Punjab Univ. J. Zool., Vol. 12, pp. 127-137, 1997

MALATHION INDUCED HAEMATOXICITY IN CHICKS OF FOWL, *GALLUS DOMESTICUS*

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Abstract: An organophosphate insecticide, malathion was administered orally to chicks in three different doses *i.e.*, 600, 400 and 250 mg/kg body wt./day for the total periods of 40 hours, 12 days and 4 weeks, respectively. The blood samples were collected following stipulated periods of 5, 10, 20 and 40 hours duration in short term experiment, at 3, 6 and 12 days in 1st long term experiment and at 2, 3 and 4 weeks in 2nd long term experiment and used for various haematological studies.
In short term experiment, significant changes were found in MCV (15% and 10% decrease) and MCHC (13.9% and 14.1% increase) at 20 and 40 hours treatments. In 1st long term experiment the Hb increased by 16%, 12% and 21% at 3, 6 and 12 days. Similar rise was also found in 2nd long term experiment. MCHC increased at 12 days (20%) in 400 mg dose and at 3 and 4 week (16 and 13%, respectively) in 250 mg dose level. The WBC count showed persistant increase in all three treatments.

Key words: Organophosphate insecticide, phosphoro-dithioate, haematology, blood morphology, haematological indices.

INTRODUCTION

The extensive use of insecticides although has lessened the human sufferings by reducing insect-borne diseases and destruction of crops by controlling the pest population but at the same time resulted in wide-spread chemical contamination of the environment. The residues of these insecticides and their derivatives persist in various components of the environment and move towards higher tiers of the food chain through biological magnification (Mugambi *et al.*, 1989; Clavijo *et al.*, 1996; Norman, 1996; Parrilla and Vidal, 1996) and have been found hazardous to animals as well as other environmental systems (Grether *et al.*, 1987; Ali *et al.*, 1988; Ali and Shakoori, 1988, 1990,1996; Shakoori *et al.*, 1988; Singh and Kavadia, 1989; Shirasaka and Konno, 1990; Jianmongkol *et al.*, 1996; Stephens *et al.*, 1996). In recent years the use of insecticides has greatly increased (Roberts, 1996), partly to boost the agricultural output and partly due to development of resistance in insects (Collar and Hink, 1987; Tang Chiong *et al.*, 1989; Bull and Pryor, 1990; Picollo De Villar *et al.*, 1990; Subramanyam and Harein, 1990; Dunkov *et al.*, 1996; Parker *et al.*, 1996; Taylor and Feyereisen, 1996., Berger and Sultatos, 1997).

Amongst these insecticides, organophosphates (OP), occupy a significant place, as far as their usage and role in the environment is concerned. Malathion is one of the most

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widely used OP insecticide against pests of crops, vegetables, fruits and stored grains (Rao *et al.*, 1989; Tang Chiong *et al.*, 1989; Bull and Pryor, 1990; Subramanyam and Harein, 1990). It is also used to eradicate the pests of public health importance. In Pakistan, it has been used against mosquitoes to control malaria by the Health Department.

Malathion is highly toxic to insects and other pests (Kao and Tzeng, 1992; Chakraborti *et al.*, 1993; Al-Shatti *et al.*, 1997) but it is considered one of the least toxic pesticide for nontarget animals (Ali and Shakoori, 1981; Husain *et al.*, 1987; Ansari *et al.*, 1987; Singh and Kavadia, 1989; Thathoo and Prasad, 1989). Poultry may be exposed to malathion and other organophosphate pesticides as these are directly applied to the skin of chicks and other birds beneath the feathers for the prevention of various parasitic infestations (Devaney *et al.*, 1982; McOrist, 1983; Pass and Jue-Sue, 1983). Moreover, poultry farms also require periodical spray to control various vector insects, the factor which is an indirect source of pesticide exposure (pass and Jue-Sue, 1983; Tang Chiong *et al.*, 1989).

Although a lot of work on different aspects of organophosphates and more specifically malathion, toxicity is available in literature, on fish, mammals and various other animals (Mukhopadhyay and Dehadrai, 1978; Paul *et al.*, 1979; Ali and Shakoori, 1981; Singh *et al.*, 1984; Mala, 1987; Richmond and Dutta, 1988; Thathoo and Prasad, 1989; Scarpato *et al.*, 1996; Hughes *et al.*, 1997), very few studies are found as far as the aves and poultry is concerned (Gupta and Paul, 1977; Gromysz-kalkowska *et al.*, 1981; Goyal *et al.*, 1986; Johnston *et al.*, 1994). Only sporadic reports on some aspects of malathion toxicity in poultry, however, exist in literature (Baron and Jhonson, 1964; Varshneya *et al.*, 1988; Rao *et al.*, 1989), while most studies are concerned with the esterase and cholinesterase inhibition (Thompson *et al.*, 1991; Busby *et al.*, 1991; Yadava *et al.*, 1991; Leons *et al.*, 1996), detailed biochemical and metabolic studies are scarce.

In the present report, the effects of malathion on the blood cellular parameters (haematology) in the chicks of *Gallus domesticus* is being reported.

MATERIALS AND METHODS

Animals and their maintenance

The broiler chicks of *Gallus domesticus* were purchased from Hybred (Pakistan) Ltd. Lahore. They were placed in cages of 5 cubic feet size, in the animal house of Zoology Department at 25 ± 1.5°C temperature. During this period, the chicks were fed on commercial poultry feed. The feed and water was provided to the chicks *ad libitum*.

Insecticide used and its administration

Toxicant used for this study was organophosphate (OP) compound,malathion [o.o-dimethyl-S-(1,2-dicarboxy ethyl) phosphoro-dithioate], 57 EC, purchased from Jaafer

Brothers (Private) Ltd., Lahore. The insecticide was administered to chicks orally after proper dilution with water.

Three sublethal doses (one strong and two weak) of malathion were used for the present series of experiments. A strong dose of malathion was administered to chicks @ 600 mg/kg body weight once and its effects were observed for a total period of 40 hours short-term experiment. Two different concentrations were used for two long term experiments. In 1st long term experiment, the chicks were administered with 400 mg of malathion/kg body wt./day for 12 days, while in the 2nd experiment, malathion was given @ 250 mg/kg body wt./day for a total period of 4 weeks.

Experimental plan

In short term experiment, a group of twenty chicks of same age group and size were administered with malathion @ 600 mg/kg body weight once, for the total period of 40 hours. The blood samples were collected from a group of four birds, following stipulated periods of 5, 10, 20 and 40 hours. Another group of four animals, processed similarly, except the insecticide treatment, was used for control experiment. For 1st long term experiment twenty seven chicks were administered with 400 mg of toxicant/kg body wt./day for a total period of 12 days. A group of 4 birds were disected at the interval of 3, 6 and 12 days. In the 2nd long term experiment, fifteen chicks were administered with malathion @ 250 mg/kg body wt./day for the total duration of 4 weeks. A group of 4-5 animals were dissected for sampling at 2nd, 3rd and 4th week. A group of 3-4 chicks processed similarly, except insecticide treatment, was used as control with each treated group. In all above experiments, the blood samples were collected after various stipulated periods, in small glass vials containing EDTA as an anticoagulent for haematological studies.

Methodolog used

The anticoagulent containing blood samples were used for the estimation of haemoglobin (Hb) contents according to Van-Kampan and Zijlstra (1961), packed cell volume (PCV = haematocrit) according to microhaematocrit method of Strumia *et al.* (1954), red blood cell (RBC) counts, white blood cell (WBC) counts according to routine clinical methods. These values were then utilized for calculating the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) according to the formulae mentioned in Dacie and Lewis (1977).

RESULTS

Malathion treatment as strong (600 mg/kg body wt. once) and week doses (400 mg and 250 mg/kg body wt./day) for the total duration of 40 hours, 12 days and 4 weeks, respectively, did not induce any severe alteration in haematological components of chicks (Tables I-III and Figs.1-3).

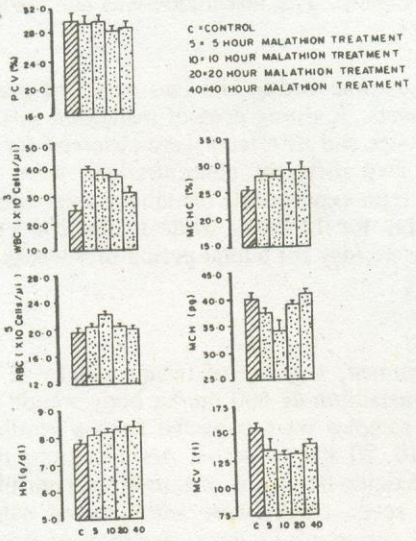

Fig. 1: **Effects of malathion (600 mg/kg body wt.) administered for a total duration of 40 hours on some blood parameters of chick. For abbreviations see Table I.**

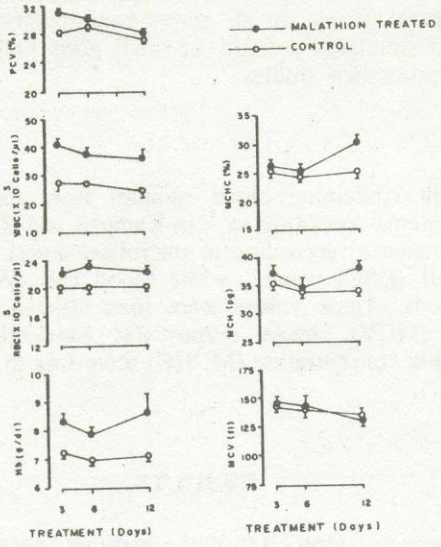

Fig. 2: **Effects of malathion (600 mg/kg body wt.) administered for a total duration of 12 days on some blood parameters of chick. For abbreviations see Table I.**

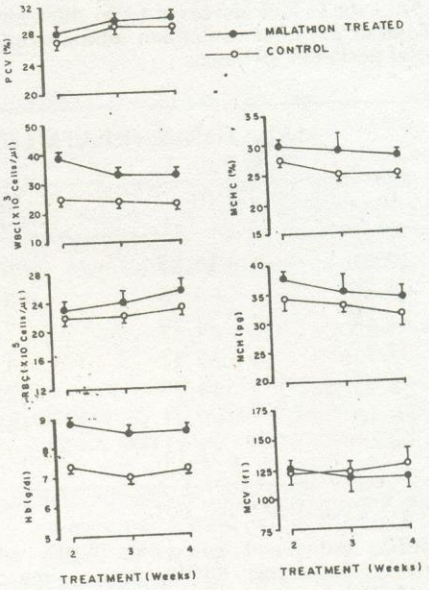

Fig. 3: **Effects of malathion (600 mg/kg body wt.) administered for a total duration of 4 weeks on some blood parameters of chick. For abbreviations see Table I.**

The Hb content, RBC count, PCV and MCH remained disturbed in short term experiment. The significant changes were found in MCV and MCHC which showed 14.65 and 9.90% decrease (MCV) and 14.10 and 13.90% increased (MCHC) at 20 and 40 hours of malathion feeding, respectively. The more pronounced effect was observed in WBC count which was increased 58% within 5 hours of insecticide feeding and retained almost same pattern until 20 hour. The count shot down more than 50% when the treatment was extended for another 20 hours (Table I; Fig.1).

During long term studies the most severely affected parameters were Hb and WBC count. The Hb content shot up by 16%, 12% and 21% in 1st long term I experiment (Table II; Fig.2) and 19%, 14% and 18% in 2nd long term experiment (Tables III; Fig.3). The uninterrupted malathion treatment for 4 weeks caused significant change in WBC count which showed 46%, 41%, 49% rise during 1st long term experiment at 3, 6 and 12 days and 52%, 39% and 40% rise during the 2nd long term experiment at 2, 3 and 4 week treatments, respectively.

In addition, PCV and MCHC also increased by 10% and 20% at 3 and 12 days, respectively after insecticide feeding in 1st long term experiment (Table II; Fig.2). The significant increase of 16% and 14% was found in MCHC in long term II experiment at 3 and 4 week of pesticide feeding. The RBC, MCV and MCH in both long term experiments and PCV in long term experiment remained undisturbed.

Table I: **Percent increase (+) or decrease (-) in different blood components of chick (*Gallus domesticus*) after malathion administration (600 mg/kg body weight) for the total period of 40 hours.**

Parameters[a]	MALATHION TREATMENT (HOURS)			
	5	10	20	40
RBC	+10.81	+15.85	+9.28	+4.49
Hb	+3.86	+4.76	+5.83	+7.41
PCV	-4.48	-4.29	-7.26	-5.65
MCV	-7.71	-5.82	-14.65*	-9.90*
MCH	-5.95	-14.77	-2.84	+2.88
MCHC	+9.10	+8.21	+14.07*	+13.88**
WBC	+58.0***	+51.15***	+47.02**	+22.72*

Student's `t' test, *P<0.05; **P<0.01; ***P<.001.

[a]**Abbreviations used:** RBC, red blood corpuscle; WBC, white blood corpuscle; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin content.

Table II: **Percent increase (+) or decrease (-) in different blood parameters of chick (*Gallus domesticus*) after malathion administration (400 mg/kg body weight/day) for the total period of 12 days.**

Parameters[a]	MALATHION TREATMENT (DAYS)		
	3	6	12
RBC	+11.26	+11.69	+10.08
Hb	+16.05**	+12.15*	+21.06***
PCV	+10.24*	+4.88	+0.88
MCV	+0.40	+01.12	-8.27
MCH	+5.0	+0.43	+10.62
MCHC	+5.06	+6.98	+20.06*
WBC	+46.37**	+41.24*	+48.62**

Student's `t' test, *P<0.05; **P<0.01; ***P<0.001.

[a]For abbreviations, see Table I.

Table III: Percent increase (+) or decrease (-) in different blood components of chick (*Gallus domesticus*) after malathion administration (250 mg/kg body weight/day) for the total period of 4 weeks.

Parameters[a]	MALATHION TREATMENT (WEEKS)		
	2	3	4
RBC	+6.10	+9.41	+7.35
Hb	+18.69**	+14.23*	+17.95***
PCV	+5.97	+1.13	+4.17
MCV	+0.13	-9.21	-3.67
MCH	+11.24	+5.83	+9.34
MCHC	+11.13	+15.93*	+13.39*
WBC	+52.20**	+38.94*	+39.92*

Student's `t' test, *P<0.05; **P<0.01; ***P<0.001.

[a]For Abbreviations, see Table I.

DISCUSSION

Malathion treatment to chicks induced some moderate type of changes when the data of three experiments, with different doses and durations, was compared with other organophosphate and organochlorine insecticides. The changes were more prominent in 1st and 2nd long term experiments. The haematological components which showed prominent alteration were Hb, MCHC and WBC count which showed significant increase in both long term experiments. All other parameters tested, remained unchanged during these treatments, except PCV which was increased at 3 day treatment only and normalized in the remaining duration.

Malathion haematoxicity was also reported in rabbits when administered @ 95 mg/kg body weight/day for 15 month duration (Ali and Shakoori, 1981). The Hb content, RBC count and PCV showed prominent decrease durinng the study while MCV increased. These findings showed that rabbits are more susceptible to malathion. However, long term (15 months) feeding may be the reason for relatively greater toxicity of malathion in rabbits at low doses when compared with chicks in the present study. In another study Shakoori *et al.* (1988) showed unchanged Hb with decrease in RBC and PVC in rats treated with pyrethroid insecticide, cypermethrin @ 420 mg/kg/day for 6 months. Other studies from this laboratory with organochlorine insecticides resulted in greater toxicity when compared with malathion (Ali and Shakoori, 1990, 1994). Contrary to this, the results in the present report with malathion indicated some-what stimulatory effect on haematopoietic system especially haemoglobin biosynthesis, with the corresponding increase in MCHC because RBC count remained undisturbed. During short term experiment, decrease in MCV and rise in MCH was an indication of fairly high compactness of the Hb in the RBC's. This may also be ascribed

to increased biosynthesis of Hb. Similar changes in MCHC were also noticed with cypermethrin in rats (Shakoori *et al.*, 1988). It has been reported in birds that hypoxia and development of dehydration may be the reason for increased Hb, RBC, MCH and PCV which is a protective mechanism to compensate the decreased uptake of oxygen under toxic stress (Coles, 1986; Campbell, 1988). These findings were also confirmed by Natarajan (1984) working with metasystox on fish. According to this report the inhibition of succinic dehydrogenase and tissue respiration due to oxygen depletion, strengthen this assumption. Although in this report, oxidative enzymes were not studied but on the basis of above report it is suspected that malathion may develop hypoxic conditions in chicks. However, this thing needs further experimentation in chicks with malathion.

The WBC count is another important haematological component, which exhibited significant alteration following malathion administration in all treatments (40 hours, 12 days and 4 weeks). Rise in WBC count is a typical response of vertebrates in case of toxic insult with a variety of toxicants. There are several reports from this laboratory which show increased WBC after treatment with different pesticides (Ali and Shakoori, 1990, 1994). Similarly Dikshith *et al.* (1980) working with phosphamidon in rabbits, Gromysz-Kalkowska *et al.* (1981) working with trichlorfon in quails and Gromysz-Kalkowska and Szubartowska (1986) working with trichlorfon in frog also showed increase in WBC which is a defensive response of the organism against foreign toxic invasion. In conclusion, increase in WBC count may be due to damaging action of malathion on blood cells and tissues which are required to counter and detoxify the effects of this toxicant and to phagosatize the dead or necrotic tissues.

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(Received: April 10, 1997)