1. INTRODUCTION

1.1. Organochlorine Use : Boon or Bane?

Pesticides have been used widely by farmers all over the world for a long time to protect plants against pests and diseases, increase the yield and hence increase their profits. However, pesticides undoubtedly present adverse effects on some components of the environment including man due to their toxicity, long persistence, and accumulation in the environment [1].

The "Age of Pesticides" arrived with the commercial introduction of DDT which has emerged as the most efficient pesticide in agriculture, forestry, and malaria control campaigns [2]. At the height of its production, over 400,000 tons of technical grade DDT were used annually of which approximately 60,000 tons were used in malaria control at the peak of the global malaria campaign [3].

Organochlorines have been introduced into Thailand for several decades and are widely used as one of the most efficient insecticides [4]. However, as organochlorine pesticide compounds are long persistent and bioaccumulating, the areas which have been applied with such chemicals are likely to be at risk. Spray residues inside and outside houses has been reported to cause some detrimental effects to animals and humans living around pesticide sprayed areas. Demonstration of organochlorine pesticide residues in bird, man, atmosphere, soil, and water by different research workers have given credence to the popular belief that these compounds are among the most hazardous widespread environmental pollutants. The use of several organochlorine insecticides is, therefore, either restricted or banned in the United States and in some other developed countries [5]. In developing countries including Thailand, DDT has remained one of the most important insecticides and is widely used to control vectors of many tropical diseases such as malaria, yellow fever, dengue, spotted fever, typhus, and others despite its toxicity and environmental hazards [4,6].

1.2. Properties of Some Organochlorine Insecticides

There are many organochlorine compounds, however, only some of them are widely used and were studied in this research, as listed in Table 1.1.

Table 1.1 Structures and names of some important organochlorine insecticides [7]

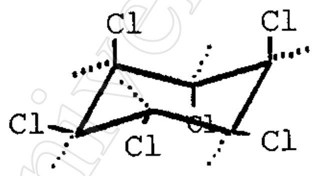
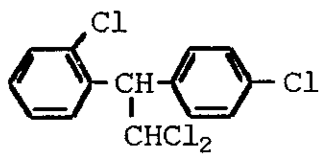
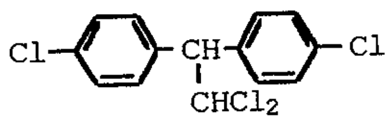
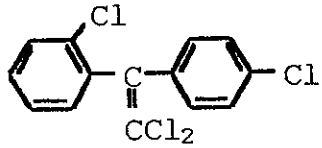
Common name	Chemical name	LD_{50} oral to rats* (mg/kg)	Structural formula
BHC (HCH)	Mixtures of stere-oisomeric 1,2,3,4, 5,6- hexachlorocyclo hexanes	500	 γ-BHC
o,p'-DDD	1,1-dichloro-2-(2-chloro-phenyl)-2-(4-chlorophenyl) ethane	3,400	
p,p'-DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane	2,500	
o,p'-DDE	1,1-dichloro-2-(2-chloro -phenyl)-2-(4-chloro-phenyl)ethylene	880	

Table 1.1 (cont.)

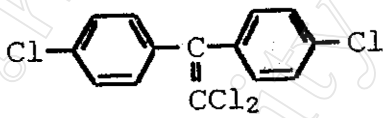
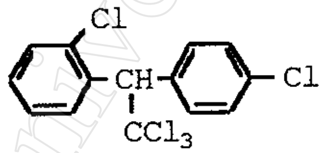
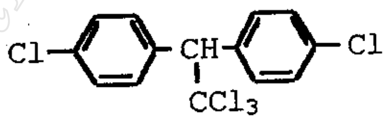
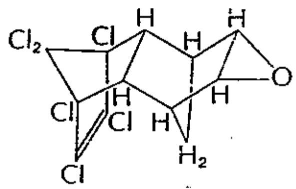
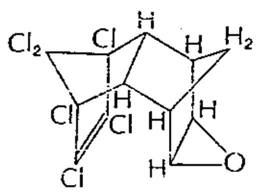
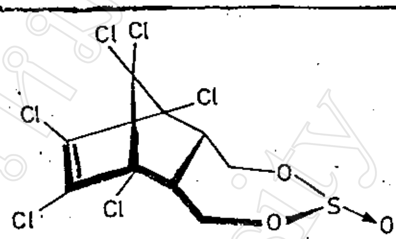
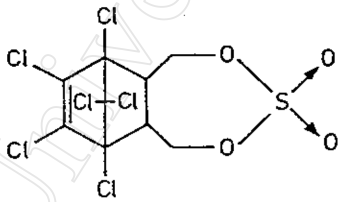
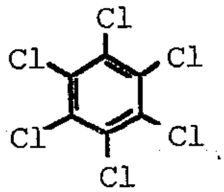
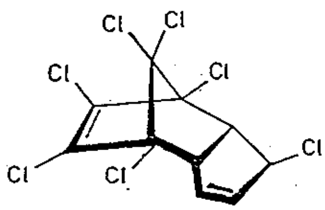
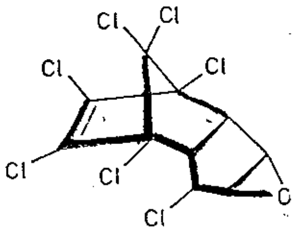
Common name	Chemical name	LD_{50} oral to rats* (mg/kg)	Structural formula
p,p'-DDE	1,1-dichloro-2,2-bis (4-chlorophenyl)ethylene	750	
o,p'-DDT	1,1,1,-trichloro-2(2-chlorophenyl)-2-(4-chlorophenyl)ethane	100	
p,p'-DDT	1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane	113	
Dieldrin	(1R,4S,4aS,5R,6R,7S,8S,8aR) -1,2,3,4,10,10-hexachloro-1,4, 4a,5,6,7,8,8a-octahydro-6,7-epoxy -1,4:5,8-dimethanonaphthalene	46	
Endrin	(1R,4S,4aS,5S,6S,7R,8R,8aR) -1,2,3,4,10,10-hexachloro-1,4, 4a,5,6,7,8,8a-octahydro-6,7-epoxy -1,4:5,8-dimethanonaphthalene	7.5	

Table 1.1 (cont.)

Common name	Chemical name	LD_{50} oral to rats* (mg/kg)	Structural formula
Endo-sulfan	C,C'-(1,4,5,6,7,7-hexachloro -8,9,10-trinorborn-5-en-2,3- -ylene)(dimethyl sulphite)	40-50	 Endosulfan I
	C,C'-(1,4,5,6,7,7-hexachloro -8,9,10-trinorborn-5-en-2,3- -ylene)(dimethyl sulphate)	40-50	 Endosulfan sulfate
HCB	Hexachlorobenzen	100	
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	90	
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetra-hydro-4,7-methanoindane	40-60	

* Source of LD_{50} oral to rats: Pryde, 1973 [5] and Matolcsy, 1988 [6]

DDT was first synthesized by Zeidler in 1874 but its insecticidal properties were discovered by Paul Muller in 1939. Hexachlorocyclohexane (or benzenhexachloride, BHC) was first made by Michael Faraday in 1825 with various isomers. The most common isomer is lindane (γ-BHC) which was first isolated in 1912, however its application as an insecticide took place at the beginning of the 1940 's. The insecticidal properties of dieldrin were first described by Dearnes *et al.* in 1949. Endosulfan was marketed in 1956 under the name of Thiodan [8].

The extreme persistence and stability of organochlorine compounds are one of their chief properties. As lipophilic compounds they tend to concentrate in membrane lipids and fat deposits, and hence, they are usually found in two main food classes such as dairy products and meat, fish, and poultry [9].

The conversion of pesticides into their derivatives is one of the important aspects in assessment of the risk of pesticide exposure because this transformation directly changes the toxicity and persistence of pesticides. Usually, p,p'-DDT is decomposed to the biologically inactive p,p'-DDE and p,p'-DDA while o,p'-DDT is similarly converted to o,p'-DDE [6]. The rapid conversion of DDT to its derivatives decreases the toxicity of DDT, e.g., LD_{50} to mosquito lava is 0.07 ppm for DDT, 0.038 ppm for DDD, and >10 ppm for DDE [5] or LD_{50} to rats of DDE and DDD are about 9 times and 34 times lesser than that of DDT, respectively (Table 1.1). In contrast to DDT, heptachlor epoxide was reported as more active than the parent compounds and more than heptachlor; and similarly, endrin is more active than dieldrin [6]. Among isomers of BHC, lindane is more toxic than α-BHC and β-BHC (LD_{50} to rats of lindane is 125 mg/kg while that of α-BHC and β-BHC is 500 and 6000 mg/kg, respectively). The toxicity of other organochlorines and their metabolites are reported as LD_{50} to rats in Table 1.1.

1.3. Organochlorine Residues in the Environment

1.3.1. Organochlorine Retention in Soil

After application, organochlorine compounds are usually resistant to be decomposed and are readily absorbed by sediments and soils which can act both as sink and as long-term sources of pesticide exposure [10].

Fuhr and Neely [11,12] stated that various types of soil showed a strong adsorptive capacity for DDT. Pesticide adsorption is closely related to the organic matter content, progressive removal of lipids, resins, polysaccharides, poly-uronides and humic matter which binds the DDT.

Moreover, organochlorines are reported as long persistent pesticides in soil [5]. The time for loss of 90% of surface-applied DDT is estimated to be about 1.5 to 2 years while 50% loss of DDT mixed into the soil occurred in 5 to 8 years, and 90% of the applied DDT is lost in 25-40 years [10]. The estimated disappearance rates of some organochlorine compounds in soil are shown in Appendix Figure 1.

1.3.2. Transport of Organochlorines to Animal Products

Since organochlorines are not water soluble, much of their residue clings to particles and is transported both by dust and by solids suspended in water. Thus, even if the concentration of DDT is very low in the surrounding environment, organisms with high fat or oil content readily absorb available DDT. Then as tiny plants and organisms are eaten in turn by next members of the food chain, levels of up to 10,000 times the background concentration can be reached. One such magnification scheme is shown in Appendix Figure 2. In general, organisms at higher trophic levels tend to contain more DDT-type than those at lower trophic levels [10,13]. By this way, food is humans' source of over 90% of average daily intake of DDT [10].

Pesticide residues in animals are governed by pesticide residue content in their food and by those factors that affect the absorption distribution, metabolism, excretion, and storage of pesticide in their bodies. Undoubtedly, rates of pesticide accumulation also vary with animal species, characteristics of pesticides, duration and concentration of exposure, and environmental conditions [8,10,14]. In chickens, organochlorines as lipophilic compounds tend to concentrate in membrane lipids, fat depots, and eggs. The whole mixed egg contains about 65% water, 12 % protein, and 11% fat. Most of the fat is in the yolk [15].

Celcil [16] reported that p,p'-DDT and p,p'-DDE concentrations content of eggs from hens which were fed daily with 5-50 ppm p,p'-DDT or p,p'-DDE for 28 weeks reached equilibrium and were approximately equal to the dietary levels of the p,p'-DDE and p,p'-DDT diets after 12 weeks. That study also found that egg is an important route for the elimination of ingested p,p'-DDT or p,p'-DDE due to more than 34% of the daily intake of p,p'-DDT and 42% of p,p'-DDE were concentrated into the eggs [16]. Organochlorine residues in fat are about 10 times greater than the amount in eggs, and are nearly equal the amount in egg fat since eggs contain approximately 10% fat [16]. Similarly, by conducting the experiment on feeding chickens with organochlorines, Waldron [17,18] and Putnam *et al.* [19] found that the concentration of organochlorine residues in eggs was directly proportional to and dependent on the concentration of organochlorines in their food. Significant pesticide contamination in eggs occurred at dietary levels of 0.1 ppm organochlorines [17]. In addition, several researchers have found the association of organochlorine content in feed with egg weight, egg shell thickness, egg shell calcium [10,16], egg production and egg hatchability [20-22] of chickens.

Many organochlorine residue analyses have been done in poultry eggs by different authors and they mostly agree that the organochlorine residue levels concentrated in eggs are significantly related with the extent of organochlorine residues in the surrounding environment [23-27]. This suggests that poultry eggs can be one of the most effective "bio-indicators" or "bio-means" for biomonitoring pesticide residues in the environment [16,23-27]. In recent years, the screening of organochlorine residues in chicken eggs, especially eggs from free-range hens, has been applied in many areas in order to evaluate the ecological side effects and potential risks of organochlorine residues in the environment [26-30]. It was also reported that the eggs from free-range hens contained much higher concentration of organochlorine residues than the eggs from commercial hens [26,27]. The ratio p,p'-DDT to p,p'-DDE was one of interesting parameters which could be applied in the organochlorine residue monitoring programs to estimate the situation of DDT use in the study areas [27-30]. This ratio varied from 0 to 2.03 in eggs from free-range hens [27]. In the organochlorine screening programs using hens' eggs as bioparameters, this ratio was found to be varied from 1.77 (1977) to 0.77 (1990) in agricultural areas in the northern part of Germany [30]. The ratio p,p'-DDT to p,p'-DDE was reported in hens' eggs from Ivory Coast, Germany to be about 0.56 in urban areas, 0.29 in national park, and 0.26 in cattle farms [28].

1.3.3. Pesticide Exposure to Humans

Incidental human exposure from the widespread use of organochlorines is a major of public health concern. In the United States, the concern for the possible adverse human health effects of pesticides really began in 1962 with the publication of Rachel Carson's book *Silent Spring* [31]. These concerns included 1) human pesticide poisoning problems, 2) chronic long-term health effects of pesticide exposures, 3) and the magnitude and significance of incidental pesticide exposures to humans [32].

The most commonly noted pesticide residues in food , in decreasing order, are those of organochlorines, organophosphates, and carbamates [8]. WHO reported that about 90% of the average daily intake of DDT of humans came from food [10]. Other exposures are from the surrounding media, like air, water, lanolin-based cosmetics, aerosols, fabrics, and tobacco smoke [10,33,34]. The monitoring of pesticide residue in food, therefore, is one of the most important approaches for assessing the risk and minimizing the potential hazards of pesticides to the environment and to human health

[7,33,35]. To protect consumer health, the joint FAO and WHO Codex Alimentarius Commission has recommended the Maximum Residue Limit (MRL) and Acceptable Daily Intake (ADI) levels of organochlorine in various food and animal feeds, as shown in Appendix Table 1 [36-38].

Organochlorines have been reported to have effects on breast cancer in women, decrease sperm concentration, and cause reproductive problems in men [39,40]. Many organochlorine residues have been detected in some parts of the human body, e.g., blood [40,41], breast milk [42,43], urinary metabolites [44], and in human adipose tissue [45].

Pesticides are toxic not only to insects, but also nontarget organisms, therefore, any pesticide regulatory decisions should be based on risk assessment and risk management [46]. Before registration or wide use of any kind of pesticides, an ecological risk assessment of the pesticide must be conducted to assess and minimize unreasonable risks to environment, taking into account the economic, social, environmental costs, and benefits of the use of pesticides [46,47].

1.4. Organochlorine Residue Analysis

For the identification of pesticide residues, metabolites, and contaminants analytical techniques which appear to have made significant advances are capillary gas chromatography (GC) and GC coupled with mass spectrometry [48-49]. Especially, GC with an electron-capture detector and capillary column is capable of detecting down to femtogram quantities of organochlorines [48]. For congener specific determination of polychlorinated organics, a two-dimensional reaction gas chromatographic system with 2 column-separation is another choice [50].

The main steps for organochlorine residue analysis are sampling, extraction, clean-up, final determination and confirmation techniques [51]. In extraction steps, for miniaturization, Frehse stated that the weight of an analytical sample can be reduced to between 2-5 g without appreciable loss of accuracy provided that the analytical sample is taken from a properly homogenized field sample and that the residue is not destroyed by such homogenization [48].

The environmental and biological samples generally can not be analyzed directly for pesticide residue due to the level of the desired residue in such matrices is too low while the interfering constituents are too high. Therefore, after extraction, clean-up should be done to eliminate or at least minimize interfering substances or undesirable co-extractives, especially fat materials, in egg analysis [52]. Clean-up techniques commonly used are column chromatography packed with silica gel [53] or florisil [53,54], thin layer chromatography, sweep-co distillation, and gel permeation chromatography [9]. In some cases of multi-residue analysis, multicolumn solid-phase extraction clean-up is done [55].

Analytical precision in residue methods is improved by the use of reference compounds (surrogate) functioning as internal standards. The limit of determination in residue analysis is required to satisfy 3 criteria: 1) it is greater than limit of detection; 2) recovery is greater than 70%; 3) and the coefficient of variation is equal or less than 20% [48]. Therefore, before final determination, it is necessary to concentrate the sample to a suitable concentration for detector response and in cases of doubt, the confirmation should be done.

1.5. Organochlorine Residue Profile in Some Matrices in Thailand

Although DDT has been banned in Thailand from production, importation, marketing, and even for agricultural purposes by the Ministry of Agriculture in 1983, the Ministry of Health is still permitting the use of DDT only for malaria eradication programs [56]. Organochlorines, especially DDT, have been legally used to control malaria-disease vectors over a large area in northern Thailand since 1953. According to the report of Malaria Center Region 2, a 5% suspension of DDT has been sprayed at least one time per year at $2 g/m^2$ residue spraying in the following Districts: Mae

Tang, Mae Rim, Sa Meung, Ging Mae Wang, Hang Dong, Chiang Dao, Fang, Mae Eye, Jam Tong, Hot, and Doi Tao. Chiang Mai Province was reported as the place with the highest death rate from malaria disease in northern Thailand.

The use of DDT for such purposes has caused significant contamination of, for example, mother's milk, human blood, and products from domestic animals living in the vicinity to DDT-sprayed areas such as fish, shrimp, meat, and poultry eggs.

The Department of Agriculture reported that organochlorine pesticides such as α-BHC, lindane, heptachlor, aldrin, dieldrin, DDT and its derivatives were found in 77 soil samples collected from different agricultural zone in Thailand during 1987-1988. Among them, p,p'-DDE and dieldrin were detected in more than 70% of soil samples. The high concentrations of organochlorines were found in 23 soil samples collected from Muang and Chiang Kham Districts in 1982 indicated the extensive use of these compounds in the study areas [57].

A survey on food contamination conducted by the Division of Food of the Department of Medical Sciences revealed that 100% of 14 eggs contained pesticides [58]. Total DDT residue levels were detected in fish to be higher than those in agricultural products but lower than those in fat or meat of domestic animals [56]. Total DDT According to the results of organochlorine analysis in 12 eggs collected randomly from Chiang Mai markets, 92% of the sample was positive with p,p'-DDT (0.002-0.032 ppm), 100% with p,p'-DDE (0.002-0.022 ppm), 58% with dieldrin (0.001-0.013 ppm) and 75% with α-BHC (0.004-0.016 ppm). Kumnerdman *et al.* reported that in 56 chicken eggs collected from the markets in the middle parts of Thailand 79% of the egg samples contained aldrin and dieldrin (0.001-0.051 ppm) while 100% of samples contained DDT and its derivatives (0.007-0.044 ppm). Other ogranochlorine residues such as α-BHC, lindane, and heptachlor epoxide were also detected in eggs of chickens, ducks, and francolins. However, most of the pesticide residue levels found in eggs from markets were less than the MRL [59].

Some organochlorine residues were also found by Feng [60] in fish, shrimp and meat collected from Chiang Mai market. These pesticide residue levels were lower than MRLs recommended by WHO and Thailand's Ministry of Public Health.

Prapamontol *et. al.* stated that DDT and its metabolites detected in the serum of villagers who lived in DDT-sprayed areas in northern Thailand were in significantly higher concentration than that found in control serum from villagers who lived in areas with no DDT use due to the long term impact of high dose exposure to DDT [41].

The Agricultural Toxic Substance Division, Department of Agriculture reported that many positive detection of organochlorine residues, especially as p,p'-DDT, p,p'-DDE and dieldrin, were in farmers' blood, and in mothers' milk. The organochlorine concentration in mothers' milk seems to be increased in 1986 compared with that in 1980.

1.6. The Aims of this Study

Briefly, a large number of pesticide contaminants may be present in the environment leading to pesticide exposure of animals, humans, and certain potential pesticide residues in the products and tissues of animals and humans. Among animals, especially free-range chickens, by frequent exposure to pesticides or even by ingesting pesticide contaminated soil and feed , the pesticide accumulation in their bodies and eggs is inevitable. Consequently, the organochlorine residue levels in eggs from free-range hens can be a good bioindicators for evaluating environmental contamination as well as pesticide residue monitoring in surrounding areas of the chickens' living space.

Moreover, chicken eggs are cheap, easily sampled, stored, and analyzed in comparison with other products from other domestic animals. Eggs are also one of the main foods in Thais' daily meals and the monitoring of pesticide residue in food is one of the most important approaches to assessing the risk and minimizing the potential hazards to the environment and to human health.

In view of these premises, the study of monitoring of organochlorine residue in hens' eggs in Chiang Mai suburban areas was conducted with the following objectives :

1. To evaluate the range of organochlorine residues in eggs of free-range hens in Chiang Mai suburban areas in comparison with the reported values of the Maximum Residue Limit (MRL) and the Acceptable Daily Intake (ADI) in eggs.

2. To provide a profile of pesticide residues for assessing the potential risk of pesticide residue to human health in the sampling areas.

3. To document the use of eggs from free-range hens as the mean for monitoring of organochlorine pesticide residues in the environment surrounding the living spaces of hens.

4. To supply the baseline data of DDT residue in free-range hens' eggs in villages where DDT was recently used.