CHAPTER I

INTRODUCTION

Problem Statement

Methyl carbamates are insecticides extensively used worldwide because they are considered to be relatively safe and non-persistent. Methomyl is classified as a methyl carbamate, which is widely used in Thailand and many agricultural countries for crop protection.

Methomyl is an acetylcholinesterase inhibitor, and its reported toxicity is mostly related to cholinergic symptoms. However, some of its toxicity may not be related to its action on acetylcholinesterase.

Some studies reported that cardiotoxicity generated by methylcarbamates is not totally mediated through their action on acetylcholinesterase enzyme. Futagawa et al. (2000) reported that the developed depressor response, leading to a decrease in cardiac contractility and/ or vascular resistance, observed in methylcarbamates-exposed rabbits was the direct effect of methylcarbamates on cardiac and vascular smooth muscle contraction.

Klotz, Arnold, and McLachlan (1997) reported that methomyl, when given alone, had a weak activity on estrogen and progeterone to activate both estrogen and progesterone-responsive reporter genes in breast and endometrial cells. However, when given together with either estrogen or progesterone, methomyl inhibited activities of these hormone by acting through pathways independent of competition for receptor binding. This study suggested methomyl as a general endocrine modulator in mammalian cells.

In addition, some *in vivo* studies showed that methomyl could generate oxidative stress. Lohitnavy and Sinhaseni (1998) reported that rats treated with methomyl showed a significant decrease in the spleen cell viability. The methomyl-induced splenotoxicity was protected by pretreating rats with N-acetylcysteine (NAC), a free radical scavenger which promotes glutathione antioxidant capacity. Therefore, the study suggested that methomyl might generate oxidative stress, which could consequently contribute to the splenotoxicity.

The possibility of oxidative damage by methomyl was also reported by Rannug and Rannug (1984) that enzyme involved in the defense against harmful reactive oxygen species (ROSs) such as superoxide dismutase, catalase, and glutathione transferase are inhibited by carbamate pesticides. This is supported by a metabolite of methomyl, which gets through glutathione conjugation, causes glutathione depletion, therefore can generate oxidative stress in biological systems (International Programme on Chemical Safety [IPCS], 1996).

Reactive oxygen species (ROSs) have long been known to be potent mediators of apoptosis in various animal and plant systems. They participate in signaling pathways during the induction phase of apoptosis and are one of several apoptogenic consequences of mitochondrial permeability transition (PT) pore, which is a critical part of apoptosis (Jabs, 1999).

Apoptosis is a major form of cell death that is used to remove excess, damaged or infected cells throughout life. It is important in normal cell turnover, the immune system, embryonic development, metamorphosis and hormone dependent atrophy, and also in chemical-induced cell death (Arends and Wyllie 1991; Ellis, Yuan, and Horvitz, 1991; Cohen et al., 1992). Loss of control of the apoptotic programme contributes to many diseases (e.g. cancers, and autoimmune diseases) (Normal and Lodwick, 1999; Bratton and Cohen, 2001).

As mentioned above, apoptosis does not occur only in mammals, it is recently found that apoptosis can also occur in plants. In plants, apoptosis of single cells or small groups of cells occurs during normal development such as sex determination, gamete development, embryogenesis, formation of fluid-conducting channels called vessels and tracheids, and the hypersensitive response to pathogen infection (Jabs, 1999; Balk and Leaver, 2001). Although little is known about apoptosis in plants, it is suggested that plant mitochondria, like mammalian mitochondria, play a key role in the induction of apoptosis (Balk and Leaver, 2001).

Effects of methomyl on apoptosis modification in plant have been reported. Male sterile maize expressing high levels of URF13 protein in its inner mitochondrial membrane was shown susceptible to methomyl. The *in vitro* studies showed that the interaction of methomyl with this protein caused pore generation in inner mitochondrial membrane, rapid swelling of the mitochondria, stimulation of NADH oxidation,

inhibition of malate-driven respiration, uncoupling of oxidative phosphorylation, and the leakage of small molecules and ions (Chaumont et al., 1995; Rhoads et al., 1994).

Because of its direct effects on mitochondrial modifying apoptosis in plant and its *in vivo* reported oxidative stress in spleen cells, methomyl is used as a model compound in this research to study the effects on mitochondria and apoptosis, including intracellular related signals both *in vitro* and *in vivo*.

This research is focused on the system related to immune response as the immune suppression from pesticides can be particularly significant in many developing countries where infectious diseases cause nearly half of all deaths (UNIDO, 1996). Human leukocytic cell lines are used to investigate effects of methomyl related to apoptosis and changes in the molecular level. Effects of its metabolite–acetonitrile, which can interfere with electron transport chain and oxidative phosphorylation–on the leukocytic cell lines are also observed. Additionally, since methomyl is previously reported to generate oxidative stress and reduce spleen cell viability in rats, changes in splenic ultrastructure and other effects on energy metabolism related to oxidative stress and mitochondrial function are investigated.

Objectives

1. To investigate apoptosis and its related cellular alterations in leukocytic cells.
2. To explain mechanisms of toxicity of methomyl in relation to spleen cell viability reduction.

Hypothesis

In addition to the effect via acetylcholine, toxicity of the insecticide methomyl detected is possibly generated directly from either methomyl or its metabolite, acetonitrile, which can interfere with mitochondria and the energy metabolism. Furthermore, methomyl may exert its direct effects through alterations in intracellular signaling transduction and cellular functions, which may result in apoptosis phenomenon seen at a certain dose range.

Contributions of the Study

1. To understand roles of intracellular signaling transduction which can provide useful information for the understanding of apoptosis in cells relevant to the immune system.
2. Better understanding of the toxicity of methomyl which may not be directly related to acetylcholinesterase inhibition.