<td>-</td>
<td>0.256(2)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.255(2)</td>
<td>0.063</td>
<td>-0.033</td>
<td>0.036</td>
<td>-0.125(2)</td>
<td>0.065</td>
<td>0.334(2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Above diagonal represents female subjects. Below diagonal represents male subjects.

1) The coefficient was significantly different from 0 (p ≤ 0.05). 2) The coefficient was significantly different from 0 (p ≤ 0.001).
As a further test of the relationship between serum total \( p,p' \)-DDE and \( p,p' \)-DDT concentrations with thyroid–stimulating hormone (TSH) and thyroxine (T4) levels did not show a statistically significant association. We only found a significant negative association of TSH with T4. A number of studies have found that the thyroid hormones may play an essential role during pregnancy, which may cause some effects on fetal neurodevelopment (Asawasinsopon et al., 2006; Chevrier et al., 2008). Another study from Spain found high TSH and low T4 levels in pregnant women to be related with reduced cognitive abilities in children aged between 10 months and 8 years (Lopez-Espinosa et al., 2009).

Interestingly, we found statistically positive correlation between serum glucose levels and serum total \( p,p' \)-DDE concentration. Recently study have showed that \( p,p' \)-DDE exposure may be a risk factor for obesity, dyslipidemia, insulin resistance and common precursors of type 2 diabetes as well as cardiovascular disease (Codru et al., 2007; Langer et al., 2007; Rignell-Hydbom et al., 2009; Lee et al., 2011). Langer et al. (2007) reported that subjects from high pollution area with high serum levels of PCB, DDE and HCB had a high proportion of impaired fasting glucose. Later in 2009, Rignell-Hydbom and colleagues found that women with the extremely high concentrations of DDE showed an increased risk of developing type 2 diabetes, in the case where diabetes were diagnosed more than six years after the baseline examination. However, we have no actual evidence that the high serum concentrations of \( p,p' \)-DDE in our subject is a risk factor for developing type 2 diabetes later in life. Further research must be continuously monitored study and needed to better understand the relationship between DDT and the risks of disease. Our result is an early warning that the environmental pollutions will lead to the bioaccumulation of POPs in the body, which is potentially harmful to human health.

ACKNOWLEDGMENTS

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Serum concentrations of organochlorine pesticides


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