CHAPTER 6

CONCLUSION

6.1 CONCLUSION

From a cross sectional study involving 577 schoolchildren from 60 schools in Peninsular Malaysia, the whole blood of the subjects was analysed for eleven organochlorine pesticides and metabolites and two organophosphorous pesticide residues. The results of the survey revealed the following levels in blood (nanogram per gram): aldrin, nd - 47.6; dieldrin, nd; endrin, nd; α-endosulfan, nd - 0.6; β-endosulfan, nd; endosulfan sulfate, nd; heptachlor, nd - 3.8; lindane, nd - 5.7; p,p'-DDT, nd - 3.4; o,p'-DDE, nd - 1.4; p,p'-DDE, nd; chlorpyrifos, nd - 10.3; diazinon, nd - 103.0. Seven percent of the subjects were found to have detectable levels of pesticide residues in their blood. There was a statistically significant relationship between the contaminated children and the ethnic groups to which they belong, as well as a correlation with site category (agricultural, industrial/urban and control). Ethnic Malays were found to be more exposed to pesticides compared to other races. In addition, subjects residing at the agricultural area were found to be significantly exposed to the chemicals. The relationship between the total mean pesticide residue concentration in blood and the length of stay at the current residence was also found to be statistically significant ($p < 0.05$). In contrast, there was no correlation between the total mean pesticide residue concentration and the length of study in the respective schools as well as the average travel time per day ($p > 0.05$). Furthermore, no correlation was observed between the mean pesticide residue concentration in blood and the rate of food consumption ($p > 0.05$).

The SPE procedure that was developed for the extraction of plasma provided consistent results and yielded high recoveries for all the analytes averaging from with coefficient variation. The procedure was relatively rapid compared to the published methods utilizing LLE. Sample clean-up for the tissues samples utilizing SPE was found to be efficient in

removing lipid contents. In addition, it was more effective and faster compared to the classical method using chromatographic columns. Hence, this method of sample clean-up gives faster operation in which a large number of samples may be processed within a given time.

In the acute exposure study, technical grade endosulfan (70α:20β) dissolved in TWEEN 80 was administered to male Sprague-Dawley rats by oral gavage at a dose level of 10 mg/kg body weight. Animals were decapitated at intervals of 0.5, 1, 2, 4, 8, 10, 24 hr and seven days following administration. Blood was sampled at each time interval and analysed for α-endosulfan, β-endosulfan, endosulfan sulfate and endosulfan diol. The concentrations of the parent compound and metabolites were also determined in the kidneys and livers seven days following administration.

Throughout the experiment, only α-endosulfan was detected in blood. The plasma concentration of α-endosulfan as a function of time was found to best fit a one-compartment model, consistent with a first-order process. Following oral administration, the highest blood concentration was observed 30 minutes after treatment, with a mean value of 75 ± 4 ng/ml. The biological half-life was determined to be 55 minutes. α-endosulfan was not detected after four hours following administration.

At the end of seven days, the kidneys and the liver were examined for the presence of the parent compound and metabolites. α-endosulfan and β-endosulfan were detected in the kidneys averaging 0.38 ± 0.17 μg/g and 0.35 ± 0.19 μg/g respectively. Endosulfan sulfate and the diol were undetected in both the kidneys and liver.

The body weight and the weights of the liver, kidneys, testes and epididymis in treated animals were statistically insignificant compared to control rats ($p > 0.05$). However, the sera testosterone levels in treated animals were found to be significantly higher than control animals ($p < 0.05$).

In the subacute study, male Sprague-Dawley rats were fed with technical grade endosulfan (70α:20β) dissolved in TWEEN 80 by oral gavage at a dose level of 5 and 10 mg/kg bw/day for 15 days. A vehicle control group received TWEEN 80 over the same treatment period. Half of the animals in the two groups were decapitated on the 16th day and the remaining half on the 30th day (15 days after the last treatment).

Following administration, the distribution of endosulfan and its metabolite, endosulfan sulfate was determined in plasma, kidneys and liver. In addition, animals were also observed for signs of toxicity. Upon termination of treatment, body weight and the weights of selected organs were determined. In addition, histological examination of the testes was carried out and sera testosterone, T3 and insulin levels determined.

No mortality was observed in control group. However, 36% of the mortality rate was observed with the high-dosed group; one rat died on the 6th and four on the 7th day of the treatment period. Obvious signs of intoxication were observed in animals treated with the higher dose compared to control animals including hypersensitivity such as excitation to touch, noise, trembling, squatting posture and bloody discharge from the eyes before death. Most animals also exhibited severe diarrhea. Animals treated with the lower dose exhibited essentially normal appearance and behavior although some animals showed signs of mild diarrhea.

On the 16th day, the α and β-isomers of endosulfan in rats receiving the 5 mg/kg dosage were detected only in the kidneys with a mean concentration of 0.95 ± 0.23 μg/g and 0.51 ± 0.15 μg/g respectively. Endosulfan sulfate was undetected in the kidneys. However, it was detected in the liver with a mean concentration of 0.09 ± 0.04 μg/g. The parent compounds were not detected in the liver. Neither the parent compound nor its metabolite was detected in plasma. On the 30th day, α- and β-isomers were undetected in the kidneys. However, endosulfan sulfate was still detected in the liver, with its mean concentration decreased to 0.05 ± 0.01 μg/g.

At a higher-dosed group (10 mg/kg), the distribution pattern of endosulfan and its metabolite followed similar trends with higher mean concentrations compared to the lower dosage. The mean concentrations of the α-isomer and β-isomer in the kidneys on the 16th day were 1.62 ± 0.64 μg/g and 0.64 ± 0.16 μg/g respectively, while the mean concentration of endosulfan sulfate in the liver was 0.09 ± 0.03 μg/g on the 16th day and 0.06 ± 0.01 μg/g on the 30th day.

Significant reduction in body weights were recorded in both treated groups compared to control animals during the treatment period. However, most animals were noted to regain their initial body weights by the end of the study. Significant histological changes of the testes in the forms of reduction of tubules diameter and the formation of clumped cells in the treated groups were observed. In addition, damage to the membrane of the tubules was also noted.

Significant changes were also observed in the levels of sera testosterone and T3 of both treated groups compared to the control animals. Animals treated with 5 mg/kg bw/day of endosulfan exhibited significantly lower levels of testosterone and T3 (105.22 ± 6.51 ng/dL and 58.79 ± 1.32 ng/dL respectively) on the 16th day compared to control animals whose concentrations of testosterone and T3 were 226.00 ± 18.00 ng/dL and 72.61 ± 2.56 ng/dL respectively. However, the animals regained their hormonal levels on the 30th day to 143.90 ± 21.22 ng/dL (testosterone) and 70.91 ± 2.93 ng/dL (T3) which were noted to be lower than control animals.

As with the higher-dosed group, animals treated with 10 mg/kg endosulfan, exhibited statistically lower testosterone and T3 levels compared to control animals on the 16th day (31.19 ± 6.72 ng/dL and 49.98 ± 3.45 ng/dL respectively). The hormone levels also showed similar trends as observed with the lower-dosed group.

The change in insulin levels in treated animals were statistically insignificant compared to control animals for both the treated groups.

6.2 RECOMMENDATION FOR FUTURE STUDY

To date, there is little information available in substantial accumulation of endosulfan in tissues. The kinetic behavior of endosulfan and the mechanism of changes observed in the present experiment need further investigation. Experiments on whether endosulfan alters thyroid function such as the induction of P-450s activities and thyroid gland histopathology need to be investigated because the latter has been proven to be the most reliable parameter for the detection of compounds that alter thyroid function by DeVito *et al.* (1999). In addition, sperm abnormality study such as the sperm morphology, sperm

count and biochemical changes in testes should be further investigated to ascertain the reproductive effects of endosulfan in rats. Pair-feeding experiments should be included in the experiment in order to evaluate the possibly sensitive effects of a chemical on the organ weight as suggested by Uemitsu *et al.* (1986). Only further investigation will lead to establishing a complete toxicological profile of endosulfan.