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Lethal toxicity of cypermethrin in combination with sevin and piperonyl butoxide in wistar rat

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ABSTRACT

Toxicological investigations are generally carried out to study the effect of a single substance. However, our environment and food include huge number of substances and their exposure to life imparts complex type of effects. This study is framed to investigate the combination effect of cypermethrin with carbamate and piperonyl butoxide (known synergist). The oral LD_{50} were recorded for each dose after 96 hours by log-dose/probit regression line method. To investigate the level of synergism of different ratio mixtures, potentiation factor and combination index were calculated. The estimated oral LD_{50} of cypermethrin was 416.98 mg/Kg body weight and Sevin was 1778.35 mg/kg body weight whereas LD_{50} of piperonyl butoxide was 11480-mg/kg body weight. The increase in magnitude of toxicity of cypermethrin is 1.95 and 5.86 for 1:1 and 1:5 ratios respectively with sevin while it is 1.29 and 3.96 for 1:1 and 1:5 respectively with PB which reveals that sevin potentiates the toxicity of cypermethrin. The study gives clue that mixture of pesticides could be more toxic in both field and house hold conditions.

Keywords: Albino rats, Cypermethrin, Combination Toxicity, Sevin.

INTRODUCTION

Synthetic pyrethroid insecticides, chemicals for agricultural pest control, are now being excessively used in India. These compounds exhibit high insecticidal efficacy and are relatively less toxic to mammals. Accidental exposure at the work place and their presence in the environment has aroused concern over their possible adverse effects on human health.

Cypermethrin is a type II pyrethroid exhibiting choreoathetosis syndrome (c s) which is characterized by hyperactive behaviour, tremors and motor in-coordination progressing to sinous writhing movements.

Toxicological investigations are generally carried out to study the effect of a single substance. However, our environment and food include huge number of substances and their exposure to life imparts complex type of effects due to collective exposure. The symptoms and injuries caused by various chemicals at once may be entirely different from the impact caused by exposure of one chemical. This study is framed to investigate the combination effect of cypermethrin, with carbamate and piperonyl butoxide.

Sevin (50% carbaryl) is a N- methyl carbamate and is used in combination with cypermethrin to compare the effect

of known synergist, piperonyl butoxide. Piperonyl butoxide is extraction of safrole and is monooxygenase inhibitor, which inhibits the enzymes of mixed function oxidase system.

EXPERIMENTAL SECTION

A.Experimental animals

Wistar albino rats [*Rattus norvegicus* Berkenhout] of almost same weight (120±10g) have been selected randomly irrespective of age and sex from inbred colony. Rats were maintained in polypropylene cages (temperature 25 ± 5^0C, relative humidity 60 ± 5% and photoperiod 12 hours /day). Each cage was provided with a water bottle for *ad libitum* water. The rats were fed on Gold Mohar rat feed (with 23.5% protein, 5% fat and 4.5% fibre) obtained from Hindustan Lever Ltd. Calcutta. The rats did not receive food for one hour prior to administration of dose.

B.Experimental chemicals

Cypermethrin [(R S)-α cyano-3 phenoxybenzyl (IRS)-cis,trans-3-(2, 2-dichlorovinyl) - 2, 2- dimethyl cyclopropane-carboxylate] (technical grade, purity- 92.7%) was obtained from M/s Rallis India Limited, Agro Chemicals Division, Vashi, Navi Mumbai, *as gratis*. *Sevin* [1- naphtyl-N-methyl carbamate] was obtained from M/s S. S. Crop. Care Limited, 10, Industrial Estate, Govind Pura Bhopal *as gratis*. It is a formulation of carbaryl (Carbaryl 50% W.D.P.). *Piperonyl butoxide* [α- [2-(2-butoxyethoxy) ethoxy] - 4,5 –methylenedioxy-2- propyltoluene] used in this experiment was manufactured by Central Drug House (P) Limited, New Delhi..

C. Experimental Protocol

Study has been based after Singh and Saxena [1] in which test compounds (cypermethrin and sevin) alone and two combinations – cypermethrin: sevin and cypermethrin: piperonyl butoxide [in two ratio (1:1) and (1:5)] for dosing were prepared by dissolving in little warm groundnut oil.

The albino rats were grouped into six groups, as follows (i) cypermethrin (ii) sevin (iii) cypermethrin: sevin (1:1), (iv) cypermethrin: sevin (1:5), (v) cypermethrin: piperonyl butoxide (1:1) (vi) cypermethrin: piperonyl butoxide (1:5). Oral LD_{50} of piperonyl butoxide has been observed to be approx 11480-mg/kg body weight, which is close to earlier reported value of Lehman [2].

Each group was further divided into five sets, each containing of seven rats. Dosages were given orally by gavage tube as per kg. body weight. Three separate experiments were carried out for LD_{50} determination for all dosages. The mortality and survival number of rats were recorded for each dose after 96 hours. The data were analyzed statistically by log-dose/probit regression line method [3]. To investigate the level of synergism of different ratio mixtures, potentiation factor [4] and combination index [5] were calculated.

RESULTS AND DISCUSSION

The mortality percentage noted after 96 hours showed a corresponding increase with the increased dose of cypermethrin and their mixtures with sevin (an esterase inhibitor) and piperonyl butoxide (monooxygenase inhibitor). Regression line and regression equation has been established and median lethal dose has been calculated for all the treatments.

The estimated oral LD_{50} of cypermethrin (technical grade) alone after 96 hours in albino rats is 416.98 mg/Kg body weight (Table-1) which is very close to 439 mg/Kg body weight [6] as oral LD_{50} of cypermethrin for rats. Perger and Szadkowski [7] reported 200-800 mg/kg body weight as range of oral LD_{50} of cypermethrin for rats. In the present investigation, the toxicity of cypermethrin has been evaluated with combination of sevin (50EC carbaryl). Sevin is a carbamate and its LD_{50} after 96 hours is 1778.35 mg/kg body weight (Table-1) which indicates that its mammalian toxicity is very low and hence has been used in combination as a synergist in the present study.

Table 1- Toxicity evaluation of Cypermethrin and Synergiesd Cypermethrin in *Rattus norvegicus*

S. No.	Compound	Regression Equation	LD_{50} (mg/kg.b.wt)	Variance	Fiducial Limits
1.	Cypermethrin	Y=5.30+3.43 (x-2.71)	416.98	0.0056	m_1=426.58, m_2=407.38
2.	Sevin	Y=5.15+5.08(x-3.28)	1778.35	0.0020	m_1=1794.32, m_2=1762.38
3.	Cypermethrin +Sevin (1:1)	Y=5.00+ 11.67(x-2.63)	426.58	0.0005	m_1=427.56, m_2=425.60
4.	Cypermethrin + Sevin (1: 5)	Y=5.10+4.63 (x-2.65)	426.62	0.0028	m_1=432.02, m_2=421.21
5.	Cypermethrin + PB* (1:1)	Y=4.93+ 16.26 (x-2.8t)	645.66	0.0003	m_1=646.55, m_2=644.76
6.	Cypermethrin + PB* (1: 5)	Y=5.35+4.50 (x-2.88)	631.04	0.0034	m_1=640.77, m_2=621.30

** Piperonyl butoxide*

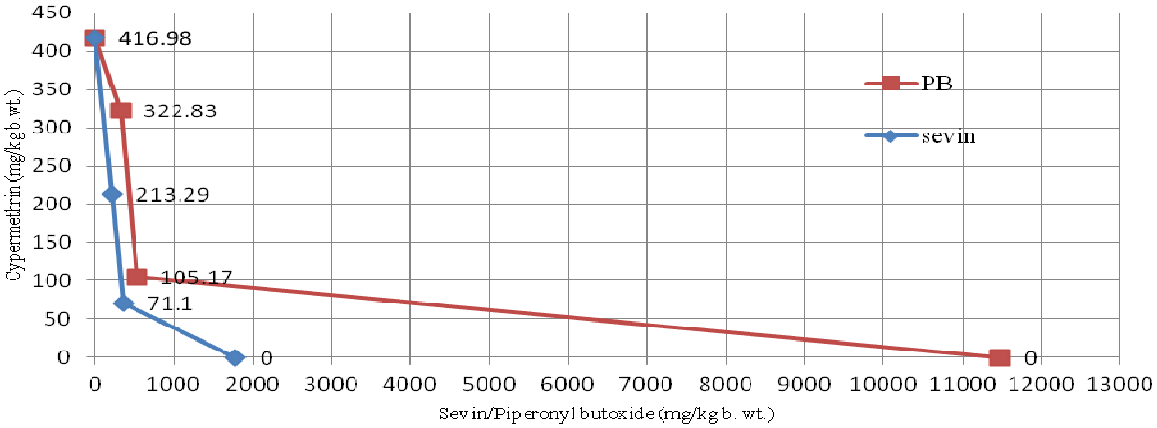

Figure 1- Isobolograms of synergised cypermethrin (with sevin and piperonyl butoxide PB)

Amount of cypermethrin in LD_{50} dose when used in mixture with sevin in 1:1 and 1:5 ratios are 213.29 mg/kg body weight and 71.10 mg/kg body weight respectively (Table–2). Thus, the increase in magnitude of toxicity is 1.95 and 5.86 for 1:1 and 1:5 ratios respectively (Table-2). It reveals that sevin potentiates the toxicity of cypermethrin and also that its potentiation is more when used in higher amount (ratios) with cypermethrin. This finding gains support by Saxena and Singh [4] who reported that sevin behaves as an esterase inhibitor for cybil (25 EC cypermethrin) and enhances the toxicity (acute LD_{50}) by a factor of 3.11 and 4.14 after 1:1 and 1:2 ratio mixture in albino rats respectively. Glickman and Lech [8], Cantalamessa [9] and Yang et al. [10] reported the increased toxicity of pyrethroids after pretreatment with esterase inhibitors.

Table2- Comparative potential and combination index of Sevin and Piperonyl butoxide on Cypermethrin toxicity in *Rattus norvegicus*

S.No	Test Compounds	LD_{50}(mg/kg.b.wt)	Amount of Cypermethrin in LD_{50} dose (mg/kg.b.wt)	Amount of Synergists in LD_{50} dose (mg/kg.b.wt)	Potentiation Factor	Combination Index (CI)
1.	Cypermethrin	416.98	-	-	-	-
2.	Sevin	1778.35	-	-	-	-
3.	Cypermethrin +Sevin (1:1)	426.58	213.29	213.29	1.95	0.69
4.	Cypermethrin + Sevin (1: 5)	426.62	71.10	355.50	5.86	0.40
5.	Cypermethrin + PB* (1:1)	645.66	322.83	322.83	1.29	0.82
6.	Cypermethrin + PB* (1: 5)	631.04	105.17	525.86	3.96	0.31
7.	PB*	11480.00**	-	-	-	-

** Piperonyl butoxide* *** Report ed by Lehmann (1951-52)*

Hydrolysis by one or more hepatic microsomal esterases is a critical step in the detoxification of synthetic pyrethroids by mammals [11] [12]. Sevin and cypermethrin both possess ester group and thus sevin behaves as a competitor for cypermethrin and may probably act as inhibitor of esterase enzymes of liver microsomes. Hence, inhibition of the esterase enzymes results in the retention of pyrethroids in the mammalian body [8], which causes the synergism of pyrethroid insecticides [11] [13].

Amount of cypermethrin in LD_{50} dose, when used in mixture with piperonyl butoxide in same 1:1 and 1:5 ratios are 322.83 mg/kg body weight and 105.17 mg/kg body weight respectively (Table-2). Potentiation factor for 1:1 and 1:5 ratio is 1.29 and 3.96 respectively (Table-2). Its efficiency also increases when used in higher ratios.

Piperonyl butoxide is a methylenedioxyphenyl synergist which acts as substrate for the microsomal enzyme – $NADPH_2$ system which metabolizes many drugs and pesticides and thus by serving as an alternative substrate (and, therefore, as competitive inhibitors) for this system, these compounds prolong the persistence of the drugs or pesticides [14] [15]. Spolzer et al. [16] reported that piperonyl butoxide inhibits the MFO enzymes and hence responsible for increased toxicity of pyrethroids in mice.

In the present investigation, the comparison is made between the synergistic potential of sevin and piperonyl butoxide on cypermethrin. Sevin enhances the toxicity of cypermethrin by a magnitude of 5.86 in 1:5 ratio mixture (Table-2) and it is the highest magnitude among the different combination and ratios in present investigation. It reveals that esterase inhibitors are better synergists for pyrethroids. Also the isobole (fig-1) for sevin depicts more curvature than piperonyl butoxide.

The present finding gains support by Cantalamessa [9] who reported that tri-o-totyl phosphate (TOTP, an esterase inhibitor) significantly increases the toxicity of cypermethrin while piperonyl butoxide (an oxidase inhibitor) does not. Glickman et al. [17] observed that inhibition of esterase activity with TOTP potentiated trans- permethrin lethality by 1.5 fold in mice while piperonyl butoxide produced no potentiation in mice.

The present finding gains support by the fact that ester cleavage is an important pathway in metabolism [18] and esterases of liver are important in metabolizing the pyrethroids [19]. Further, detoxification of cypermethrin in rat and mouse liver is mainly done by esterases and oxidases by factors of 17 and 4 respectively [20]. This difference also reveals that esterase inhibition may increase the accumulation and retention of pyrethroids in the body compared to oxidase inhibition.

CONCLUSION

Thus, the findings of present study establish that sevin is a better synergist compared to conventional- the piperonyl butoxide and may give clue for establishing new formulations of pesticides for mammalian pest control operations in both field and house hold conditions.

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