CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

In recent years, the total amount of the use of pesticides has increased worldwide. In Thailand, the Department of Agriculture (DOA) reported that pesticides were increased 1.2 fold in 5 year time from 110,000 tons in 2008 to 134,000 tons in 2013. The major abundant was herbicides, insecticides and fungicides, respectively (DOA, 2013). Many fungicides used in agriculture have toxic effects to the environment and living organisms when applied improperly. Although some methods of fungicide residue analysis have been reported, there is still a need for more rapid and cost effective procedures to serve general public especially consumer group.

Benzimidazole fungicides which the main compound is carbendazim are systemic pesticides widely used in agriculture for pre- and post-harvest protection of crops against fungal diseases (Veneziano et al., 2004) and structure of carbendazim is shown in Figure 1.1. Carbendazim is a systemic broad spectrum fungicide and used to control fungi causing diseases in fruits, vegetables and many field crops. It is absorbed through roots and leaves and acts by inhibiting both the germination and growth of fungal mycelia (Al-Ebaisat, 2011). Carbendazim is toxic to humans, animals and plants. The toxicity produces rapid effects on meiotic spermatocytes and latent effects on spermatids, leading to morphological abnormalities and failure of spermatogenesis (Akbarsha et al., 2000). Recently, it has been found that subchronic

administration of carbendazim induced testicular alterations, spermatogenic inactivity and embryotoxicity (Jia et al., 2002). Over the years, research in the field of carbendazim residue analysis as well as other pesticides (Taylor et al., 2002 and Michel and Buszewski, 2004) in food has experienced a continuous expansion of the number of techniques available for determining their content in different fruits and vegetables. However, it is still of interest to develop a high sensitive sample and low cost analytical method (Hu et al., 2005).

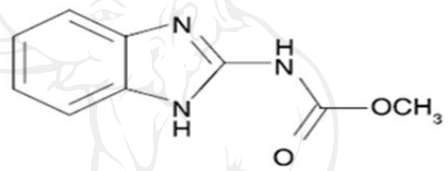

Figure 1.1 Structure of carbendazim

The most commonly used analytical method for the analysis of carbendazim is liquid chromatography with UV (Veneziano et al., 2004 and Al-Ebaisat, 2011), diode-array (Wu et al., 2009), fluorescence (Blasco et al., 2006 and Nemeth-Konda et al., 2002) or mass spectrometric detections (Romero-Gonzalez et al., 2008). Different extraction solvents include acetone (Pan et al., 2008), acetonitrile (Hiemstra et al., 2007), methanol (Veneziano et al., 2004), ethyl acetate (Romero-Gonzalez et al., 2008 and Juan-Garcia et al., 2007), dichloromethane (Singh et al., 2007), followed by homogenizing, then shaking by using sonication (Juan-Garcia et al., 2007). Solid-phase extraction (SPE), using C_{18} bonded silica procedure, was employed for isolation

(Codex, 2000). Pan et al. (2008) reported that HPLC condition was required mobile phase modifiers such as methanol-water or acetonitrile-water (Hu et al., 2008) to improve the peak shape and/or resolution and the application of sorption of interferences on SAX/PSA dual-layer solid phase extraction was impressive.

Dithiocabamates (DTCs) are important organosulfur compounds and their wide use fungicides for protection of crops, fruits, vegetables, seed, and ornamental plants. DTCs can decompose or metabolize to carbon disulfide (CS_2) and ethylenethiourea (ETU) (Choua et al., 2004; Shukla and Arora, 2001). In Thailand, the most imported dithiocabamates (DTCs) including mancozeb, propineb and zineb between 2007 and 2012 (DOA, 2012) and the determination of DTC residues (i.e. mancozeb, propineb, and zineb) in fruits and vegetables where the CS_2, evolved, following heating under acidic condition, is extracted into a layer of isooctane which is then analysed for CS_2 by GC-FPD (Cald et al., 2006; Cesnik and Gregoric 2006; Vryzas et al., 2002). Widely used methods for determining DTCs or metabolites (CS_2) residues in vegetables and fruits included spectrophotometric (Caldas et al., 2001), gas chromatography (Cesnik and Gregoric, 2006), and high performance liquid chromatography (Kazos et al., 2007).

The most imported DTC fungicides in Thailand was mancozeb (Panuwet et al., 2012) and it is a contact fungicide and used on a wide variety of crops in agriculture and horticulture. Generally, mancozeb has low acute toxicity but can been effect in neuropathology, thyroid toxicity, and developmental toxicity in long-term (Caldas et al., 2006). The structure of mancozeb is shown in Figure 1.2.

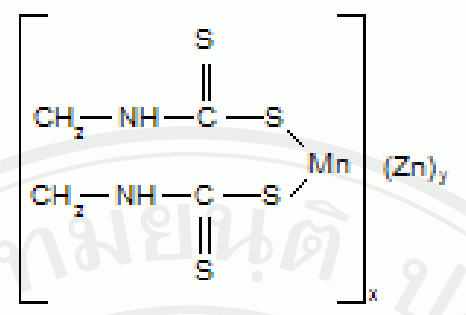

Figure 1.2 Structure of mancozeb

In recent statistics from the Bureau of Occupational and Environmental Diseases in 2012, blood samples of 152,846 farmers were tested for pesticides residues in their body. It was found that 30% of farmers (46,016 people) had pesticides residues in their body which is qualified unsafe and it also showed that the main source of having pesticides in the body was the consumption of contaminated products. Moreover, the recorded data in 2011 illustrated that 36% of general Thai public had risk in getting pesticides which was 4% higher than farmers in the same surveyed year. From the reported data, several health issues need to be concerned, such as illness and death due to the pesticide exposure, cancer initiation and reproductive system problem.

Basically, there are four major steps in human health risk assessment including hazard identification, dose-response assessment or risk identification, exposure assessment, and risk characterization. The first and the second steps are processed following the Codex Alimentarius Commission (Codex) which is evaluated the hazard in both quality and quantity way. The Joint Meeting of Food and Agriculture Organization and the World Health Organization (FAO/WHO) on Pesticide Residues (JMPR), is responsible for reviewing residue and analytical aspects of the pesticides in foods. The JMPR is in charge to establish the acceptable daily intake (ADI) which

is a measure of the amount of a specific substance in food that can be ingested on a daily basis over a lifetime without an appreciable health risk. The ADI of carbendazim is 0.03 mg/kg bw/day (Wu et al., 2009). Therefore, the result of human health risk assessment from contaminated carbendazim residue vegetables may be useful for food safety policy.

1.2 The objectives of the present study

This research study included 2 sections as following.

Section 1: To develope a method for detecting carbendazim and dithiocabamates fungicide

1.1 To develop a method for detecting carbendazim fungicide in vegetables using HPLC-UV

1.2 To develop a method for detecting dithiocarbamate fungicide in vegetables using GC-FPD.

Section 2: To assess the health risk of exposure to benzimidazole and dithiocarbamate fungicide residues in vegetables

1.3 Keywords and definition

1.3.1 Carbendazim (methyl-2- enzimidazole carbamate) is systemic fungicide with both protective and curative activity against a wide range of fungal diseases.

1.3.2 Mancozeb or manganese ethylene bis(dithiocabamate) is group of dithiocarbamate and used to protect many fruit, vegetable, nut and field crops including potato blight, leaf spot, scab and rust.

1.3.3 Vegetables in the present study include cauliflower, ginger, kale, cucumber, yard long bean, guinea-pepper, chili, pepper and tomato.

1.3.4 Health risk assessment include hazard identification, dose-response assessment or risk identification, exposure assessment, and risk characterization.

1.4 Conceptual frame works

In this study, the method will be developed for determination of carbendazim and mancozeb residues in plants from Chiang Mai city, Thailand. Then, the method will be applied for detection in plants. The conceptual frame works will be conducted as shown in Figure 1.2. Eight types of plants will be purchased from five fresh markets in Chiang Mai city from August-October 2011 and 54 vegetable samples including cauliflower, ginger, kale, cucumber, yard long bean, guinea-pepper, chili and tomato were purchased and transported to Toxicology laboratory, Research Institute for Health Sciences, Chiang Mai University.

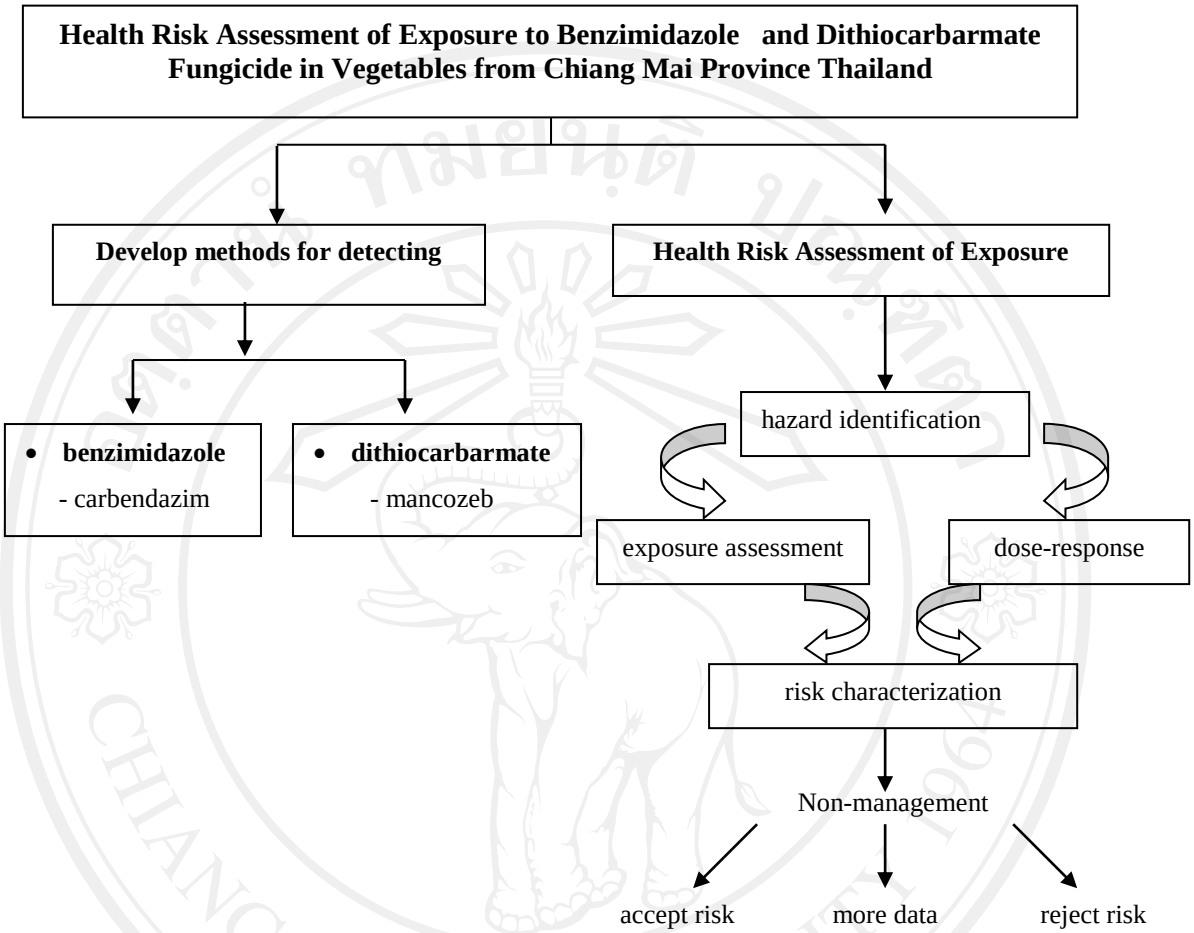

Figure 1.3 Block diagram of the study