# Chapter III

# Results

## 1. Anticonvulsant activity

Anticonvulsant activity of the tested compounds were evaluated in mice using two standard primary screening models, MES and PTZ tests. While NSS and PEG 400 (0.1 ml/25 g B.W. i.p.) given to control groups of mice exhibited no protection in both MES and PTZ models. Anticonvulsant activity of the tested compounds, VPM, was demonstrated in MES model but not PTZ model.

### 1.1 Anticonvulsant activity against MES

Like VPA, VPM demonstrated anticonvulsant activity against electroshock in a dose-dependent manner exhibiting the $ED_{50}$ of 138, 119 and 107 mg/kg B.W at the pretreated time of 15, 30 and 60 min, respectively (Figure 5, 8 and Table 2). The corresponding values of $ED_{50}$ exhibited by VPA were 250, 230 and 242 mg/kg B.W. (Figure 6,8 and Table 2).

### 1.2 Anticonvulsant activity against PTZ

In contrast to VPA which exerted its protection effect against convulsion induced by PTZ giving the $ED_{50}$ of 77 mg/kg B.W. (Figure 7), VPM in the doses up to 400 mg/kg. B.W. did not protect any experimental animals against chemoshock in this model (Table 2). Moreover, despite of lack of anticonvulsant effect, all mice receiving VPM in the dose of 400 mg/kg. B.W. slept.

## 2. Toxicity

### 2.1. Lethality

The most frequent adverse effects observed in mice receiving high doses of VPM and VPA (500-900 mg/kg B.W. i.p.) were ataxia, sedation and hypnosis. Lethality was observed within the period of 72 hours, however, death occurred mostly within 24 hours. The median lethal dose ($LD_{50}$) of VPM and VPA were 631 and 685

mg/kg B.W., respectively (Figure 9). However, in terms of safety, VPM possessed higher relative safety margin ($LD_{50}/ED_{50}$) than VPA (5.89 vs 2.97, Table 2).

2.2 Rotorod test

Normal mice as well as mice receiving either NSS or PEG400 (0.1 ml/25 gm. B.W.) were able to maintain their equilibrium for at least 2 min on the rotating rod in one of two successive trials. Neurological impairment indicated by an inability of the animals to maintain their equilibrium was observed in mice after the administration of VPM and VPA. The median neurotoxic dose($TD_{50}$) of VPM and VPA were found to be 151 and 309 mg/kg. B.W., respectively (Figure 10,11 and Table 2). However, both tested compounds exhibited rather similar protective index (PI = $TD_{50}/ED_{50}$). They are 1.41 and 1.34 for VPM and VPA, respectively(Table 2).

2.3 Locomotor activity test

Both compounds, VPM(100 and 200 mg/kg B.W. i.p.) and VPA(100 and 250 mg/kg B.W.), depressed locomotor activity of mice significantly different from those receiving NSS. However, no significant differences was observed between either the effect of PEG400 and VPM or the effect of VPA and PEG400 (Figure 12 and 13).

2.4 Barbiturate sleeping time

As illustrated in Figure14, PEG400 (0.1 ml/25 gm. B.W.) tended to slightly prolonged barbiturate sleeping time but no statistically significance was noted between the effects of NSS and PEG400. In comparison to PEG400, the barbiturate sleeping time was significantly prolonged by VPM (100 and 200 mg/kg. B.W.) as well as VPA(100 and 250 mg/kg. B.W.).

2.3 Hypnotic effect

The hypnotic effect previously observed in 1.2 was systematically evaluated in mice using loss of righting reflex to indicate the onset of hypnotic effect. VPM demonstrated a hypnotic effect in dose-dependent manner, the higher the dose the fast onset (Appendices, page 62). As shown in Figure 15, the median hypnotic dose ($HD_{50}$) of VPM was found to be 250 mg/kg. B.W.

3. Effects on some cortical amino acid neurotransmitter levels in freely moving rats

The excitatory neurotransmitters in question are glutamate and aspartate whereas GABA and glycine are the ones with inhibitory effect. Alteration of amino acid neurotransmitter's levels was expressed as percentage of change from basal value which was determined from three consecutive samples before the administration of the tested substances. Qualitative and quantitative determination of the amount of the amino acids were accomplished by HPLC as exemplified by HPLC chromatogram in Figure 16.

In control groups, the effect of PEG400 on spontaneous release of cortical aspartate, glutamate, glycine and GABA was not statistically different from those of NSS (Figure17, 18, 19 and 20). VPA in the dose of 100 mg/kg B.W. did not exert any significant effect on the levels of aspartate, glutamate, glycine and GABA. Nevertheless, in higher dose (250 mg/kg. B.W.), VPA did reduce the spontaneous release of glutamate but not aspartate, significantly different from the PEG400 treated group (Figure18 and 17). No significant effect of high dose of VPA (250 mg/kg B.W.) was noted on the levels of inhibitory amino acids, glycine and GABA(Figure19 and 20).

Low dose of VPM (100 mg/kg. B.W.) did not exhibit significant effect on any of the amino acids measured except a significant reduction in the level of glycine (Figure19). In addition, VPM in the dose of 200 mg/kg B.W. (Figure18) significantly reduced only the level of glutamate.

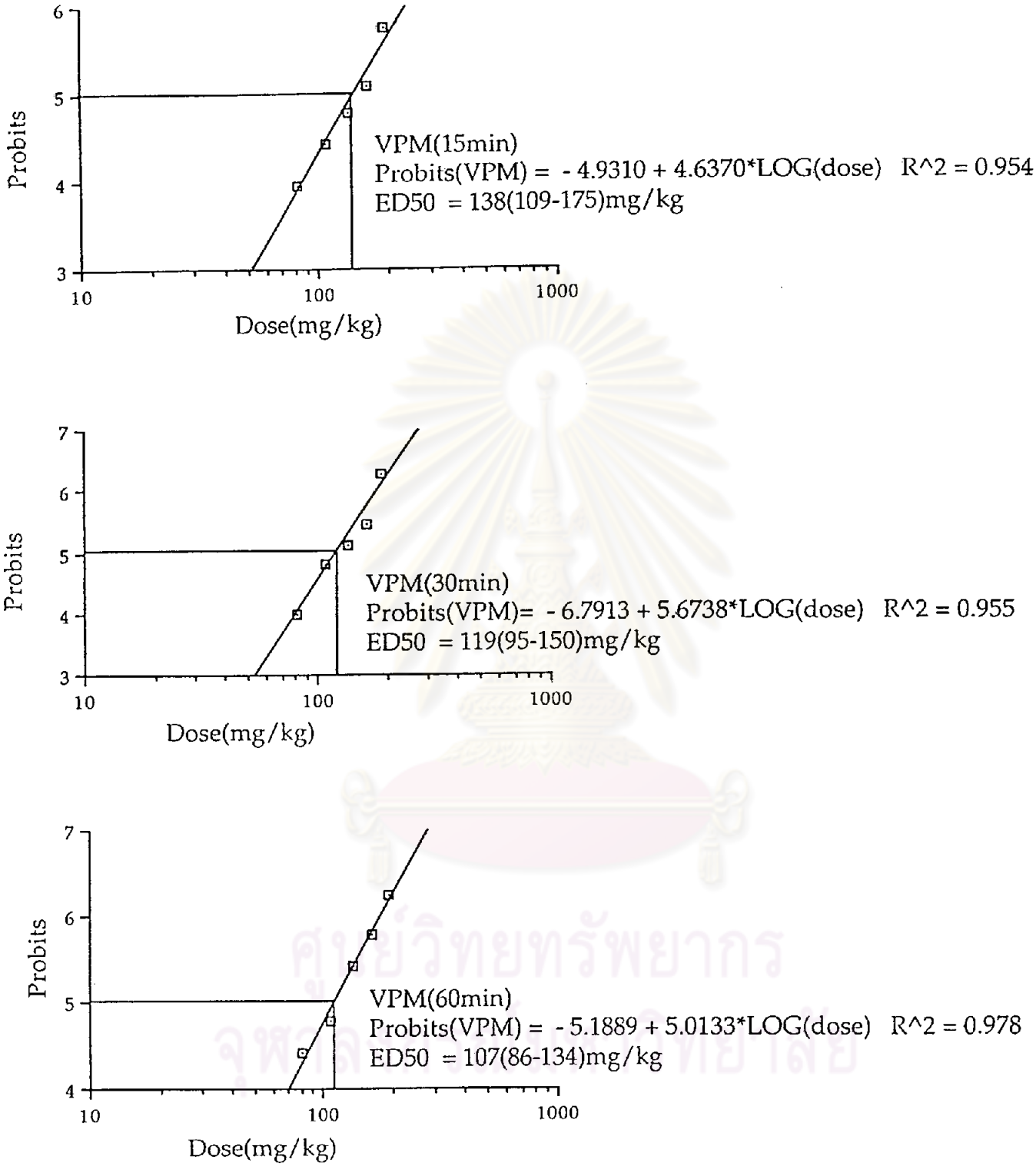



Figure 5 Log dose response curves of VPM (i.p.) against MES in mice at 15, 30, and 60 min pretreated times

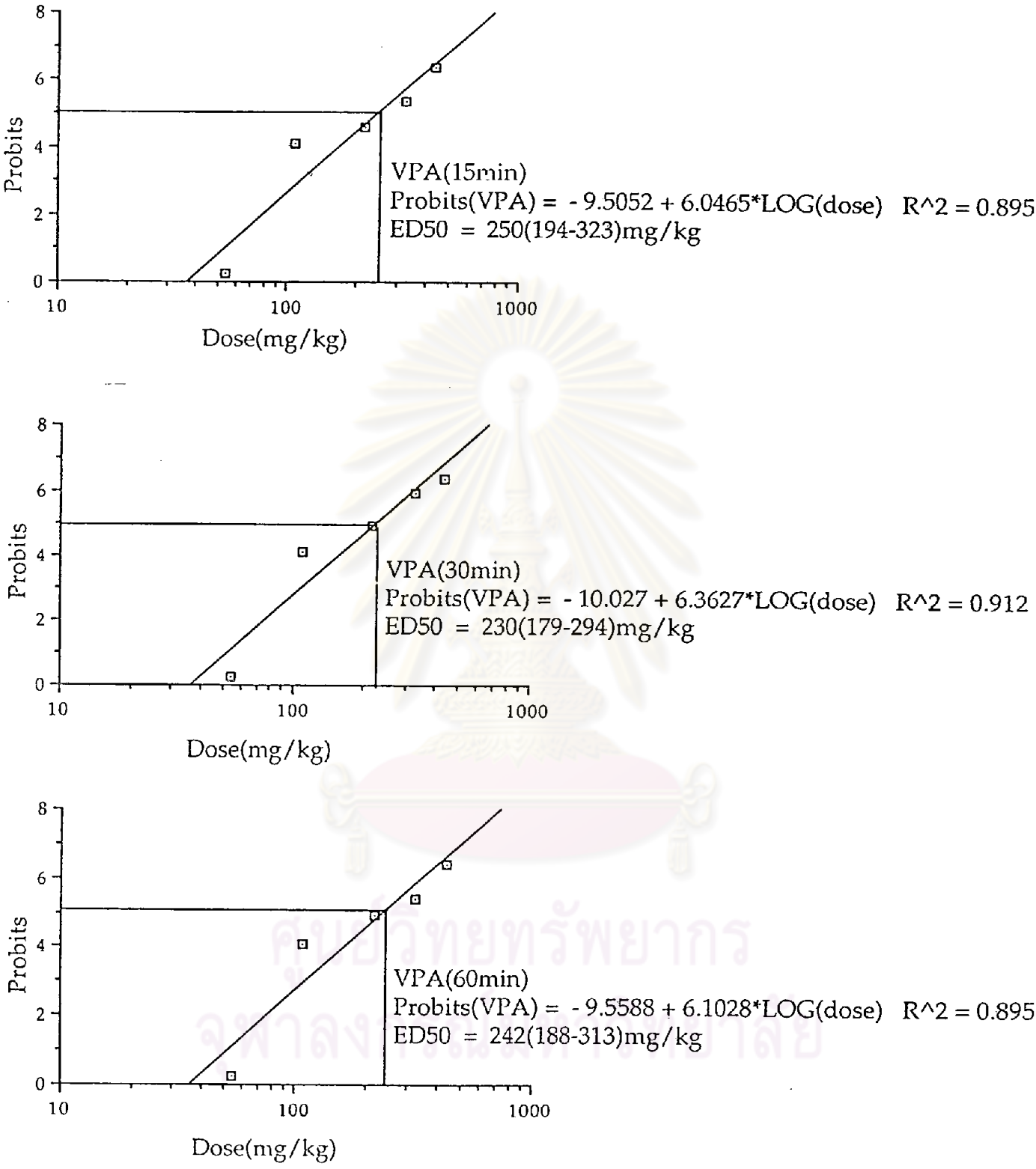
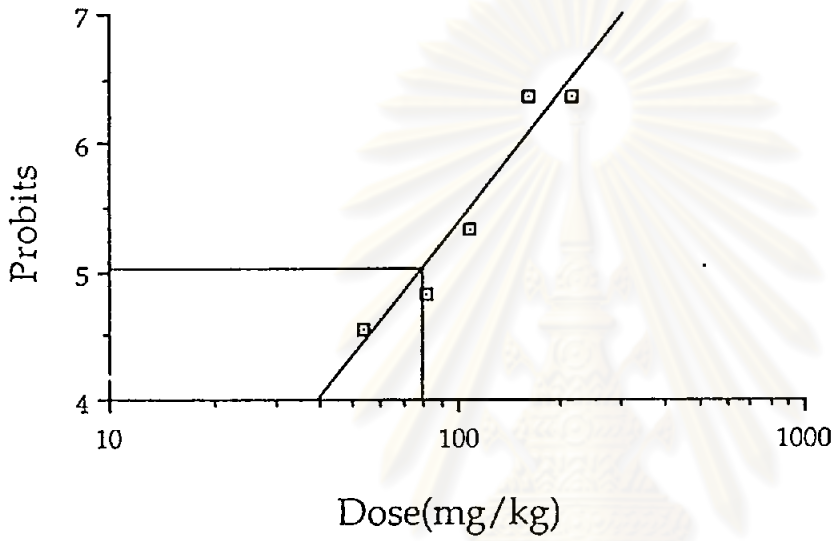



Figure 6 Log dose response curves of VPA (i.p.) against MES in mice at 15, 30, and 60 min pretreated times




Probits(VPA)= - 1.4913 + 3.4317*LOG(dose) R^2 = 0.937

ED50 = 77(55-109)mg/kg

Figure 7 Log dose response curves of VPA (i.p.) against PTZ in mice at 30 min pretreated times

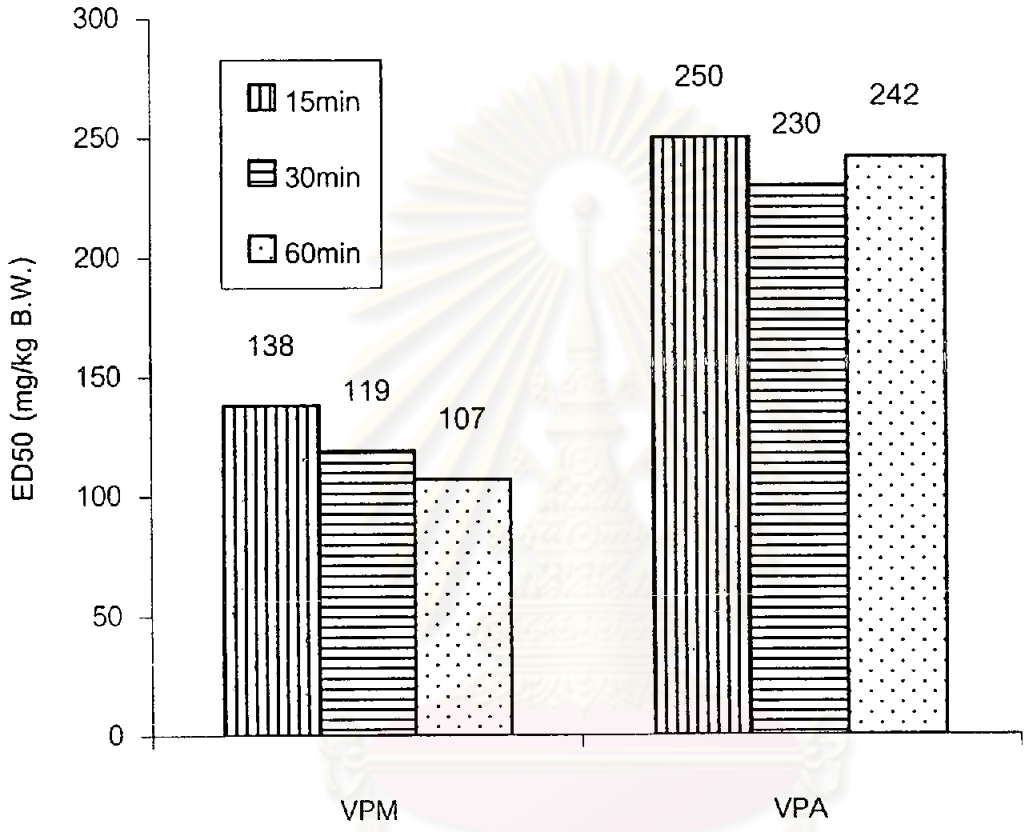



Figure 8 Comparison of $ED_{50}$ at various pretreated times of VPM and VPA (i.p.) against MES in mice

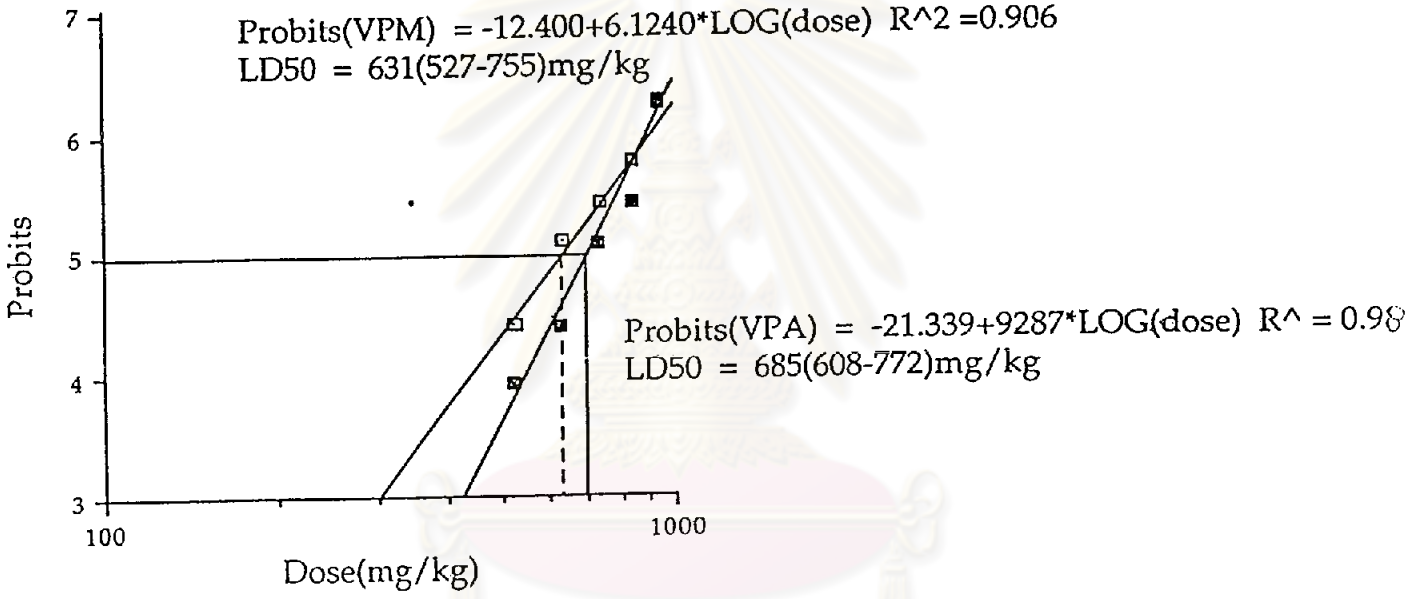



Figure 9 Log dose response curves of acute toxicity(lethality) of VPM and VPA (i.p.) in mice.

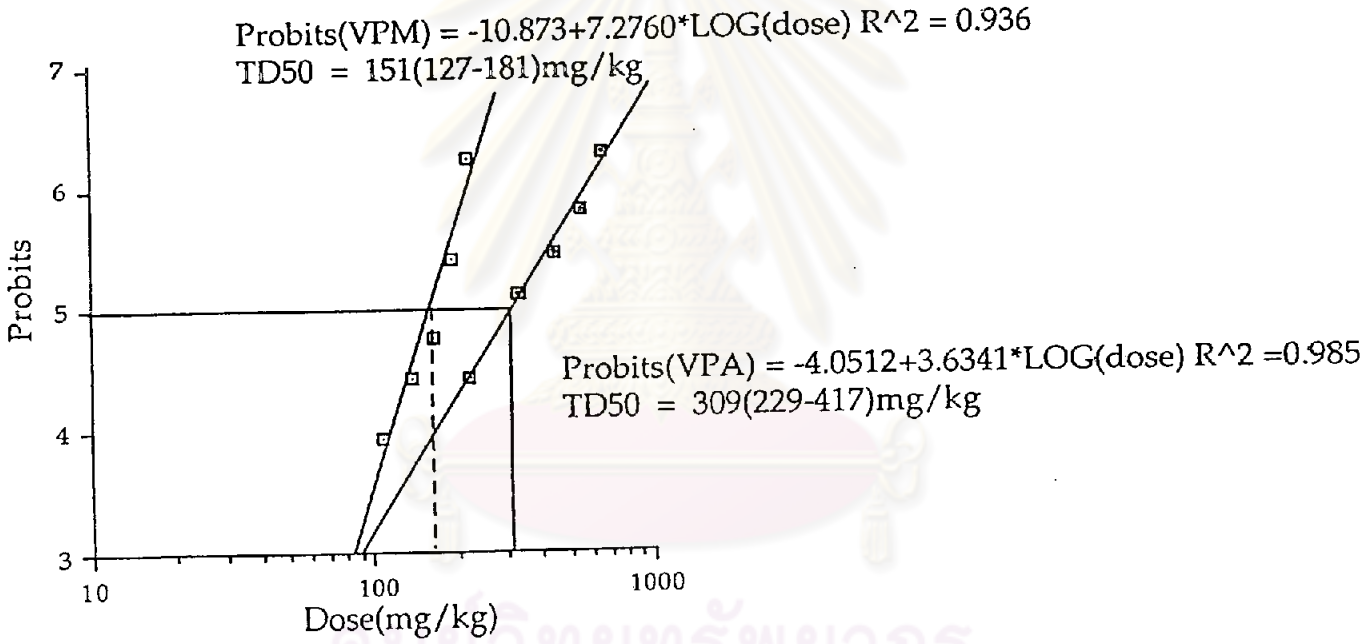



Figure 10 Log dose response curves of neurotoxicity (Rotorod test) exhibited by VPM and VPA (i.p.) in mice.

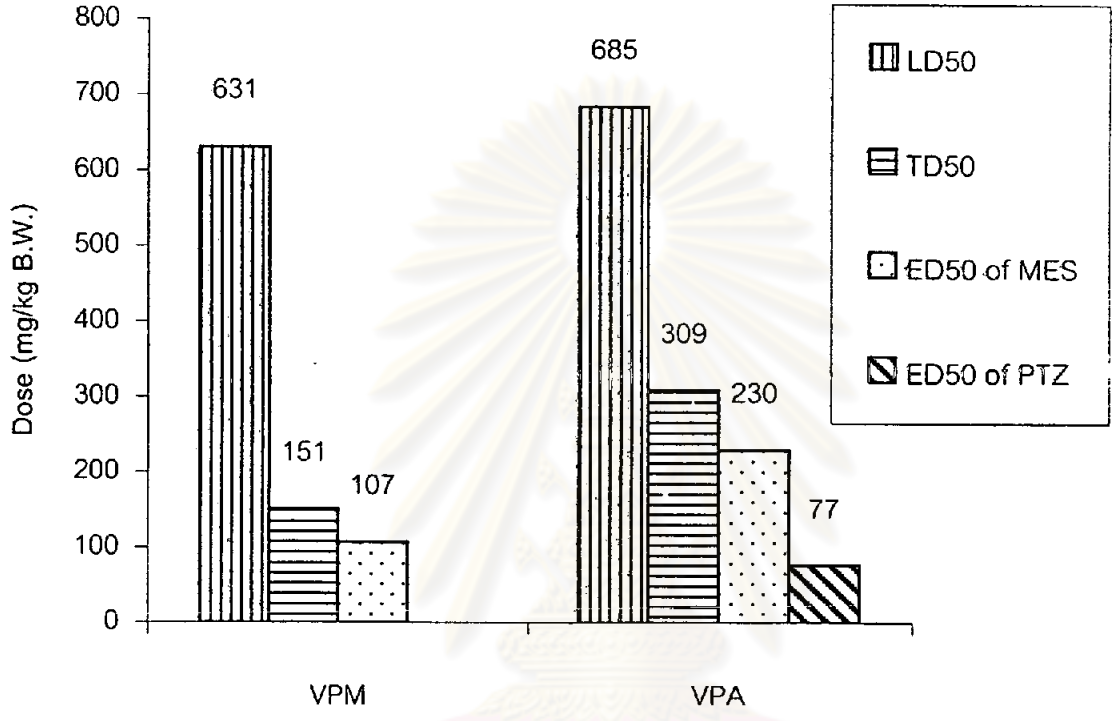
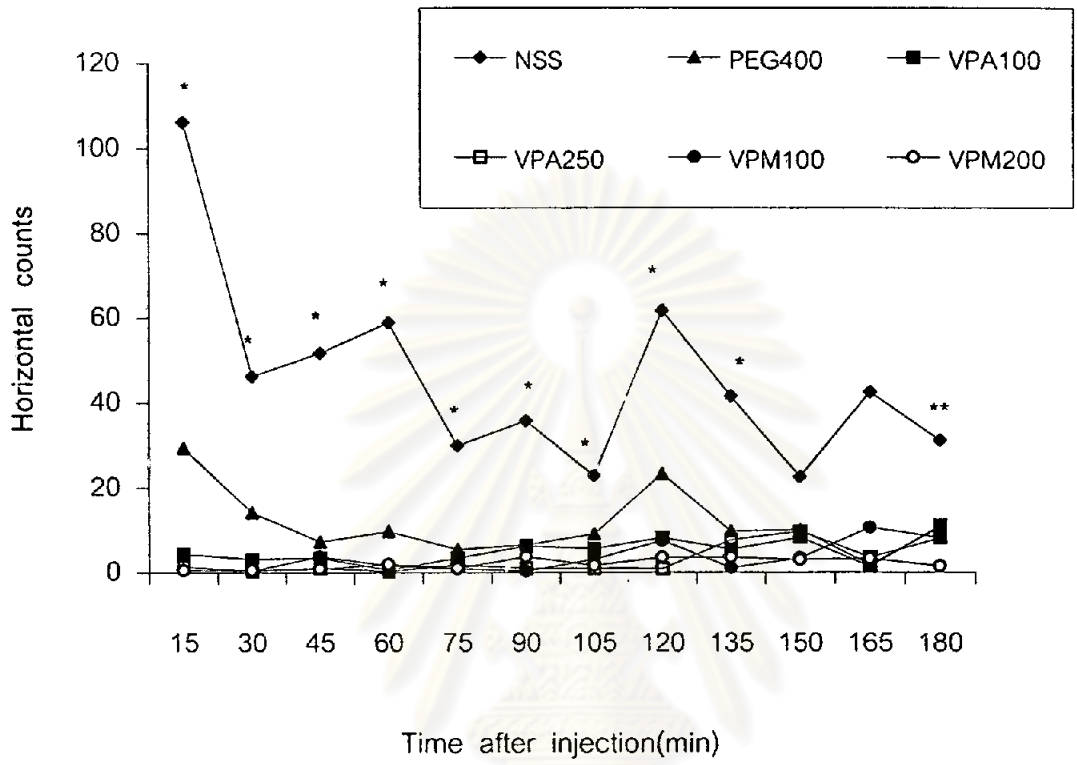



Figure 11 Illustration of the LD50, TD50 and ED50 elicited by an intraperitoneal administration of VPM and VPA in mice in MES and PTZ models

Table 2 ED50, $TD_{50}$, $LD_{50}$, PI ($TD_{50}$ / $ED_{50}$), and relative safety margin ($LD_{50}$/ $ED_{50}$) of an intraperitoneal administration of VPM and VPA in MES and PTZ models.

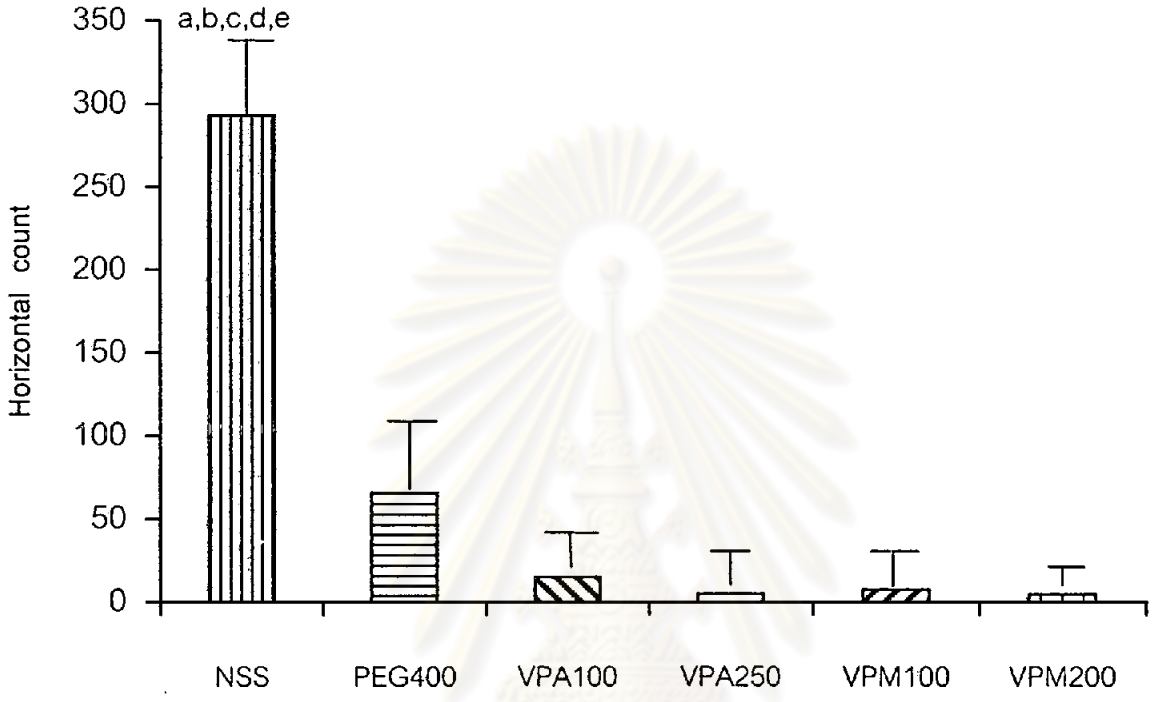
| Parameter | Animal model | VPM | VPA |
|---|---|---|---|
| $ED_{50}$ (mg/kg B.W.) | MES | 107 (86-134) | 230 (179-294) |
| | PTZ | - | 77 (55-109) |
| $TD_{50}$ (mg/kg B.W.) | Rotorod | 151 | 309 |
| PI | MES | 1.41 | 1.34 |
| | PTZ | - | 4.01 |
| $LD_{50}$ (mg/kg B.W.) | - | 631 | 685 |
| Relative safety margin | MES | 5.89 | 2.97 |
| | PTZ | - | 8.89 |




*p<0.05 denotes statistically significant difference from PEG400,VPA100,VPA250,VPM100,VPM200

**p<0.05 denotes statistically significant difference from VPM200

Figure12 Effects of an intraperitoneal administration of VPM and VPA on horizontal counts (Mean±SEM) of locomotor activity in mice at various times




[a] p<0.05 denotes statistically significant difference from PEG400

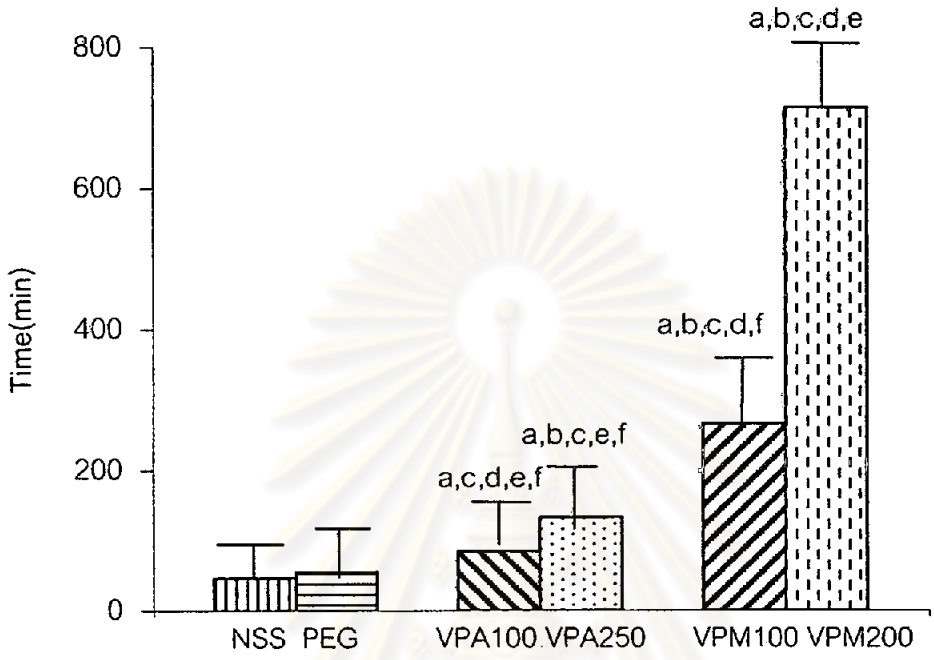
[b] p<0.05 denotes statistically significant difference from VPA100

[c] p<0.05 denotes statistically significant difference from VPA250

[d] p<0.05 denotes statistically significant difference from VPM100

[e] p<0.05 denotes statistically significant difference from VPM200

Figure13 Effects of an intraperitoneal administration of VPM and VPA on total horizontal counts (Mean±SEM) within 75 min of locomotor activity in mice




[a]p<0.05 denotes statistically significant difference from NSS

[b]p<0.05 denotes statistically significant difference from PEG400

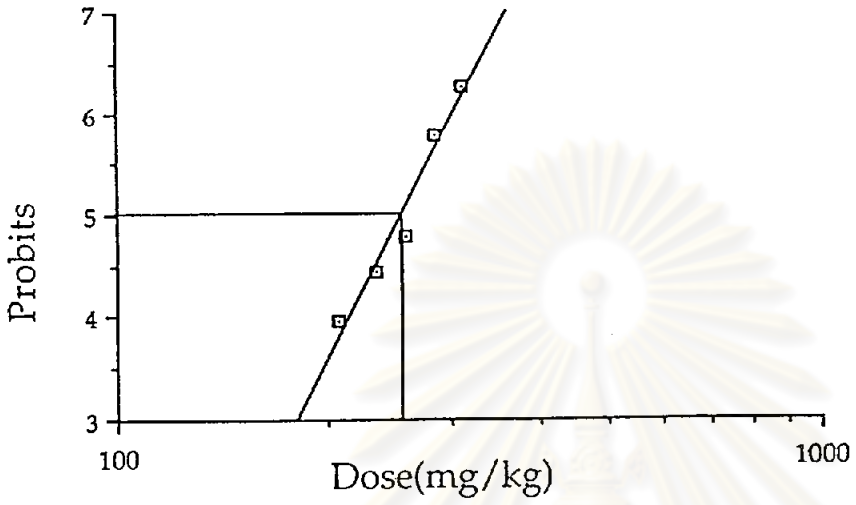
[c]p<0.05 denotes statistically significant difference from VPA100

[d]p<0.05 denotes statistically significant difference from VPA250

[e]p<0.05 denotes statistically significant difference from VPM100

[f]p<0.05 denotes statistically significant difference from VPM200

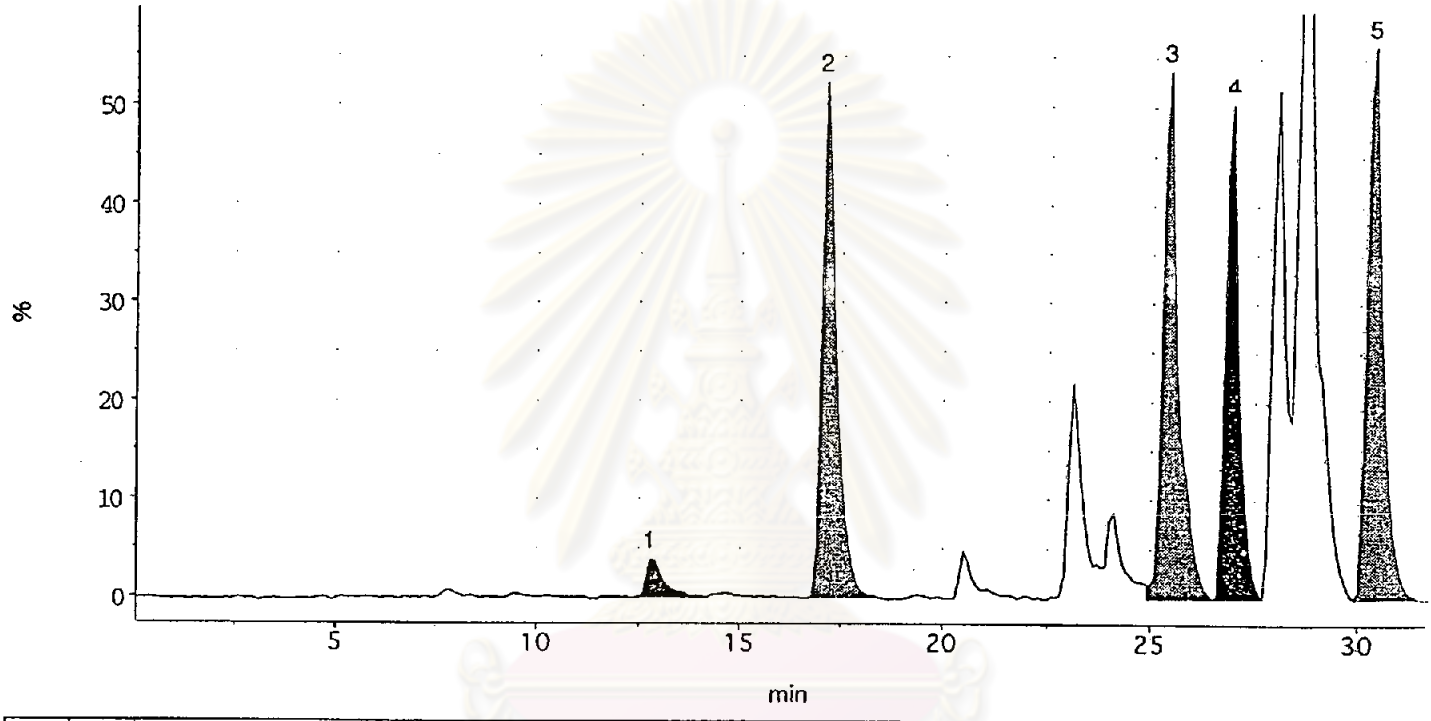
Figure14 Effects of an intraperitoneal administration of VPM and VPA on barbiturate sleeping time in mice




Probits(VPM) = - 27.185 + 13.420*LOG(dose) R^2 = 0.959

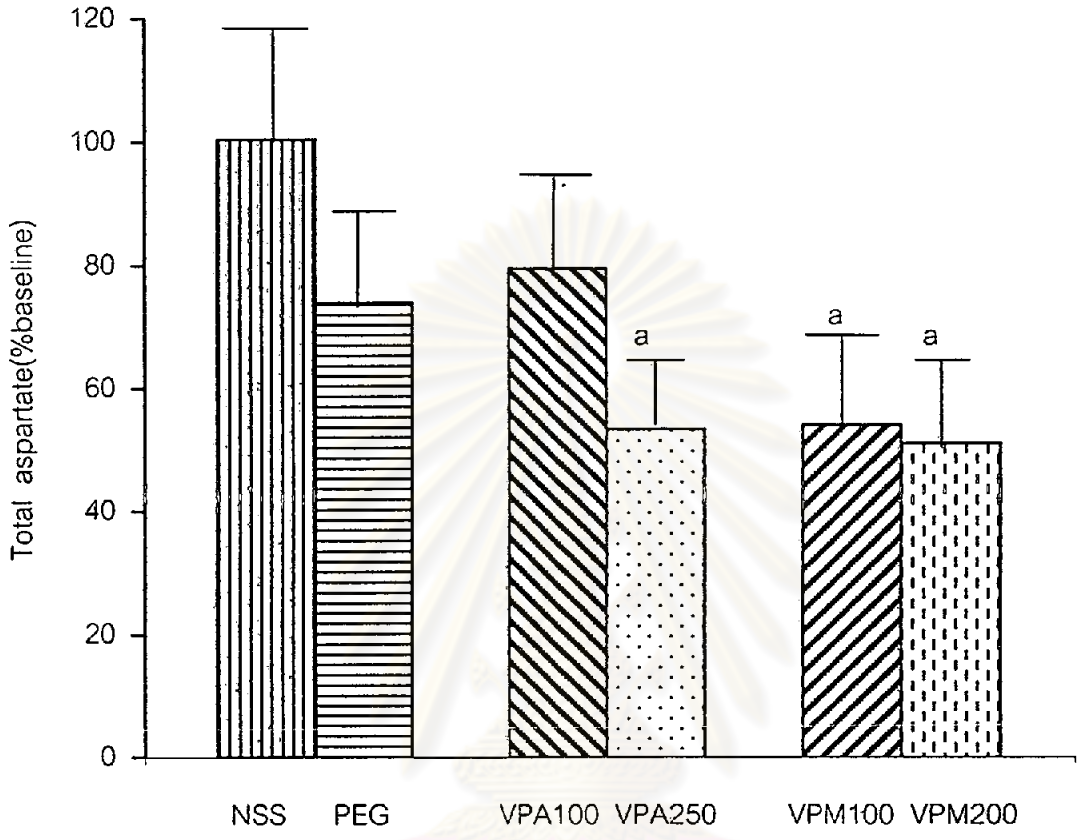
HD50 = 250(227-275)mg/kg

Figure 15 Log dose response curves of hypnotic effect by VPM (i.p.) in mice.



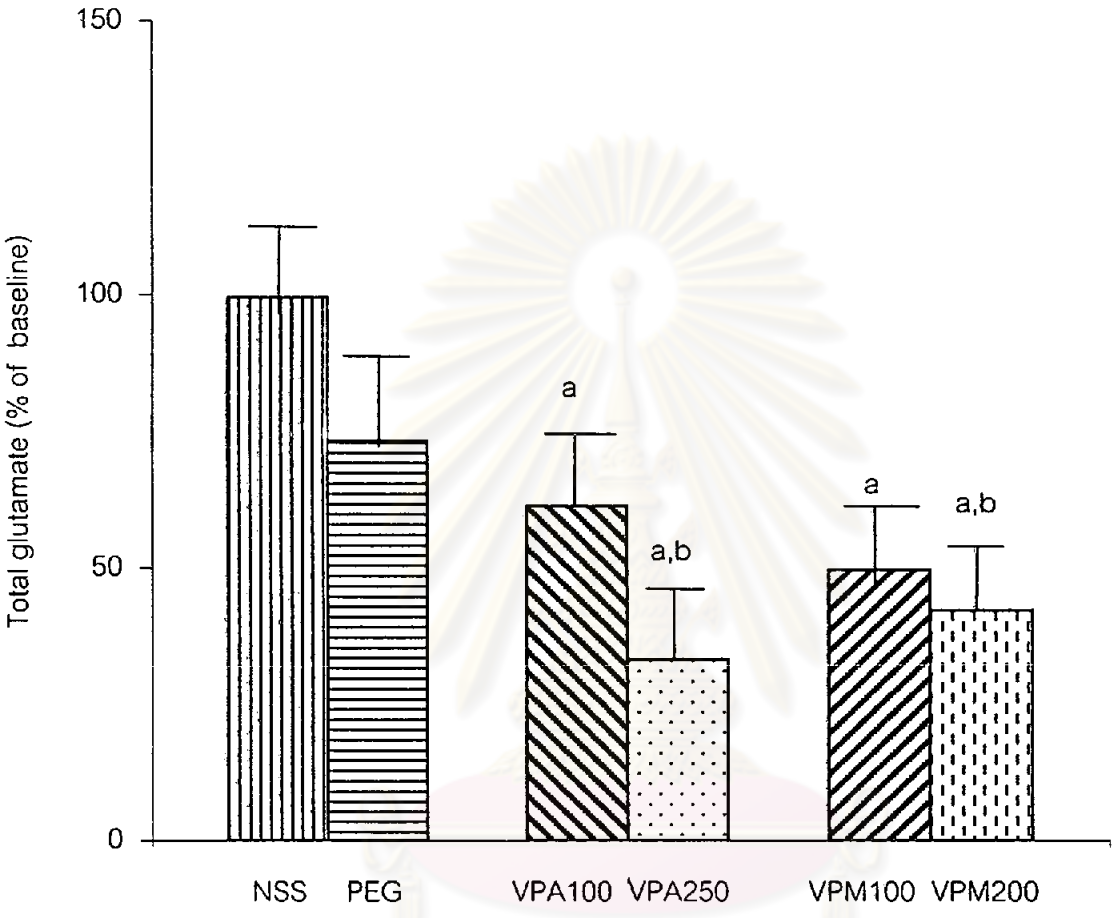

| Peak | | Name | $t_R$ (min) | Start (min) | End (min) | Area | Height (%) | Norm (%) | Peak Type | Amount |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | Asparate | 12.8 | 11.6 | 14.2 | 2.13 | 2.46 | 2.44 | BB | |
| 2 | | Glutamate | 17.1 | 16.8 | 18.5 | 20.727 | 33.10 | 23.72 | BB | |
| 3 | | Homoserine | 25.4 | 24.9 | 26.5 | 22.760 | 33.89 | 26.04 | DD | |
| 4 | | Glycine | 26.9 | 26.5 | 27.7 | 19.000 | 31.78 | 21.74 | DD | |
| 5 | | GABA | 30.3 | 29.9 | 31.4 | 22.779 | 35.51 | 26.06 | DB | |
| | | | | | | 87.40 | 136.74 | 100.00 | | |

Figure 16 HPLC chromatogram of OPA-derivatizes amino acid from the rat cerebral cortex




[a]p<0.05 denotes statistically significant difference from NSS

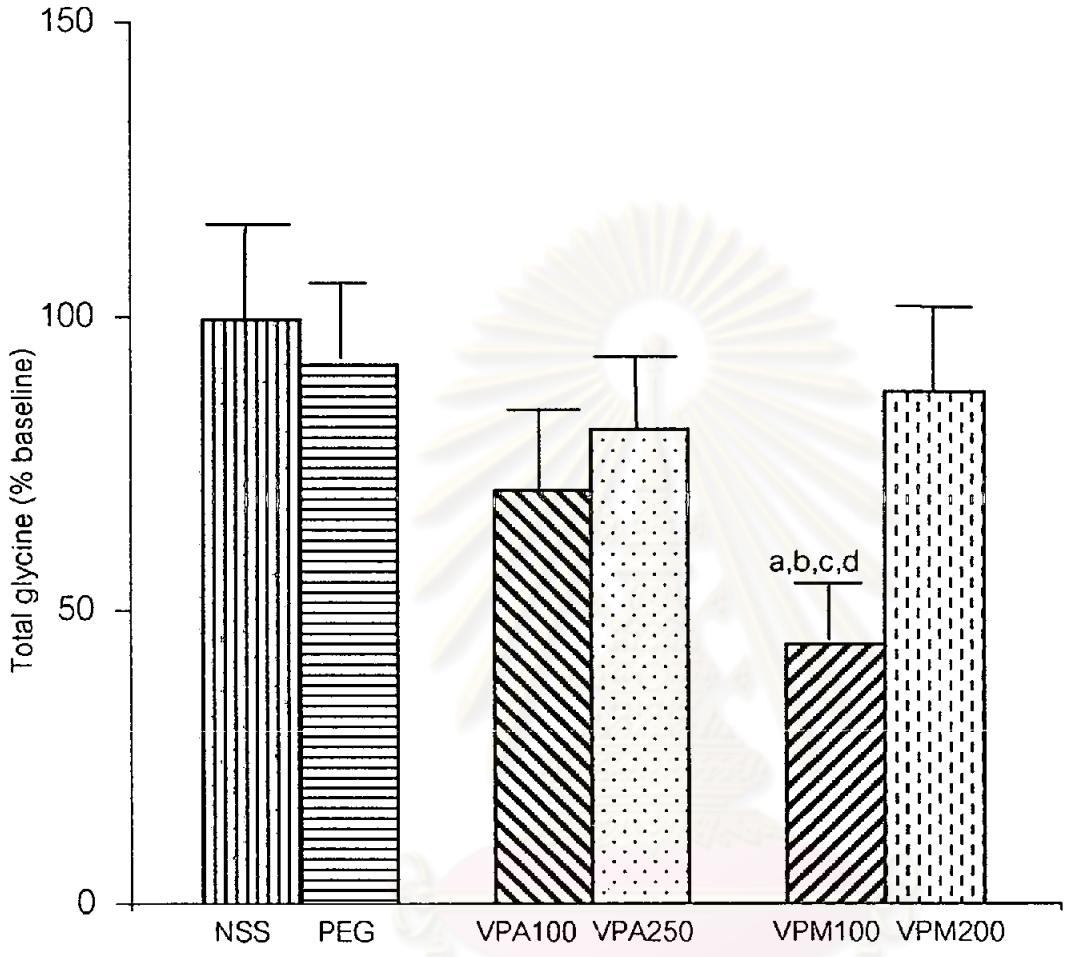
Figure17 Effects of an intraperitoneal administration of VPM and VPA on the total amount of the rat cortical aspartate levels in the dialysate collected for 3 hours




[a] $p<0.05$ denotes statistically significant difference from NSS

[b] $p<0.05$ denotes statistically significant difference from PEG400

Figure 18 Effects of intraperitoneal administration of VPM and VPA on the total amount of the rat cortical glutamate levels in the dialysate collection for 3 hours



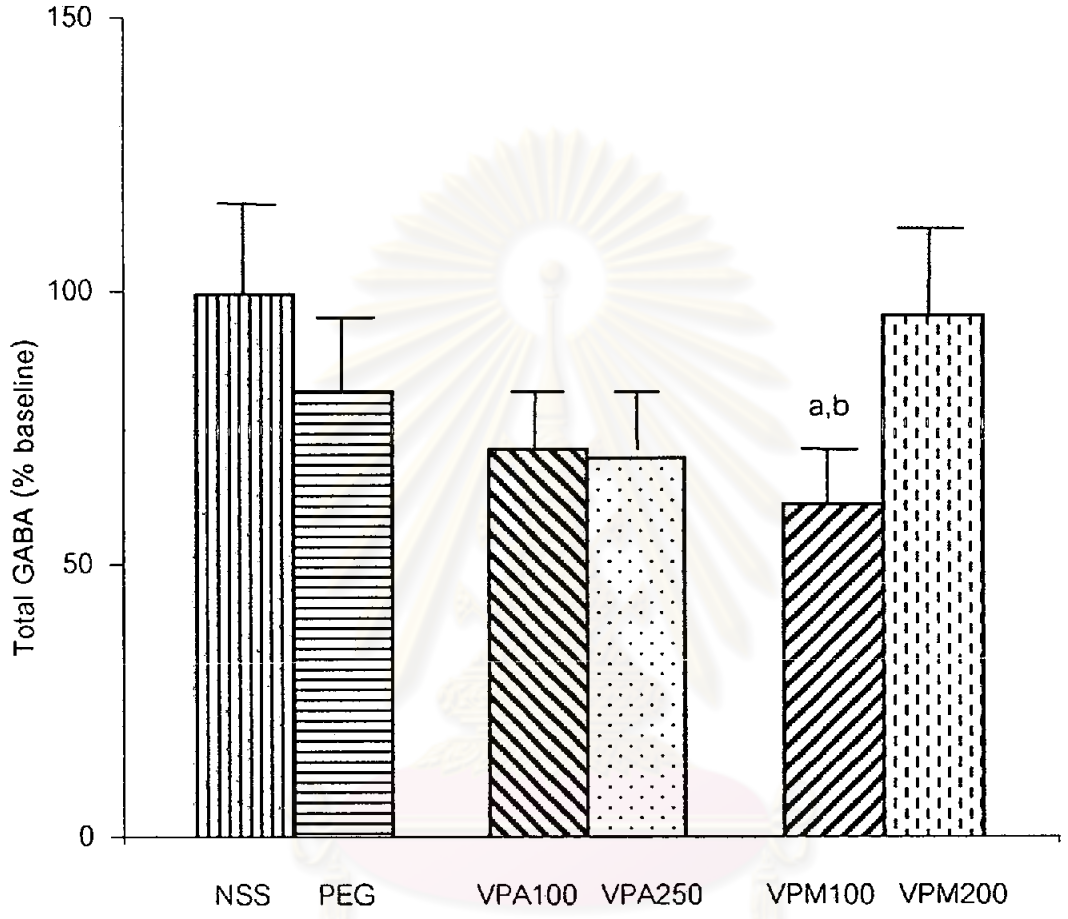

[a]p<0.05 denotes statistically significant difference from NSS

[b]p<0.05 denotes statistically significant difference from PEG400

[c]p<0.05 denotes statistically significant difference from VPA250mg

[d]p<0.05 denotes statistically significant difference from VPM200mg

Figure 19 Effects of an intraperitoneal administration of VPM and VPA on the total amount of the rat cortical glycine levels in the dialysate collected for 3 hours




[a]p<0.05 denotes statistically significant difference from NSS

[b]p<0.05 denotes statistically significant difference from VPM200mg

Figure 20 Effects of an intraperitoneal administration of VPM and VPA on the total amount of the rat cortical GABA levels in the dialysate collected for 3 hours