4. DISCUSSION

Following solid phase extraction (SPE) residues of carbaryl in water and soil samples were determined by HPLC with UV-VIS detection. Octadecyl (C_{18}) sorbent was employed in the solid-phase extraction to extract and concentrate the analytes from the water and soil samples. Clean-up and preconcentration on selective C_{18}-SPE cartridges is a simple and rapid single pretreatment step. The method is superior to conventional liquid-liquid extraction in terms of accuracy, precision, analysis time and less toxicity.

HPLC conditions for the analysis of carbaryl are listed in Table 2.1. Optimization for maximum absorption of carbaryl at different wavelengths was done. In general, one would analyze the substance of his interest at the maximum ultraviolet (UV) absorption, which in this instance clearly occured at 220 nm, as shown in Table 3.1 and Figure 3.1.

Values of number of theoretical plates (N) of the HPLC column used in this work are shown in Table 3.2. The average value of N was found to be about 4260, indicating that the HPLC column efficiency was still in a good condition

The limit of detection in this experiment was found by injecting low concentrations of standard carbaryl onto the HPLC column to find out the noise level which was 0.0004 µg/ml, as shown in Figure 3.4. The height of this noise level was measured and the detection limit was obtained as two times of this value and it was found to be 0.0008 µg/ml, as shown in Figure 3.5.

Peak areas obtained were plotted against carbaryl concentrations as shown in Figures 3.6 and 3.7 for the linearity range of carbaryl with high and low concentrations, respectively. The linearity range for carbaryl at 220 nm was found to be 0.04 to 120 μg/ml, as shown in Table 3.3 and Figure 3.7. This linearity range was adequate as it covered most carbaryl concentrations that might appear in soil and water samples.

In addition to sensitivity, the HPLC method for carbaryl detection developed in this work was found to require a relatively shorter analysis than other methods described. For example, six samples could be prepared within two hours for chromatographic analysis which subsequently needed only 7 minutes per run. Therefore, the method adapted in this study was found to be economical as well as extremely sensitive. This method could also be used for quantitation of carbaryl, its degradation products as reported by Ward et al [7]. The method described, therefore, has potential for a variety of application for studies in environmental toxicology.

An investigation to minimize the volume of carbaryl eluent was done after the mobile phase had been chosen. The mobile phase used was ACN/1 mmole ammonium acetate buffer (1:1) while ACN/water (1:1) was used as the eluent for carbaryl. After the carbaryl standard was spiked into sample matrices and passed through the solid phase extraction process, then the carbaryl was eluted with that eluent and it was collected in 4 fractions of 1 ml each. The peak areas of carbaryl in different fractions of eluent are listed in Table 3.4 and shown as histograms in Figures 3.8 and 3.9 for two different concentrations of carbaryl standard spiking. It was found that the second fraction contained the highest amount of carbaryl and the last fraction (3.0-4.0 ml of eluent) had a very low amount of carbaryl. From these results, peak areas of carbaryl of two fractions (3.5 ml following with 1.0 ml) were designed for comparison. Since the peak area of carbaryl eluted by 3.5 ml eluent is about 50-70 times greater than the area resulted from the elution by 1 ml eluent, the optimized volume for carbaryl elution could be deduced as 3.5 ml.

Repeatability of the injection by autosampler was also tested by making multiple injections of the same carbaryl standard concentration. The results shown in Table 3.6 reveal that the standard deviation were 6.63 x 10^3 and 321 with the variation coefficients of 0.50 and 0.27 % for peak areas and peak heights, respectively. These values indicate a very narrow change of detector response for the same concentration of standard used.

Confirmation test was done to ascertain that the "sought for" analyte was carbaryl by calculating the ratios of peak areas at two different wavelengths, as shown in Table 3.7. Both standard and samples were injected at 220 and 230 nm under the same conditions and with the same injection volume (20 μl). After that the ratios of peak areas at these two wavelengths were calculated. The results were in the range of 4.8-5.1 which represented an acceptable range due to the fact that the same substance should result in the same value of peak area ratio.

In sample preparation steps using C18 solid phase extraction, percent recoveries of the extraction process are listed in Tables 3.8 and 3.9. Percent recovery work of carbaryl in both soil and water samples after spiking and passing through the SPE was done in order to determine the efficiency of the C_{18} SPE cartridge for carbaryl extraction. For soil samples, recoveries range was found to be 86 to 95 % with 2.5 standard deviation and 2.7 in %Cv, as shown in Table 3.8. Percent recovery range was found to be 92 to 97 % with the standard deviation of 2.3 for water samples, as shown in Table 3.9. This method could therefore be used as the standard operation method for extraction of carbaryl from sample matrices in this work.

Field studies were conducted to determine residue levels of carbaryl in water and soil samples. The first experiment was done at Ban Sop Pao in Lamphun Province, the location shown in Figures 2.1 and 2.2. The area for sample collection was 4 x 20 m^2, as shown in Figure 2.3. Both water and soil samples were designed to be collected randomly on the day before spraying, right after spraying, and 1, 2, 3, 4 and 8 days after spraying. Physical and chemical parameters such as pH and temperature were measured and recorded every time when sample collections were made, as shown in Tables 3.10.

Moisture content, organic matter and pH of the soil were measured in the laboratory and the results as shown in Tables 3.11, 3.13 and 3.15, respectively for the first experiment. Moisture content of the soil was found to be quite high within the range of 16.6-23.7 % as it was still the rainy season. Organic content in soil samples was found to be in the range of 8.8-9.5 % representing a narrow range of changing. pH of the soil was relatively constant over the periods of soil collection.

The results of the residue assays for carbaryl in soil samples from treated kale field at Ban Sop Pao in Lamphun Province are summarized in Table 3.17 and Figure 3.10. Detectable residues of carbaryl were present in every soil samples. As mentioned in the sampling design, the application of carbaryl solution with concentration of 3 g/l for 10 liters volume was done by using a sprayer. The carbaryl formulation with the tradename Sevin 85WP was 85% WP 1-napthyl methylcarbamate. Thus 30 g of the Sevin used contained 25.5 g carbaryl. Carbaryl concentration at the soil surface of this 80 m^2 area should be 5.0 mg/kg soil. Details of this calculation are given in Appendix 2. The surface soil sample collected on the day of carbaryl application was found to have the carbaryl concentration of 0.18

mg/kg which was about 28 times less than what it should have been. This is because most of carbaryl was coated on the outer surface of kales. Similarly, carbaryl concentration, of soil samples at the second depth was found to be as low as 0.09 mg/kg on the day of spraying. This can be explained that carbaryl solution itself could not yet leach to the deep layer of soil after a short period of time after spraying.

Figure 3.10 shows amount of carbaryl in soil samples which was detected the days after spraying. The fluctuation in carbaryl concentration caused by many factors such as rain, sunlight, temperature and irrigation system. Since carbaryl is moderately soluble in water (120 mg/l at 30 °C) [8], it could be easily leached from the outer surface of vegetable leaves directly to soil by watering. As can be seen in Figure 3.10, carbaryl concentration in surface soil samples (0-15 cm) increased in three steps on the first, third and fourth day, respectively after carbaryl application. This phenomenon could have been resulted from the plant watering. It should be noted that the vegetable grower did water the vegetable fields in the evening of the application day and the second day after. The third step of increased carbaryl concentration was caused by overnight raining before sample collections. For deeper level soil samples, the interassay data of carbaryl concentration hardly showed significant change properly because of the soil composition. As mentioned in the background information of the study site (Appendix 2), soil samples at the surface and at the deeper levels could be classified as clay and silty clay, respectively. Thus the percentages of clay and silt are very much higher than sand, providing the capacity to maintain high water content for the surface soil particles. The probability of water to assimilate to the lower level soil is quite low. This could be a reason to explain why the carbaryl concentrations detected in soil at the second depth were found to be more or less in the same range. The slight fluctuation of the data migth have been caused by contamination from soil surface during sample collection and to a lesser extent by external factors such as rain, sunlight or temperature.

However, the complexity of problems increases even more when the behavior of chemicals in soil is taken into consideration. The ability of soil particles to adsorb and bind chemicals for long periods of time can result in very slow degradation rates, compared to rates in plants and animals [2]. This also could be accountable for a higher concentration of carbaryl in surface soil sample on the eighth day after application than the concentration on the day of carbaryl spraying.

The amounts of carbaryl detected in water samples were very low as shown in Table 3.18 and Figure 3.11 compared with those found in the soil samples. Water samples collected promptly after carbaryl application yielded the highest value of carbaryl concentration (1.1 μg/l) in comparison with carbaryl concentrations from water samples on other days. Figure 3.11 shows that carbaryl concentration in water samples decreased from 1.1 μg/l on the day of application to 0.13, and 0.07 μg/l on the first and second day after application, respectively, and none was detected on the third day after application. The amount of carbaryl was found to have increased to approximately 1 μg/l on the fourth day after application which happened to concide with the fact that there was a heavy overnight raining before sample collection. On the last day of sample collection (8 days after carbaryl application), none of the carbaryl was detected in water sample. From this data, it may be stated that the degradation rate of carbaryl in water is faster than that in soil. However, direct transfer of carbaryl into water supplies must be avoided.

To determine if the reproducibility of the method was adequate, five analyses were carried out for each sample of soil and water in a single day. Results of soil at two depths are given in Tables 3.19, 3.20 and those of water samples are given in Table 3.21. The interassay data yielded %Cv from 2.6 to 9.7 for surface soil samples, 4.0 to 12 for soil samples at the second depth (15-30 cm), and 2.7 to 6.6 for water samples. These values suggest that the carbaryl concentrations in each subsample analyzed were not so different and therefore the method used is good enough for analyzing the carbaryl. In addition, water samples were found to be homogenized than soil samples because of a lower range of % Cv.

Due to the worldwide increase in the use of pesticides, the presence of insecticide residues is found not only on the crops to which they are applied but also, owing to leaching into and run-off from the soil, in ground water and surface water, respectively. From the ecological point of view, the risk assessment for any potential hazard to the living things should be considered. If the highest carbaryl concentration in water samples in this experiment was considered in terms of risk assessment, a likely event could be that water was consumed by a rat. Knowing that maximum residue limit (MRL) of carbaryl in drinking water is 0.1 μg/l, as recommended in Germany, and that carbaryl has an oral LD_{50} of 500 mg/kg in female rats, one can do simple calculations to find out if the carbaryl concentration found here had any effect on rats. Suppose a 0.5 kg female rat consumed 0.5 liter of water contaminated by 1.1 μg/l carbaryl, the highest concentration found in this experiment. The maximum amount of carbaryl which could be consumed by a female rat would be 500 mg. But half a liter of this contaminated water would contain only 0.55 μg carbaryl, which is several thousands times less than the oral LD_{50}. It is quite apparent that the highest amount of carbaryl found in this work would have no effect to rats.

Risk assessment for higher level organisms such as dogs or human beings can be done in a similar manner. A human acceptable daily intake (ADI) for carbaryl recommened by FAO/WHO in 1973 was 0-0.01 mg/kg body weight [8]. Normally, human daily intake for drinking water is about 2 liters. If this amount of drinking water was contaminated by 1.1 μg/l carbaryl, the amount of carbaryl taken by human would be 2.2 μg at most. If the weight of the man who had consumed the contaminated water was 60 kg, the highest amount of carbaryl which could be taken would be about 0.60 mg. But the amount of carbaryl from 2 liters of the contaminated water was 2.2 μg which is, of course, much lower than 0.60 mg, the recommened ADI. This means that an average man could drink this contaminated water without any effect to him.

The fact that the carbaryl concentration remaining in soil surface samples collected on the last day was still high and the graph pattern did not appear conclusive yet (Figure 3.10) prompted the author to embark on the second study site at Saraphi District, Chiang Mai Province to study the persistence of carbaryl in soil surface without any contribution from vegetables to clarify the problem of high concentration level residue in soil sample. Soil sample was designed to be collected in such a way that sample collection could be made on the day before carbaryl spraying, right after spraying, 1, 2, 3, 5, 7, 11, 15, 22, 29,40 and 60 days after spraying. In application of carbaryl, the concentration of spray solution used was 3 g/l with 1 liter volume. The insecticide used for application was the same as that used in the first experiment. As the content of the insecticide was 85 %WP 1-napthyl methylcarbamate, 3 g insecticide contained 2.6 g carbaryl.

The area of sampling site was 2 m x 4 m which was evenly sprayed with 2.6 g carbaryl on the soil surface. The soil samples were collected randomly from 10 spots throughout the area with a 10 cm i.d. core sampler which amounted the sample about half a kilogram each. Carbaryl concentration found in soil samples on the day of application could have been about 5.0 mg/kg (Appendix 2). As the result shown in Table 3.22, carbaryl concentrations found in soil samples on the day of application and the first day after were 6.8 and 5.7 mg/kg, respectively, which appeared to be higher values than the expected value.This could have been attributed by the technique of application of the inexperinced worker, causing an uneven distribution of the carbaryl throughout the plot. Consequently, when soil samples were randomly collected from 10 spots and mixed together, it could be that the number of spots in the higher concentration area was greater than the spots in the lower concentration one and resulted in unusally high amounts of carbaryl content. Additionally, the calculation is based on the approximate values of amounts of soil taken each time and the inner diameter of core sampler. Therefore, the theoretical value of carbaryl concentration found on the day of spraying does not represent the actual concentration.

The physical and chemical parameters of the second experiment including soil moisture content, soil organic matter and soil pH were measured in the laboratory and the results are shown in Tables 3.12, 3.14, and 3.16, respectively. Most of the moisture content values in soil samples were quite low compared with the first experiment because of dry weather. Thier values were in the range of 2.6 to 8.3 %. Organic matters in soil and pH of the soil presented of each day of collection were not so much different.

Percentage of organic matters in soil was in the range of 6.7 to 7.5 while pH of the soil was in the range of 5.5 to 6.5.

Each soil samples was divided into 6 subsamples, as shown in the diagram in Figure 2.6. One subsample was spiked with standard carbaryl to enable comparisons to be made with the other five subsamples and to serve the work on percent recovery. The summary of statistical data was shown in Table 3.23. Percent recoveries represent the efficiency of the method used, Therefore, percent recoveries in this experiment with the range 82-99 % appeared to be acceptable values. Percentage of variation coefficient (%Cv) and standard deviation (SD) can tell the fluctuation of the data collected. Thus, %Cv of 5.4 to 10 and SD of 0.06-0.39 illustrated that there were not so big different values for the samples analyzed each day.

Figure 3.12 shows the degradation rate of carbaryl in soil. As mentioned, the persistence of carbaryl in soil is rather long because soil particles adsorb and bind this chemical for long periods of time. When an insectide reaches the soil, its fate is dependent on a number of conditions, including soil type, pH, organic content, moisture content and the exposure to environmental parameters, such as wind, sunlight, rain, temperature and humidity.

Seasonwise, the second experiment was carried out in winter starting from 15th December 1993 to 14th Febuary 1994. During this time the weather was dry and cool and there was strong sunlight during the daytime. Without any rain and due to dry weather, soil moisture was found to be very low compared with that of the first experiment. As illustrated in Figure 3.12, the carbaryl concentration can be seen to decrease without any fluctuation.

Quality assessment can be considered for checking of the precision of the experimental data. It is the process of using external and internal quality control measurements to determine the quality of the data produced in this experiment. Precision is a measure of the closeness with which multiple analyses of a given sample agreeable with each other. Precision assessment can be done by replicate analyses. Table 3.24 shows the percentage of variation cofficients for the steps of analysis. Results from this table can be concluded that %Cv for the HPLC auto-injection was the lowest (0.5 %) which was followed by %Cv for SPE plus injection (2.7 %). On the other hand, variation coefficients of the whole process (including homogenization SPE and injection) for surface soil samples, soils at the second depth and water samples in the first experiment were 5.2, 7.8 and 4.6%, respectively. This suggests that homogenization of soil samples was the weakest point of the whole analysis whereas %Cv for homogenization of water samples was less than soil due to their different states. For the second experiment, percentage of variation coefficient was found to be 7.2 for the whole analysis.

The amounts of carbaryl in soil samples from the second experiment were very much higher than those obtained from the first experiment because there were no vegetables taking part on the soil surface in the second experiment. The carbaryl degradation rate was found to be very fast in the first three days after spraying due to the fact that the soil surface was directly exposed to the air and sunlight. From the graph of carbaryl degradation in Figure 3.12, the half life of carbaryl may be calculated via the equation below :

$$t_{1/2} = \frac{-0.693\,t}{2.303 \log (N_t/N_0)}$$

where, t = time (day)

N_t = amount of carbaryl on day t

N_0 = amount of carbaryl on the spraying day

By calculation where t equals to 3 days, N_t and N_0 are equal to 2.5 and 6.8 mg/kg, respectively, the half life of carbaryl was found to be approximately 2 days. This means that the starting carbaryl concentration would be decreased down to half amount in 2 days. The degradation rate of carbaryl during the first few days after application was found to be very fast.This is quite typical of carbamate insecticide. Because of such characteristics, carbamate insecticides tend to be more widely used than the other types of pesticides nowadays. It should be noted here, however, that in Germany carbaryl was banned in 1986 because its well-documented effects to the living things and also the environment could not be tolerated in that country.

Although carbaryl contamination in soil generally will not directly cause any effect to higher level of living organisms such as human beings or animals, it can be leached from the soil into ground water and surface waters. Because of the proven or suspected toxicity of many of these pesticides, the monitoring of such pesticides is needed from both the regulatory and the consumer's points of view.