

## ACUTE NEUROBEHAVIOURAL TOXICITY OF PHOSPHAMIDON AND ITS DRUG-INDUCED ALTERATION

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**Abstract:** Phosphamidon, a systemic organophosphate insecticide, (1.4 mg/kg - dose  $\frac{1}{4}$ th of LD<sub>50</sub> given ip), produced several autonomic, neurological and behavioral effects in mice with peak effects being at 15 min. Similar dose in rats also abolished conditioned avoidance response. Pre-treatment with atropine, iproniazid, alpha-methyl-p-tyrosine, p-chlorophenylalanine or thiosemicarbazide reduce many of these effects. This suggests that phosphamidon toxicity involves the central cholinergic, adrenergic, serotonergic and GABAergic systems in addition to peripheral cholinergic effects.

**Key words:** phosphamidon      organophosphate toxicity      atropine      iproniazid  
alpha-methyl-p-tyrosine      p-chlorophenylalanine      thiosemicarbazide

### INTRODUCTION

Phosphamidon (PMD) is a broad spectrum organophosphate insecticide which is coming in use. It is metabolised by microsomal mixed function oxidases (1) in the living system to become an active anticholinesterase (AChE) agent (2). Its lethal effects are attributed mainly to AChE inhibition in various tissues (3, 4). Little is known about its effects on the central nervous system. Since the compound may produce occupational hazard, the present paper describes the acute toxic effects of PMD on some neurophysiological functions and their possible mechanisms.

### METHODS

The study was conducted in male swiss mice (20- 26 g) and wister rats (150-200 g) maintained on pellet diet and water ad libitum. The drugs used were: Technical grade phosphamidon (2-chloro-2-diethyl carbamoyl-1-methyl vinyl dimethyl

phosphate, Ciba-Geigy Ltd. 85%); atropine sulphate (Aldrich); iproniazid phosphate (Sigma); (DL)-P-Chlorophenylalanine (Sigma) and thiosemicarbazide (S.D. Laboratories). They were diluted in normal saline and were administered, ip (volume : 0.1 ml/ 10 g for mice and 1ml/200 g for rats).

**Median lethal dose (LD<sub>50</sub>) :** The ip LD<sub>50</sub> of PMD was determined (5) using 5 groups of 10 animals each with doses ranging from 4 to 8 mg/kg, deaths being observed upto 24 hr.

**Acute neurobehavioral toxicity :** The animals were treated (ip) with PMD (1.4 mg/kg in mice and 1.5 mg/kg in rats i.e.  $\frac{1}{4}$ th of LD<sub>50</sub>), respectively. The autonomic (diarrhoea, urination, piloerection, lacrymation, salivation and ear skin color), neurological (restlessness, passivity, tremor, convulsion, and forced locomotor activity) and behavioral (spontaneous motor activity, exploratory behavior and conditioned avoidance response) effects were assessed as described by Turner (6) at

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15 min, the peak effect of PMD as determined in separate experiments. The parameters were studied in mice except that the conditioned avoidance response (CAR) was assessed in rats.

### Drug pre-treatment

In some experiments, the animals were pre-treated (ip) with either atropine (10 mg/kg, 20 min. prior), iproniazid (100 mg/kg, 24 hr prior), alpha-methyl-p-tyrosine (AMPT, 200 mg/kg, 4 hr prior), P-chlorophenylalanine (PCPA, 200 mg/kg, 12 hourly for 3 days) or thiosemicarbazide (TSC, 5 mg/kg, 4hr prior) followed by PMD administration.

The data was analysed by unpaired student's 't' test.

### RESULTS

PMD produced a dose dependent mortality in mice and rats. LD<sub>50</sub> (ip) was  $5.7 \pm 0.7$  in mice and  $6.1 \pm 0.8$  mg/kg in rats. Peak effect following small dose in mice was at 15 min and the animals were apparently free from toxic symptoms by 12 hr. All autonomic parameters, passivity and tremors were significantly stimulated whereas, behavioral parameters were severely depressed (Table 1). Atropine pre-treatment markedly protected against the autonomic, neurological and behavioral toxic effects of PMD. However, animals were not completely free from toxic symptoms. Iproniazid was partially, but significantly, effective in overcoming the behavioral toxicity of PMD. Moreover, passivity was completely overcome, and increased

TABLE I : Neurobehavioral Toxicity of Phosphamidon (PMD) alone and after Pretreatment with various Drugs

S. No.	Parameter	Saline	Phosphamidon	Atropine + PMD	Iproniazid + PMD	AMPT + PMD	PCPA + PMD	TSC + PMD
<b>A. AUTONOMIC</b>								
1.	Diarrhoea	1.3 ± 0.9	4.5 ± 1.3*	1.3 ± 0.7*	2.2 ± 0.6*	0.8 ± 0.6*	1.5 ± 0.5*	1.4 ± 1.1*
2.	Urination	0.5 ± 0.5	1.5 ± 0.7*	0.3 ± 0.5*	1.3 ± 0.5	0.6 ± 0.7*	1.0 ± 0.4	0.7 ± 0.6
3.	Piloerection	0.2 ± 0.5	1.5 ± 0.5*	0 ± 0*	0.6 ± 0.5*	1.1 ± 0.5	1.8 ± 0.6	1.6 ± 0.7
4.	Lacrymation	0 ± 0	0.7 ± 0.4*	0 ± 0*	0.5 ± 0.5	0.6 ± 0.5	0 ± 0*	0.5 ± 0.5
5.	Salivation	0 ± 0	0.3 ± 0.5*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
<b>B. NEUROLOGICAL</b>								
6.	Restlessness	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.5*	0 ± 0	0.5 ± 0.5*	0 ± 0
7.	Passivity	0 ± 0	2.6 ± 0.5*	0 ± 0*	.0 ± 0*	0.4 ± 0.1*	0 ± 0*	0 ± 0*
8.	Tremors	0 ± 0	1.1 ± 0.5*	0 ± 0*	0.7 ± 0.5	0 ± 0*	1.1 ± 0.3	0.7 ± 0.7
9.	Convulsions	0 ± 0	0 ± 0	0 ± 0	0.8 ± 0.4*	0 ± 0	0 ± 0	0.5 ± 0.5*
<b>C. BEHAVIORAL</b>								
10.	Motor activity	45.0 ± 9.0	3.0 ± 1.0*	43.0 ± 6.0*	12.0 ± 4.0*	8.0 ± 3.0	22.0 ± 10.0*	39.0 ± 8.0*
11.	Exploration	16.3 ± 1.4	0.9 ± 0.9*	14.6 ± 1.7*	24.7 ± 3.5*	2.0 ± 1.3	12.3 ± 2.5*	12.4 ± 3.1*
12.	Conditioned avoidance response (in rats)	47.0	10.0*	29.0*	23.0*	17.0*	40.0*	27.0*

Values are mean scores (± S.D.) from 10 mice in case of parameters 1 to 11 and from 8 rats for parameter 12.

Saline acted as control for PMD treated group which in turn served as control for pretreated groups.

\*Difference significant as compared to control (P<0.05).

See text for abbreviations of pre-treatments.

degree of restlessness was observed. AMPT effectively antagonized diarrhoea, urination, passivity, tremor and partially restored the motor activity and CAR. Other parameters did not show any significant change. PCPA and thiosemicarbazide pretreatment had similar effects imparting protection against the behavioral toxicity of PMD, with some favourable effect on diarrhoea and passivity as well (Table I). Righting reflex, forced locomotor activity and ear skin color did not show any change at any time with the insecticide alone or in combination with either drug treatment.

### DISCUSSION

PMD exhibited no species difference with regard to its acute toxicity in mice and rats. That the effects of sublethal doses were maximum by 15 min is expected, since its absorption and metabolism into an active compound (2) reaches its maxima by this time. Brain acetylcholine (ACh) can return to normal or near normal levels within 8-12 hr, despite consistent AChE inhibition by an organophosphate compound (7). The return of brain ACh level towards normal correlates fairly well with the disappearance of intoxication symptoms, but shows little relationship with cholinesterase activity. However, more subtle signs of behavioral impairment, like avoidance performance deficit, could still be observed after brain ACh had returned to normal levels (7). The marked increase in autonomic parameters after PMD treatment was obviously due to increased peripheral cholinergic function. Presumably, the central cholinergic actions of these compounds lead to induction of passivity tremor and convulsions in mice as suggested by other workers (8, 9). The increased ACh levels in the brain leads to severe depression (10) and this may be the basis of neurological and behavioral toxicity, since atropine predictably antagonised PMD effects. However, the functions were not completely restored, indicating involvement of other systems in genesis of PMD toxicity syndrome. Iproniazid pre-treatment did not alter the

peripheral cholinergic effects of the insecticide, but CNS depressant action was counteracted due to its anti-depressant action induced through monoamine oxidase inhibition which leads to increased catecholamine levels in the various brain regions controlling different functions. AMPT, norepinephrine synthesis inhibitor, significantly prevented diarrhoea, tremor and passivity, but had little effect on motor activity, exploration and CAR probably because they are primarily under dopaminergic control (11). This indicates some adrenergic link in the mediation of central cholinergic functions. However, the exact mechanism is not known. Animals depleted of 5-hydroxytryptamine (5-HT) with PCPA showed no diarrhoea or lacrymation and the behavioral parameters i.e. motor activity, exploration and CAR were only partially suppressed, after insecticide treatment. As a result of suppressed serotonergic system in the brain, the adrenergic activity is increased (12) which may be responsible for the observed effects. Similar effects were seen when the norepinephrine levels were increased as a result of MAO inhibition by iproniazid. However, there is good evidence that the serotonergic system is affected indirectly, due to cholinergic excess, as well as directly after treatment with an OP compound (13).

Involvement of GABAergic system was evident from experiments with thiosemicarbazide, the GABA-synthesis inhibitor. PMD produced little CNS depression, thus restoring alertness, motor activity, exploration and CAR to a great extent. The observed effects are due to inhibition of the inhibitory transmitter GABA, leading to CNS stimulation. Hence, it could be contemplated that adequate quantities of GABA (and 5-HT) might be helpful in suppressing PMD toxicity.

The results suggested that the toxicity of PMD results from complex interaction between the central cholinergic, adrenergic, serotonergic and GABAergic systems in the brain, in addition to its peripheral cholinergic effects.

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