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# TOXICITY OF POLYTRIN-C ON THE PSYCHOPHARMACOLOGICAL OBSERVATIONS IN WISTAR RAT.

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## ABSTRACT

The work has been designed to asses the neuro toxicity of a combination pesticide through behavioral parameters [Psychopharmacological observation] and instrumental analysis such as spontaneous motor activity for 28 days. The mixed formulation selected for the study, polytrin-C was used for sub acute and acute toxicity studies on Wister rats weighing around 90-120 gms of either sex.. Acute studies were carried out to fix the LD 50 dosage of the selected pesticide. Based on the LD 50 values the sub acute doses were fixed [1/40$^{th}$, 1/20$^{th}$, 1/10$^{th}$ of the LD 50 Dosage]. It is evident from the results that 1/20 and 1/10 of LD 50 dosed animals of either sex showed marked differences in the behavioral patterns when compare to 1/40 of LD 50 dose animals. Due to the synergetic effect neurotoxicity symptoms of combination pesticide results show a different toxicological profile when compare with the toxicity of constituent pesticide.

## KEY WORDS

Neurotoxicity, Psychopharmacology, polytrin-C, Pesticides

## INTRODUCTION

Man lives in the world of chemicals. Chemicals are produced in widespread commercial usage particularly as pesticides, medicines and industrial chemicals. Although these chemical are generally beneficial they have their negative aspects too. Many of these chemicals may pollute the environment and some eventually find their way to humans through contaminated food, water and air. It is now established that organophorous and carbamates are toxic because of their interference in the normal synaptic transmission of nerve impulse. The Ops after entering the body of an organism reaches the cholinergic sites of the nervous system and inhibit the activity of the AChE by binding at its active sites. AchE inhibition, thus leads to the accumulation of Ach at nerve endings which in turn cause the disruption of the nervous activity resulting in excitation, paralysis and finally the death of the organism.

Nowadays organophosphate esters are widely used as pesticide and have substantially replaced many of more persistent chlorinated

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hydrocarbon insecticide[1]. Recently natural pyrethroids have replaced organophosphates because of their low mammalian toxicity and lack of persistence in the environment[2,3]. Neuro toxicity is one of the major effects induced by the organo-phosphates[4] and pyrethroids[5,6], since lot of data available regarding neuro-toxicity of constituent pesticide [organophosphates or pyrethroids] ,but less information available regarding the toxicity of combination pesticide. It has been stated that organophorous pesticides like chlorpyrifos and profenos are compatible with pyrethroids like cypermethrin[2]. Data on particular combination of insecticides are scanty and general principles are necessary for predictive purposes. The possible types of toxicological interaction between two insecticides are (1) additive, (2) synergistic and (3) antagonistic or they may act independently of one another. Often, compounds of same toxic effects act additively which in case of insecticides with different toxic effects; the combined effect is less than additive. It has been shown that some pairs of Ops exhibit greater than additive toxic effects when administered together. Data on neurotoxicity of combination pesticides are not available and it is only logical to believe that the neurotoxicity of combination pesticides may differ from that of individual pesticides. hence the present work has been designed for 28 days sub acute studies to asses the neuro toxicity of a combination pesticide through psychopharmacological observation[7].

## MATERIALS AND METHODS

Wister rats [either sex]weighing around 90-120 gms selected for the present study and they were acclimatized and fed ad libitum with standard rat pellets obtained from Lipton India Ltd Bangalore. The new combination pesticide polytrin –C [organo phosphate profenofos 40% + pyretheroids cypermethrin 4% ] obtained from Aventis Bombay.

LD 50 studies [**Acute Toxicity]**

Acute Toxicity studies were carried out using forty rats was randomly divided into four groups, each group consisting of 10 animals [five females and five males]. The allocation of the groups and fixation of doses of the combination pesticides[8] in the different groups were carried as given below.

**Compound Name:** Polytrin C

**Details of Groups and Dosage**

Group I: Control [only distilled water]
Group II: 75-mgs/kg body-weight
Group III: 150 mgs/kg-body weight
Group IV: 300-mgs/kg-body weight

The control group [G1] received distilled water by oral intubations [single administration] at a dose of 10ml/kg body weight. Groups II, III and IV received different doses by oral gavages, [single administration] of the pesticide dissolved in distilled water. The animals were observed 14 days after dosing and basing on the mortality, the LD50 was calculated using Finney's probit analysis[9].

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***Sub acute Studies***

Psychopharmacological studies reveal behavioral changes observed depending biochemical and physiological changes at various morphological sites within the body due to the pesticide toxicity. Similar to acute studies, sub acute psychopharmacological sub acute studies [28 days] were carried out by randomly dividing the animals into four groups and each group consisting of 5 males and 5 females. Group I the untreated control receiving only distilled water at a dose of 10 ml/kg body weight. The doses of the combination pesticide in the sub acute studies were based on the LD 50 values obtained from acute studies. Group II received 1/40$^{th}$, Group III-1/20$^{th}$, Group IV-1/10$^{th}$ of the LD 50 Dosage.

***The allocation of group and doses were fixed as follows***

Group I Control [distilled water]
Group II 8.5 mg/kg body weight
Group III 17 mg/kg body weight
Group IV 34 mg/kg body weight

The above mentioned dosages of the compounds were dissolved in distilled water and administrated by oral gavages once daily for 28 days. During the period of study in-life parameters like body weight and feed consumption were recorded. The psychopharmacological parameters to assess the Neuro toxicity of the combination pesticide evaluated during the live phase of the sub acute studies[10]. Hence psychopharmacological symptoms such as restlessness, convulsions and skeletal movements like ataxia, catalepsy, gripping strength and righting reflex were studied in the compound treated with reference to the respective untreated control[11]. The above observations were made on the 7$^{th}$, 14$^{th}$, 21$^{st}$ & 18$^{th}$ days using the Actophotometer [Inco] and Digital Rotorod apparatus[12].

## RESULTS

In the 28-day sub acute study with polytrin-C [profenos 40% + cypermethrin 4% ] in Wister rats, a decrease in feed consumption and a related decrease in body weight[13,14] compared to control was observed from the third week of treatment. Only mild behavioral changes were observed and severe signs of neurotoxicity like tremors and convulsions were not observed in the polytrin-C treated groups [TABLE 1 & 2] indicating polytrin-C to be less neurotoxic combination pesticide because in the study polytrin-C treated rats did not show any significant change in motor activity and muscle relaxant property exhibited in female animals of highest dose (34 mg/kg b.w.) Psychopharmalogical parameters reflect the magnitude of neutoxicity and in the present study, an incidence of the behavioral changes was observed from the third week in rats treated with Polytrin-C did not induce any abnormal behavioral changes in male rats till the third week of treatment and the fourth week in female rats Psychopharmacological investigation revealed only minor behavioral abnormalities in rats treated with

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poytrin-C at the doses of 17 and 34 mg/kg b.w., from the third week of the study. A decrease in spontaneous motor activity [table 3] and muscle relaxant property [table 4] was observed mainly in the highest dose group (34 mg/kg b.w.) while only minor inconsistent (not time related) changes were observed in other groups.

**Table 1**
***Behavioral parameters of rats treated with different concentration of Polytrin-C.***

| Groups | Weeks | Behavioral Parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alertness | | Passive/Active | | Grooming | | Aggressiveness | | Restlessness | | Tremors | |
| | | M | F | M | F | M | F | M | F | M | F | M | F |
| I | 01 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 02 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 03 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 04 | + | + | A | A | + | + | N | N | N | N | - | - |
| II | 01 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 02 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 03 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 04 | + | + | A | A | + | + | N | N | N | N | - | - |
| III | 01 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 02 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 03 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 04 | + | + | P | P | + | + | - | - | N | N | - | - |
| IV | 01 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 02 | + | + | A | A | + | + | N | N | N | N | - | - |
| | 03 | - | - | A | P | + | + | N | N | N | N | - | - |
| | 04 | + | - | P | P | + | - | N | N | N | - | - | - |

M: Male A: Active +: Presence of activity N: Normal F: Female
P: Passive -: Absence of activity

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**Table 2**

***Behavioral parameters of rats treated with different concentration of Polytrin-C.***

| Groups | Weeks | Behavioral Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Convulsions | | Ataxia | | Catalepsy | | Gripping Strength | | Righting Reflex | |
| | | M | F | M | F | M | F | M | F | M | F |
| I | 01 | - | - | - | - | - | - | + | + | + | + |
| | 02 | - | - | - | - | - | | + | + | + | + |
| | 03 | - | - | - | - | | - | + | + | + | + |
| | 04 | - | - | - | - | - | - | + | + | + | + |
| II | 01 | - | - | - | - | - | - | + | + | + | + |
| | 02 | - | - | - | - | - | - | + | + | + | + |
| | 03 | - | - | - | - | | - | + | + | + | + |
| | 04 | - | - | - | - | - | - | + | + | + | + |
| III | 01 | - | - | - | - | - | | + | + | + | + |
| | 02 | - | - | - | | - | - | + | + | + | + |
| | 03 | - | - | - | - | - | - | + | + | + | + |
| | 04 | - | - | - | - | - | - | + | + | + | + |
| IV | 01 | - | - | - | | | - | + | + | + | + |
| | 02 | - | - | - | - | - | - | + | + | + | + |
| | 03 | - | - | - | - | - | - | + | + | + | + |
| | 04 | - | - | + | - | - | + | + | + | + | - |

M: Male F: Female +: Presence of activity -: Absence of activity

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**Table 3**
***Motor activity (counts/15min) in Wistar rats treated with different concentration of polytrin-D.***

| Group | Sex | Week | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| I Control | Male | 199.2[a] ± 3.78 | 208.0[a] ± 2.99 | 171.1[a] ± 7.04 | 186.0[a] ± 6.82 |
| | Female | 208.1[a] ± 4.08 | 199.0[a] ± 4.88 | 163.4[a] ± 4.68 | 211.6[a] ± 4.07 |
| II 8.5 Mg/kg b.w. | Male | 202.4[a] ± 5.03 | 178.6[b] ± 5.17 | 185.2[a] ± 3.96 | 190.0[a] ± 4.06 |
| | Female | 188.2[b] ± 3.38 | 173.2[b] ± 3.12 | 196.4[b] ± 6.06 | 184.0[b] ± 7.12 |
| III 17.0 Mg/kg b.w. | Male | 198.1[a] ± 3.45 | 187.0[b] ± 5.53 | 180.5[a] ± 1.92 | 173.2[c] ± 3.01 |
| | Female | 164.6[c] ± 2.88 | 171.0[b] ± 4.46 | 166.4[a] ± 2.28 | 153.2[c] ± 3.81 |
| IV 34.0 Mg/kg b.w. | Male | 178.6[b] ± 3.11 | 163.0[c] ± 3.32 | 146.2[b] ± 4.78 | 132.0[d] ± 2.94 |
| | Female | 168.2[c] ± 2.61 | 152.0[c] ± 3.15 | 134.1[c] ± 2.17 | 123.5[d] ± 3.08 |

Values are presented as mean ± Standard Error
Values (sex-wise) carrying similar superscripts are not statistically significant ($p>0.05$).

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**Table 4.**
***Gripping strength (counts) in Wistar rats treated with different concentration of Polytrin-D.***

| Group | Sex | Week | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| I Control | Male | 34.2[a] ± 1.81 | 31.0[a] ± 0.94 | 29.4[a] ± 1.87 | 32.0[a] ± 1.02 |
| | Female | 29.0[a] ± 1.13 | 32.1[a] ± 1.26 | 27.0[a] ± 1.91 | 30.3[a] ± 1.17 |
| II 8.5 Mg/kg b.w. | Male | 28.0[b] ± 1.72 | 30.0[a] ± 1.11 | 32.0[a] ± 1.32 | 31.2[a] ± 2.04 |
| | Female | 29.4[a] ± 0.81 | 30.5[a] ± 1.13 | 26.0[a] ± 0.94 | 28.0[a] ± 1.09 |
| III 17.0 Mg/kg b.w. | Male | 33.0[a] ± 1.17 | 27.0[b] ± 1.22 | 28.2[a] ± 0.84 | 25.0[b] ± 1.04 |
| | Female | 31.0[a] ± 0.91 | 29.0[b] ± 0.91 | 26.8[a] ± 1.11 | 24.0[b] ± 1.81 |
| IV 34.0 Mg/kg b.w. | Male | 28.5[b] ± 1.01 | 27.0[b] ± 1.24 | 24.8[b] ± 0.87 | 23.0[b] ± 1.07 |
| | Female | 26.2[b] ± 1.14 | 23.0[c] ± 1.06 | 19.0[b] ± 1.18 | 17.0[b] ± 2.01 |

Values are presented as mean ± Standard Error
Values (sex-wise) carrying similar superscripts are not statistically significant ($p>0.05$).

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## DISCUSSION

In the present study there was a decrease in the body weight gain in rats treated with polytrin-C at the dose of 17 and 34 mg/kg b.w., which was similar to the observation in two different 2-year studies carried out with cypermethrin in rats[15,16]. But opposed to the findings (mentioned earlier) of these 2-year studies with cypermethrin, the present study with the combination pesticide polytrin –C did show clinical signs of intoxication.

Clinical signs of neurotoxicity reported by earlier studies include impaired ability to walk abnormal gait, splayed hind limbs, lethargy, piloerection, ataxia, paralysis, hypersensitivity, gross disorientation and convulsions[17] mild signs of neurotoxicity like lethargy, hypersensitivity and lack of grooming were observed in the high dose group (34 mg polytrin-C/kg b.w.) in the present study but severe signs of neurotoxicity like ataxia, tremors, convulsions and paralysis were not observed in the polytrin-C treated groups.

From the 28- day study polytrin-C, it was clear that the combination pesticide exhibited comparatively lesser toxicity than the constitute pesticides. This phenomenon could be due to the lower availability of profenofos or cypermethrin when administered as a combination or could be due to the relatively lower toxicity of the combination pesticide polytrin-C compared to the constitute insecticides profenofos and cypermethrin.

## SUMMARY

The combination pesticides are gaining popularity in pest control programmes as they exhibit a broad spectrum of activity coupled with better efficiency and economy. But for the registration of these pesticides, acute toxicity data is sufficient and therefore the long-term toxicity of these compounds remains unexplored. Alternatives are required for long-term studies as they are difficult to carryout, time consuming and expensive. From this study it can be understood that a well designed sub-acute study clubbed with neurotoxicological assessment can provide a major part of the information observed from the long term study[18]. The study also revealed that the combination pesticide behave differently and exhibit a different toxicological profile when compared with the toxicity of the individual pesticide in the combination. Therefore it is very necessary that all the toxicological studies have to be carried out (as for a new pesticide) for a combination pesticide before it can be brought to the field. Similar studies can also help to identify combination pesticides that have lesser mammalian toxicity compared to the individual pesticides used in the combination while retaining or obtaining better efficiency.

The present study was carried out to evaluate the short-term sub acute (28 days) toxicity and neurotoxicity of Polytrin-C in Wistar rats. The acute toxicity of the combination pesticide was evaluated to arrive at the doses for the sub acute study. Sub acute psychopharmalogical studies (28 days) such as spontaneous motor activity and muscle relaxant property were also observed to estimate the neurotoxicity of these pesticides.

Rats administered polytrin-C [profenofos 40% + cypermethrin 4%] for 28 days at the doses of 8.5, 17 and 34 mg/kg b.w., showed a decrease in feed consumption and a related decrease in body weight compared to control, from the third week of treatment. Psychopharmalogical investigation revealed only minor behavioral abnormalities in rats treated with polytrin-C at the doses of 17 and 34 mg/kg b.w., from the third week of the study. A

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decrease in spontaneous motor activity and muscle relaxant property was observed in the highest dose group of 34 mg/kg b.w., while only minor inconsistent (not time related) changes were observed in other group.

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