4. DISCUSSION

4.1 Organochlorine Residue Analysis

With an HP-608 capillary column operated under GC conditions following the recommendation of the US-EPA 608 method for organochlorine analysis, the peaks of p,p'-DDD and o,p'-DDT were not chromatographically separated. These two compounds have such similar mass chromatograms that they could not be clearly differentiated by GC-MS. So far in this study, attempts to separate them were not made since they posed no major problem. Therefore, the compound found with retention time of 17.930 minutes was reported as the sum of o,p'-DDT and p,p'-DDD. Nevertheless, prior studies on organochlorine residues reported that p,p'-DDD was presented in eggs of free-range hens much more frequently than o,p'-DDT [26,27]. This is confirmed by the organochlorine analysis results of Dr. Joachim Krueger in the University of Saarland, Germany. His results showed that among fifteen eggs sent to Germany for intra-laboratory test, only 20 precent of egg samples contained o,p'-DDT while p,p'-DDD was detected in 50 percent of egg samples (Table 3.6).

In order to separate these two compounds, a new capillary column or an adjusted temperature program could be attempted. According to the manual of J & W Scientific Products [63], the DB™-608 capillary columns is able to resolve all of the chlorinated pesticides targeted in the US-EPA method 608. The other excellent confirmation columns are DB™-5, DB™-1701, DB™-17 which could separate completely two peaks of o,p'-DDT and p,p'-DDD [63]. Especially, DB™-1701 exhibited a completely different elution pattern (as shown in Appendix Figure 4) making it a good complement to other capillary columns including HP-608 column.

Regarding the reproducibility of electron-capture detector to organochlorine compounds, the ECD response as the peak height of p,p'-DDT compound is less reproducible than that of other organochlorines as indicated by the high coefficient of variation (Table 3.5). This may be due to the active sites in the injection port which may result in the facile degradation of p,p'-DDT at high temperature (e.g. 250°C in the injection port, 300°C in the oven).

4.2. Eggs from Free-Range Hens as the Mean for Biomonitoring of Organochlorine Residues in the Environment

It is apparent that total DDT residue in eggs from free-range hens have much higher concentration than those in the eggs from enclosed hens ($p<0.01$, Appendix 2.8), when comparing the findings with other available data, as shown in Table 4.1. This is consistent with earlier findings of Hashemy- Tonkabony (1978) in Iran [26] and Mugambi (1986) in Central Kenya [27].

Table 4.1 Comparison of dieldrin and total DDT residues in eggs from free-range hens (native breed chickens) and enclosed hens (commercial types) in Thailand

Source of eggs	No. of eggs analyzed	Dieldrin (mg/kg egg) Mean ± SD (range)		total DDT (mg/kg egg) Mean ± SD (range)	
Free-range hens	64	0.010 ± 0.010 (0.-0.092)	a	1.60 ± 3.19 (0.004-18.7)	a
Commercial types					
Chiang Mai market*	12	0.008 ± 0.008 (0.001-0.013)	a	0.019 ± 0.016 (0.002-0.055)	b
Central part of Thailand [58]	56	(0.001-0.051)		(0.007-0.044)	

* Organochlorine residues in the eggs from Chiang Mai market were analyzed by the author and her colleges in 1994 using the same method and GC system

The higher concentration of organochlorine residues detected in the eggs of free-range hens may be due to the free-range chickens consuming the additional feed contaminated with pesticides, e.g. worms, insects and ants in the environment besides the feed obtained from farms. Most of organochlorine pesticides are lipophilic compounds and extremely persistent. They can be present in biological materials even 5 to 15 years after the last application of the pesticide [5,10]. Hence, organochlorine accumulation in eggs from free-range hens is inevitable, and evidently, organochlorine residue levels in these eggs can reflect an extensive organochlorine contamination of the environment around the hen living space. The findings confirmed again the function of poultry eggs as a good "bio-sample" to biomonitor and evaluate the potential risk of organochlorine residues in surrounding environment [23-30].

There are many factors that may affect organochlorine accumulation rates in chicken eggs. For example, different strains of hens, age of hens, and different chemical compounds may differ considerably in their susceptibility toward the addition of chemicals in their diet [10,13,14]. These probably are explanation for the significant higher concentration of total DDT and cis-heptachlor epoxide residues found in the eggs from 3-year old hen compared with those from both 1 and 2-year old hens (Table 3.7). The 3-year old hen might have a longer period of pesticide exposure than the younger ones. Unlike DDT and cis-heptachlor epoxide, dieldrin residue in eggs of the 3-year old hen was not higher than that of 1-year old hen. Organochlorine residues in eggs from 2-year old hen were lower than those from other hens. It should be noted that in this study the 1 and 3-year old hens were of the same strain and feather color (black) while the 2-year old hen was brown. The eggs of the 2-year old hen were also bigger than those of other hens. In addition, chemical properties of dieldrin and cis-heptachlor epoxide are quite different from DDT and its derivatives [6]. There are several factors directly affecting the levels of organochlorine residues in eggs such as nutrition and ecological factors (e.g. duration and concentration of organochlorine exposure) [10,13,17-19], and perhaps most important, the absence and presence of some factors, owing to biological variability [13,26]. However, research on these factors were limited in this study.

Generally speaking, in order to study and evaluate the risk of pesticide residues and pesticide exposure to the environment, biomonitoring, or more precisely, measurement of biological parameters such as animal products and human tissue is being used more frequently than environmental monitoring [44]. Undoubtedly, this technique can simultaneously assess all resources and accumulate responses, and it may require fewer samples [44]. Thus, the ability of using eggs from free-range hens to monitor organochlorine residues in the environment is feasible and recommended. When compared with other kinds of matrices, eggs show many advantages, e.g. cheap, easily sampled, stored and analyzed. Eggs are also one of main sources of daily foods, therefore, the obtained data of organochlorine residue levels in eggs could be used for evaluating and minimizing the potential risk of pesticide exposure to human health. Especially, in the long term biomonitoring programs, this kind of studies could supply the reliable, efficiency and integrated results. However, the study reported here could not avoid some limitations, e.g. limited number of egg-samples, lack of replication of sample collection (i.e. only one time of survey), thus, there is a need to conduct more such organochlorine screenings in hens' eggs in large areas and long period.

4.3. Organochlorine Residues in Eggs from Free-Range Hens in Chiang Mai Suburban Areas

Inferring from the study which is designed to determine factors affecting organochlorine residues in chicken eggs, the author tried to minimize the fluctuation of organochlorine residues in eggs caused by sampling technique by limiting the variations of age and variety of hens during the survey. Of 64 eggs collected randomly from Mae Rim, Hang Dong, Muang and San Kampaeng Districts during the survey, sixty seven percent and seventy five percent were from black hens and from the more or less 1-year old hens, respectively (detailed information of hens and eggs are presented in Appendix Tables 3,4,5, and 6).

The mean concentration of total DDT and the ratio of p,p'-DDT to p,p'-DDE were both found highest in Mae Rim District, with 67% of samples exceeding the ERL (Table 3.8). These reasonable findings could be attributed to the extensive use of DDT in Mae Rim. Indeed, the total amount of DDT (75 WP) used yearly in Mae Rim to control mosquitoes was likely increased in recent years (Appendix Table 8). In Mae Rim area, some eggs were collected from the villages which have been sprayed continuously with DDT since 1953 (at least one time per year) such as Pang Hai, Muang Kha (reported by Malaria Center Region 2). These villages are far from urbanized areas and located in the forestry and mountainous areas with a high risk of malaria disease.

Besides Mae Rim, Hang Dong is another area in which the DDT is still used. Though there is no significant difference of the mean values of total DDT residue in eggs between Mae Rim and Hang Dong Districts ($p>0.05$), the DDT exposure in Mae Rim District was, in fact, much more extensive and intensive than that in Hang Dong District. This was supported by considering the ratio of p,p'-DDT to p,p'-DDE which is much lower in Hang Dong District with only 37% of cases exceeding 1 (Table 3.8), indicating the limitation of DDT use in this area. In addition, the annual amount of DDT (75 WP) sprayed in Hang Dong District seems to be decreased according to report of Malaria Center Region 2 (Appendix Table 8).

The mean total DDT residue in eggs from Muang District was below the ERL with only one egg exceeding this level (Table 3.8). In reality, the eggs collected in Muang District came from 2 sources: residential area and forestry area (Doi Suthep National Park). The eggs from residential area contained less total DDT residue than those from forestry area (see detailed data in Appendix Table 10). Moreover, among six eggs collected from Doi Suthep, three egg samples (coded as HCK1, HCK2, HCK3) obtained from Hmong hilltribe villagers contained higher concentration of total DDT residue than any egg surveyed in Muang District. One egg (HCK2) was even found to have the total DDT residue exceeding the ERL. So far , there is no report about the history of DDT application on Doi Suthep. However, informal interviews with villagers revealed that the ability of pesticide application, including DDT, in the hilltribe villages was significantly possible. It was also realized that the chickens in this village were usually mainly fed with cabbage grown by the villagers. This cabbage might contain high concentration of pesticide residues resulted from the villagers' careless pesticide application.

The mean concentration of total DDT found in San Kampaeng eggs was lower than the ERL and there was no egg exceeding this level. All the p,p'-DDT to p,p'-DDE ratios found in San Kampaeng eggs were less than one (Table 3.8). In comparison with Mae Rim and Hang Dong Districts, Muang and San Kampaeng Districts represent for urban and craft-industrial areas with high density of population and low risk of malaria disease. Therefore, the use of organochlorine, especially DDT, in the public health program or in agricultural purposes is limited in these areas. Practically, the people living in these areas are aware of the detrimental effects of DDT, and hence, they have shifted to use other pesticides which may cause less harmful impacts than DDT. This was supported by the higher number of positive eggs containing dieldrin as well as the higher concentration of dieldrin found in eggs from Muang and San Kampaeng when compared with the values from Hang Dong and Mae Rim District (Table 3.9). It was also noted that in San Kampaeng District, instead of using DDT which is long persistent and mark bioaccumulation to control disease vectors, the public health center is now in the process of applying Cypermethin 10E (Sundat, Singapore), a kind of pyrethroid pesticide which is toxic to insects but far less persistent and almost no bioaccumulation [62].

In contrast, people living in forestry and mountainous areas used to be careless in their pesticide application. Besides the annual amount of DDT applied legally by the Thai Government, the extra amount of DDT used by villagers is considerable. The survey in Mae Rim and Hang Dong Districts, and Doi Suthep found that many farmers applied pesticides without following any proper guidance. Some of them did not even know what kind of pesticide they were using. In these areas, there are several factors leading to indiscriminate DDT spraying such as high risk of mosquito-borne disease, lack of living facilities to protect themselves to avoid "mosquito attacks", and especially, the economic benefits achieved from DDT use for agricultural purposes.

Besides DDT and its derivatives, dieldrin and cis-heptachlor epoxide were detected in 50% and 23% of 64 egg samples, respectively. However, all of the residue levels of dieldrin and cis-heptachlor epoxide were below the ERL (Table 3.9), and were not significant different among four surveyed areas.

It is found in this study that the residues levels of p,p'-DDT were significantly associated with those of p,p'-DDE ($p<0.001$). This is in line with prior studies suggesting that p,p'-DDT is rapidly decomposed to p,p'-DDE by the action of high temperature or strong ultraviolet light [6]. Consequently, the ratio of p,p'-DDT to p,p'-DDE can be used as a bio-index to estimate the situation of DDT use in the survey areas in long term biomonitoring programs of organochlorine residues in the environment. The increase tendency of the p,p'-DDT p,p'-DDE ratio in long term biomonitoring programs indicates that DDT remains to be used in survey areas [27,30]. The high ratio (should be >1) is expectedly found in the areas with recent history of DDT application whereas the lower ratio can be obtained from the areas where DDT is no longer used. This is evidenced by the high ratio of p,p'-DDT to p,p'-DDE (1.7±1.1) found in Mae Rim District (Table 3.8). It is necessary to note that this ratio should be used only in the long term organochlorine residue biomonitoring programs. For a few egg analyses this ratio could result in "confuse conclusion".

4.4. Comparison of the Present Study with Other Reports

So far, no reported study like the present investigation has been done in Chiang Mai. However, from the available literature it seems that the mean value of total DDT residue in eggs of free-range hens in the present study is higher than that reported from other egg-screening done elsewhere as shown in Table 4.2.

Table 4.2 Comparison of organochlorine residue levels found in eggs from free-range hens in Chiang Mai suburban areas with other reported levels

Study area	No. of eggs	Dieldrin residue Mean ± SD (range)	DDT and its derivatives Mean ± SD (range)	%≥ ERL of DDT
Chiang Mai, Thailand	64	0.01 ± 0.01 (0.001-0.092)	1.60[1,2] ± 3.19 (0.004-18.7)	40
Embu and Meru, Kenya [27]	156	0.610[1,2] ± 2.06 (0.010-14.90)	1.15[1] ± 1.81 (0.020-10.3)	42
Tehran, Iran [26]	78	0.168[1,2] (0.018-0.31)	0.440 (0.110-4.78)	22
Ivory-coast [28]	200	ND	0.253 ± 0.531 (0.001-4.62)	14
Southwest Cameroon [29]	30	0.001 ± 0.003 (0-0.015)	0.141 ± 0.408 (0.004-1.99)	10

[1] Mean ≥ ERL of WHO [36]

[2] Mean ≥ MRL of Thailand [62]

It can be seen from Table 4.2 that the mean total DDT in this study is approximately 10 and 1.4 times higher than that reported in Southwest Cameroon and in Kenya, respectively. More alarming, the mean total DDT in eggs from Chiang Mai suburban areas was three times exceeding the ERL of WHO, and was twelve-fold the MRL of Germany. It even exceeded the MRL of Thailand which is already the highest (1.5 mg/kg egg) [62]. Particularly, the mean concentration of total DDT in eggs from Mae Rim District is two times exceeding the MRL of Thailand, and the correspondent value from Hang Dong District is 1.3 times higher than this limit. These findings are the warnings to all concerned to be more aware of the risk of organochlorine residues in eggs in the survey areas, particularly, and in the environment, in general.

The higher DDT residue level and the lower dieldrin residue level reported here in comparison with reported levels from other countries proved the extensive use of DDT instead of dieldrin in the study areas.

4.5 Risk Assessment to Consumers

In Thailand the number of eggs produced by free-range hens (native breed chicken) is not as numerous as from commercial hens. Most villagers raise free-range chicken for both egg and meat production which mainly were the villagers' food sources not used for marketing. The question is, therefore, proposed that whether or not we should be aware of DDT residue levels in these eggs which are 2-fold the MRL of Thailand.

Evaluation of the potential health hazard is difficult since it is uncertain to what extent the levels reported here can cause chronic toxicity in mammals and, indeed, what manifestations may result from such exposure. Practically, all these organochlorine compounds are acutely neurotoxic, and have been reported effecting on breast cancer in women, causing decrease of sperm concentration and reproductive problems in men [40,42-45].

To protect consumer health, the joint FAO and WHO Codex Alimentarius Commission has recommended the ERLs and the ADI levels of organochlorines in various foods and animal feeds (Appendix Table 1). The ADI for man, expressed on a body-weight basis, is the amount of a pesticide that can be consumed daily without appreciate risk and it should not be exceeded for prolonged periods [37-38]. Taking this approach, the present results were compared with the ADIs for a male adult with 70 kg and a child with 15 kg in body weight. The average dieldrin content of each egg was one-eighteenth of the ADI for an adult and one-fourth of the ADI for a child while the mean DDT content of each egg was about one-twentieth of the ADI for an adult and one-fifth of that for a child. More alarming, the highest total DDT content in the eggs surveyed was one-second and one-half the ADI for an adult and for a child, respectively. In addition, it has to be recognized that eggs are not the only source of organochlorine residues in the diet of adults and children since these chemicals are also found in other foods, e.g. meats, vegetables, and fruits.

4.6 Recommendation to Present Use of DDT in Thailand

The present findings indicate that there is a need to review the use of some organochlorine insecticides, especially DDT, in the survey areas. Man must learn how to live with pesticides. Before using any pesticide there is a need to know whether it is likely that in the future the pesticide will become more dangerous and cause more damage to the environment. Organochlorine pesticides have been proved to cause adverse impacts on humans. Organochlorines are also not the only choice to control mosquito vector disease since some studies have reported the behavioral resistance of mosquitoes to DDT [3,4,65]. Therefore, the extensive use of DDT for fighting mosquito-borne diseases in Thailand should be reviewed and other safer alternatives to DDT should be seriously considered.

The pesticide regulatory decisions should be based on risk assessment and risk management. All efforts must be urged in order to attain the stated goal which is no more DDT use within next five years by Malaria Center Region 2. The implementation of alternative pesticides, e.g. Cypermethrin or Permethrin, is necessary. These pyrethroid pesticides were reported as larvicides and pesticides controlling adult mosquitoes which are biodegradable, rapidly inactivated in man, and low mammalian toxicity [3,64]. The amount of DDT remaining in stores should be properly disposed of (i.e. properly treatment). Besides chemical control, biological control is another choice, e.g. the introduction of the mosquito predators such as *Toxorhynchites amboinensis* (a culicidae mosquito predator) or *Gambusia affinis* (a fish). However, biological control is a intricate method which success depends on several parameters and used to be doubtful. One of the most efficient methods which is strongly recommended to the Thai Government is environmental control or environmental sanitation. This low cost and simple method can eliminate mosquito breeding areas and, therefore, considerably reduce mosquito populations. In addition, appropriate policies should be launched to improve the living standards of people living in remote areas with high risk of mosquito-borne diseases. The Thai Government should provide them the protective facilities against mosquitoes, e.g. sleeping nets, blankets, safe insect repellent gels, insect repellent lamps, and medicines. Public education, training on pesticide application process , educational campaigns to awake and enhance the public awareness of the detrimental effects of pesticides on the environment and human health should be emphasized in the sustainable development plan of the country. Last and perhaps most important of all is the implementation of integrated pest management (IPM) concept which attempts to rationalize the use of naturally operating regulatory mechanisms (predators, parasitoids), agricultural practices, selective application, and the results of population monitoring and surveillance. In the areas where DDT was really necessary to eliminate mosquitoes as the officers of Malaria Center Region 2 stated, the authorities ought to be very careful of DDT residue spraying. The side effects of DDT house spraying such as toxicity of DDT residues, bad odor, death of domestic animals, over dosage spraying, and disfigured whitish house walls should be considerably concerned. People living in the DDT heavily sprayed areas must be fully awake to the risk of DDT exposure. Villagers, especially children need to avoid contacting with DDT residue powders and need to minimize the amount of their DDT daily intake to be lower than the ADI levels of WHO. Villagers can consume the commercial eggs instead of the native breed eggs or villagers can keep their domestic animals in cages and feed them with "DDT free feed" in order to produce "clean animals" and "clean animal production". The possible transport of DDT residue from DDT-sprayed areas to DDT-unsprayed areas, to animals, and to other components of the food web or the environment should be significantly limited. Also very important is the suitable dosage of DDT spraying. The DDT dosage applied in Thailand was at the highest level recommended by WHO as stated by officer of Malaria Center Region 2.