CHAPTER IV

RESULTS

1. Determination of relationship between serum LDH activity and LDH isoenzyme changes and mothomyl toxicity in rats.

Signs of toxicity

The rats given methomyl at a single oral dose of 3, 5 and 7 mg/kg developed the signs of toxicity within 5 minutes after dosing. The severity of toxic signs depended on the dose level. These acute signs were known as results of cholinesterase inhibition, for example; nicotinic effects: generalized tremor, generalized muscular weakness, muscular fasciculation; muscarinic effects: increased bronchosecretion, difficult respiration; and chewing movement. These effects persisted for about 30 minutes to 1 hour. Subsequently, the severity declined and completely recovered within 2 hours. No animals receiving methomyl died.

All rats receiving 5 repeated oral doses 5 mg/kg of methomyl survived the feeding period. The animals showed the same clinical signs of toxicity as observed in the single dose treatment groups. Nevertheless, these signs became less marked after day 3 of dosing.

There was no weight loss after dosing and no significant diffirence in weight gain between control groups and the rats receiving a single dose and repeated doses of methomyl. The average weight gain of the rats was 7.4 g/day.

Haematologic tests

The treatment groups of an acute dose (3, 5, and 7 mg/kg) and 5 repeated doses of methomyl did not showed any significant change in hematocrit, white cell count and differential count.

The hematocrit and leukocyte count of the rats in this study were 35.9-46.2 g% and 3.8-6.8 ($x10^3/mm^3$) respectively. The differential count was as follows: lymphocyte 85-94%, neutrophil 2-14%, monocyte 2-5%, eosinophil 0-3% and basophil 0-2%.

Total LDH activity

The total LDH activity in the plasma of control rats in the acute and repeated dose study were 147-186 U/l (at 95% CI, n=21) and 138-172 U/l (at 95% CI, n=24) respectively. The total plasma LDH activity of each groups at various time points after dosing are summarized (table 14 and 15). The number of rats which had increased total LDH activity at various dose levels and various time points after dosing are shown in table 16.

On the first day after single oral dosing of methomyl, all treatment groups significantly increased in total LDH activity when compared with the controls (figure 17). One of six rats treated with high dose (7 mg/kg) of methomyl showed extremely increase of LDH activity (2778.6 U/l) on day 3 following dosing. After that, the enzyme activity in the plasma seemed to decrease to the normal level.

The rats fed 5 mg/kg of methomyl for 5 days exhibited slightly changes in total LDH activity on day 5 and 7 after dosing, but not significant (figure 18).

Table 14. The total LDH activity of rats treated with a single dose of methomyl (3-7 mg/kg) at various time points after dosing.

Group / Time (after dosing)	Control	Methomyl 3 mg/kg	Methomyl 5 mg/kg	Methomyl 7 mg/kg
Day 1	161.4±17.7	341.1±8.9*	296.4±10.7*	211.4±37.1*
Day 3	146.4±16.3	108.9±11.8	242.9±57.0	668.6±527.8
Day 5	178.6±27.4	137.1±20.9	156.0±22.2	137.2±18.0
Day 7	184.3±20.7	176.2±6.8	148.6±15.4	165.5±30.2

Values are expressed as mean ± S.E. (n=2-6).
Two-way ANOVA was used coupled with Duncan's multiple range test for statistical analysis.
* Significant increase when compared with the controls ($p<0.05$).

Table 15. The total LDH activity of rats treated with 5 daily doses of 5 mg/kg of methomyl at various time points after dosing.

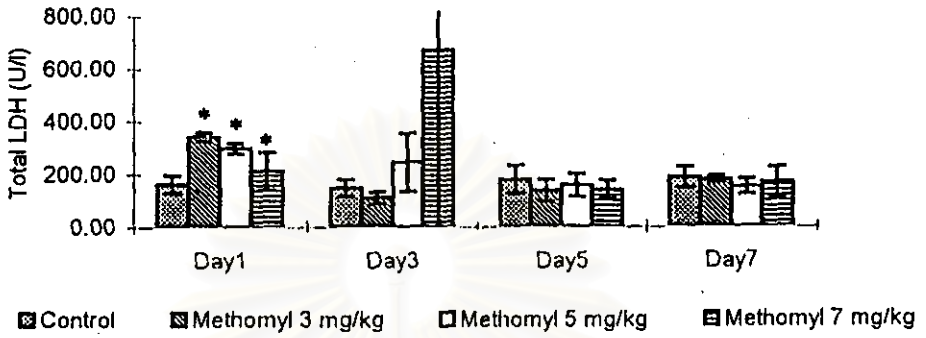
Group / Time (after last dosing)	Control	Methomyl 5 mg/kg/d (5 doses)
Day 1	130.0±17.3	124.3±25.6
Day 3	154.3±9.6	140.0±6.0
Day 5	177.2±19.8	232.9±99.2
Day 7	177.2±11.4	204.3±54.9

Values are expressed as mean ± S.E. (n=5-6).
Two-way ANOVA was used coupled with Duncan's multiple range test for statistical analysis.

Table 16. The number of rats fed a single dose and repeated doses of methomyl with increased total LDH activity.

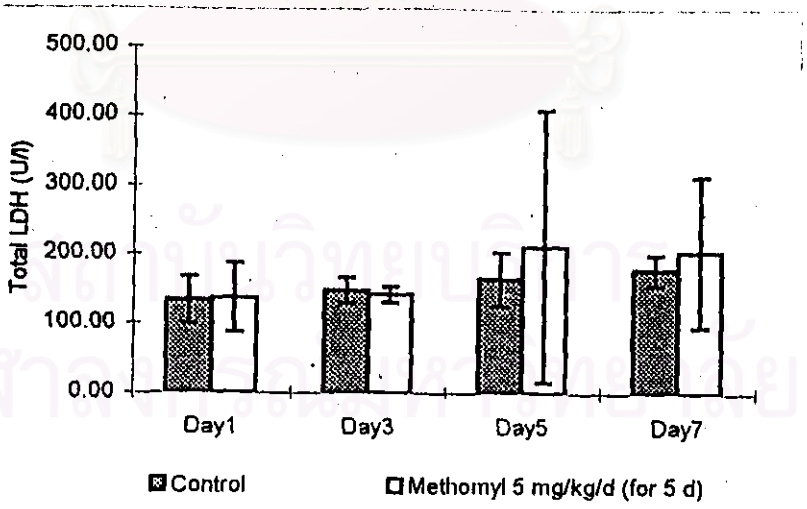
Group / Time (after dosing)	Levels of increased total LDH activity*	Single dose treatment			Repeated dose treatment
		Methomyl 3 mg/kg	Methomyl 5 mg/kg	Methomyl 7 mg/kg	Methomyl 5 mg/kg/d (5 doses)
Day 1	1	1 / 4	2 / 4	2 / 4	1 / 6
	2	3 / 4	-	-	-
	3	-	-	-	-
	4	-	-	-	-
Day 3	1	-	-	-	-
	2	-	1 / 6	-	-
	3	-	1 / 6	-	-
	4	-	-	1 / 6**	-
Day 5	1	-	1 / 6	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	1 / 6***
Day 7	1	-	-	1 / 6	1 / 6
	2	-	-	-	-
	3	-	-	-	1 / 6
	4	-	-	-	-

* 1 = total LDH >1.5-2 fold of normal values; 2 = total LDH >2-2.5 fold of normal values; 3 = total LDH >2.5-3 fold of normal values; 4 = total LDH >3 fold of normal values.
** The highest total LDH activity of rats fed a single dose of methomyl = 2778.6 U/l.
***The highest total LDH activity of rats fed 5 daily dose of methomyl = 664.30 U/l.



* Significant increase when compared with the controls ($p<0.05$).
(Two-way ANOVA was used coupled with Duncan's multiple range test for statistical analysis.)

Figure 17. Total LDH activity in plasma of rats receiving an oral dose of methomyl at various dose levels (3-7 mg/kg) at different time points after dosing (mean $\pm$ 1.96S.E. ; n=2-6).



(Two-way ANOVA was used coupled with Duncan's multiple range test for statistical analysis.)

Figure 18. Total LDH activity in plasma of rats receiving 5 repeated doses of selected dose (5 mg/kg/day) of methomyl at various time points after dosing (mean $\pm$ 1.96S.E. ; n=5-6).

LDH isoenzymes pattern

LDH isoenzymes in this study are expressed as relative percentage of total LDH. LDH isoenzymes in the plasma of control rats are as follows: LDH-1 13.8%, LDH-2 4.2%, LDH-3 4.3%, LDH-4 5.6%, LDH-5 72.1% for single dose study and LDH-1 15.5%, LDH-2 3.9%, LDH-3 3.3%, LDH-4 5.7% LDH-5 71.5% for repeated dose administration of methomyl (figure 19). The results (mean±S.E.) are summarized in table 17 and 18.

Rats given an oral dose of 7 mg/kg of methomyl showed significant increase in LDH-3 and LDH-4 on day 3 after dosing (figure 20).

However, in the repeated dose study, there was no significant difference between control and treatment groups.

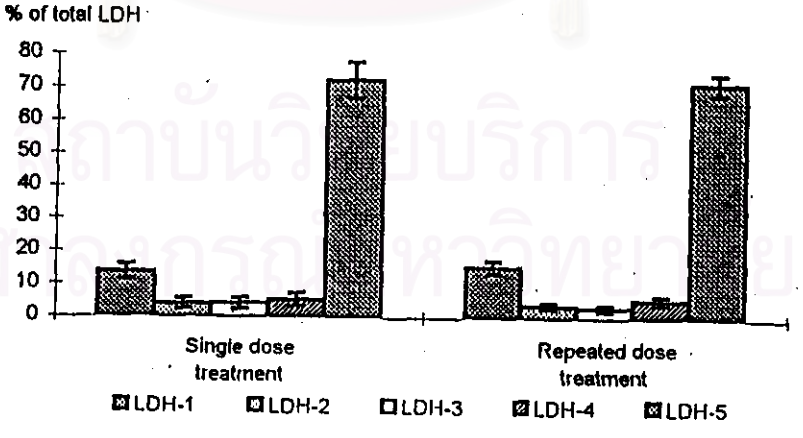


Figure 19. LDH isoenzymes in the plasma of control rats in acute and repeated dose administration (mean±1.96S.E.).

Table 17. LDH isoenzymes in the plasma of rats treated with an oral dose of methomyl (3-7 mg/kg) at various time points after dosing (mean±S.E.; n = 3-6).

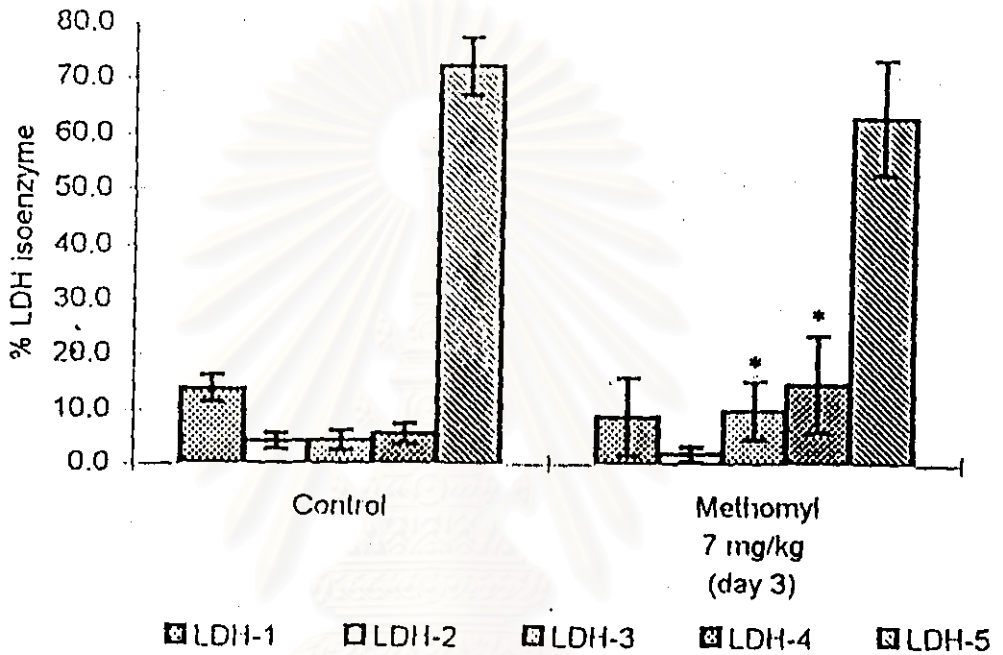
Day(s) after dosing	Groups*	% of total LDH activity				
		LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
Day 1	C	13.8±1.6	5.6±3.2	5.4±2.6	6.4±2.1	68.9±8.6
	M3	9.0±2.0	1.9±0.6	2.8±1.6	5.2±1.8	81.1±3.7
	M5	6.8±1.8	2.7±1.1	5.1±2.2	6.8±3.1	78.7±7.1
	M7	9.2±2.3	1.2±0.0	3.0±1.6	6.3±1.4	80.3±5.1
Day 3	C	17.8±2.8	3.5±1.6	4.7±1.9	5.2±1.8	68.0±5.6
	M3	21.6±3.5	2.4±0.8	1.2±0.1	1.8±0.2	73.3±4.2
	M5	14.0±2.6	2.3±0.2	1.6±0.7	4.6±1.3	77.6±1.6
	M7	8.8±3.6	2.0±0.7	10.0±2.7**	14.7±4.5**	63.0±5.3
Day 5	C	12.2±1.9	3.6±1.5	5.3±2.0	8.3±2.5	70.9±5.1
	M3	17.9±2.7	3.1±1.5	2.5±0.8	4.2±0.9	72.3±3.5
	M5	14.8±1.6	4.6±1.7	3.9±1.9	6.0±1.5	70.7±3.4
	M7	12.7±0.1	2.4±1.0	2.7±1.0	5.5±1.3	76.7±3.0
Day 7	C	11.5±2.1	3.8±1.1	2.3±1.3	2.8±0.5	79.5±3.1
	M3	11.3±1.0	2.4±0.7	2.0±0.5	6.0±1.4	78.4±2.4
	M5	12.2±1.9	3.2±1.3	5.2±2.0	6.8±2.5	72.7±4.0
	M7	12.2±1.2	7.3±4.1	1.6±0.3	5.3±0.4	73.5±3.9

* C=Control; M3=Methomyl 3 mg/kg; M5=Methomyl 5 mg/kg; M7=Methomyl 7 mg/kg.
** Significantly increase of LDH isoenzymes when compare with the controls ($p<0.05$).
(Two-way ANOVA was used coupled with Duncan's multiple range test for statistical analysis.)

Table 18. LDH isoenzymes in the plasma of rats treated with 5 daily doses of 5 mg/kg of methomyl at various time points after dosing (mean±S.E.; n = 4-6).

Day(s) after dosing	Groups*	% of total LDH activity				
		LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
Day 1	C	15.1±1.9	2.4±0.6	1.1±0.2	3.1±0.9	78.3±2.0
	M5	11.7±1.6	3.7±1.4	4.5±2.0	6.0±1.7	74.2±3.4
Day 3	C	13.7±1.5	4.8±0.9	4.5±1.2	7.4±1.0	69.7±1.5
	M5	14.9±2.3	2.3±0.6	4.0±1.9	6.4±2.6	72.5±2.0
Day 5	C	14.2±2.6	4.7±1.2	5.3±0.9	7.2±1.4	68.7±4.6
	M5	17.3±3.8	3.8±0.9	3.5±1.4	4.7±1.3	70.8±5.0
Day 7	C	19.2±1.8	3.8±0.8	2.3±0.7	5.2±1.3	69.5±1.3
	M5	15.0±0.9	3.2±1.3	3.2±1.5	6.0±1.2	72.7±3.3

* C=Control; M5=Methomyl 5 mg/kg/day for 5 days.



* Significant difference when compared with the controls ($p<0.05$).
(Two-way ANOVA was used coupled with Duncan's multiple range test for statistical analysis.)

Figure 20. Comparison of the LDH isoenzymes between control and 7 mg/kg methomyl-treated rats (mean±1.96S.E.).

GPT activity

Rats receiving single dose (3-7 mg/kg) and 5 doses of methomyl (5 mg/kg/day) had no increase in SGPT activity when compared with the control groups (Appendix F).

Organ weight and relative organ weight

In an acute dose administration of methomyl (3, 5, and 7 mg/kg), there was no significant differences among control and test groups when measuring the organ weight and the relative weight of spleen, heart and kidney. However, there was significantly lower weight and relative weight of liver in all treatment groups on day 1, 3, and 5 after dosing and the 7 mg/kg methomyl-treated group on day 7.

The rats treated with repeated doses of 5 mg/kg methomyl for 5 days showed slightly decreased in the relative weight of liver in the test group on day 1 and day 3 after last dosing, but not significant. We evaluated that the weight and relative weight of liver in treatment groups cannot be considered abnormal when compared with the controls. There was no significantly changes in the relative weight of other organs. The data are summarized in Appendix G.

Histopathologic alterations

Microscopic examination of all tissues (liver, spleen, heart and kidney) showed the results within normal variation in both of the single dose and repeated dose administration of methomyl. There were slightly histopathologic changes observed in liver and spleen of the rats receiving a single dose of 5 and 7 mg/kg of methomyl on day 1 and day 3 as well as the rats treated repeated doses on day 3, 5, and 7. However, according to the expert pathologist's opinion, these was no significant difference when compared with the controls. The microscopic alterations were characterized by an increase in incidence of mild (+1) focal necrosis of liver and mild (+1) folicular hyperplasia of spleen.

2. Determination of splenotoxicity in methomyl-exposed rats.

Signs of toxicity

The rats given a single oral dose of methomyl (6 and 8 mg/kg) developed the signs of toxicity rapidly in a dose-dependent manner similar to those in the first study. Certain rats pretreated with N-acetyl-L-cysteine (NAC) had diarrhea and the cholinergic signs were unobvious when dosed with 8 mg/kg of methomyl.

Erythrocyte and spleen cholinesterase activity

Erythrocyte cholinesterase activity of rats treated an acute dose of 6 and 8 mg/kg of methomyl slightly decreased, but not significant. Whereas the rats pretreated with NAC showed the cholinesterase activity quite similar to the control groups (table 19).

The 6 and 8 mg/kg treatment groups of methomyl and NAC-pretreated group did not showed any significant change in cholinesterase activity in the spleen when compared to the control groups.

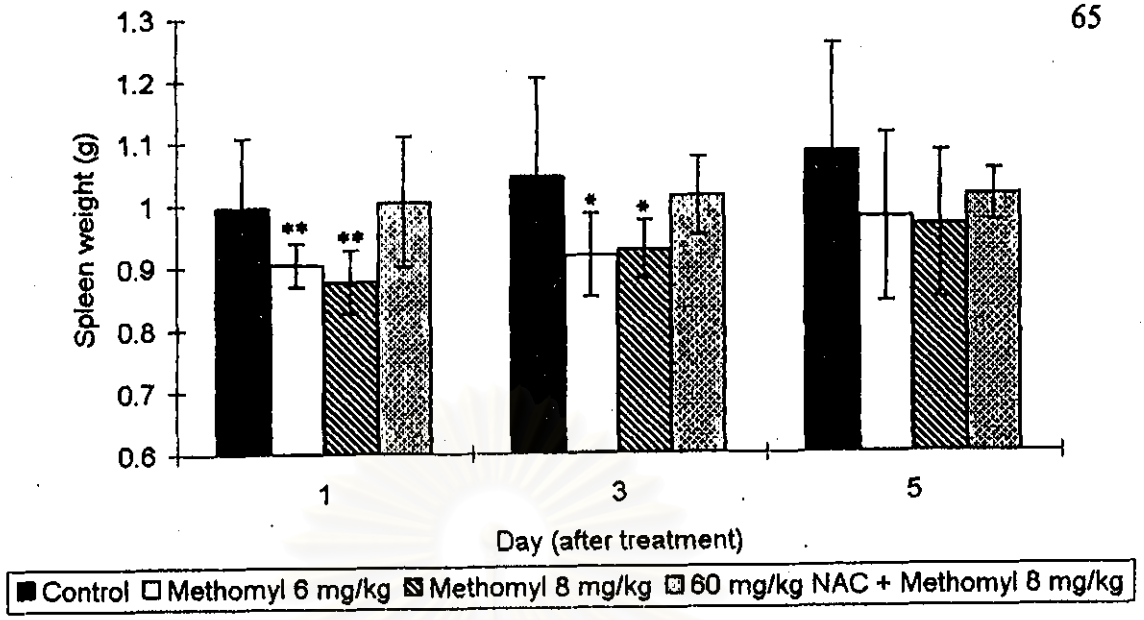
Spleen weight and Splenocyte viability

On the first day after treatment, the spleen weight, relative spleen weight, and splenocyte viability of rats receiving 6 and 8 mg/kg methomyl significantly decreased when compared to the control groups, but these reductions did not occur in the NAC-pretreated group (table 19 and figure 21, 22). These reductions declined on day 3 and 5, indicating the reversibility of these effects.

Table 19. The erythrocyte cholinesterase inhibition, spleen weight, relative weight and splenocyte viability of rats treated with 6, 8 mg/kg of methomyl, the rats pretreated with 60 mg/kg NAC following 8 mg/kg methomyl, and the control groups at various time points after dosing (mean ± S.D.; n = 4-8).

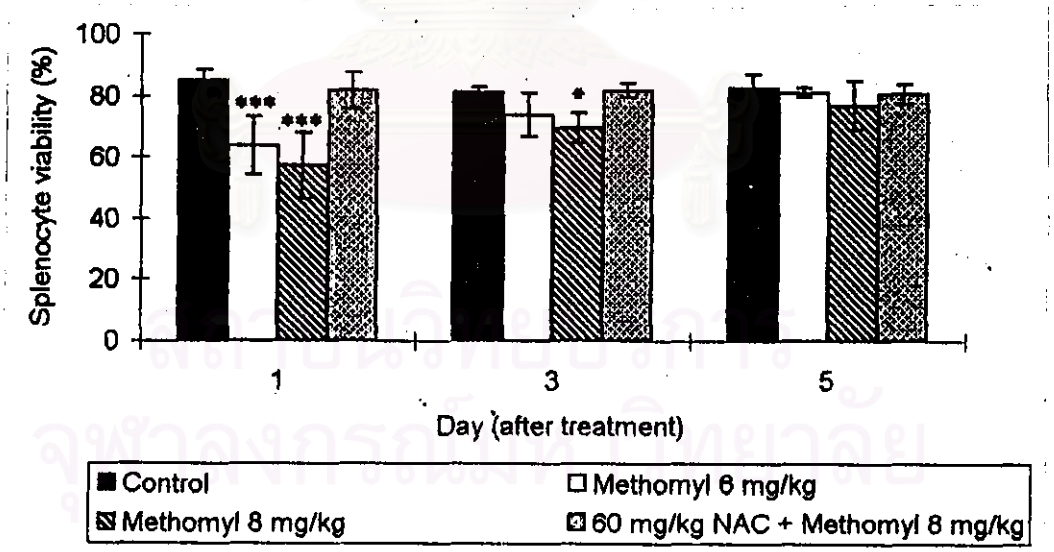
Day	Group	RBC ChE activity (U/g Hgb) (n=8)	Spleen weight (g) (n=8)	Relative weight (% of body weight) (n=8)	Splenocyte viability (%) (n=4)
1	C	8.34 ± 1.77	0.995 ± 0.112	0.48 ± 0.05	84.8 ± 3.7
	M6	7.15 ± 1.18	0.904 ± 0.035**	0.45 ± 0.03	63.5 ± 9.5***
	M8	7.58 ± 1.73	0.877 ± 0.051**	0.44 ± 0.04*	57.2 ± 10.8***
	NAC+M8	7.69 ± 1.62	1.004 ± 0.104	0.49 ± 0.04	81.5 ± 5.9
5	C	9.41 ± 3.45	1.043 ± 0.160	0.49 ± 0.06	80.9 ± 1.8
	M6	8.21 ± 1.55	0.917 ± 0.067*	0.43 ± 0.03**	73.5 ± 7.0*
	M8	8.57 ± 2.99	0.927 ± 0.047*	0.44 ± 0.02*	69.3 ± 4.9
	NAC+M8	9.64 ± 2.18	1.012 ± 0.062	0.48 ± 0.02	81.4 ± 2.3
7	C	8.01 ± 1.86	1.082 ± 0.176	0.45 ± 0.06	82.1 ± 4.5
	M6	7.60 ± 1.62	0.977 ± 0.135	0.42 ± 0.04	80.7 ± 1.7
	M8	7.47 ± 1.31	0.965 ± 0.118	0.43 ± 0.06	76.5 ± 7.9
	NAC+M8	8.20 ± 2.79	1.012 ± 0.041	0.44 ± 0.03	80.1 ± 3.2

C = control; M6 = methomyl 6 mg/kg; M8 = methomyl 8 mg/kg; NAC = 60 mg/kg N-acetylcysteine
* $p<0.10$, ** $p<0.05$, *** $p<0.01$ (Student's t-test was used for statistical analysis).



* p<0.10, ** p<0.05, *** p<0.01 (Student's t-test was used for statistical analysis).

Figure 21. Spleen weight of the rats treated with an acute dose (6 and 8 mg/kg) of methomyl and the rats pretreated with 60 mg/kg of NAC following an acute dose of 8 mg/kg of methomyl at various time points after dosing (Mean ± SD; n = 8).



* p<0.10, ** p<0.05, *** p<0.01 (Student's t-test was used for statistical analysis).

Figure 22. Splenocyte viability of the rats treated with an acute dose (6 and 8 mgkg) of methomyl and the rats pretreated with 60 mg/kg of NAC following an acute dose of 8 mg/kg of methomyl at various time points after dosing (Mean ± SD; n = 4).