CHAPTER II
Mortality and Behaviour
INTRODUCTION

Studies on the mechanism of action of insecticides and also on the causes of their selective toxicities to different animals have started with the appearance of synthetic organic insecticides. In assessing the biological activity of an insecticide, it is usual to carry out the appropriate tests under carefully controlled conditions so that the only experimental variable is the amount of chemical employed. Since biological activity follows a normal dose-response relationship, a genetically homogenous population of test organisms standardised with respect to age and sex will respond in a relatively fixed and predictable manner to a chemical administered by a particular route under specified physical conditions like temperature, etc. Even small variations in any of these parameters can lead to marked changes in biological response through effects on penetration, distribution, metabolism of target site interaction of the chemical concerned. The discrepancies sometimes found in the results of similar tests conducted in different laboratories are undoubtedly often the result of the dose-response relationship.

Another and potentially more important way in which the biological activity of a chemical may be modified is through the prior or simultaneous exposure of test organisms to another chemical agent. The effect resulting from various
combinations of chemicals are often dramatic so that sometimes they are unpredictable and are commonly referred to as interactions. If the net result of the interaction is enhancement of biological activity, the effect is said to be synergistic, whereas if the activity is decreased, the effect is described as antagonistic. Purely additive toxic effects resulting from combinations of chemicals having the same or opposite pharmacological actions are not usually considered as interactions.

It is probable that a variety of synergistic and antagonistic interactions have long been known and that the beneficial aspects of the latter were first recognised in the cause of early attempts to develop antidotes for various toxic agents present in the environment. In view of the enormous number and diversity of such chemicals, the theoretical potential for some type of unforeseen interaction appears to be considerable.

Since the introduction of the synthetic organic pesticides, there has been a rapid increase in the use of chemicals of high biological activity for pest control. Some of the chemicals used for these purposes have acute toxicities for a wide range of animals, including man. Others, while not being so toxic are highly resistant to degradation in the environment and may accumulate in man and other animals (Sahai and Chauhan, 1977).
Excessive use of pesticides affects air, water, land environment, human health and wild life. Pesticide application results in their entry into different sources of water through the action of wind or rain (Amminikutty and Rege, 1977).

Several studies in the last few years (Burdick et al., 1964; Wurster, 1968; Reinert, 1969 and Peakall, 1970) have shown a variety of environmental problems attributable to the wide spread use of DDT. There are some reports on effects of toxicity of Sevin on fish (Koundinya and Ramamurthi, 1979).

The amphibians which are adapted to live at shallow margins of streams and ponds or which live on exposed surface of the ground seem affected most by current means of applying chemicals (Rudd, 1964).

Amphibia are often exposed to pesticides applied directly to their habitat or as a result of run off from the treated agricultural lands. Although amphibians are usually not affected by concentrations of pesticides that kill fish (Cope, 1965) or small invertebrates (Sanders and Cope, 1966), they are susceptible to some of the commonly used organic pesticides.

Observations on various amphibians species after field applications of insecticides provides only preliminary data on the response of these organisms after exposure to a particular toxicant. Herald (1949) observed the effects of
DDT on behaviour patterns of adult frogs and toads after a series of field sprayings. Ponds treated at 0.6 lbs/acre of DDT were sufficient to cause toxicity symptoms and subsequent death of many frogs 24 hours after spray treatment. The studies of Mulla (1963, 1966) and Mulla et al. (1963) reported on the effects of field application of several common insecticides on the bullfrog, Rana catesbeiana and the toad Bufo boreas and Scaphiopus hammondii.

Boyd et al. (1963) conducted bioassays with adult cricket frog, Acris crepitans and A. gryllus. They found that the cricket frogs living near areas heavily treated with DDT for several years tended to be less susceptible than individuals having had no prior contact with insecticide.

Ferguson and Gilbert (1968) determined the relative toxicities of five insecticides to adult cricket frogs, A. crepitans, A. gryllus and Bufo fowleri, and found that toads were generally more tolerant of the insecticides than cricket frogs. However, no such data are available on these lines in the Indian bullfrog, Rana tigrina. Hence a study of acute exposures to DDT and Sevin on the frog, Rana tigrina was undertaken.

MATERIALS AND METHODS

The animals were collected from areas near Nanded and brought to the laboratory. Healthy frogs weighing between 80-85 gms were selected and were divided into different
groups, each containing ten animals. During experimentation, the methods recommended by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) were followed. The experimental groups were acclimatized to laboratory conditions and the laboratory temperature was 27°C.

The insecticide solutions were prepared in acetone and then they were diluted with water to get the test solution of required concentration. The test solutions of DDT ranged from 0.1% to 0.9% while that of Sevin were from 0.001% to 0.011%.

Static bioassays, without aeration and with the toxicant added to the test medium at the beginning of the test were used to determine the toxicity of two insecticides. The bioassays were conducted in 20 litres test chambers, containing 8 litres of dechlorinated water or insecticide solution.

After appropriate toxicity range of the test chemicals were determined by preliminary testing, nine concentrations of DDT ranging from 0.1% to 0.9% were prepared. Similarly eleven concentrations of Sevin ranging from 0.001% to 0.011% were prepared. The control animals were exposed to the highest concentration of solvent to which the frogs in other test solutions were exposed. Observations on survival were made at 24, 48, 72 and 96 hours. $L_{C50}$ (concentration required for 50% mortality) was calculated according to Litchfield and Wilcoxon (1949) graphical method.
RESULTS

(a) **ACUTE TOXICITY WITH DDT**: Table 1 gives the acute toxicity data with various concentrations of DDT to which the frogs were exposed.

**Symptoms**

Sub-groups 2 and 3: The symptoms noted in these sub-groups were un-coordinated movements. There was no mortality noted in sub-group 2, but 10% mortality was observed in sub-group 3 within 96 hours.

Sub-group 4: The symptoms similar to the symptoms observed in sub-groups 2 and 3 were noted. Here the un-coordinated movements were followed by hyperactivity, and 20% mortality was found within 96 hours.

Sub-groups 5 and 6: Hyperexcitability, extension of legs, general tremulousness, repeated falling on back and righting efforts, followed by loss of locomotion were the symptoms observed in the animals of these sub-groups. There was 50% and 70% mortality within 96 hours in the sub-groups 5 and 6 respectively.

Sub-groups 7 and 8: The symptoms similar to the symptoms observed in sub-groups 5 and 6 were noted and the mortality was 80% and 90% respectively within 96 hours.

Sub-groups 9 and 10: The animals exhibited the same symptoms as that of sub-groups 7 and 8. The mortality recorded within 96 hours in these two sub-groups was 100%. 
<table>
<thead>
<tr>
<th>Sub-group No.</th>
<th>Number of animals</th>
<th>DDT concentrations (%)</th>
<th>Mortality noted after time intervals of</th>
<th>% mortality within 96 hrs.</th>
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<td>10</td>
<td>10</td>
<td>0.9</td>
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In sub-groups 2 to 4, the onset of symptoms was observed in about 45 minutes, whereas in sub-groups 5 and 6 in about 30 minutes and in sub-groups 7 to 10 in about 20 minutes.

(b) ACUTE TOXICITY WITH SEVIN: Table 2 gives the acute toxicity data with different concentrations of Sevin.

Symptoms

Sub-groups 2 to 5: The symptoms observed in these sub-groups were un-coordinated movements followed by hyperexcitability. But there was no mortality within 96 hours.

Sub-groups 6 and 7: The symptoms were similar to those observed in sub-groups 2 to 5. There was 20% and 30% mortality in these sub-groups.

Sub-groups 8 to 12: Hyperexcitability, extension of legs, tremulousness, repeated falling on back and righting efforts, loss of motion, loss of sensitivity to stimuli were the symptoms observed. The sub-group 8 showed 50% mortality within 96 hours, while there was 60%, 80%, 100% and 100% mortality in sub-groups 9, 10, 11 and 12 respectively.

In sub-groups 2 to 7 the onset of symptoms was noted in 30 minutes, whereas in remaining sub-groups in about 15 minutes.

The control animals (sub-group 1) used for both the insecticides were quite normal and healthy throughout the experiment.
<table>
<thead>
<tr>
<th>Sub-group No.</th>
<th>Number of animals</th>
<th>Sevin concentrations (%)</th>
<th>Mortality noted after time intervals of</th>
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<td>% mortality within 96 hrs.</td>
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<td>1</td>
<td>10</td>
<td>Control</td>
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<td>12</td>
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$L_{C50}$: Figures 1 and 2 show the graphical representation of DDT and Sevin respectively. The $L_{C50}$ for DDT was 0.4% and for Sevin was 0.007%.

**DISCUSSION**

Most toxicological studies begin with acute toxicity tests to learn the kind and duration of illness associated with fatal injury and to learn the magnitude of the smallest dosages necessary to produce the effects. The acute toxicity is basic to toxicological investigations. Death is normally an easily detected and obviously important adverse effect. Experimentally, 50% effect is the most reproducible measure of the toxicity of a toxic agent to a group of test organisms, and 96 hours is often a convenient, reasonably useful exposure duration.

The straightforward static test has been widely adopted by many authorities (Doudoroff *et al.*, 1951; Henderson and Tarzwell, 1957; Henderson and Pickering, 1958; Henderson *et al.*, 1959) and the determination of median lethal concentration ($L_{C50}$) for the organism to a particular test is necessary for determination of safe concentrations.

The bioassay results have shown that the toxicity of insecticides to the frog, *Rana tigrina*, was a function of concentration and duration of exposure. It is evident from the results that DDT is less toxic than Sevin.
Fig. 1: Percent mortality of the frog, Rana tigrina, exposed to various concentrations of DDT.
Fig. 2: Percent mortality of the frog, *Rana tigrina*, exposed to various concentrations of Sevin.
Acute toxicity of DDT to fishes has been reported by many workers (Kinter et al., 1972; Holden, 1973; Leadem et al., 1974; Pillai et al., 1976). Some studies have also been done on frogs (Herald, 1949; Mulla, 1963; Kaplan and Overpeck, 1964; Ferguson and Gilbert, 1968).

The toxicity studies reveal that the insecticides bring about the mortality in fishes at a lesser concentration than that is needed for frogs.

The amphibians seem to be somewhat more resistant to insecticides than fishes (Rudd, 1964), which is also evident from the results presented here. This susceptibility can be related to the nature of the skin (Muirhead-Thomson, 1971).

The question of physiological and behavioural effects of sublethal exposures to pesticides has recently been closely studied (Muirhead-Thomson, 1971). There is evidence that the behaviour of animals in response to pollutants may be an important factor in determining the toxicity of such chemicals in nature (Summerfeld and Lewis, 1967).

It is generally recognised that fish respond to toxic chemicals by increased opercular movements (Belding, 1929) and Burton et al. (1972) demonstrated that acute zinc poisoning in Salmo gairdneri involved a modification of gaseous exchange process at the gills with subsequent hypoxia at tissue levels. On the other hand, death in mammals has been related to biochemical lesions leading to ventilation impairments and asphyxiation (De Chandole et al., 1953).
Behavioural responses after exposure to insecticides are in agreement with those observed earlier in fishes (Carter, 1971; Wildish et al., 1971), and frogs (Sanders, 1970). The first sign of poisoning is un-coordinated movements of the test animal. The stages following this symptom are called the DDT jitters, i.e., tremulousness of the entire body and limbs. They are also true for Sevin. These stages in sequence are: (1) un-coordinated movements (2) extension of limbs (3) general tremulousness (4) hyperexcitation (5) repeated falling on back and righting efforts.

The differences in time required for exhibition of these symptoms is in agreement with the findings of Roeder and Weiand (1946, 1948). According to them, the action of low doses of insecticides starts at a sensory cell, then reaches the central nerve synapses and finally influence the motor neurons, while high concentrations directly act on the motor nerves.

In fishes, anti-acetylcholinesterase activity of insecticides was studied with reference to the inhibition of brain and serum ChE (cholinesterase) (Wildish et al., 1971; Alsen et al., 1973). However, these studies could not establish a linear relationship between the death of the fish and degree of brain and serum ChE inhibition. But Miyamoto et al. (1963 b) suggested that inhibition of brain ChE is more important for appearance of toxic symptoms with
insecticides. The findings recorded here show that the activity level of AChE decreases as the concentration of insecticide increases (vide Chapter III).

SUMMARY

1. Acute toxicity studies of the insecticides DDT (organochlorine) and Sevin (carbamate) were conducted on the bullfrog, *Rana tigrina*.
2. The toxicity of insecticides to the frog was a function of concentration and duration of time.
3. DDT was less toxic when compared with toxicity of Sevin.
4. There was 50% mortality within 96 hours in 0.4% test solution of DDT and 0.007% test solution of Sevin.
5. The symptoms of poisoning were in the sequence:
   (a) un-coordinated movements (b) extension of limbs (c) general tremulousness (d) hyperexcitation (e) repeated falling on back and righting efforts.
REFERENCES


Herald, E.S. 1949 Effects of DDT-oil solutions upon amphibians and reptiles. *Herpetologica*, **5**: 117-120.


