Combination of Organophosphorus Pesticides Applied in Agricultural Fields
Introduction

The application of Organophosphorus pesticides (OPs) for controlling insects, pests has recently shown a tendency to a combination of two or more pesticides for diverse reasons such as for increasing their effectiveness, for controlling more than one pest at a time, and for increasing the storage life of OP [1]. There are different varieties of pests found in agricultural fields in India. Since application of one pesticide is not sufficient to kill all the pests. So application of a mixture of pesticides can play a vital role in controlling a wide variety of pests. Some of the mixtures [2] which have been applied in Indian agricultural fields are Methyl parathion (Mpara) + Monocrotophos (Mcp), Methyl parathion (Mpara) + Malathion (Mlth), Chlorpyrifos (Clpf) + Dimethoate (Dmt) and Malathion (Mlth) + Dichlorvos (Dclv). We have treated rats with these four mixtures for 30 days at 1/20 LD₅₀ dose of each pesticide. Significantly it has been found that rats treated with Mpara + Mcp mixture died within 3 days of application. So this mixture proved to be fatal for rats. But rats treated with other three mixtures survived. After 30 days of treatment, rats were sacrificed and acetylcholinesterase (AChE) activities were measured from four different parts of brain. From the results it can be suggested that which mixture has minimal effect on mammalian brain.

Sollmann [3] used the terms synergism and antagonism in the limited sense of strictly algebraic summation or subtraction of effects. For cases of effects greater than would be expected, he used the term potentiation, or supplemental synergism.

Examples of some factors which affect the toxicity of chemicals are well known: temperature, nutritional state, disease state, age, emotional state, sex, etc., all of which must be taken into consideration in evaluating the toxicity of a chemical, whether it is a drug or a pesticide. All of these also are examples of synergism or antagonism.

The significance of the interaction of pesticides with other pesticides can be evaluated on relation to public health considerations on the use of pesticides. The first type, discovered by Food and Drug Administration, involved
potentiation between two Organophosphates, EPN and Malathion [3]. Numerous other examples of more than additive toxicity have been reported by Casida et al. [4].

The initial biological reaction involves the conversion of thiophosphate (P=S) to (P=O). This reaction has been shown to be catalyzed by liver microsomal enzymes. Once the compound has been converted to the oxygen analogue, several reactions can occur. The most important of these reactions are:

(1) inhibition of cholinesterase enzymes in blood and other organs;
(2) inhibition of aliesterase enzymes in blood and various organs;
(3) hydrolysis to an inactive compound.

The first two of these involve a biochemical lesion, whereas the third is a detoxification reaction. The degree to which a compound inhibits cholinesterase or aliphatic esterase is strongly dependent on the specific structure and spatial arrangement of the substituent radicals.

In Malathion, the dithiophosphate is converted to the oxygen analogue and because of these aliphatic ester groups in the side chain, is rapidly detoxified by aliesterase before it can inhibit cholinesterase. Rather being an inhibitor, Malathion can be looked upon as a substrate for this enzyme, since the rate is so rapid.

When the biological consequence of the simultaneous administration of Malathion and another compound which preferentially inhibits aliesterase, such as EPN or TOCP, it is apparent that the oxygen analogue of Malathion would now accumulate and exert its toxic effect by inhibiting cholinesterase. This is potentiation, or supplemental synergism. It was observed that the LD$_{50}$ of an equitoxic mixture is 1/12 of the LD$_{50}$ of the individual compounds.

EPN is not only the compound that would inhibit and potentiate the toxicity of Malathion: others are TOCP, Delnav®, Dipterex and most triaryl phosphates and phosphates.

The significance of this type of potentiation is obvious. From an industrial point
of view, the use of potentiating compounds could conceivably give rise to unforeseen accidents. The presence of residues of potentiating compounds on food items eaten at the same meal or closely related in time can give rise to greater toxicity than would be anticipated if cholinesterase inhibition is employed as the most effective index of toxicity and the basis of tolerance. All these complications have been evaluated by profession of toxicology and considered in establishing our current food tolerances and labeling restrictions [3].

A study of the toxicity of Delnav® in combination with other organic phosphates was undertaken to determine whether this compound was capable of producing potentiation of the toxicity of other insecticides having the same pharmacologic actions. The technique employed to detect potentiation was administration orally of a series of dosages of an equitoxic mixture of each component of the mixture simultaneously to male Sprague-Dawley (200-250g). However, to determine the ratio for preparing equitoxic mixtures it was necessary to determine the oral LD_{50} for each compound in this strain of rats.

It is recognized that strictly additive toxicity in a mathematical sense rarely occurs. In reality, additive toxicity for pharmacologically related compounds can be expected to result in ratios of expected to observed values that are less than unity in most cases. Among the factors responsible for this are differences in rate of absorption and metabolism. For strictly additive toxicity to occur, the maximal action of each component must coincide with respect to time. The results of this study indicated that Delnav® has some degree of additive toxicity when administered simultaneously with most, if not all, of the other compounds. In no cases did the toxicity of the combinations deviate sufficiently from the expected additive toxicity to indicate significant potentiation. However, when Delnav® is administered 4 hours prior to Malathion, significant potentiation is produced [4].
Materials and Methods

Materials:

1. Animals: Healthy male Albino Charles Foster rats (~100g body weight) were treated with *ad libitum* food and water in 12 hours day and night cycle for 30 days along with pesticide. In each cage (0.91m x 0.91m x 0.61m), there were six rats, which were kept at 27°C in the laboratory in good condition having adequate arrangement of ventilation. The rats were kept in all clean day and night light controlled, temperature controlled animal house following all rules and regulations provided by our animal ethics committee. The food served to the rats included soaked grams, wheat and water. The trays of each cage were daily cleaned with disinfectants. The rats were divided into 5 groups for four different mixtures at 1/20 lethal dose of each of the pesticide in the mixture. Group 1 was considered as the control and fed with food and water. The rats of group 2, 3, 4 and 5 were fed 1/20 LD<sub>50</sub> dose of pesticide (dissolved in palm oil) orally for the mixtures - Mpara + Mcp, Mpara + Mlth, Clpf + Dmt and Mlth + Dclv respectively for 30 days.

2. Pesticides: Organophosphorus pesticides like Methyl parathion, Malathion, Dimethoate, Chlorpyrifos, Monocrotophos and Dichlorvos were used

Objective to select the specific pesticides under experiment are given as follows:

Methyl parathion: controls chewing and sucking insects in a wide range of crops, including cereals, fruit (citrus), vines, vegetables, ornamentals, cotton and field crops.

Malathion: controls Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera in a wide range of crops, *e.g.*, cotton, pome, soft and stone fruit, potatoes, rice, vegetables- used extensively to control major arthropod disease vectors (Culicidae) in public health programmes, ectoparasites of cattle, poultry, dogs and cats, human head and body lice, household insects and to protect stored grains.

Dimethoate: controls a wide range of insects, *e.g.*, aphids, thrips, planthoppers and white flies on ornamental plants, alfalfa, apples, corn, cotton, grape fruits, grapes, lemons, oranges, pears, pecans, sunflower, sorghum, soyabean, tangerines, tobacco,
tomatoes, watermelons, wheat and other vegetables used as a residual wall spray in farm buildings for house flies—administered to livestock for control of botflies.

Chlorpyrifos: controls cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants and lice—used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops as well as on lawns and ornamental plants and directly on sheep and turkeys, for horse site treatment, dog kernels, domestic dwellings, farm buildings, storage bins and commercial establishments.

Monocrotophos: controls insects on cotton, peanuts, sugarcane, tobacco, ornamental conifers, ornamental flowering plants, ornamental woody shrubs and ornamental deciduous trees.

Dichlorvos: controls household and public health pests, stored product insects, hornflies, houseflies, face flies, stable flies, gnats and mosquitoes on lactating dairy animals and beef cattle, mushroom flies, aphids, spider mites, caterpillars, thrips, white flies in glass house crops and outdoor fruit and vegetables.

3. Common Laboratory Reagents: Common laboratory reagents used in the present experiments were of analytical reagent grade purchased either from E. Merck, Sigma or SRL.

4. Chemicals like acetylthiocholine iodide, 5,5'-dithiobisnitrobenzoic acid, Polin were obtained from Sigma Chemical Company.

Methods:

(A) Collection of tissue

After 30 days of treatment, rats were sacrificed in a death trap by chloroform treatment for 5 minutes. The death trap was previously kept saturated with chloroform vapour before each treatment. The head of the anaesthetized rats were removed with the help of an autoclaved pair of scissors and the brain was taken out. The four different parts of their brains namely hypothalamus, striatum, cerebellum and cerebrum were cut apart and then collected at 0°C for homogenisation. Homogenisation was carried out in phosphate buffer (0.1 M Na₂HPO₄ and KH₂PO₄, pH 8.0). Brain homogenates of all the four different parts of rat brain were centrifuged at 8000xg. From the supernatants specific
spectrophotometric AChE assay were performed and effect of mixture of pesticide on four parts of rat brain were observed.

(B) Assay of brain Acetylcholine esterase enzyme

The supernatants of hypothalamus (H), striatum (S), cerebellum (CR) and cerebrum (C) were used to assay AChE activity after incubating them with phosphate buffer (0.1 M Na$_2$HPO$_4$ and KH$_2$PO$_4$; pH 8.0), distilled water and substrate — S-acetylthiocholine iodide (Sigma) for 1 hour. After 1 hour the reaction was stopped with 15% PCA (perchloric acid). Then 3 minutes centrifugation was done. AChE activity was measured from the colour produced in the supernatant with DTNB (5,5' bisdithionitrobenzoic acid, Sigma) dissolved in phosphate buffer (0.1 M Na$_2$HPO$_4$ and KH$_2$PO$_4$; pH 7.5) at 420nm [5]. AChE activity has been expressed in Δ OD/mg pr/hr.

(C) Estimation of protein

Protein was estimated by following the method of Lowry et al. [6].
Results and Discussion

The AChE activity of the three different combinations has been represented in Table 1. The percentage inhibition of AChE activity has been found to be lower in all the four regions in Milth + Dclv combination compared to the other two combinations.

From the bar diagrams it is evident that the AChE inhibition in case of combination of pesticides are not following additive or subtractive mode. Even they could not be utilized in the predicting formula because the biological effect is different here due to combination of pesticides. For finding out this effect will need another thesis. Statistical calculation was not satisfactory to establish a relationship due to lack of combination of pesticides used in fields. We have not used any data from literature. We have obtained the AChE activity from our own laboratory work.
SUMMARY OF CHAPTER 3

1. Combination of pesticides not showing algebraic additive or subtractive property in cases of inhibition of AChE.
Comparison of AChE activity (ΔOD/mg pr/hr) in various regions of rat brain after 30 days of treatment:

- Cerebrum
- Cerebellum
- Hypothalamus
- Striatum

The graphs illustrate the AChE activity levels under different conditions: Control, Clpf, Dmt, and Clpf + Dmt. The x-axis represents the treatment groups, and the y-axis represents the AChE activity levels.
Comparison of AChE activity (\(\Delta OD/\text{mg pr/hr}\)) in Cerebrum of rat brain after 30 days of treatment

Comparison of AChE activity (\(\Delta OD/\text{mg pr/hr}\)) in Hypothalamus of rat brain after 30 days of treatment

Comparison of AChE activity (\(\Delta OD/\text{mg pr/hr}\)) in Cerebellum of rat brain after 30 days of treatment

Comparison of AChE activity (\(\Delta OD/\text{mg pr/hr}\)) in Cerebrum of rat brain after 30 days of treatment
References


CHAPTER 3

Table 1: AChE activity (Δ OD/mg pr/hr) in different parts of rat brain after 30 days of treatment with three types of pesticide mixtures at 1/20 LD₅₀

<table>
<thead>
<tr>
<th></th>
<th>Hypothalamus</th>
<th>Striatum</th>
<th>Cerebellum</th>
<th>Cerebrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.189±1.173</td>
<td>17.762±1.831</td>
<td>2.706±0.137</td>
<td>1.847±0.073</td>
</tr>
<tr>
<td>Mlth+Mpara</td>
<td>2.013±0.218†</td>
<td>4.997±0.175*</td>
<td>1.373±0.029*</td>
<td>0.826±0.024*</td>
</tr>
<tr>
<td>Clpf+Dmt</td>
<td>2.508±0.202*</td>
<td>4.429±0.645*</td>
<td>1.270±0.060*</td>
<td>1.227±0.084*</td>
</tr>
<tr>
<td>Mlth+Dclv</td>
<td>3.567±0.167*</td>
<td>10.215±1.188*</td>
<td>2.145±0.115*</td>
<td>1.789±0.166</td>
</tr>
</tbody>
</table>

† p<0.05; * p<0.02; ‡ p<0.01; †† p<0.001

Results are expressed as Mean ± SEM of 6 rats in each cage.