Quantitative Biochemical Lesions of Malathion Dipping in the Domestic Fowl (*Gallus domesticus*)

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**ABSTRACT:** The impact of various concentrations (0 to 1.5%) of malathion dipping of White Leghorn hens upon cholinergic phenomena (AchE) in plasma and red blood cells (RBC) at different time intervals (0, 6, 12, 24, 72, 120 and 168 h), nucleic acids (DNA and RNA) and protein constituents at 168 h was studied in WLH hens. Malathion produced a significant inhibition (P<0.01) of AchE activity in plasma as well as RBC. The inhibitory response was more marked in RBC but persisted for a longer duration in plasma. Malathion did not induce any change in DNA content in liver and brain tissue. RNA content in liver was significantly decreased, control Vs. 1.5% treatment group (P<0.01), 0.5% Vs. 1.5% (P<0.01) and 1% Vs. 1.5% (P<0.05) Malathion dipping failed to produce any significant alteration either on total serum protein, albumin (A1), globulin (G) and A1/G ratio or total protein. Glycoprotein and stialic acid content of liver and brain tissue. Plasma tyrosine concentration was significantly increased (P<0.05) upon the impact of Malathion dipping in the poultry birds. It appears that malathion treatment of chickens induces the metabolic rate.

**Key Words:** Malathion dipping, Cholinesterase, DNA, RNA, Protein constituents, Plasma, R.B.C. Liver, Brain, Poultry birds.

**INTRODUCTION**

Malathion (o, o- dimethyl dithio phosphate diethyl mercapto succinate), a prominent organophosphate pesticides, is extensively used in commercial poultry industries of tropical countries to control external parasites (ticks, lice, mites etc.) of the poultry birds in the form of dip or spray. This family of pesticides have been replaced the organochlorine compounds because of their rapid breakdown in water and their low environmental persistence (Dembele et al 1999). The toxicity of organophosphate pesticide is primarily due to powerful inhibitor of true and pseudo cholinesterase (ChE) activity through phosphorylation of serine hydroxyl group of the esteratic site in insects or vertebrate (Krishnamurthy, 1983, Haubruge and Toutent, 1997). This causes the increase of acetylcholine level at the nerve synapse resulting continual stimulation of the fibres and showed symptoms like tremors convulsion etc. which ultimately produces biochemical lesions in target and host organism.

Indiscriminate use of pesticide causes widespread harmful effect in avian and mammalian bio-system. Abbott et al. 1964 have reported the Arial spraying of Malathion reduced the hatchability of embryonated chick egg. While Kulezyeki (1975) observed that dichlorovos spraying adversely affected the embryonic development in pheasants. Sokkar et.al.(1975) Showed retarded egg production in laying hens after being dipped in diazinon.

Karan et.al. 1980,1980b, 1981) have also reported that sumithion causes adverse effect on ovary, thyroid and egg quality in WLH hens. In our earlier communication we have reported that malathion dipping causes marked alteration in different enzymic profile and gross parameter related to carbohydrate, protein and lipid metabolism in poultry birds (Pal et.al. 1989, 1991, 1995, Pal and Kushwah, 1990, 1997,1998) The present work was designed to assess further, the biochemical lesions of malathion dipping poultry birds pertaining to nucleic acids, protein constituents and especially to monitor the kinetics of cholinesterase inhibition in RBC and plasma for exploiting this enzyme as biomarker to diagnose the organophosphate toxicity in poultry industry.

**MATERIALS AND METHODS**

**Animals**

Adult White Leghorn hens (14 months of age) of K-strain were procured for experimental studies from All India coordinated Research Project of Poultry for eggs, Department of Poultry science, Jawaharlal Nehru Krishi Vishavavidyalaya, Jabalpur. These were housed in individual cage and were maintained on uniform husbandry conditions and were provided water *ad libitum.*

**Pesticides**

Malathion (o, o -dimethyl dithiophosphate diethyl marcapto succinate) 50% E.C. was obtained from Artee Minerals (Pesticide Division), New Delhi. Artee Malathion 50% E.C. contains 50% Malathion and 50% solvent and auxiliaries.

**Treatments**

Twenty-four birds were divided into four groups, each comprising of six birds, and one group was kept untreated (control). Birds from the experimental groups were exposed to pesticide by dipping them in the malathion solutions of varying concentrations (0.5, 1.0 & 1.5%), keeping the head and neck out side the solution where as birds of control group were dipped in the same manner in fresh water.

**Collection and Preparation of Sample**

Blood samples were collected from the wing vein after dipping at interval of 0, 4, 6, 12, 24, 72, 120 and 168 hours with heparinized syringe. Plasma and RBC were separated by routine procedures by spinning at 3000 rpm at 4°C and were used separately for the assay of acetycholinesterase activity. After 168 hours of dipping, birds were exsanguinated keeping them fasted overnight.
Liver and brain tissue were quickly dissected out, washed with chilled normal saline, blotted dry with dry piece of filter paper weighed and homogenized in chilled sucrase solution (0.25M) and concentration was made to 10% (w/v).

Methods

The acetylcholinesterase activity (Acetyl hydroxylase E.C. 3.1.1.7) was assayed by the method of Hestrin (1949) modified by Augustinasso (1957). Nucleic acids were isolated by the methods of Schmidt and Thaunhauser (1945) and were estimated as per the method of De Dekan and De Dekan (1959) as described by Iswara et.al. (1972).

Plasma tyrosine was determined by the method of Udenfriend and Copper (1952) Sialic acid content was estimated by the method of Reinhold (1950) as outlined (1965). The protein content of the plasma and RBC was estimated by the method of Aminoff (1961). Glycoprotein was determined using method of Suzuki (1965). The protein content of the plasma and RBC was estimated by the method of Reinhold (1950) as outlined by Chaykin (1966). Enzyme activity was expressed in umoles of acetylcholine hydrolysed/mg protein/minute.

Statistical analysis

The data were analyzed by complete randomized design described by Snedecor and Cochran (1968).

RESULTS

The data summarized in Tables 1 and 2 showed changes in acetylcholinesterase activity in the plasma/RBC of WLR hens after dipping in Malathion solution of graded strength (0.5, 1 and 1.5%) at intervals of 0, 4, 6, 12, 24, 72, 120 and 168 hours. At the lowest concentration of Malathion in dip solution plasma AchE activity was decreased significantly (P < 0.05) of 6 hours, the inhibitory effect being accentuated at 12 and 24 hour. Thereafter, recovery of enzyme activity was noted up to 168 hour. When the concentration of malathion in dip solution was increased to 1%, the inhibitory response persisted up to 168 hr. notwithstanding the trend towards recovery of enzymatic activity up to 12 hr, upon increasing the concentration of malathion to 1.5%. The extent and pattern of the inhibition response remained virtually same as recorded with 1% concentration. However, a significant (P < 0.05) suppression of plasma AchE activity started earlier i.e. of 4 hour. At the lowest concentration (0.5, 1 %) the inhibitory response was non significant. Thus results showed that the exposure of hens to malathion, inhibition of plasma AchE activity but response was not strictly dose dependent. Exposure of hens to Malathion in dip solution caused marked inhibition of red blood cell AchE activity (Table 2). At the lowest concentration (0.5%) of the pesticide the inhibitory response ensued at 6 and 12 hour (P < 0.01) but at 24 hour the enzymatic activity tended to recover. By 168 hr, the enzymatic activity had recovered to the level of control value. Upon increasing the concentration of Malathion to 1% the pattern of alteration in enzymatic activity level remained unchanged. At the maximum concentration (1.5%) of malathion, a highly significant inhibitory response in red blood cell AchE from 4 to 120 hr. (at all levels, P < 0.01) was observed and the enzyme activity tended to recover only after 168 hour. These findings indicate that regardless of the concentration of Malathion in the dip solution, the maximum inhibition of enzymatic activity occurred of 6 and 12 hour in the RBC, where as in plasma such response of inhibition was recorded at 12 & 24 hr. It was more susceptible to the in vivo inhibitory action of Malathion than the plasma. However, inhibition was persisted longer in fluid medium, possibly due to continued release of Malathion from the tissue storage sites as part of homeostatic mechanism.

The data on the impact on nucleic acid in liver and brain tissue of birds are presented in Table-3. The pesticide did not induce any significant change in DNA content in either tissue. But RNA concentration in liver was significantly decreased, control vs. 1.5% treatment group (P=0.01), 0.5% vs 1.5% (P<0.01) and 1% vs. 1.5% (P<0.05). RNA content in the brain tissue was unaffected. Data pertaining to protein constituents are presented in Table-4. The protein constituents are practically unaffected barring tyrosine concentration, which was decreased significantly (P<0.05).

DISCUSSION

The activity of acetylcholinesterase (AchE) controls the action of acetylcholine, the neurotransmitter. The inhibition of AchE leads to the accumulation of acetylcholine and prevents the transmission of nerve impulse across the synaptic gap. The resulting disturbance in electrophysiology causes loss of muscular coordination, induction of convulsions and death (Jones et al., 1977). Discovery and the existences of isomers of AchE and their relative rates of inhibition by the organophosphates compounds, are likely identify the receptor site for binding acetylcholine and the multifaceted membrane bound AchE which are undoubtedly important aspect of target -enzyme inhibition interaction (O’Brien, 1970 O’Brien et al., 1974). Oral administration of Malathion in indigenous chicken produced symptoms of toxicity characteristic of anticholinesterase, no pathogonomic lesions could be detected. Where as ingestion of the insecticide up to 800 mg/kg caused rapid inactivation of plasma cholinesterase to the extent of 50% within 12 to 48 hrs (Gupta and Paul, 1971). The result of present study indicate that following exposure to the usual concentration (1.5%) of malathion used for dipping adult hens in commercial poultry houses for control of ectoparasites, a significant inhibition of plasma AchE activity occurred as early as 4 hr, and persisted up to 168 hour. Limited comparative reports on the inhibition pattern of AchE activity in RBC and plasma is available in regard to Malathion dipping of chicken. Pal and Kushwah (1998) reported that inhibition of AchE activity in brain tissue after 168 hr, of Malathion dipping in poultry birds. Inhibition of this enzyme activity in plasma and RBC mammalian species viz. albino rats (Hanzel et. al., 1954; Zeratisean et.al., 1961), Guinea pigs (Hanzel et al., 1954) and human (Morphy and Duboc's 1957) have been reported after Malathion intoxication. A comparative study of the selective tolerance of chicken and rabbits to malathion (Stanley et
al., 1957) revealed that chicken are marked by more sensitive that rabbits and the AchE inhibition was of the order of 62.9% in the avian as compared to only 8.6% in the mammalian species.

Results of the present study advance our knowledge on the susceptibility of chicken to the action of Malathion after dipping exposure. Therefore, the assay of AchE activity plasma/RBC may be used as marker enzyme to diagnose the Malathion toxicity in Poultry industry.

Nucleic acids seems to be the most sensitive indices to access the extent of cellular damage upon the impact of pesticide because of the relative constancy of nucleic acid per somatic cell nucleus (West et al., 1970). In general, both DNA and RNA concentrations were tended to decrease in either issue following dipping exposure to malathion and the response being significant (P<0.01) only for RNA in liver at 1.5% concentration of pesticide. No comparable report is available in the literature. However, malathion ingested at the rate of 50 mg/kg failed to induce any perceptible change in the nucleic acid concentration in the liver of treated rats (Gupta et al., 1974). In respect to parallel effect of chlorinated insecticides the reports are conflicting. Thus, where as an increase in total liver protein and RNA concentration was recorded after DDT intoxication but dieldrin (1-5 PPM) had no effect in liver protein concentration, growth rate and body weight of rats (Kohli et al.1975). Similarly, Manciucalea and Girgea (1975) reported the increase value of hepatic DNA and RNA content following exposure to small doses of fenchlorofos and heptachlor in chicks. On the contrary, a decrease value of DNA and RNA concentration in liver tissue was recorded following lindane intoxication in the chicken (Manciucalea, et al., 1977). Similarly, a reduce DNA content in blood was also recorded in sheep following thiodan intoxication.

It may however, be noted that the in vivo action of organophosphorus compound vary substantially from that of chlorinated hydrocarbons. The difference in observation may be due to the differential action of different types of pesticide in different species of animals, Richardson (1981) opined that lipid soluble toxic compound might be dissolved in the lipid compartment of neural membrane and thereby changing the morphology and function. Kurkure et al. (1993) has observed that the retarded body weight and liver weight along with congestion and granular degeneration of hepatocytes upon the effect of endosulfan in chicks. The alteration of histarchitecture viz. satellitosis, neurophagia and glial nodule formation in liver and brain tissue were recorded upon the inoculation of endosulfan (2 ug) in developing chick embryo (Pushpanjali, 1994). These observation indicates that, at least, one of the reason for the observed decrease in nucleic acids concentration in the present study.

The data presented in Table-4 indicates the absence of any significant change in concentration of total protein, albumin, globulin and A/G ratio. Dudeja et. al. (1978) reported that oral administration of Malathion at 3000 and 5000 ppm was lethal to immature chicken. At this dose level insecticide induced hyper globunimia, hypoalbuminimia and increased total plasma protein concentration. In the present study no significant change occurred probably due to homeostasis. Estimation of plasma tyrosine content reflects the thyroid status of chicken (Gulati and Nangia 1973). In the present study plasma tyrosine was found to be enhanced following dipping exposure to Malathion, but the response was significant (P<0.05) only when concentration of Malathion in dipping solution was 1.5%. This might suggests Malathion dipping stimulate he basal metabolic rate (BMR). The in vitro study of Malathion showed a dose dependent increase in hepatic total ATPase activity of rats (Pal and Kushwah,1990). We have also reported that malathion dipping also causes reduction of hepatic glycogen concentration concomitant with enhanced glucose concentration in blood (Pal et al., 1991) this suggestive of tissue glycogenolysis. Besides, enhanced blood lactic acid concentration (P<0.05) with widening of lactate/pyruvate ratio attests to acceleration of the reaction of anaerobic glycolysis as part of in vivo tissue response to Malathion. A pronounced hyperglycaemic response was also recorded in chicken (Uppal, 1970), in rats (Kohli et al. 1975) and in Indian water buffalo calves (Gupta et al. 1981) following administration of Malathion. We have reported earlier that plasma tyrosine level showed a rising trend against endosulfan exposure rats maintained low protein diet (Pal et.al. 1989). Thus, the increase value in plasma tyrosine in hens upon exposure to malathion dipping is meaningful (present study) in regard to BMR. The unaltered value of structural components like glycoprotein and sialic acid in brain tissue indicates that Malathion dipping at usual concentration did not produce any degenerative changes in the brain tissue.

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REFERENCES


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Table 1. Changes in acetylcholinesterase activity\(^@\) in the plasma of WLH hens following dipping in Malathion solution of varying concentration (μ mole acetylcholine hydrolysed/minute/ml)

<table>
<thead>
<tr>
<th>Concentration of Malathion (50% E.C.) in the dipping solution</th>
<th>Sampling after Malathion dipping (hr.)</th>
<th>0</th>
<th>4</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>72</th>
<th>120</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td></td>
<td>0.36 ±0.07</td>
<td>0.29 NS</td>
<td>0.23</td>
<td>0.19</td>
<td>0.17</td>
<td>0.27 NS</td>
<td>0.27 NS</td>
<td>0.28 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03(-20)</td>
<td>(-36)</td>
<td>(-46)</td>
<td>(-52)</td>
<td>(-25)</td>
<td>(-24)</td>
<td>(-21)</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td></td>
<td>0.35 ±0.03</td>
<td>0.27 NS</td>
<td>0.23</td>
<td>0.23</td>
<td>0.16</td>
<td>0.17</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03(-23)</td>
<td>(-33)</td>
<td>(-54)</td>
<td>(-52)</td>
<td>(-50)</td>
<td>(-45)</td>
<td>(-37)</td>
<td></td>
</tr>
<tr>
<td>1.5%</td>
<td></td>
<td>0.34 ±0.01</td>
<td>0.25 NS</td>
<td>0.20</td>
<td>0.15</td>
<td>0.16</td>
<td>0.16</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02(-26)</td>
<td>(-40)</td>
<td>(-53)</td>
<td>(-53)</td>
<td>(-52)</td>
<td>(-47)</td>
<td>(-42)</td>
<td></td>
</tr>
</tbody>
</table>

Average of six birds ± S.E; Figures in parentheses represent the percentage of enzyme inhibition.
NS - Not Significant , * Significant (P<0.05), ** Significant (P<0.01)

Table 2. Changes in acetylcholinesterase activity\(^@\) in the red blood cells of WLH hens following dipping in Malathion solution of varying concentration (n mole acetylcholine hydrolysed/min/mg of protein)

<table>
<thead>
<tr>
<th>Concentration of Malathion (50% E.C.) in the dipping solution</th>
<th>Sampling after Malathion dipping (hr.)</th>
<th>0</th>
<th>4</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>72</th>
<th>120</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td></td>
<td>1.5283 ±0.1942</td>
<td>1.3814 NS</td>
<td>0.8978</td>
<td>1.1340</td>
<td>1.2564</td>
<td>1.4402</td>
<td>1.4212</td>
<td>1.4666</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3579 (-9.61)</td>
<td>(-41.25)</td>
<td>(-25.79)</td>
<td>(-17.79)</td>
<td>(-5.76)</td>
<td>(-7.00)</td>
<td>(-4.03)</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td></td>
<td>1.4617 ±0.1755</td>
<td>1.1301 NS</td>
<td>0.9724</td>
<td>0.8931</td>
<td>1.2900</td>
<td>1.2264</td>
<td>1.4692</td>
<td>1.4589</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0922(-22.68)</td>
<td>(33.47)</td>
<td>(-38.89)</td>
<td>(-13.11)</td>
<td>(-16.09)</td>
<td>(-0.51)</td>
<td>(-19.19)</td>
<td></td>
</tr>
<tr>
<td>1.5%</td>
<td></td>
<td>1.4792 ±0.2661</td>
<td>1.1156 NS</td>
<td>0.8798</td>
<td>-0.9659</td>
<td>1.0148</td>
<td>1.1528</td>
<td>1.1986</td>
<td>1.3193</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0656(-27.58)</td>
<td>(-40.52)</td>
<td>(-35.68)</td>
<td>(-39.39)</td>
<td>(-22.06)</td>
<td>(-18.96)</td>
<td>(-10.80)</td>
<td></td>
</tr>
</tbody>
</table>

Average of six birds ± S.E; Figures in parentheses represent the percentage of enzyme inhibition.
NS - Not Significant , * Significant (P<0.05), ** Significant (P<0.01)
Table 3: Changes in nucleic acid content in the liver and brain of WLH hens following dipping in Malathion solution of varying concentration (Analysis of variance).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DNA</th>
<th>RNA</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brain tissue</td>
<td>Liver tissue</td>
<td>Brain tissue</td>
<td>Liver tissue</td>
</tr>
<tr>
<td>Concentration</td>
<td>3</td>
<td>128.8257</td>
<td>273.4476</td>
<td>135.5694</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>350.9246</td>
<td>227.1864</td>
<td>114.5647</td>
</tr>
</tbody>
</table>

Average (µ g/g wet tissue)

<table>
<thead>
<tr>
<th>Concentration of malathion (50% E.C.) in the dipping solution</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain tissue **</td>
<td>56.2562</td>
<td>66.0282</td>
</tr>
<tr>
<td>Liver tissue **</td>
<td>29.5291</td>
<td>63.1861a</td>
</tr>
</tbody>
</table>

S.E. ±7.6477 ±6.1534 ±4.9085 ±4.1497 ±4.1497

C.D. 5% -- -- -- 12.234
C.D. 1% -- -- -- 16.6935

Figures having the same superscripts do not differ significantly; Figures in parenthesis represent the percentage of increase/decrease; NS - Not significant, ** - Significant (P<0.01)

Table 4. Changes in some protein constituents in liver, brain and blood of WLH hens after 168 hrs. of dipping exposure in malathion solution of varying concentration (Analysis of variance).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D.F</th>
<th>Serum (gm/100 ml serum)</th>
<th>Plasma (mg/100ml)</th>
<th>Liver (mg/gm wt tissue)</th>
<th>Brain (mg/gm wt. tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Protein</td>
<td>Albumin</td>
<td>Globulin</td>
<td>A/G ratio</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td></td>
<td>4.026</td>
<td>1.975</td>
<td>2.051</td>
<td>0.883</td>
</tr>
<tr>
<td>0.5%</td>
<td></td>
<td>4.216</td>
<td>1.799</td>
<td>2.417</td>
<td>0.798</td>
</tr>
<tr>
<td>1.0%</td>
<td></td>
<td>3.917</td>
<td>1.752</td>
<td>2.165</td>
<td>0.841</td>
</tr>
<tr>
<td>1.5%</td>
<td></td>
<td>3.858</td>
<td>1.773</td>
<td>2.083</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.47)</td>
<td>(-8.9)</td>
<td>(+15.1)</td>
<td>(-9.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.12)</td>
<td>(-5.5)</td>
<td>(+7.7)</td>
<td>(-4.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.12)</td>
<td>(+1.6)</td>
<td>(+10.7)</td>
<td>(+6.0)</td>
</tr>
<tr>
<td>S.E.</td>
<td></td>
<td>±0.249</td>
<td>±0.184</td>
<td>±0.243</td>
<td>±0.166</td>
</tr>
</tbody>
</table>
| C.D. (5%)           |     | ±1.436

Figures having the same superscripts do not differ significantly; Figures in parenthesis represent the percentage of increase/decrease; * - Significant (P<0.01), NS - Not significant