CHAPTER V

DISCUSSION

Methomyl has high oral toxicity. A low dose at 3 mg/kg of technical methomyl was able to cause marked signs of cholinesterase inhibition within 5 minutes after dosing. However, these toxic effects recovered rapidly due to its rapid metabolism and elimination. In repeated dose study, there was no cumulative toxicity; oppositely, the signs appeared declining in the next doses.

Total LDH activity and LDH isoenzymes in methomyl-exposed rats

In the present study, the increased total plasma LDH activity in rats treated with a single dose of methomyl was observed on day 1 and 3 after dosing and then the enzyme activity returned to normal on day 5 and 7 indicating the reversibility of this effect. This reversibility of the enzyme may be due to the short half-life of LDH or the cessation of effects because of the rapid excretion of methomyl.

Subsequent LDH isoenzymes assays showed that LDH-4 significantly increased in the rats receiving high dose (7 mg/kg) of methomyl on day 3. In rats, LDH-4 was predominant in spleen (Wilkinson, 1965) and pulmonary vascular endothelium (Schultze et al., 1994). The elevations of total LDH, LDH-4 in serum in this study and the earlier study of Kaplan and Sherman (1977) which showed the increase in the incidence and severity of extramedullary hometopoiesis in the spleen and the vacuolization of epithelial cells in rats fed 400 mg/kg methomyl in diet for 12 months suggest that the spleen and /or vascular endothelial cell are possible target organs of methomyl toxicity.

The histopathological examination also revealed the slightly changes in the liver and spleen of rats treated with methomyl. These alterations may involved injury which also reflects in other parameters measured such as significant changes of LDH-3, LDH-4 and the decrease in relative weight of rat liver. However, according to the expert pathologist's opinion, there was no significant difference when compared with the control groups. Furthermore, there was no significant change in GPT activity of plasma in any groups, suggesting that the methomyl may not be related to hepatic cell injury.

Correlation between alteration of LDH isoenzymes and toxicity of methomyl

As previously mentioned, this study showed the possible correlation between LDH-4 and methomyl toxicity in rats. However, these effects were not seen after a period of time which may indicate reversibility of the effects after the dosing regimens chosen in this study. Moreover, There are limited studies indicating the distribution of LDH isoenzymes in various organs of rats. Further studies of the precise sources of LDH-4 should be conducted and the effects of methomyl to other organs should be observed as well.

Methomyl-induced splenotoxicity

The splenotoxicity testings were carried out in this study in the higher doses of methomyl (6 and 8 mg/kg) by determination of splenocyte viability and spleen weight of rats. Significant reductions in the splenocyte viability and spleen weight of both treatment groups were observed in a dose-related manner on day 1 and day 3. Proposed mechanisms of splenotoxicity induced by methomyl are:

1) Direct effect of methomyl on splenocytes. A study of Amer, Fahmy and Donya (1996) revealed that methomyl caused chromosomal aberration in mouse spleen cells after 1 mg/kg intraperitoneal injection.

2) Effects on vasculature of spleen There are many evidences of vasculature injury induced by vasodilators such as fenoldopam mesylate, minoxidil, and phosphodiesterase III inhibitors (Boor et al., 1995; Bugelski et al, 1989; Yuhas et al., 1985). Their vasodilation effect was believed to be a major cause of enzyme leakage. The over-vasodilatation produced exceed critical wall tension and subsequently medial necrosis occurred (Boor et al., 1995).

Acetylcholine (Ach) is known as a potent vasodilator via nitric oxide pathway (Fukaya and Ohhashi, 1996; Furchgott and Zawadzki, 1980). The accumulation of Ach generated by cholinesterase inhibition may cause over-vasodilatation and subsequently result in vascular injury. Moreover, NO itself is cytotoxic and therefore overproduction of NO may also cause cell death. More detailed possible mechanism of methomyl-induced splenotoxicity should be further explored.

Roles of N-acetylcysteine on methomyl-induced splenotoxicity

N-acetyl-L-cysteine (NAC) is an antioxidant which acts like glutathione. In this study, NAC was found to be a protective agent of splenotoxicity in rats pretreated with 60 mg/kg of NAC. The free radicals produced by inflammatory cells, for exmple, polymorphonuclear (PMN) cells within the injured area, can be scavenged by NAC. However, the pathologic findings did not suggest inflammatory reaction. Another possible explanation is that the several agents with antioxidant properties such as dithiothreithol, phenylhydrazine, potassium borohdride and phenidone can inhibit the relaxation of vascular smooth muscle induced by NO (Collins, 1991). Therefore, NAC may relieve the over-vasodilatation and cytotoxic effect induced by NO.

CHAPTER VI

CONCLUSION

Methomyl is a highly toxic carbamate insecticide which widely used in Thailand. The mechanism of toxic action is inhibition of acetylcholinesterase enzyme that causes cholinergic signs such as lacrimation, bronchosecretion, muscular fasciculation, muscular weakness and respiratory disorders both in human and laboratory animals.

From this study, we have found that the determinations of total LDH activity, LDH isoenzymes, spleen weight, and relative spleen weight were affected in rats treated with a single dose and repeated doses of methomyl. However, no significant difference in weight gain and haematological values between the control groups and the treatment groups was observed.

The rats receiving an oral dose of all test groups showed significant increase in total LDH activity on the first day and the highest enzyme activity occured only in the rats receiving 7 mg/kg of methomyl on day 3 after dosing. Then, the level of LDH activity declined to normal level that showed the reversibility of this effect. These high LDH activities were concurrent with significantly increased LDH-3 and LDH-4. This altered isoenzymes profile may substantiate that the spleen may be a target organ of methomyl toxicity even in short term repeated exposure. This is also supported by the reductions of spleen weight and splenocyte viability in rats treated with 6 and 8 mg/kg of methomyl on day 1 and day 3 after dosing. The possible mechanisms were proposed in the previous chapter. Such proposals should be further investigated.

Interestingly, N-acetyl-L-cysteine (NAC), an antioxidant which acts like glutathione was found to be a protective agent of methomyl-induced splenotoxicity in the rats pretreated with 60 mg/kg of NAC. This may be due to the antioxidant properties of NAC which can act as a scavenger of free radicals released from inflammatory cells within the injured area. However, the pathologic findings which did not reveal the inflammaotry reaction suggest the second explanation that NAC relieved the over-vasodilatation and cytotoxic effect produced by NO via acetylcholine overstimulation.

In conclusion, the present study showed splenotoxicity of methomyl in rats and the possible correlation with increased LDH-4. However, there are limited studies indicating the distribution of LDH isoenzymes in various organs of rats. Further studies of the precise sources of LDH-4 should be established and the effects of methomyl to other organs should be observed. The exact mechanism of methomyl-induced splenotoxicity and the protective effect of spleen from methomyl by NAC should be further explored as well.