Discussion
4. DISCUSSION:

It has not been possible to predict upon the basis of chemical structure whether or not a compound will cause a typical type of toxicity. However, O-O-diethyl O-(3,5,6-trichloro-2 pyridyl) phosphorothioate are generally suspected for the inhibition of acetyl cholinesterase activity. Like all the organophosphate insecticide, chlorpyrifos affects the nervous system. In laboratory animals chlorpyrifos can also cause delayed effects on the nervous system. Chlorpyrifos toxicity studies have demonstrated various degrees of sensitivity among animals. Birds are also not exceptional to this. During normal course of treatment for control of several pest species, a significant amount of the pesticide are very likely to enter into the avian system. Thus the pesticide affects the overall dynamics of development, metabolism and reproduction in the avian model. From different studies related to chlorpyrifos toxicity (Morgan, 1989; Chambers and Carr, 1993; Bushnell et al., 1993; Sakai, 1990 and Richardson, 1993) it can be concluded that:

1. Symptoms developed out of chlorpyrifos treatment are common to all organophosphate insecticides.
2. Chlorpyrifos can cause acute toxicity.
3. The inhibition of acetylcholinesterase activity is more persistent than that caused by the other organophosphates.
4. Also inhibits enzymes other than acetylcholinesterase.
5. Single dose of chlorpyrifos can cause delayed neurotoxicity.
6. In laboratory test chlorpyrifos has caused reproductive problems.

It is wellknown that the pesticides affect incidence on embryonic mortality at hatching and injection of pesticides during the period of organogenesis causes increase of embryonic mortality (Muto et al., 1992). In the present experiment the percentage of death is higher (90.90 %) in highest dose aT16 day of incubation. The percentage of death varies from 72.23 % to 81.97 %, 79.03 % to 82.69 % and 90.56 % to 90.90 % in three doses respectively. Dan and Medda (1990) also observed more or less same percentage of mortality in chick embryos treated with an organophosphorus insecticide. The present experiment also shows that increased mortality rate is related with the increasing concentration and increasing time of exposure. Along with this percentage of abnormal embryo are also gradually decreases with the increasing concentration
DISCUSSION

and time of exposure suggesting lethality of abnormal embryo caused by the treatment. The loss of neuromuscular control (Crawford et al., 1985) as well as the property of this particular pesticide in cholinesterase inhibition (Smithson and Sanders, 1978; Zaazau et al., 1973; Gupta et al., 1984; Datsov et al., 1985 and Cox, 1994) with a failure to maintain a series of changes in the body might be the cause of lethality.

Water losses from the embryo in excess of 10% of fresh mass resulted in embryonic dehydration and low hatching rate and (Davis and Acker man, 1987). The present study also shows significant water loss due to the treatment in all days except 12 days of incubation. This might be explained as a change in osmoregulation and thus the pesticide helps in retaining the moisture content of the embryo. Possible decreased rate of sodium transport can not be ruled out as Hoyt (1979) suggested water and ion content of the other compartments other than allantoic fluid was preserved in the embryo. The important feature of this study is also a decrease in mean body weight in comparison to control. It is also related with the dose and time. Loss of body weight suggest that chlorpyrifos has prominent effect on metabolic process and are fairly in agreement with the reports of Cavanagh (1964); Sacramento (1993) and Muto et al. (1992). Water loss from the cells and intercellular space is a factor for such reduction in body weight. Diminishing rate particularly for T1 and T3 in 16 day of incubation with lapse of time suggests a tendency to recover from the pesticidal effect, which may be due to the biodegradation of the pesticide. Simultaneously, the lengths of the body including brain and axial length have also been shortened with the loss of body weight, except on axial length for 12 and 16 days in T1, which is not significant. The average body length of the embryo due to the treatment of pesticide is reduced by 19.63 % to 69.64 % which is statistically different from that of control. Brain length is also reduced by 2.65 % to 77.37 % over control. The inhibition of growth might be a consequence of reduction in length. The ratio of neck and trunk length reveals the more drastic shortening of the neck region. The notochord is actually involved in elongation of the embryonic axis. Notochordal folding (Garrison, 1984; Garrison and Wyttenbach, 1985) and subsequent failure of the notochord to function effectively in axial elongation are the prime cause for shortening of the neck. Severe contraction of the axial musculature due to neuromuscular blocking agents (Meiniel, 1981; Meneely and Wyttenbach, 1989) which favour membrane depolarization induces the formation of short neck.
DISCUSSION

It has been reported that malformations of the embryo are the results of the treatment of the pesticide in early stage of embryonic development (Romanoff, 1972). Reports of malformations like short neck, muscular hypoplasia, parrot beak micromelia, abnormal feathering, microphthalmia, and anophthalmia are available due to pesticide (Landauer, 1975; Moscioni et al., 1977; Schom and Abbott, 1977; Schom et al., 1973; 1979; Seifert and Casida 1978; Dan et al., 1995 and Sahu and Ghatak, 2001) in some avian species. The other reports of malformations like cervical lordosis, scoliosis and anophthalmia are also available in quail and pheasant embryos (Varnagy et al., 1981a, b; 1982). The severity and incidence of course differs among species. Present study of pesticidal effects in producing anomalies like abnormal feathering, microphthalmia, deformed beak, exposure of the brain, reduced development in size deserve mentioning. Inhibition of kynurenine formamidase and subsequent suppression of nicotinamide adenine dinucleotide (NAD) levels in the embryo (Procter and Casida, 1975; Procter et al., 1976; Moscioni et al., 1977 and Kitos et al., 1981) or due to the disturbance of the cholinergic nervous system (Landauer, 1975; Meiniei, 1976) may results in the abnormalities of the embryo.

Organophosphorus pesticide has been shown to produce unexpected effects in producing disturbances in the blood picture. The results of the present haematological study justify the statement that chlorpyrifos has also exhibit some degree of relation with the intensity and direction of change. The change that is considerable reduction in the number of erythrocytes, haemoglobin level and packed cell volume agree with the result of studies by a large number of authors using organophosphorus compounds (Ali and Shakun, 1981; Gromysz- Kalkowska et al., 1981; Szubartowska, 1983; Gromysz- Kalkowska and Szubartowska, 1986; al- Qarawi et al., 1999; Khalaf- Allah, 1999; Szubartowska and Gromysz- Kalkowska, 1992). The reduction in the number of erythrocytes in chlorpyrifos intoxication may be the result of haemorrhages and clotting. Extensive haemorrhaging to the pulmonary parenchyma or veiling clots in the arterioles as suggested by Zilolo et al., (1973) due to the pesticide intoxication and also haemolytic action (Gromysz- Kalkowska et al., 1981) of organophosphorus pesticides also deserve mention. The present study also shows an increase in mean corpuscular volume in all doses except in T2 and T3 for 14 days of incubation, suggesting an intensified compensating activity of the haematopoietic system in response to the haemolytic action of chlorpyrifos. The results are in full agreement with the study of Gromysz- Kalkowska et al., (1981 and 1984) on quails intoxicated with organophosphate pesticide. The fall of erythrocyte count and haemoglobin level during different
developmental period due to chlorpyrifos intoxication with a simultaneous tendency to fall of the
haematocrit value may be related with the intensive decrease of reticulocyte count at this time.

Davidsohn and Henry (1979) indicated that MCH and MCV are correlated in magnitude in
mammals suffering from various anemias. In Japanese quail these two parameters change
synchronously after haemorrhage. However, both MCH and MCHC decreased due to the
chlorpyrifos exposure in the present study. Though the present study does not cover the
percentage of reticulocyte, it is presumed that decreased rate of reticulocyte may have contributed
to non elevation of MCH and MCHC.

Present experiment clearly indicates that exposure of embryo to pesticide induces a
marked chemically specific leukocyte response. The effect of chlorpyrifos on leukocyte in
developing chick is a lymphocyte leukocytosis with neutrophilaenia. It would seem the decrease in
total leukocyte count and also changes in leukogram should be attributed to the effect of
chlorpyrifos as a chemical stressor. This can be inferred from the fact that organophosphorus
compounds activate the pituitary-adrenal axis (Vartic et al., 1972; Brzezinski, 1973; Wojcik,
1975).

In leukocytes, a considerable increase in the number of lymphocyte and a decrease in the
number of neutrophil except in T3 of 14 and 16 days of incubation are observed during different
period of epigenesis due to the pesticide intoxication. The number of eosinophil is also increased
excepting on 16 days of development, which shows a decreased value over control. It would seem
that the main cause of high susceptibility to intoxication is due to the disturbances in the response
of the organism to stress in the form of the pesticide.

Changes in the number of monocytes in the present study are on the increasing side. The
increase in the number of monocytes in all doses during different period of development points out
the mobilizing activity of the reticuloendothelial system. This assumption can be confirmed by the
results of the high increase rate of phosphatase activity in the present study. Present data also
confirms the result of Dormfest et al., (1983) who observed an increased number of monocytes in
rats intoxicated with phenylhydrazine (PHZ). The rise in monocytes may be an indication of
enhanced activity of reticuloendothelial system. With the increase of monocyte, basophil number is
also increased in the present study, during different period of incubation in all doses of treatment
with the pesticide. This indicates that pesticide caused a general granulocytopoiesis.
Collectively this leukocyte data suggest that chlorpyrifos can be used to stimulate lymphocytopoiesis in developing chick embryo. The changes observed in the blood during intoxication by pesticides within a short time after their administration may lead to the conclusion that the developing chick embryo are susceptible to intoxication.

A significant reduction in the quantity of serum protein is observed in the present study. The reduction may be associated with the observed haematological responses and attributed specifically to the toxic effect of chlorpyrifos on developing embryo. The result of present study confirms the report of Gomes et al., (1999).

Serum acid phosphatase activity of embryo treated with chlorpyriphos has been significantly increased over the control. Again, the data shows a dose dependent effect, i.e. an increase in dose produced an increase in enzymatic activity. This indicates a possible in vivo labilization of lysosomal membranes with the release of acid phosphatase (Vijayendra Babu and Vasudev, 1984). This finding is also supported by the fact that the pesticide chlorpyrifos has caused liver damage available in the histopathological findings of the present study, which in turn lead to the release of acid phosphatase. The activities of phosphatase enzymes are of significance in certain pathological conditions (Oser, 1971). Shaikila et al. (1993) observed increased level of acid phosphatase activity in the liver of fish exposed to pesticide. Increased acid phosphatase activity in the gills of exposed fish was observed by Borah and Yadav (1996). Abou-Donia (1978) and Abou-Donia and Graham (1978) also observed increased acid phosphatase activity in hens treated with an organophosphate pesticide.

Alkaline phosphatase activity registered a rise in serum in chick embryo following exposure to chlorpyrifos during different developmental period except on 12 day of incubation, which is not significant. A great increase in the activity of alkaline phosphatase is due to the strong toxic effect of the pesticide, which may be the result of increased permeability of plasma membrane (Bak et al., 1976). The increased activity could be attributed to a hyperglycemic effect of the pesticide (Latner, 1975) as a result of glycogenolysis and consequent increase of phosphatase activity to meet energy requirements in order to counter the stress.

The serum transaminases, particularly SGOT showed an increase activity by reaching maximum value at the end of the treated periods. The recorded GOT values are also higher than GPT in the present study. Ahmed (1986) also observed similar type of activity in rat brain due to the exposure of dieldrin. The present study aims to throw light on the effect of the chlorpyrifos on
the activity of the transaminase (GOT and GPT) during the experimental period. However, in low
dose treatment GOT activity remains low than control. Serum enzymes are considered as a test of
liver function (Campbell and Ofurum, 1986). So the activity of the enzymes may be a hepatotoxic
effect and is related with the intensity of cellular damage. Present study of the transaminase
activity is very interesting, since it shows both increase and decreased activity and sometimes
there is no tress of GOT and GPT in the T2 dose. Stress condition helps to induce the release of
corticosteroid and adrenaline from adrenal gland, but the process of transamination thus increases.
Therefore, the increase of transaminase might have some correlation with the cellular damage and
or stress condition.

These experiments clearly indicate that direct injection of chlorpyrifos at different doses
into the fertile chicken eggs after 120 hours of incubation induces a marked effect on the blood
protein system. However, different doses have different response to the individual blood protein
parameter. Particularly in haemoglobin fractions, the pesticide brings about reduction maximally
35% by loss of three protein types in 12 day embryo at highest doses, resulting a dose specific
response. Similar dose related response in reduction of plasma protein types are also observed to
a tune of 70.37% in the same developmental period with the higher doses, while the same higher
dose treatment did not cause maximum reduction in the transferrin pattern. On the other hand, the
embryos exposed to lower dose of pesticide the rate of D, E fraction for 12 and 14 day, B, C
fractions for 16 day vary significantly compared to controls so far as haemoglobin proteins are
concerned. Similarly, to this protein the rate of fraction C and D for 12 and 14 day respectively and
B and F for 16 day vary significantly showing higher value than the controls in T2 group, but in the
highest dose treatment it appears that rate of fraction F and B are significantly higher in 12-day
and 14 day embryo respectively, whereas in 16 day embryo fractions B, C and D are significantly
higher. Fractions A & B and fractions E & F are only present in controls in case of 12-day and 14-
day embryo respectively in the T3 group.

From these experimentally obtained results, it appears that the level of haemoglobin of
developing embryo varies with the effect of pesticides. Haemoglobin biosynthesis seems to occur
in maturing erythroid cells of chick (Colro, 1975) through haemosome formation (Brunner et al.,
1983 a, b) and the rate of haemoglobin synthesis is enhanced in peripheral blood (Brunner et al.,
1991). The pesticide thus might effect the synthesis mechanism in erythroid cells. The results
obtained at different concentrations suggest some effects of the pesticides on blood physiology and their consequences may likely affect the haemoglobin pattern in the electrophoretograms.

The present study of the plasma protein in the developing embryo exposed to different concentration of pesticide could be generalized as follows:

a) The protein bands in treated species show a variation with regard to the number and molecular weight and there is a maximum reduction of 70.37% in the number at highest dose, particularly in 14-day embryo, compared to controls.

b) Both high and low dose treated groups showed a decrease in the rate of different protein fractions during different period of development.

c) If the results obtained at low concentration (T1) are compared to controls, it is possible to see

12 day:
Fraction B and C : The rate of fraction is significantly higher.
Fraction D : The differences are not significant.

14 day:
Fraction D and E : The rate is also significantly higher.
Fraction F to H : Only present in controls.

16 day:
Fraction A and B : Shows significantly higher rate.
Fraction F to H : Only present in controls.

d) In T2, it appears that the rate is distinctly high for fraction B, C and C than that of control during 12 and 14 day respectively. Fraction G, H and F to H are not detected in this period. On 16-day fraction H appear only in pesticide exposed group.

e) In highest dose treatment it may be observed that:

12 day:
Fraction A : Only present in controls.
Fraction C : Appears in significantly higher rate.

14 day:
Fractions A, B and D : Show significant high rate.
Fraction F to H : Only present in controls.

16 day:
Fraction H : Only present in controls.
DISCUSSION

Fraction C and D

As a general rule, the presence of more fraction and general reduction during developmental period are noted. It seems that genotoxic potential of the pesticide might have impaired the genetic loci controlling mechanism of plasma protein.

The different types of transferrin examined in this study with regard to their mobility on SDS- PAGE are different. The maximum number reduction of 45% occurred in the highest dose treated groups of 16 day embryo. The results could be summarised as follows:

- an increases in the rate of E, F fractions and B, C fractions in 12 day of both T1 and T2, whereas in T3 the rate of fraction D is significantly higher. Fractions A, B and J are not found in the highest dose treatment. Fraction G is available in control for 12 day.

- at lower dose the fraction B, C decrease their rate, while an increase in the rate of D, E fractions are observed in the same 14 day. Significant decrease of B fraction is observed in the same day of T2, but in the highest dose fractions D, E and F appear in significantly high rate. It seems that the pesticide operates on the protein synthesis system by stopping the synthesis of B fraction in T2.

- H and I fractions appear only in embryo of 16 day, but are not detected in controls. Loss of E, F and J fractions are available in 16 day embryo of T2 group, while in T3 groups fractions A, B and C are only present in control.

The data for molecular weight so far available in the controls are within the range and can be comparable with the data observed for serum transferrin (Baldwin, 1990; Aisen and Listowsky, 1980). The protein types are distributed in the gel as a separate multiple bands. This data is consistent with the report of Chung and McKenzie, (1985) in which several bands were found for horse transferrin. Genetic polymorphism in the transferrin loci (Tsuji et al., 1984; Chung and McKenzie, 1985) is the probable cause of multiple banding patterns of the transferrin. The electrophoretic patterns are changed due to the action of pesticide, which reflects in differences in polypeptide sequence between transferrin types.

The present study of the serum protein fraction in the developing embryo exposed to different concentration of pesticide could be accounted as follows:

The protein bands in treated groups showed a variation with regard to the number and molecular weight and there was:
DISCUSSION

a) a reduction of maximum 56.25% in the number of fractions in highest dose (T3) in 14 day embryo compared to control.

b) both high and low treated groups showed a decrease in the rate of different protein fractions during different period of development.

c) If the results obtained at low concentrations (T1) are compared to controls it is possible to see:

12 day:
Fraction B : The rate of fraction is significantly higher.
Fraction G : Only present in controls.

14 day:
Fraction B and D : The rates are also significantly higher.
Fraction F : Only present in controls.

16 day:
Fraction C : The rate is higher.
Fraction E : Appears significantly.

d) In second dose (T2) treatment it has been observed that:

12 day:
Fraction B : The rate of fraction is higher.
Fraction D : Lower rate of fraction.
Fractions F & G : Only present in control.

14 day:
Fraction B : The rate of fraction is slightly higher.

16 day:
Fractions E & G : Only present in treated groups.

e) In highest (T3) dose treatment it appears that:

12 day:
There are distinctly present 4 fractions as C to F.
Fractions A and B is totally absent.
Fractions D and E show high rate of fractions.

Whereas, 14 day shows:
Complete loss of low molecular weight proteins present in the fractions E and F.
Distinct high rate of fractions are observed in fractions A, B and C.
Likewise, 16 day embryo shows:
High rate of fractions for A and C.
Fraction E is not detected.

As a general practice the presence of more fractions and general reduction during developmental period are noted. It also appears that genotoxic potential of the pesticide might have impaired the genetic loci controlling mechanism of plasma protein.

It has been known that effects of insecticide in animals cause toxicity and eventually may leads to death. The death is possibly due to damage and resultant dysfunction of vital organs. The prominent effect of the toxicant in the present study is observed in the vital organs of the embryo. The prominent effects of toxicant in the present study are observed in the vital organs of the embryo.

In the present study section of liver shows abnormal features due to the treatment of chlorpyrifos. Treatment with the higher dose shows severe reactions in the liver. Remarkable observation in the liver is the absence of nuclei in the cord cells. This may be due to the strong toxic effect of this toxicant for which the cell membrane might have ruptured or the nucleus degenerated, causing a cell vacuolated. Histopathological changes such as pyknotic nuclei or the degeneration might be the result of deranged state of metabolism (Dalela et al., 1978). Insecticides are known to cause pathological changes of hepatic parenchyma (Earl et al., 1971; Uppal and Ahmed, 1977). The present investigation of liver damage is also in conformity of the work performed by Tomar et al., (1995).

The present work also shows also abnormalities in the structure of kidney due to the treatment. The less number of glomerulus as well as tubular degeneration in the form of loss of brush border in proximal convoluted tubules are the major reactions in the sections of kidney. Thus exposed to the pesticide reveals severe intensity of histopathological changes. The results are in conformity with the findings of Ernest (1980), which may be a result of deranged state of metabolism (Dalela et al., 1978).

Though the treated embryo apparently looks normal, some biochemical defects may be there, which ultimately leads to abnormality, in proper functioning of the individuals.