CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

Pesticides are useful for controlling, insect pests of plants and animals. However, these chemicals are harmful for human beings and animals. Pesticides today include insecticides, miticides, fungicides, herbicides, defoliants and rodenticides. Insecticides kill insects by ingestion, by contact or by inhalation. The insect pests cause a huge damage to humans by destroying or damaging food, materials and crops (1,2). The major chemical categories of insecticides are as follows: Organophosphorus, synthetic pyrethroids, carbamate and organochlorine (3). Most insecticides are in the organophosphorus group. In Thailand, organochlorine insesticides are now rarely used. They have been replaced by carbamate and organophosphorus insecticides, such as chlorpyrifos, dichlorvos, prothiofos and methyl malathion. Organophosphorus insecticides are used in agriculture for control of pests, weeds and plant diseases of cotton, rice, nuts, vegetables, fruits etc. Organophosphorus were developed for their effects on insect, which are similar to their effects on humans. Some are very poisonous and have even used in World War II as nerve agents. These insecticides were commonly used in the past but at present many have been replaced due to their hazard to health their effects and stability in the environment. Compounds of dangerously high mammalian toxicity are included in this group, reflecting the interest of using them to make chemical weapons during the war years. Most organophosphorus are toxic to the nervous system by disrupting an enzyme which regulates acetylcholine, a neurotransmitter. The action

of the organophosphorus insecticides depends upon inhibition of the enzyme acetylcholinesterase. The neurotransmitter substance, acetylcholine, functions in various parts of the nervous system in both insects and mammals. Inhibition of the enzyme, this reaction, in contrast to the normal acetylating by acetylcholine, is less readily reversible to regenerate the active enzyme. Toxicity can cause illness or death. Neurotoxicity is restricted to the nervous system and teratology to embryonic stages (4). Royal Project Foundation (RPF) has been trying to persuade the highland farmers to minimize use of pesticides in agriculture by introducing IPM (Integrated pest management) to them. However, selected effective pesticides are still recommended to them when other methods cannot control the pest. Royal Project's vegetables and fruits are routinely checked at planting plots 1-2 days before harvesting. The produces are randomly rechecked at the local packing house and once more at the analytical laboratory of Plant Protection Center, RPF, using GT pesticide test kit (personal contact information). Such test kit can detect only some pesticide residues in carbamate and organophosphorus group but not in quantitative amount.. The test kit can be used in the laboratory with inextensive training. The test may result in false positive and negative readings. The strong colored vegetables may interfere with reading. The results cannot tell the amount of each pesticide found as the high performance liquid chromatograph (HPLC) and gas chromatograph (GC) does. Analysis of vegetables for pesticide residue is complex because of the matrix. In order to, obtain accurate result, be efficient in analyzing pesticide residue, several types of sample preparation have been made. The qualitative and quantitative determination of pesticide in aqueous samples is usually performed by liquid- liquid extraction (LLE) and solid-phase extraction (SPE) method (5). Both procedures require several steps for sample preparation, where the amount of solvent must be used. Solid phase microextraction (SPME) is considered to be a fast, selective

and solvent-free direct extraction of the analytical technique which can combine both extraction and pre-concentration in one step (6).

1.2 Previous analytical methods for organophosphorus pesticide

Previous analytical methods used for determination of organophosphorus pesticide upon the level of sensitivity required and examples of analyses of different samples using particular method for determination of OPPs are shown in Table1.1.

Pesticides are used in agriculture for three main purposes; to produce a larger yield of crop, with higher quality and to reduce the input of labour and energy into crop production. Insecticides are widely used in agriculture because of their powerful activity against insects. Analytical procedures have been proposed to determine and control insecticides in vegetables. Of these procedures, GC and HPLC methods were used along with using LLE and SPE for cleaning up.

Very polar organophosphorus compounds could not be extracted from water by using commonly available SFE cartridges. In addition, GC analysis on these compounds was found to be troublesome due to their polar and thermolabile character. The development of an alternative highly sensitive and selective method for the determination of the above mentioned, very polar organophosphorus in water, was based on LC-MS. One ml of water samples were directly injected onto an RP18 HPLC . The detection limits were in the range of 0.01-0.03 µg/l and compared to conventional GC methods. The developed LC-MS for analysis of polar organophosphorus was a more favourable alternative to GC (20). There were report on analysis of several organophosphorus and carbamate pesticide residues in the bodies of honeybees using GC and gel permeation chromatography (GPC) for

cleaning up. Freeze-dried insect samples were blended with diatomaceous earth, then eluted with methylene chloride. The samples were cleaned up by GPC and a cyclohexane-ethylacetate eluant. The extraction process was rapid and the results were good. It reduced cost and consumption of solvents, thereby safeguarding the health of the analyst and the environment (21). Organophosphorus pesticide residues in food matrixs were analyzed. An automated high-performance gel permeation chromatography (HPGPC) system with high performance column was used for cleaning up the wheat extracts. Using fast temperature programming with a conventional GC oven, the analysis time was reduced compared to the conventional GC technique (22). Flow injection analysis (FIA) was developed and applied to monitor organophosphate and cabarmate pesticides in spiked samples of tap water, drinking water and fruit juices, by a biosensor with photothermal biosensor detection. The biosensor was used for making or direct detection of pesticides in spiked samples without any pretreatment steps. Limits of detection for organophosphate from 1 ng/ml to 4 µg/ml (23). A simple and fast miniaturized automated matrix solid-phase dispersion method for the sample preparation and quantitative extraction of organophosphorus pesticides and a pyrethroid in orange was developed. Only 25 mg of sample and 100 µl of organic solvent were used per one analysis. The extracts were analysed by GC-MS without any purification. LOD was 4-90 µg/kg. The recoveries were 83-118% and RSD were 10-13%. The method for analysis of apple, pear and grapes was also studied and equally good results were obtained (24).

1.3 Previous analytical methods for organophosphorus pesticide using solid phase microextraction

Solid phase microextraction (SPME) is a direct extraction of the analytes with the use of a small- diameter silica fiber coated with a polymeric stationary phase. The fiber was then housed in a syringe assembly for protection. Using SPME can eliminate the separate concentration step from the LLE and SPE methods. SPME involves the analytes diffusion directly into the fiber and the analytes will be concentrated there. The fiber is then transferred directly into the injection port of the gas chromatograph where all the analytes are thermally desorbed and deposited at the head of the GC column. All steps of the sample preparation such as extraction, concentration, derivatization and transfer to the chromatograph, are integrated in one step and in one device. SPME methods have been developed for a variety of applications, including environment, drugs, blood, urine, water, soil, air and food (25, 26).

SPME, a sample preparation technique, having advantages on solvent-free extraction, and simplicity, has been discussed in several papers. (27, 28, 29), SPME can be used for determining pesticides in human body fluids, including serum and urine samples. The procedures were applied to the samples obtained from exposed and non-exposed subjects (28 : 217). The analysis of fire ant pesticides in water was accomplished by combining SPME with either gas chromatography/quadruple ion trap mass spectrometry (GC/MS) or high-performance liquid chromatography / quadruple ion trap MS (HPLC/MS). The method was developed to be sensitive, and each result was obtained from only 10 min extractions from standards in water. The results were shown to be linear in the range of 100 ng/l-100 µg/l (30). The analyte

mixture contained organonitrogen, organochlorine and organophosphorus in groundwater samples and soil samples. These analyte mixtures were determined by solid-phase microextraction and gas chromatography-mass spectrometry. Both polyacrylate (PAC) and polydimethylsiloxane (PDMS) coated fibers were used to extract the analytes directly from the samples over the concentration range 1-100 µg/l. The limits of detection were determined to be at the ng/l to sub ng/l levels, depending on the selectivity of the coating for each analyte. Limits of detection (LOD), equilibration times and precision of some analytes are listed in Table 1.2 (6 : 932).

Table 1.1 Various analytical method for the determination of organophosphorus pesticide from different samples

Sample	Preparation	Analytical method	Detection limit	Recovery	Reference
Beef fat	Solid phase extraction (SPE)	GC-FID	6-31ng/l	122% ±13%	(7)
Olive oil	Low-temperature clean-up	GC-NPD	-	77-104%	(8)
Food, biological and environmental samples	Supercritical fuid extraction (SFE)	TLC and HPTLC	0.05 – 0.5μg/ml 0.1-1 μg/ml	-	(9)
Food	Accelerated solvent extraction system (ASE)	GC-FPD	-	80-90%	(10)
Water	SPE	GC-FPD	-	>70%	(11)
Food	Organic extraction	GC-MS-MS	0.5 μg/kg	-	(12)
Soil	Microwave - assisted extraction (MAE)	GC-ECD	-	89.4 - 97.8%	(13)

Table 1.1 (continued)

Sample	Preparation	Analytical method	Detection limit	Recovery	Reference
soil	Soxhlet extraction	GC-NPD, GC-MS	5 -9 ng/g	-	(14)
water	SPE	GS-MS	-	-	(15)
Standard solution	SPE	GC-MS	-	-	(16)
Food	Column chromatography	GC, HPLC	-	75-90%	(17)
Food	Extraction	Immunoassay	0.2-0.4 ng	-	(18)
Food	-	Colorimetric	1 μg	-	(19)

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Table 1.2 Limits of detection, equilibration times and precision of some analyte

Analyte	LOD (ng/l)		equilibration (times/min)		precision (%RSD)	
	PAC	PDMS	PAC	PDMS	PAC	PDMS
Methylparathion	3	3	90	30	5	7
Chloropyrifos	1	1	90	120	8	8
Prothiofos	1	1	60	90	17	7
Ethylparathion	1	1	90	60	4	12

Headspace solid-phase microextraction (HS-SPME) was developed for the determination of organochlorine pesticides and their metabolites in sandy soil samples. The developed procedures involving temperature effect, absorption time, soil matrix and the addition of different polarity solvent were optimized. Results were compared to those achieved from using soxhlet extraction standard method. PDMS and PDMS-divinylbenzene showed good extraction efficiency. Linear of range between 0.2-4 ng/g soil was observed. The RSD was lower than 25%. LOD were between 0.06-0.65 ng/g, which were lower than those obtained from using soxhlet extraction. The recoveries were in the range of 68% to 127% (31).

The pesticide families; organochlorine, organophosphorus, triazine, thiocarbamates, substituted uracils, urea derivatives and dinitroanilines were among others analyzed in samples of different matrix complexity such as water, food, soils and biological fluids. The samples were analyzed using SPME for sample preparation coupled with GC or HPLC (32).

Fire ant pesticides in water were analyzed by combining SPME with either GC/MS or HPLC/MS. SPME is a fast, selective, and solvent –free extraction technique that accomplishes both extraction and pre-concentration events in a single step. The methods were developed to be sensitive, each obtained only 10 mins extractions from standards in water. Quantitative analyses were performed for chlorpyrifos, fenoxycarb, and avermectin. The method achieved poor precision for hydramethylnon (30 : 11).

Chinese herbal formulation was determined for organochlorine pesticide residues by using SPME coupled with GC-MS. PDMS fiber was used to extract organochlorine pesticides. The optimal experimental procedures for the adsorption and desorption of pesticides were evaluated. Detection limits were reached at below ng/g levels and comparison between SPME and soxhlet extraction showed that SPME has less than one order detection limit for pesticide residue determination (33). SPME associated with microwave assisted extraction (MAE) is an extraction technique to be used for extracting organochlorine pesticides in medicinal plants. The associated SPME and MAE, using water as the extracting solvent, showed good results for the medicinal plants (34). Pesticides in spiked water samples were concentrated by SPME technique and were desorbed from the SPME fiber coating with supercritical carbon dioxide as the desorption solvent, prior to their analyses with HPLC. Linearity was found between the tested concentration range from 0.1 to 5.0 mg/l, with the RSD between 7.4 to 12.0% (35). The herbicide profoxydim had fast degradation in methanolic, and was able to form an aqueous solution depending on the pH and the lithium salt. SPME-HPLC –UV was analysed

thermally on labile analytes in aqueous matrixs. The fibers were applied on-site at a flooded, bare soil field, and then shipped back to the laboratory, desorbed from the fiber, and determined by HPLC-UV analysis. Conventional techniques, such as LLE and SPE, require additional shipping and handling costs and time-consuming for multiple sample preparation steps. These techniques have potential for sample degradation and a loss in accuracy. Carbowax-SPME-HPLC achieve a high sensitivity for polar analytes, a limit of detection in the lower µg/l range and a precision less than 10 %RSD (36).

Organophosphorus pesticides spiked in water sample solutions were extracted with SPME and were desorbed by supercritical fluid carbon dioxide ($SFCO_2$) before on-line introduction into HPLC. Five µl of $SFCO_2$ injected into the HPLC system were dissolved in the methanol/water (80/20) as mobile phase. LOD were from 60 µg/l to 600 µg/l. RSD were from 8.6 to 13.5%.

Human fluids, including serum and urine sample, using direct immersion SPME and GC/ECD/FPD analysis were investigated for organochlorine and organophosphorus pesticides. The advantage of using SPME is reduction of procedure in sample treatment. Quantitative of SPME for the determination of pesticides in human fluids required dilution of samples prior to SPME, in order to reduce matrix influence on the extraction of pesticides. Recoveries were over 70% with RSD in the range of 1-20%. LOD were in the range of 0.1-0.4 ng/ml for organophosphorus pesticides in urine samples (28 : 220).

The analysis of organic molecules with lower molecular mass, such as drugs, metabolites, endogenous substances and poisons in body fluids were used in SPME-GC-MS and SPME-LC. The methods were good for increasing samples

in each analysis throughout which were found to improve the quality of analytical methods (26 : 167). Sarin is a organophosphorus pesticide, a methylphosphonate nerve agent and a chemical warfare agent. Very fast sampling can be achieved for conventional air. Air sampling using SPME typically use fibers that contain the phenol-based polymer (BSP3). SPME sampling in non-equilibrium mode has been used in combination with GC for rapid field analysis of a number of atmospheric constituents (37). The fused silica fiber was coated with sol-gel and was carried out as follows. The polyimide coating fiber was burnt off from the outer surface using a cigarette lighter. The burned residues were then carefully removed from the fiber tip using a tissue paper soaked with methanol (38). The bare fiber was kept submersed in a 0.1 M NaOH solution for 30 mins, rinsed with deionized water and dried in the open air for 30 mins. The fiber used, was 100 µm PDMS or 33 µm polymeric fullerene, was treated with 98% sulfuric acid and washed thoroughly with methanol. The bare fiber was then dipped into the sol solution and was kept submersed for a controlled period of time determined by the composition of the sol solution and the desired thickness of the coating to be created on the fiber. This procedure requires repeating to create thicker coatings (39,40).

Polyorganosiloxane is produced with two substituted organic groups on each silicon atom as a linear silicone polymer. If a mixture of monochlorosilane, dichlorosilane is hydrolyzed, the resulting product is dimethylsiloxane and trimethylsiloxane (41, 42).

The chemically bonded stationary phases were prepared in the following manner, and 10 g of glass beads were dried in a laboratory oven at 200°C for

24 hours. The hot glass beads were then placed into a PTFE-lined autoclave containing 50 cm^3 of a 0.5 M solution of organosilane reagent in a dry toluene. The autoclave was closed and placed in the oven at 100 °C for 10 hours. On completion of the reaction, the autoclave was cooled to room temperature, and the packing was washed five times by decantation in benzene (43). Bonded stationary phase for use in liquid chromatography(LC) was prepared on a variety of silica substrate materials. Monomeric phase was prepared by reaction with dimethyloctadecylchlorosilane under conditions. Silicas were added to a solution of 100 ml of carbon tetrachloride containing a 10 fold excess of silane. The slurry was refluxed for 4 hours, filtered, washed, and dried (44).

1.4 Purpose of the study

1. To develop SPME fiber for analysis of organophosphorus insecticide residue.

2. To develop technique for analysis of organophosphorus insecticide residue in vegetable by using SPME/GC-PFPD.

3. To analyze organophosphorus insecticide residue of mevinphos, methylparathion, prothiofos and profenofos in tomato samples using SPMS/GC-PFPD compared with using AOAC.

4. To analyze organophosphorus insecticide residue of mevinphos, methylparathion, prothiofos and profenofos in vegetables.