

# CHAPTER I

## INTRODUCTION

### 1.1 Problem Definition

In recent times, the consumer behavior has shifted towards natural products especially herbs. The herbs that can be consumed should have no poisonous residues nor should they cause any side effects to consumers' health. In essence, they ought to have medicinal properties to cure medical problems in much the same way as the present day medicines. For all these advantages, the demand for herbs and extracted substances is tremendously increased.

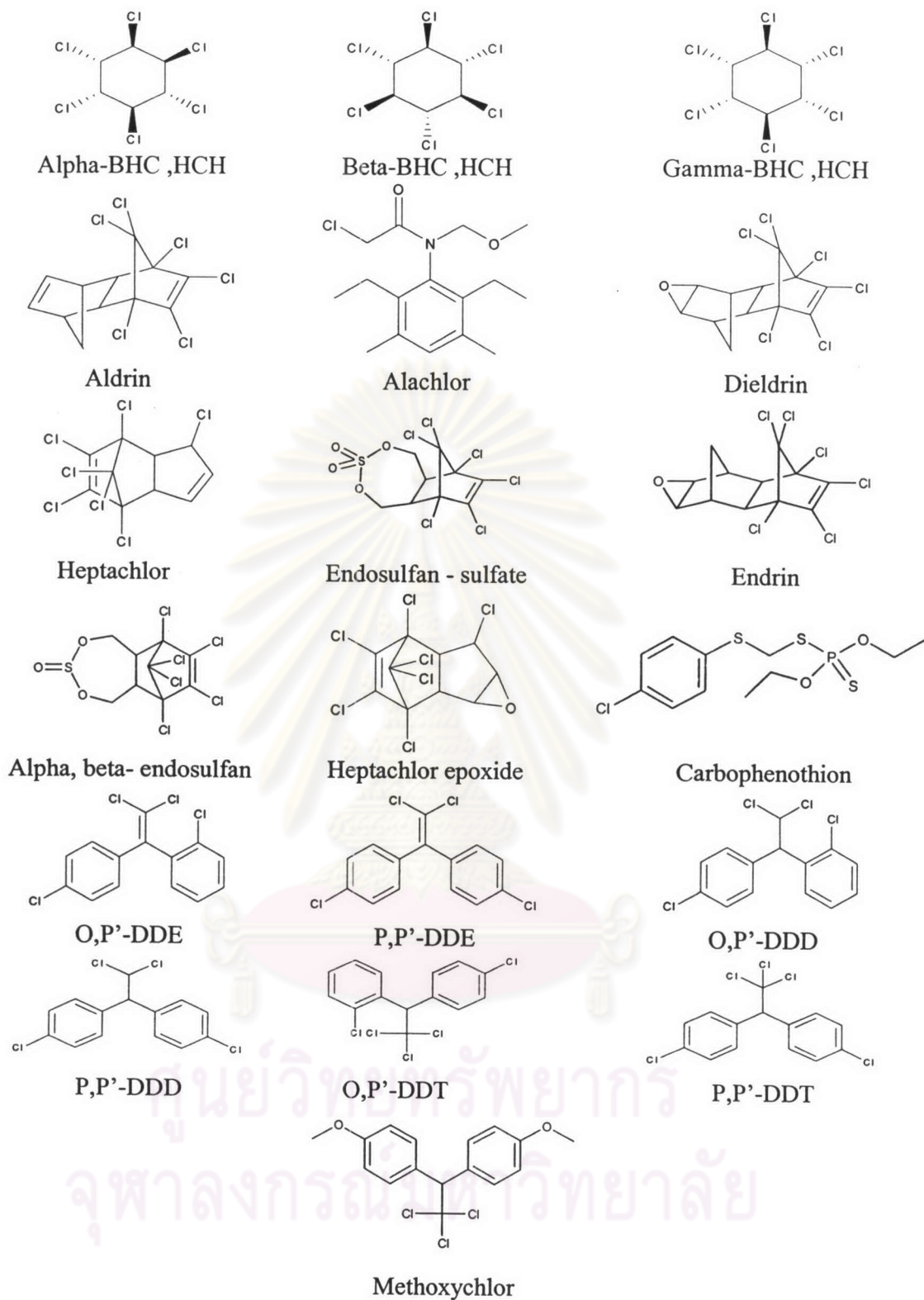
*Curcuma Longa* (curcumin) is one of the most useful Thai herbs. A number of contemporary research studies have dealt with the biological essay about curcumin, which contains a lot of medicinal properties. It is as effective as anti-inflammatory, anti-bacterial and anti-oxidant agents that can be used in place of modern medications. It also finds its place in food and cosmetic products. What is more, curcumin can help guard the liver against toxic compounds and lower cholesterol in serum.

In nature, curcumin grows in the tropical areas such as India, Indonesia and Thailand. For Thai people, curcumin is one of the economic herbs that bring in a considerable amount of family income per year. Therefore, the sheer magnitude of curcumin for export is needed and the demand for it is usually much more than the amount that can be locally produced. One method used by the farmers to increase the curcumin is the use of pesticides to protect the crop production from pests. Thus, there is a high risk that curcumin can be contaminated with pesticide chemicals. After 1937, Paul Mueller found dichlorophenyltrichloroethane (DDT), which has the property of

pesticide, and later various synthetic and powerful pesticides were developed and produced. After the Second World War, a lot of pesticides have been widely used.

Nowadays, many countries have used several kinds of pesticides, which are harmful not only to pests but also to human beings since all pesticides have an adverse effect on human health. For example, the obvious symptoms that can be seen in human include headache, ataxia and vomit. The severity of the symptom depends on the quantity of pesticide being consumed, inhaled and/or absorbed through the skin. The United States Pharmacopoeia Convention, Inc. (USP) defined the Maximum Residual Limits (MRLs) of pesticides contaminant in food for organochlorine pesticides at 0.05 – 3.00 ppm (mg/kg). In 1972, the US Environmental Protection Agency (US EPA) abandoned the use of DDT in the United States because of its harmful adverse effects. In 1974, the US EPA banned the use of 3 organochlorine pesticides: chlorden, dieldrin and heptachlor. Since 1987, almost all organochlorine pesticides have been banned. In Thailand, the use of 81 pesticides have been prohibited, especially those in the group of organochlorine, for instance, DDT. The reason behind this is that they are highly damaging both to human and the environment since they are water insoluble and can be collected by and be present in the environment for a long time. With the above knowledge in mind, before exporting curcumin to other countries, the residual organochlorine pesticides must be determined and extracted if they are present. This is because Thai farmers are likely to increase the use of various kinds of pesticides, including those being banned, to stop pests and to protect their crops. The prolonged period that organochlorine pesticide residues can be present in both the crop and environment is the important reason for scientists to determine if curcumin to be exported as well as that to be consumed locally is free from contamination.

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**Figure 1.1** Structures of 24 organochlorine pesticides

## 1.2 Literature Reviews

Organochlorine residues may be recovered from agricultural or other biological samples by exhaustive extraction with a variety of solvents <sup>(1)</sup>. Analytical methods are needed to screen, quantify and confirm pesticide residues in fruits and vegetables. Multi-residue methods (MRMs) and single residue methods (SRMs) generally consist of the same basic steps, but the MRMs are more preferable than the SRMs for the analysis of pesticides, because the MRMs provide the capability of determining different pesticide residues in a single analysis <sup>(2)</sup>. The MRMs concept is raised to a higher dimension when a single extract is examined with more than one chromatographic determinative step, each providing the coverage of residues in a different class, e.g. chlorinated hydrocarbon, organophosphates and carbamates <sup>(3)</sup>.

The MRMs is potentially capable of determining any residue extracted by its extraction step. Each determinative step in MRMs provides coverage for a particular group of residues in the extract, and each clean-up step is designed to purify the extract sufficiently to permit accurate determination. Over 30 years ago, among the more widely used MRMs were those of Mills <sup>(4)</sup>; Mills, Onley and Gaither <sup>(5)</sup>; Storherr <sup>(6)</sup>; Luke <sup>(7)</sup>; and Krause <sup>(8)</sup>.

A sample preparation by multi-residue methods for pesticides in fruits, vegetables, soils and water, began in 1963—Mills, Onley, Gaither-MOG procedure. The extraction was done with acetone or acetonitrile, partitioning with petroleum ether, cleaning up with florisil, and the detection with GC-ECD and paper and thin layer chromatography. The scope of the method did not include the more polar organonitrogen and organophosphorous pesticides <sup>(9)</sup>.

The method of the Association of Official Analytical Chemists (AOAC), typifies the international recognized MRMs. The method has made it possible to determine many extractions and laborious clean-up. The methods, generally consist of an extraction step with a water miscible solvent, followed by a clean-up step with an organic solvent of limited water capacity to achieve the removal of interferences present in the sample extract, and/or solid phase clean-up with silica or florisil. Finally, analytic

determination is performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) with selective detectors <sup>(10)</sup>. These methods detect approximately 325 pesticides and pesticide-related compounds and most of them have undergone rigorous multi-laboratory calibration studies, such as those needed to obtain the official acceptance by the AOAC <sup>(11)</sup>. However, its continued use still presents some disadvantages, such as (i) the amount of chemicals and toxic solvents used (ii) their inefficiency as a screening method. The methods are too complex, and they did not allow the generation of relevant data in time to prevent contaminated foods from entering the marketplace, because the procedures were time-consuming and laboriously intensive. In addition, the newly developed groups of pesticides are getting more and more polar and/or thermodegradable, and they should be incorporated into the existing MRMs.

To avoid the general drawbacks of the classical methods, methylene chloride partition is applied after two petroleum ether partitions. More polar pesticides such as merinphos can be determined with a newly developed thermoionic detector (TID) for phosphate and nitrogen residues. In 1972, modification was made to analyze even more polar compounds; particularly methamidophos. The petroleum ether partitioning was eliminated. Water was not added to acetone to obtain a better recovery of the water-soluble compounds. TID is used to analyze P and N containing residues. ECD is used after florisil clean-up. In 1975, the acetone in partitioning step was replaced by petroleum ether to eliminate the precipitate occurring during the analysis of some fruits. Acetone extraction was usually preferred since it was suitable for both non-polar and polar pesticides <sup>(12 - 13)</sup>, as demonstrated in different comparative studies performed by GC and HPLC. Acetone has low toxicity. It is easy to purify, evaporate and filter. And, above all, it is cheap. Fruit and vegetable extracts in acetone are usually cleaner than those obtained with other solvents of similar polarity. The National Food Administration of Sweden <sup>(14)</sup> also used acetone extraction which was followed by partitioning with hexane-dichloromethane, and twice with dichloromethane. After the clean-up method on an SX-3 permeation chromatography column, residues were determined by GC using ECD, NPD, FPD and FID. In Germany, pesticides analysis in fruits and vegetables is mainly performed with MRMs. This method was developed to obtain extracts suitable for GC determination with selective detectors, mainly ECD, NPD and FPD. Fruits and vegetables are

extracts with acetone-dichloromethane, and pesticide residues are detected after the clean-up by gel permeation chromatography (GPC) and mini-silica gel column fractionation in up to six fractions. The data about elution and recoveries of more than 400 pesticides and their metabolites as well as a few common pollutants are well documented<sup>(15-16)</sup>.

In 1981, the flame photometric detector (FPD) that could be used as P or S mode with the Hall electrolytic conductivity detector (ELCD) which could be used in a halogen or nitrogen detection mode was developed. So that the florisil clean-up step for determination of the chlorinated pesticides could be eliminated for the first time after the use of ELCD in halogen mode.

In 1982, the process of simultaneous extraction and clean-up in chromatographic column mixing or blending aqueous samples with silica gel or alumina was introduced to remove lipids prior to liquid-liquid partitioning and florisil clean-up.

A rapid and efficient multi-residue extraction procedure using ethyl acetate and sodium sulfate, followed by GPC on a SX-3 column, was first reported by Roos et al.<sup>(17)</sup>. Recoveries better than 90% were obtained for OCPs and OPPs. The ethyl acetate and sodium sulfate extraction without further clean-up was applied as screening method for the analysis using GC-FPD and GC-NPD. Interfering chromatographic peaks were decreased and the analysis time and use of solvent reduced, resulting in cheaper analysis.

In 1985, the applications of GPC for a clean-up of more than 300 pesticides were reported that they could be used to simultaneously clean chemical residues. In the same year there appeared the development of Solid Phase Extraction (SPE) C-18 bonded silica gel cartridges, XAD resins, Tenax-GC cartridges that were applied for extraction and preconcentration of pesticides from air and water samples. To replace classical LLP, and to reduce analysis costs and pollution, the SPE method has been developed. In this process, the compound is isolated from a liquid sample by differences in the relative solubilities between a liquid mobile phase and a stationary phase.. The modified Mill's method, consisting of acetonitrile extraction and clean-

up on C-18<sup>(18)</sup> was developed by The California Department of Food and Agriculture (CDFA).

The pH of the filtrate is adjusted to neutral with phosphate, and the acetonitrile layer is separated from the aqueous layer by a salting out process. This method was evaluated by analysis for seven OCPs at 0.1 – 0.2 µg/g in six representative fruits and vegetables using GC and HPLC. SPE cartridges, containing normal or reversed-phase supports, have become available commercially and offered the potential of simplifying the purification of the initial extract as well as reducing the amount of solvent consumed. C-18 commercial cartridges were examined for the clean-up of crop extracts on the determination of fungicide and OCPs.

About 1984 – 1988, there was a development of “two-dimensional” capillary GC, which involved the use of two columns of different polarity and multiple detectors, hence eliminating the need in extensive clean-up. In 1993, Cairns method was used by means of the SPE cartridge for clean-up. The change was necessary in accordance with the change in technology, which had gone from packed GC column to open-bore columns, which were more easily affected by the non-volatile sample extractives that were deposited in the injection port. Two SPE were introduced. One used C-18 SPE to remove non-polar sample compounds by a reverse phase clean-up. The other uses a strong and weak anionic extraction SPE cartridge to remove acidic phenols and saccharine. Methyl carbamate, phenylureas, and benzimidazoles were determined without additional clean-up step.

Matrix solid-phase dispersion (MSPD) is a new extraction and clean-up technique, that has been developed to avoid the general drawbacks of the LLE, such as the use of large amounts of solvent, the occurrence of troublesome emulsions with certain fruit or vegetable matrices and their slowness<sup>(19)</sup>. This method constitutes a significant advance in simplicity and efficiency that make it possible to screen more samples. Kadenski et al. demonstrated the applicability of MSPD to a large number of fruit and vegetable matrices for pesticide residues. In most cases, samples were added with distilled water, if necessary, for proper blending. Plant material was mixed with florisil and, after that, extracted with methylene chloride-acetone or ethyl acetate. The

analytical performance of the method and the MRLs were established by the European Union.

Recently, Supercritical Fluid Extraction (SFE) has been recognized, in the field of pesticide residue analysis. Lehotay et al. <sup>(20)</sup> demonstrated in an initial study, that the extraction of various pentachloronitrobenzene pesticides from vegetables by SPE was clean enough for direct injection to GC-MS in EI mode. The selection of the appropriate SPE conditions such as CO<sub>2</sub> density, temperature modifier, type of solid-phase used for trapping the analytes, and elution solvent was manipulated to overcome most chromatographic method. In their later work, the same authors used a SPE multi-residue method for the determination of 46 pesticides of different polarities and physico-chemical properties from fruits and vegetables, followed by GC-MS. Recoveries obtained were over 80% except for methamidophos, which was not recovered at all.

For the development of instrumental analysis, in recent years, capillary columns have almost completely replaced the packed column owing to their high resolving power, which allows the separation of a large number of pesticides with similar physico-chemical characteristic <sup>(21)</sup>. The most frequently used detectors include ECD, NPD, FPD and MSD. The last one has become the standard confirmatory technique MIP-AED, which allows the specific detection of many elements, has recently been applied to the determination of pesticides. In the past 30 years, the ECD has been the detector mostly used in pesticide residue analysis. It presents a very high sensitivity to other halogenated pesticides but its selectivity is rather poor. A new detector introduced in 1989 is the AED, used for its selective detection of the elements fluorine, chlorine, bromine, iodine, phosphorous, sulfur and nitrogen. Mass Spectrometer Detector can be employed to achieve selective detection, by full scanning or selective ion monitoring, of target pesticides in the presence of the complex matrix. Quantification is usually achieved by the technique of selected ion monitoring (SIM). With this technique selectivity is also improved. In addition, a multi-residue method for screening OPP residues in fruit and vegetable samples, with an ion trap mass spectrometer in the chemical ionization mode, has been developed.



Solid phase microextraction (SPME) is a sample preparation technique that, since its development by Pawliszyn and co-workers in the early 1990s<sup>(22 - 24)</sup>, has received increasing attention. Advantages of this new solvent – free extraction technique, as simplicity or capability of injecting the whole extracted sample, have been discussed in several papers<sup>(25 - 28)</sup>. Development of an adequate SPME procedure for pesticide determination should allow us to achieve a reduction in sample manipulation, even eliminating the need for clean – up steps. Several application of SPME to pesticide determination in biological samples can be found for organochlorine<sup>(29)</sup> and organophosphorous pesticides<sup>(30 - 31)</sup>, as well as for herbicides<sup>(32)</sup>. Headspace approach has been preferred in order to avoid interferences from the sample matrix. However, when the headspace approach is applied, most of non – volatile pesticides are excluded from the determination, and consequently the scope of the developed procedures is more limited than those based on direct immersion of the SPME fibre.

High-performance liquid chromatography (HPLC) is being extensively used in pesticides chemistry and related areas where the chemicals of interest are frequently of thermal unstable for GC separation.

Immunoassay (IA) provides rapid, sensitive, and cost-effective analysis for variety of pesticide residues. IA cannot complete in term of the information about the sample composition. The main disadvantage is that only one compound at a time can be determined. Up to now IA has not been reported for pesticide residue determination in fruits and vegetables<sup>(33)</sup>.

Although many papers deal with pesticide residues in different matrices, very few concerns powdered spices. In 2000, levels of 14 organochlorine pesticides in spices powder that not Curcumin were extracted with n-hexane-dichloromethane (4:1) and the extracts were cleaned in a single step on a cartridge packed with silica and florisil, quantified by GC-MS in SIM mode. Recoveries were measured from 60% for dieldrin and endrin to 97% for other pesticides<sup>(34)</sup>.

### 1.3 The Purpose of The Study

The sample preparation and quantitative analysis of organochlorine pesticides in each country have different standard methods. One of the reasons for this is trade-barrier. In Thailand, there has been no standard method for Curcumin sample preparation prior usually the analysis of contaminated organochlorine pesticide residues. The several problems involve in sample preparation and quantitative analysis of organochlorine pesticides. Contamination in Curcumin include chemical substances such as volatile oils, coloring matters, lipids, protein and minerals. This matrix is very complex and requires a more challenging clean-up process for trace analysis. Curcumin contains a large amount of volatile oils and compounds from those of low to more polarities. Obviously, this leads to the fact that the sample preparation of pesticides from Curcumin is more likely to be difficult too.

At present, the development of high performance sample preparation technique and analytical method to investigate pesticides contamination in the sample concentrates on the ability of determining low-concentration levels of several compounds. Thus, the multi-residue method seems to be most suitable for this purpose. The aim of this work was to develop a simple and rapid sample preparation method for determination of 17 organochlorine pesticides in *Curcuma Longa*. (Curcumin), by Gas Chromatography (GC) and  $\mu$ -Electron Capture Detector ( $\mu$ ECD). Moreover, this work will extend its investigation to cover the Turmeric powders (Curcumin) in the Thai markets.

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