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DDT in Human Milk from Chiang Mai Mothers: A Public Health Perspective on Infants' Exposure

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The insecticidal properties of DDT were discovered in 1939, and it has since enjoyed world wide popularity. DDT has primarily been used in agriculture for controlling insect pests, and in public health programs against typhus and malaria (Turusov et al. 2002). Consequences of the stability and lipophilic nature of DDT are accumulation in adipose tissues of both animals and humans, and a high persistence in the environment. Its intensive use has led to ubiquitous contamination of the environment, and exposure can occur through diet as well as through environmental and occupational exposure (Kannan et al. 1997, Rivero-Rodriguez et al. 1997, Longnecker et al. 2001). DDT is potentially carcinogenic and causes alterations in the reproductive and the nervous system (IARC 1991). Therefore many industrialised countries banned the use of DDT in the 1970's, but in developing countries it is still used mainly in the vector-control programs against malaria (Turusov et al. 2002). The presence and persistence of DDT worldwide is still a problem of great relevance to public health. Sampling of body fluids such as serum and breast milk has shown to be adequate indicators of body burdens of DDT, and a further advantage is that analysis of breast milk is a non-invasive technique (Al-Salah et al. 2002). In numerous studies worldwide, residues of DDT have been detected in breast milk (Schmidt 1999). In lactating women breast milk is the major route of excretion of DDT (Raum et al. 1998), and this has caused much public concern raising questions on whether breast-feeding might be harmful to infants. Breast milk is considered the best nutrition for the growing infant, since it provides all the necessary nutrients and antibodies in adequate combination. Breast-feeding is also an important social factor in the relationship between mother and child (Schlaud et al. 1995).

DDT was used for the first time in Thailand in 1949, and has been used widely in both agriculture and public health programs to fight primarily malaria. In 1983 the use of DDT in agriculture was banned in Thailand, and in the year 1994 its use was banned in public health programs, though allowed under certain circumstances until the year 2000.

The focus and purpose of the present study was to explore the contamination level of DDT in Chiang Mai mothers' milk, in order to compare the trend of contamination against previous studies, and investigate if breast-fed infants in the region were exposed to health hazards via the breast milk.

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MATERIALS AND METHODS

In total 30 physically healthy mothers, between 18-35 years of age, who had been residents in districts of the Chiang Mai Province, except Chiang Mai City district, for at least 10 years were enrolled 6 weeks postpartum, when they came to the Postpartum Care Unit at the Maharaj Nakorn Chiang Mai hospital for check up.

Breast milk samples of 10 mL were collected from each participating mother, between August 8th and August 27th 2002. A nurse collected the milk samples between 9-12 a.m., with the use of a manual breast pump. The samples were stored in 10 mL tubes in a box with ice, and afterwards taken to the toxicology laboratory at the Research Institute for Health Sciences, CMU, for chemical analysis.

Short questionnaires on age, ethnicity, place of residence, length of residence, occupation, length of education, number of pregnancies, number of live births, sex of current infant, number of children breastfed and breast feeding length of former children were used to obtain knowledge and personal data about the mothers.

To determine the fat concentration of the breast milk samples, a creamatocrit method described by Lucas et al. (1978) was used. The following conversion formula was used for the calculation (Silprasert et al. 1987)

$$\text{Fat (g/l)} = (6.24 \times \text{creamatocrit (\%)}) - 3.08$$

The DDT residues were determined using a slightly modified version of the method described by Prapamontol and Stevenson (1991). This method involved a one-step solvent extraction of 2 mL milk-aliquots with 10 mL ethyl acetate – methanol – acetone (2:4:4) followed by 20 min ultrasonication and 15 min centrifugation. The supernatants were further cleaned by solid-phase extraction using C_{18}-bonded silica cartridges, then cleaned with concentrated sulphuric acid, dried with nitrogen gas and re-dissolved in isooctane. Finally, the supernatants were spiked with aldrin as an internal standard before analysis.

The GC analysis were carried out on a HP 5890 II gas chromatograph, equipped with a fused silica capillary column (HP-1: 25m x 0.32 mm id with 1.05 μm film thickness), a ^{63}Ni electron-capture-detector and a computerised data handling system (HP Chemstation, Revision A.06.X0). Operating conditions: injection port: 200°C; column (temperature programming): 80°C for 1 min, then change to 190°C with 30°C/min ramp rate, then change to 250°C with 4°C/min ramp rate and hold at 250°C for 15 min; detector temperature: 300°C. Oxygen free nitrogen (99.999 %) was used as make-up gas and helium gas (99.993 %) was used as carrier gas.

The percent recoveries for *p,p'*-DDT and *p,p'*-DDE ranged from 80 to 130 %. The limit of detection of the method was 5 ng/mL whole milk for *p,p'*-DDT and 3 ng/mL whole milk for *p,p'*-DDE. One series of pooled milk (no. = 4) were analysed each day and one aliquot of pooled milk was analysed along with each batch of milk samples for internal quality control. The Intra-batch coefficient of variations were 12.7 % for *p,p'*-DDT and 5.9 % for *p,p'*-DDE, whereas the inter-batch variations were 25 % and 27 % for *p,p'*-DDT and *p,p'*-DDE, respectively. These quite high inter-batch variations are due the fact that the last 2 batches of pooled milk showed very high levels. To test if the results from the last two batches of mothers' milk, no. 17-30, were reliable, comparisons of the mean values of aldrin, *p,p'*-DDT and *p,p'*-DDE were made between sample no. 1-16 and 17-30.

The 30 mothers' milk samples are individual samples, and therefore their values of pesticide residues are not known beforehand. But there was no significant up-going trend it was concluded that the results of the mothers' milk samples were reliable.

Statistical analyses were performed using the Statistical Package of the Social Sciences (SPSS for Windows version 10.0)

RESULTS AND DISCUSSION

A total of 30 women, mean age of 27.2 years, entered the study. The mean length of residence in the Chiang Mai Province was approximately 26 years, and their mean length of education was 9.1 years. Each woman had approximately breast fed 1.5 children and the average fat concentration was 17.2 g fat per L breast milk.

Table 1. DDT and metabolites in whole milk in the 30 samples above the LOQ (C_{mL} (ng/mL)).

C_{mL} (ng/mL)	Mean	Median	SD	Min	Max	Frequency %
p,p'- DDT	10.3	8.0	10.0	0.0	38.2	77
p,p'- DDE	75.5	67.1	40.8	9.6	170	100
Total- DDT	86.7	75.3	47.9	9.6	208	100

Table 1 showed the mean, median, standard deviation of the mean (SD), minimum, maximum and frequency (%) of the compound detected in samples.

In whole milk mean and maximum levels of *Total*-DDT at 86.7 and 208 ng/mL were determined. *p,p'*-DDT was detected in 77 % of the samples, whereas *p,p'*-DDE was detected in 100 % of the samples.

To get an impression of the environmental impact of DDT, the contamination levels are presented on fat-basis in table 2 and 3, allowing a non-biased comparison between samples despite varying fat content in the milk.

Table 2. DDT and its main metabolites in fat in the 30 milk samples above the LOQ (C_f (µg/g)).

C_f (µg/g fat)	Mean	Median	SD	Min	Max
p,p'- DDT	0.8	0.6	1.1	0.0	5.3
p,p'- DDE	6.0	3.9	5.4	0.7	24.7
Total- DDT	6.9	4.8	6.3	0.9	30.1

Horizontally, the mean, median, standard deviation of the mean (SD), minimum and maximum are listed.

The mean levels of residues were 0.8 µg/g fat, 6.0 µg/g fat and 6.9 µg/g fat for *p,p'*-DDT, *p,p'*-DDE and *Total*-DDT, respectively.

The Student's t-test for independent samples was used to test if the mean of the samples with a fat concentration lower than 10 g/L breast milk was different from the mean of the samples with a fat concentration above 10 g/L. The women with the lowest concentration of fat in their breast milk (<10 g fat/L), had significantly higher levels of DDT measured on fat-basis, but the DDT concentrations in whole milk were not significantly higher. The Student's t-test for independent samples also showed that the levels of DDT in mother's milk were not associated with the age of the women, the number of children they had given birth to or the number of children they had breastfed.

Table 3. Residues of *Total*-DDT in breast milk fat in various countries.

Total- DDT (µg/g fat)						
Country	Year	N	Mean	Median	Max	Reference
North Thailand	**2002**	**30**	**6.9**	**4.8**	**30.1**	**Present Study**
Indonesian	2001	10	0.5	n/r	n/r	Burke et al. 2003
China	2000	54	3.6	n/r	n/r	Wong et al. 2002
Hong Kong	1999	132	2.9	n/r	n/r	Wong et al. 2002
North Thailand	1998	25	15.0	n/r	n/r	Stuetz et al. 2001
United Kingdom	1997/98	156	0.5	0.3	4.8	Harris et al. 1999
Sweden	1997	40	0.1	n/r	n/r	Norén and Meironyté 2000
North Germany	1995/97	246	0.2	0.2	1.5	Schade and Heinzow 1998

n/r; not reported n/r; not reported

The left column lists the countries. Horizontally, the parameters are: year, size of the study (N), and levels of *Total*-DDT in µg/g fat. When possible, average, median, and maximum values are noted. The right column states the references.

In Northern Thailand, the contamination levels in breast milk are remarkably higher than both the industrialised and Asian countries. There are several explanations for this. In the studies from the various countries different analytical methods have been used which may have an impact on the test results. Also, the studies are not always representative since groups with high risk often are preferred, and it is not homogenous groups of women that are compared in terms of age, number of children, lactation period etc. Furthermore, the relatively small cohorts that are used in several of the studies may not be considered adequate and reasonable data-bases for statistical analysis. However, there is no reason to doubt the high levels in Asia, but it is important to keep the uncertainties in mind.

DDT's damaging impact was overlooked or down played in Thailand since there was no better alternative in the struggle against huge public health problems such as malaria. As late as 1998 the malaria centres in Chiang Mai province decided to test the use of pyrethroids as a substitute for DDT as a vector control agent. It was decided that only deltamethrin should be used from the year 2000 onwards to control the malaria-carrying mosquitoes in the urban areas of the districts (Stuetz et al. 2001). According to the WHO (WHO 1999), use of other pesticides as an alternative to DDT would have increased the costs of malaria control dramatically, and would not have been affordable in some countries. Substitution of DDT would have led to a decrease in the covering of control programs, so if DDT was not to be used, vast populations in the malarious areas of the world such as Northern Thailand would be condemned to the frightening ravages of endemic and epidemic malaria (Al-Salah et al. 2002).

The levels found in the present study are far lower than the levels measured in Northern Thailand in 1998, which was to be expected because of the difference in study-base. The study-base from 1998 consisted of mothers' living in a specific area, which had a reputation of high use of DDT, whereas the present study-base consisted of mothers' living in Chiang Mai province, except the city district.

In Northern Thailand and the rest of the Asian countries, the mothers' primary sources of pesticide exposure are directly through public health programmes fighting diseases such as malaria. DDT had been widely used in spraying huts etc. in an attempt to control the vectors that spread the disease. Another possibility for exposure was through farming labour. It is possible that DDT is used illegally in agriculture in Northern Thailand, thus the produced foodstuffs may also be a major source of exposure in the Chiang Mai province. This situation may be similar in other Asian countries. In the industrialised countries, the primary source is fatty foodstuffs and imported food crops from non-industrialised countries.

In order to assess the infants' actual exposure in Northern Thailand, the Estimated Daily Intake (EDI) of DDT via the mothers' whole milk we assumed that the children consume 130 mL of milk per kg of body weight per day.

Table 4. Infants' Estimated Daily Intake of DDT and its metabolite.

EDI (μg/kg/day)	Mean	Median	SD	Min	Max	ADI	PTDI
p,p'- DDT	1.3	1.0	1.3	0.0	2.0	-	-
p,p'- DDE	9.8	8.7	5.3	1.3	22.2	20	-
Total- DDT	11.3	9.8	6.2	1.3	27.0	20	1

Horizontally, the parameters are: mean, median, standard deviation of the mean (SD), minimum and maximum, and in the columns furthest to the right the acceptable daily intake (ADI) and provisional tolerable daily intake (PTDI) values are shown.

The estimated daily intake of *Total*-DDT for infants through mothers' milk in this study exceeded the ADIs as recommended by the WHO in 1984, but at the maximum levels only (FAO/WHO 1984).

In 1994 the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) converted the ADI to a provisional tolerable daily intake (PTDI) for DDT (FAO/WHO 2000). The term 'provisional' reflects the lack of reliable data on the consequences of human exposure to DDT. The PTDI level indicates that the children in Northern Thailand are exposed to very high intakes of DDT, which might be health damaging, in particular considering the special vulnerability of children (WHO/EEA 2002).

In both Northern Thailand and the industrialised countries, breast milk is the primary source of exposure for the infants, because breast milk is the primary nourishment during the first months of an infant's life. Breastfeeding is beneficial in many ways and should be promoted, despite the fact that due to high contamination levels, there is a risk of causing possible health damage to some of the exposed infants.

The ratio DDE/DDT may provide an estimate of DDT use over time, as DDT is broken down slowly to DDE. Thus the higher the ratio the longer time since exposure to DDT.

Table 5. Ratio between DDE and DDT in breast milk in Northern Thailand.

C_{ml} (ng/mL)	Year	*p,p'*- DDE	*p,p'*- DDT	DDE/DDT	Reference
North Thailand	2002	75.5	10.3	7.3	Present Study
North Thailand	1998	287.5	90.9	3.2	Stuetz et al. 2001

The left column lists the country. Horizontally, the parameters are: year, mean concentration of *p,p'*-DDE and *p,p'*-DDT, DDE/DDT ratio and the right column state the references.

In the present study the DDE/DDT ratio was 7.3 compared to the ratio found in Northern Thailand in 1998 at 3.2.

Even though the two study-bases are not directly comparable, this difference in ratio may indicate that the use of DDT has declined. It would be interesting with newer studies with similar study-bases to the ones used in Northern Thailand in 1998 and 2002, in order to make it clear whether the use has in fact declined, or if DDT is used illegally, and therefore still being a health hazard to young infants.

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