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RESEARCH ARTICLE

TOXIC EFFECT ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN RANA CYANOPHLICTIS (SCHNEIDER) (COMMON INDIAN FROG) EXPOSED TO SEVIN INSECTICIDE

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ABSTRACT

The carbanyl compounds (Sevin insecticide) 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 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 Sevin insecticide (25 ppm and 50 ppm) treatment. In the brain of Sevin insecticide 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 hrs of treatment the percent reduction in total lipid content in Sevin insecticide treated liver was 60% and 65.45% and in brain 66.66% and 75% was observed respectively. . From this experiment it was observed that Sevin insecticide has a strong potential to reduce hemoglobin, WBC and RBC in Rana cyanophlictis.

INTRODUCTION

Sevin is the trade name for a widely used synthetic insecticide containing the active ingredient carbaryl. Carbaryl belongs to the chemical class called carbamates. As insecticides go Sevin is only moderately toxic to mammals and is still widely used in gardens and landscapes. It is, however, highly toxic to honey bees and many other beneficial insects and mites. Sevin is sold as a powder (dust), granule, and liquid concentrate. Uses include vegetable gardens, landscape plants, lawns, tree fruits and small fruits and the target pest list is broad and includes many common insect and mite pests. While Sevin insecticide can be used safely we believe there are now safer alternatives that also avoid the harmful effects that broad spectrum chemicals, like carbaryl, can have on beneficial.

The widespread application of pesticides has attracted the attention of ecologists to under-stand 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. How-ever in natural communities, species can experience both direct and indirect effect. Anthropogenic chemicals are pervasive in nature and biologists are faced with challenge of under-standing how these chemical impact ecological community. A diversity of pesticides and their residues are present in a wide variety of aquatic habitats [26,22,2]. 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 [6,21]. 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 [17,3], 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

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studies can only examine direct effect. However in natural communities, species can experience both direct and indirect effects.

Worldwide amphibian diversity and population numbers have been reported to be declining [38, 39]. Pesticides are sometimes implicated yet few studies have been conducted to determine if pesticides actually present a hazard to them [15]. In addition, most published studies on the effects of pesticides on amphibians have been conducted on embryo and tadpole life stages [16, 8,9,31,5]. Only one study has been conducted on the effects of Sevin insecticide (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 Sevin insecticide had been applied to. *Plethodonglutinosus*showed significant inhibition of cholinesterase activity after 3 days of exposure to a 5.6 kg/ha application of Sevin insecticide.[4].*Plethodoncinereus*did not show this effect, thus indicating variations in speciessusceptibility to sevin insecticide . In the 1980's, sevin insecticide was applied annually to 4,486,000 ha in the United States [35]. It is used most commonly in the control of mosquitoes, flies, household insects, animal ectoparasites, and human lice. Sevin insecticide 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 [29]. Sevin insecticide is lipophilic and readily taken up through the skin, respiratory system, or gastrointestinal tract, with absorption enhanced if Sevin insecticide is in the liquid form [14]. The predominant mechanism of organophosphate toxicity is inhibition of acetylcholinesterase in thenervous system causing accumulation of acetylcholine [11].

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 [10].Humans occupationally exposed to organophosphorus compounds, including Sevin insecticide, have marked impairmentof neutrophil chemotaxis.[1] 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 [40,30]. The main objectives of the present investigation is to find out the toxic effect of Sevin insecticide on total protein, total lipid and total carbohydrate content in brain and liver of Rana cyanophlictis as well as to observe the changes in hematological parameters in Indian Toad exposed to Sevin insecticide.

MATERIALS AND METHODS

Both male and female toads Rana cyanophlictis of various size (male body weight ranging from 20-66 gm and female body weight ranging from 12-100 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 Sevin insecticide, 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 Sevin insecticide) and another is for experiment (with Sevin insecticide).

One ml of Sevin insecticide 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 the samples were sacrificed by pitching and both liver and brain tissue were dissected out to estimate the protein, lipid and carbohydrate content. The blood was collect-ed to estimate the Hb, WBCs and RBCs in both experimental set and control set.Total pro-tein, [24], Lipid [13] and Carbohydrate [34] contents were estimated in the Brain and liver of Rana cyanophlictis at 24 hrs, 48 hrs, 72 hrs 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.

RESULTS

Total protein content

In Sevin insecticide treated samples after 24 hrs exposure the reduction in protein content in liver was found to be 22.22% and 30.55%. In the Brain tissue the reduction was 75% and 44% in the treated Sevin insecticide samples at concentrations of 25 and 50 ppm respectively. At 48 hours 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 hours 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 hrs 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 de-sired concentrations of Sevin insecticide respectively (Table 1).

Table 1 Shows the protein content in both liver and brain tissue in Rana cyanophlictis exposed to 25 ppm and 50 ppm of the Sevin insecticide data in parentheses reflects the percent decrease over control in the protein content

Exposure	Control		25 ppm		50ppm	
Duration in hour	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%)

Subjected to two way ANOVA a significant difference was observed between the exposure period (F1 0.05 = 6.02) as well as between the concentrations (F2 0.05 = 92.46) in case of liver tissues whereas a non significant difference was observed between the exposure period (F1 0.05 = 2.96) in brain tissue. However, between concentration significant difference was observed (F2 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 Sevin insecticide treatment, for 24 hrs the lipid content was found to be 56.36% and 61.81% in Sevin insecticide treated liver respectively. In the brain of Sevin insecticide treated toad the reduction was 64% and 68 % respectively. At 48/ hrs 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 hrs of treatment the percent reduction in total lipid content in Sevin insecticide 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 Sevin insecticide the lipid content was found to be 61.81% and 65.45% respectively. In case of Sevin insecticide 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 (F1 0.05 = 3.47) where as between the concentrations significant difference was noticed (F2 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. (F1 0.05 = 11 and F2 0.01 = 1461).

After 25 ppm and 50 ppm of Sevin insecticide 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 (F1 0.05 = 11.67) and between the concentrations (F2 0.01 = 939.50). Whereas, the data obtained from the treated brain non significant difference was found between exposure periods (F1 0.05 = 1.37) however, a significant difference was noticed between the concentrations F2 0.01 = 781.25).

Hemoglobin content

After treatment with 25 ppm and 50 ppm of Sevin insecticide 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 Sevin insecticide treated blood was found to be 7.89% and 9.21%.

Table 2 Reflect the Lipid content in both liver and brain tissue in Rana cyanophlictis exposed to 25 ppm and 50 ppm of Sevin insecticide. The data in parentheses reflects the percent decrease over control in the Lipid content.

Exposure	Control		25ppm		50 ppm	
Duration in hour	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%)

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 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).

respectively and after 96 hour of exposure to the desired

Table 3 Reflect the Carbohydrate content in both liver and brain tissue in Rana cyanophlictis exposed to 25 ppm and 50 ppm of. Sevin insecticide The data in parentheses reflects the percent decrease over control in the carbohydrate content

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 4 Reflects the Hb, WBC and RBC content in Rana cyanophlictis exposed to 25 ppm and 50 ppm of Sevin insecticide. The data in parentheses reflects the percent decrease over control in the Heamatological parameters.

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%)

When the data were subjected to two-way ANOVA a significant difference was observed between the exposure periods (F1 0.05 = 6.55) as well as between the concentrations (F2 0.05 = 97.80)

WBC Content

From the experiment it was observed that the WBC content of Rana cyanophlictis was also reduced drastically. After 24 hr of exposure to 25 ppm and 50 ppm of Sevin insecticide 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 concentration. At 72 hour the percent inhibition of 10.88% and 18.54% was recorded respectively and after 96 hour of exposure Sevin insecticide 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 (F1 0.05 = 2.88) whereas between concentrations a significant difference was observed (F2 0.05 = 31.43).

RBC Content

From the experiment it was observed that the RBC content of Rana cyanophlictis was also reduced drastically like that of WBC content. After 24 hrs of exposure to 25 ppm and 50 ppm of Sevin insecticide 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 Sevin insecticide concentration. At 72 hour the percent inhibition of 3.27% and 4.42% was recorded 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 (F1 0.05 = 4.68) whereas between concentrations a significant difference was observed (F2 0.05 = 8.83).

Rana cyanophlictis (Female and male)

DISCUSSION

The carbanyl compounds (Sevin insecticide) 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 a carbaryl 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 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 carbanyl 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 Sevin insecticide (25 ppm and 50 ppm) treatment.

The reduction in total protein contents after pesticide application in different insects was reported by many workers. See [27, 19., 31, 33, 1, 2, 37].The protein reduction the liver and kidney of reptiles was also reported [20,12]. 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 Sevin insecticide in the liver tissues of treated animals. The treated animals being under Sevin insecticide 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, Sevin insecticide 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 concentra-tion 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 [25,36]. The release of granulocytes from bone marrow as a result of stress induced stimulation mediated by corticosteroids a stress hormone may be the possibility [41]. 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 [7,8] 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 Sevin insecticide has a strong potential to reduce hemoglobin, WBC and RBC in Rana cyanophlictis.

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