Chapter 1

Toxicity on Biochemical and Hematological Parameters in *Bufo melanostictus* (Schneider) (Common Indian Toad) Exposed to Malathion

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Additional information is available at the end of the chapter

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1. Introduction

The widespread application of pesticides has attracted the attention of ecologists to understand the impact of the chemical on natural communities have a large number of laboratory-based single species studies of pesticide, such studies can only examine direct effect. However in natural communities, species can experience both direct and indirect effect. Anthropogenic chemicals are pervasive in nature and biologists are faced with challenge of understanding how these chemical impact ecological community. A diversity of pesticides and their residues are present in a wide variety of aquatic habitats [1,2,3]. While pesticides have the potential to affect many aquatic taxa, the impacts on amphibians are of particular concern in the past decade because of the apparent global decline of many species [4,5,6]. The lists of possible causes of amphibian declines are numerous and pesticides have been implicated in at least some of these declines. Pesticides occur in amphibian habitats [7,2], amphibians living with insecticides in these habitats exhibit physiological signatures of these pesticides and declining population are correlated with greater amounts of upwind agriculture where pesticide use is common. While these correlative studies suggest that pesticides may affect amphibian communities, there are few rigorous experiments to confirm that pesticides are altering amphibian communities.

The widespread application of pesticides has attracted the attention of ecologists that struggle to understand the impact of the chemical on natural communities have a large number of laboratory-based single species studies of pesticides, such studies can only examine direct effect. However in natural communities, species can experience both direct and indirect effects.

World wide amphibian diversity and population numbers have been reported to be declining [8,9,10]. Pesticides are sometimes implicated yet few studies have been conducted

to determine if pesticides actually present a hazard to them [11]. In addition, most published studies on the effects of pesticides on amphibians have been conducted on embryo and tadpole life stages [12,13,14,15,16]. Only one study has been conducted on the effects of malathion (diethyl mercaptosuccinate, S-ester with O, O-dimethyl phophorodithioate) on amphibians in a post-metamorphic life stage. Two woodland salamander species (*Plethodonglutinosus*and *P. cinereus*) to substrates which malathion had been applied to. *Plethodonglutinosus*showed significant inhibition of cholinesterase activity after 3 days of exposure to a 5.6 kg/ha application of malathion.[17].*Plethodoncinereus*did not show this effect, thus indicating variations in speciessusceptibility to malathion. In the 1980's, malathion was applied annually to 4,486,000 ha in the United States [18]. It is used most commonly in the control of mosquitoes, flies, household insects, animal ectoparasites, and human lice. Malathion has been element labeled and applied to fields to study its potential translocation and bioaccumulation; and small rodents, insects and birds had detectable levels 1 yr after treatment [19].Malathion is lipophilic and readily taken up through the skin, respiratory system, or gastrointestinal tract, with absorption enhanced if malathion is in the liquid form [20]. The predominant mechanism of organophosphate toxicity is inhibition of acetylcholinesterase in thenervous system causing accumulation of acetylcholine [21]. This causes hyper excitability and multiple postsynaptic impulses generated by single presynaptic stimuli. Minimal work has been conducted on effects of organophosphorus compounds on disease susceptibility. At intraperitoneally injected doses above 230 mg/kg the mice showed chromosomal abnormalities at 6 hr post-injection [22].Humans occupationally exposed to organophosphorus compounds, including malathion, have marked impairmentof neutrophil chemotaxis.[23] In addition, these workers had increased frequency of upper respiratory infections which increased with the number of years of exposure to organophosphorus compounds. Organophosphorus compounds can also affect immune function of macrophages and lymphocytes in culture [24,25].

The main objectives of the present investigation is to find out the toxic effect of malathion on total protein, total lipid and total carbohydrate content in brain and liver of *Bufomelanostictus*as well as to observe the changes in hematological parameters in Indian Toad exposed to Malathion.

2. Materials and methods

Both male and female toads (*B. melanostictus)* of various size (male body weight ranging from 21-65 gm and female body weight ranging from 13-100 gm) were collected during night time and the test samples were brought into the laboratory and immediately transferred to the glass container supplemented with mud and sand to provide a natural habitat to the Indian toad. The samples were feed with liver and earthworm along with adequate water. The samples were maintained at room temperature for a period of seven days for acclimation to the laboratory condition and then used for experimentation in the eighth day.

To study the effect of Malathion, ten toads were placed in each glass container irrespective of sex and size and sorted out in to two groups of each experiment i.e., one set is for control (without

Malathion) and another is for experiment (with Malathion). One ml of Malathion in concentrations of 25 ppm and 50 ppm (in acetone as solvent) each were injected subcutaneously in the abdominal region of the Test samples species with the help of an insulin syringe.

After which thesamples were sacrificed by pitching and both liver and brain tissue were dissected out to estimate the protein, lipid and carbohydrate content. The blood was collected to estimate the Hb, WBCs and RBCs in both experimental set and control set.Total protein, [26], Lipid [27] and Carbohydrate [28] contents were estimated in the Brain and liver of *Bufomelanostictus* at 24 h, 48h, 72 hr and 96 hrs post-treatment with the test chemical. Sahli'shaemoglobinometer was used to estimate of haemoglobin RBC count was done by Neubaurs improved double haemocytometer using Hayem's solution as diluting fluid whereas for WBC count instead of Hayem's solution, Turk's fluid (W.B.C. diluting fluid) was used. A batch of untreated (control) sample was also kept for comparison purposes.

The data obtained were analysed by using SPSS 10.0 package (SPSS INC, USA) and Two-way ANOVA test was applied to find out the significant difference between the exposure period and concentrations.

3. Results

Total protein content

InMalathion-treated samples after 24h exposure the reduction in protein content in liver was found to be 22.22% and 30.55%. In the Braintissue the reduction was 75% and 44% in the malathion-treated samples atconcentrations of 25 and 50 ppm respectively. At 48 hour of exposure the reduction in protein content was 31.42% and 40% in liver where as in brain the reduction was 73.33% and 80%. Similarly during 72 hour of exposure the reduction in protein content was 34.28% and 42.85% in liver whereas in brain the reduction was 82.35%. During 96 h duration the reductions in protein content in the liver were recorded as 42.85 % and 48.57%. In brain the decrease was 82.35% and 88.23% in the treated samples at the desired concentrations of malathion respectively (Table 1).

Exposure Duration in hour	Control		25 ppm		50ppm	
	Liver	Brain	Liver	Brain	Liver	Brain
24	0.36±0.021	0.18±0.008	0.28±0.016 (22.22%)	0.05±0.021 (72.22%)	0.25±0.014 (30.55%)	0.04±0.014 (77.77%)
48	0.35±0.014	0.15±0.008	0.24±0.014 (31.42%)	0.04±0.08 (73.33%)	0.21±0.016 (40%)	0.03±0.024 (80%)
72	0.35±0.014	0.17±0.021	0.23±0.016 (34.28%)	0.03±0.094 (82.35%)	0.20±0.014 (42.85%)	0.03±0.007 (82.35%)
96	0.35±0.021	0.17±0.021	0.20±0.014 (42.85%)	0.03±0.014 (82.35%)	0.18±0.014 (48.57%)	0.02±0.008 (88.23%)

Table 1. Shows the protein content in both liver and brain tissue in *B. melanostictus* exposed to 25 ppm and 50 ppm of malathion. The data in parentheses reflects the percent decrease over control in the protein content

Subjected to two way ANOVA a significant difference was observed between the exposure period ($F_{1\ 0.05} = 6.02$) as well as between the concentrations ($F_{2\ 0.05} = 92.46$) in case of liver tissues whereas a non significant difference was observed between the exposure period ($F_{1\ 0.05} = 2.96$) in brain tissue. However, between concentration significant difference was observed ($F_{2\ 0.05} = 374.22$)

Total lipid content

Total lipid content was estimated in the liver and brain of the treated organisms. After 25 ppm and 50 ppm of Malathion treatment, for 24 h the lipid content was found to be 56.36% and 61.81% in Malathion treated liver respectively. In the brain of Malathion treated toad the reduction was 64% and 68 % respectively. At 48 h exposure the decrease in lipid content in liver was 58.18% and 63.63% where as in brain it was 65.21% and 69.56%. Simultaneously, during 72 h of treatment the percent reduction in total lipid content in Malathion treated liver was 60% and 65.45% and in brain 66.66% and 75% was observed respectively. At 96 hour of treatment with 25 ppm and 50 ppm of Malathion the lipid content was found to be 61.81% and 65.45% respectively. In case of Malathion treated brain of the test samples the reduction was found to be 69.56% and 78.26% (Table 2). Subjected to two way ANOVA test a non significant difference was observed between the exposure duration ($F_{1\ 0.05} = 3.47$) where as between the concentrations significant difference was noticed ($F_{2\ 0.01} = 3256.06$) in case of liver tissue. Simultaneously the data obtained from the treated brain a significant difference was found between exposure period and the concentrations. ($F_{1\ 0.05} = 11$ and $F_{2\ 0.01} = 1461$).

Exposure Duration in hour	Control		25 ppm		50ppm	
	Liver	Brain	Liver	Brain	Liver	Brain
24	55±0.81	25±1.42	24±1.41 (56.36%)	9±1.63 (64%)	21±1.41 (61.81%)	8±1.41 (68%)
48	55±0.41	23±0.81	23±2.82 (58.18%)	8±1.41 (65.21%)	20±1.41 (63.63%)	7±1.42 (69.56%)
72	55±0.71	24±0.82	22±0.81 (60%)	8±1.63 (66.66%)	19±1.41 (65.45%)	6±0.81 (75%)
96	55±1.63	23±0.85	21±0.021 (61.81%)	7±1.63 (69.56%)	19±1.41 (65.45%)	8±0.81 (78.26%)

Table 2. Reflect the Lipid content in both liver and brain tissue in *B. melanostictus*exposed to 25 ppm and 50 ppm of malathion. The data in parentheses reflects the percent decrease over control in the Lipid content.

Total carbohydrate content

In this present experiment, when the toads were exposed to the desired concentrations of the test chemical for different time interval a drastic reduction in total carbohydrate content in liver as well as in brain tissue was observed. After 25 ppm and 50 ppm of

Malathion treatment, for 24 h the carbohydrate content was found to be 45.58% and 54.41% in liver tissue respectively. In the brain tissue the reduction was 55.28% and 57.14 % respectively. At 48 h exposure the decrease in carbohydrate content in liver was 53.96% and 57.14% where as in brain it was 60.52% and 63.15%. Simultaneously, during 72 h of treatment the percent reduction in total carbohydrate content in liver was 60% and 61.53% and in brain 60.6% and 63.63% was observed respectively. At 96 hour the carbohydrate content in both liver and brain was found to be 60.93%, 64.06% and 66.66% respectively in both the concentrations (Table 3). When the data obtained in case of liver and were analyzed by two way ANOVA test a significant difference was observed between the exposure duration ($F_{1\ 0.05}$ = 11.67) and between the concentrations ($F_{2\ 0.01}$ = 939.50). Whereas, the data obtained from the treated brain non significant difference was found between exposure periods ($F_{1\ 0.05}$ = 1.37) however, a significant difference was noticed between the concentrations $F_{2\ 0.01}$ = 781.25).

Exposure Duration in hour	Control		25 ppm		50ppm	
	Liver	Brain	Liver	Brain	Liver	Brain
24	0.68±0.008	0.35±0.008	0.33±0.036 (45.58%)	0.16±0.021 (55.28%)	0.31±0.021 (54.41%)	0.15±0.014 (57.14%)
48	0.63±0.016	0.38±0.008	0.29±0.012 (53.96%)	0.15±0.008 (60.52%)	0.27±0.016 (57.14%)	0.14±0.014 (63.15%)
72	0.65±0.016	0.33±0.016	0.26±0.021 (60%)	0.13±0.094 (60.60%)	0.25±0.021 (61.53%)	0.12±0.008 (63.63%)
96	0.64±0.008	0.36±0.016	0.26±0.021 (60.93%)	0.12±0.014 (66.66%)	0.23±0.016 (64.06%)	0.12±0.008 (66.66%)

Table 3. Reflect the Carbohydrate content in both liver and brain tissue in *B. melanostictus* exposed to 25 ppm and 50 ppm of malathion. The data in parentheses reflects the percent decrease over control in the carbohydrate content

Hemoglobin content

After treatment with 25 ppm and 50 ppm of Malathion in different time interval the bloods from the test samples were collected and hemoglobin was measured. From the result it was observed that during 24 hr of exposure the percent reduction in hemoglobin content was 26% and 6.57 %. At 48 hr of treatment the percent reduction in hemoglobin in malathion treated blood was found to be 7.89% and 9.21%. After 72 hour of exposure a reduction of 7.89% and 10.52% in the hemoglobin content was observed for 25 ppm and 50 ppm concentration respectively. A decrease of 8% and 10.66 % was found after 96 hour of exposure (Table 4).

When the data were subjected to two-way ANOVA a significant difference was observed between the exposure periods ($F_{1\ 0.05}$ = 6.55) as well as between the concentrations ($F_{2\ 0.05}$ = 97.80)

Exposure Duration in hour	Control			25 ppm			50ppm		
	Hb	WBC	RBC	Hb	WBC	RBC	Hb	WBC	RBC
24	7.6±0.081	4.96±0.94	7.65±0.47	7.2±0.16 (5.26%)	4.76±0.47 (4.03%)	7.55±1.69 (1.30%)	7.1±0.16 (6.57%)	4.28±0.94 (13.70%)	7.54±1.88 (1.43%)
48	7.6±0.081	4.96±1.69	7.64±0.94	7±0.16 (7.89%)	4.68±1.88 (5.64%)	7.54±1.88 (1.30%)	6.9±0.14 (9.21%)	4.22±0.47 (14.91%)	7.43±0.47 (2.74%)
72	7.6±0.21	4.96±0.94	7.63±1.69	7±0.14 (7.89%)	4.42±0.94 (10.88%)	7.38±0.94 (3.27%)	6.8±0.21 (10.52%)	4.09±0.47 (18.54%)	7.29±0.47 (4.45%)
96	7.5±0.081	4.96±0.94	7.62±2.49	6.9±0.14 (8%)	4.09±0.47 (17.54%)	7.15±1.69 (6.16%)	6.7±0.17 (10.66%)	3.84±0.47 (22.58%)	7.08±0.47 (7.08%)

Table 4. Reflects the Hb, WBC and RBC content in *B. melanostictus*exposed to 25 ppm and 50 ppm of malathion. The data in parentheses reflects the percent decrease over control in the Heamatological parameters.

WBC Content

From the experiment it was observed that the WBC content of *B.melanostictus*was also reduced drastically. After 24 hr of exposure to 25 ppm and 50 ppm of malathion the decrease in the WBC was found to be 4.03% and 13.70%. Similarly at 48 hr a drastic reduction of 5.64% and 14.91% in the WBC content of toad was found at 25 ppm and 50 ppm of malathion concentration. At 72 hour the percent inhibition of 10.88% and 18.54% was recorded respectively and after 96 hour of exposure to the desired concentrations of the test chemical the reduction in WBC content was found to be 17.54 % and 22.58%.

Subjected to two-way ANOVA, non-significant difference was observed between the exposure periods ($F_{1\ 0.05} = 2.88$) whereas between concentrations a significant difference was observed ($F_{2\ 0.05} = 31.43$).

RBC Content

From the experiment it was observed that the RBC content of *B.melanostictus*was also reduced drastically like that of WBC content. After 24 hr of exposure to 25 ppm and 50 ppm of malathion the decrease in the RBC was found to be 1.30% and 1.43%. Similarly at 48 hr a drastic reduction of 1.30% and 2.79% in the RBC content of toad was found at 25 ppm and 50 ppm of malathion concentration. At 72 hour the percent inhibition of 3.27% and 4.42% was recorded respectively and after 96 hour of exposure to the desired concentrations of the test chemical the reduction in RBC content was found to be 6.10 % and 7.08%.

Subjected to two-way ANOVA, non-significant difference was observed between the exposure periods ($F_{1\ 0.05} = 4.68$) whereas between concentrations a significant difference was observed ($F_{2\ 0.05} = 8.83$).

4. Discussion

The organophosphates are compounds widely used as insecticides and chemical welfare agents. Although extremely toxic in some cases, these materials are generally short lived in

the environment compared to halogenated organics and related compounds. The toxicity of an organophosphate is related to its leaving group, the double bonded atom, usually O or S and the phosphorous ligands, the groups surrounding the phosphate in the compound. The metabolic replacement of sulphur by oxygen in the liver or other detoxicification organ activates the sulphur containing organophosphate into a much more potent form. The extreme toxicity of these compounds is due to their ability to bind to the amino acid serine, rendering it in capable of participating in a catalytic reaction within enzyme as the further blocking of the active site by the organophosphate residue.

The decrease of total protein content in both liver and brain is may be due to less incorporation of amino acids in the translation process i.e., a reduced incorporation into any kind of proteins and pesticides disturb the protein synthesis. In the present study the total protein content in both liver and brain in Indian Toad decreased after malathion (25 ppm and 50 ppm) treatment.

The reduction in total protein contents after pesticide application in different insects was reported by many workers. See [29, 30, 31, 32, 33, 34, 35].The protein reduction the liver and kidney of reptiles was also reported [36,37]. The present investigations also appear to be in line with the earlier findings. The present results therefore confirm the findingsin this respect.

Carbohydrates are less sensitive as compared to lipids. A reduction in the glycogen concentration in the treated groups could have happened due to activation of glycogenolytic enzymes like phosphorylase system leading to decrease in glucose concentration by malathion in the liver tissues of treated animals. The treated animals being under malathion stress, the stress hormone (epinephrine) released from the adrenal medulla possibly have acted on the liver tissues vide circulation leading to glycogenesis, mediated by adenylatecyclase, cAMP, protein kinase and finally the activated phosphorylase system. From this present investigation it was observed that, malathion has a strong potential to reduce the carbohydrate content in liver and brain tissue of treated toad.

In the current study, the decreased total lipid content may possibly due to either decreased lipogenesis or suppressed translocation/transportation of lipid to plasma. The effect of the doses of whole body treated seems to have acted in same way to depress lipogenolysis possibly by denaturing or by inactivating some of the lytic enzymes, or by hampering the transportation of these molecules to other steroidonesis tissues via the plasma pool due to alternation in membrane functions. Therefore the enhanced level of cholesterol concentration may have contributed to an overall decrease in the total lipid pool of the liver tissue of the treated animals.

The stress induced changes in the total leucocytes count and differential count in mammals have been reported [38,39]. The release of granulocytes from bone marrow as a result of stress induced stimulation mediated by corticosteroids a stress hormone may be the possibility [40]. The lymphocytes which constitute the dominant leucocytes type in toads appear to decrease. Such as decrease in the lymphocytes count may either be due to rupture or degradation of some of these aged circulating immuno competent cells.

The heamolysis of Red blood cells have been reported in various physical and chemical stress [41,42] Under such condition the total circulation red cell population is expected to show a decline in number. The observed decrease in the circulating red cell count can be accounted for the possible mechanisms such as decrease production of renal erythropoietin which stimulates the bone marrow and spleen to release more erythrocytes. From this experiment it was observed that malathion has a strong potential to reduce hemoglobin, WBC and RBC in *Bufomelanostictus*.

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