

# **SYNOPSIS (SUMMARY) OF THE THESIS**

## **TERATOLOGICAL EVALUATION OF FORMULATED DICOFOL AND DELTAMETHRIN IN CHICK EMBRYO**

Submitted for the award of the degree of  
**Doctor of Philosophy**

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## **INTRODUCTION**

Dicofol [2,2,2-trichloro-1,1-bis (4-chlorophenyl-) ethanol] an organochlorine pesticide, is very effective against red spider mite and has been approved for the use on agricultural crops such as apples, cotton and citrus cultivates, tomatoes, walnuts, mint, cucurbits, beans and peppers etc. and also non-residential lawns. It was first introduced in 1957 by US-based multinational company named as Rohm and Haas. Today, it is manufactured in countries like India, Spain and Israel and sold under a number of trade names such as Kelthane, Colonel, Decofol and Acarin etc. (Extoxnet, 1996).

Deltamethrin, [(S)-a-cyano-3-phenoxybenzyl-(1R)-cis-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carbo-xylate] is a type II synthetic pyrethroid (manmade analogues of naturally occurring pyrethrins found in the flowers of *Chrysanthemum cinerariaefolium*) which was synthesized in 1974 and since then, it has been applied for a range of commercial crops and recreational uses and by extension controls a variety of pests (Extoxnet, 1995). It is used as an active ingredient in the number of commercial insecticides such as Butoflin, Cislin, Crackdown, Decis and K-Otek and mostly used for growing cotton. It is primarily applied to commercial crops such as corn, coffee, hops, artichokes, maize, cereals, fruits and stored products (WHO, 1990).

The current study was undertaken to investigate the teratogenic effect of commercial formulations of insecticide dicofol (Colonel-S) and deltamethrin (Decis) in the developing chick embryo. Chick embryo has been proved to be a promising animal model for preliminary screening of various toxic chemicals including pesticides.

## **REVIEW OF LITERATURE**

Analytical screening of wildlife tissue samples for organochlorine chemicals rarely includes dicofol, and this may explain why, compared with other organochlorine, the relative hazard of dicofol to wildlife populations is poorly known (Clark *et al.*, 1995). Dicofol has been reported to be 'highly' to 'very highly' toxic to a range of aquatic organisms, including fish,

invertebrates and estuarine/marine organisms (EPA, 1998). In birds, dietary concentrations of dicofol between 1 to 10 mg/g (wet weight) fed to captive adult females found to cause eggshell thinning, reduced hatching success, or reduced fertility in eastern screech-owls (*Otus asio*) (Clark *et al.*, 1995) and American kestrels (*Falco spar-veruis*) (MacLellan *et al.*, 1996).

Deltamethrin belongs to the most recent group (fourth generation) of synthetic pyrethroids (Datta and Kaviraj, 2003) and because the rate of its detoxification in mammal is very high than in insects (Patro *et al.*, 1997), it is considered quite safe to mammals. But earlier studies with deltamethrin, provided evidences to suggest that this pyrethroid insecticide have various degrees of toxicological impacts in different experimental animals such as fish (Datta and Kaviraj, 2003; Koprucu and Aydin, 2004; Ural and Saglam, 2005; Koprucu *et al.*, 2006; Velisek *et al.*, 2006; Sharma and Ansari, 2011; Amin and Hashem, 2012), Japanese quail (Martin, 1990), freshwater mussel (Koprucu and Seker, 2008), *Daphnia magna* (Xiu *et al.*, 1989) and South American toad (Salibian, 1992) at the concentrations much lower than those recommended for its safer use.

The avian egg and its developing embryo have been widely used to test toxicity and mechanism of teratogenesis for a longer period of time than any of the other assayed considered so far (Collin, 1987). Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environment-friendly chemical plant protection techniques (Keseru *et al.*, 2004). Daresté (1877), Ancel (1950), Ridgeway and Karnofsky (1952) and Karnofsky (1955) were among the pioneers who referred to study on chick embryo. Recently, several studies have been conducted on mechanism of teratogenesis of various pesticides such as cypermethrin (Anwar, 2003), dimethoate (Alhifi *et al.*, 2004), flufenoxuron (Rachid *et al.*, 2008), bendiocarb (Petrovova *et al.*, 2010), chlorpyrifos and cypermethrin (Uggini *et al.*, 2010), endosulfan (Mobarak and Al-Asmari, 2011) and lufenuron (Pinakin *et al.*, 2011) to demonstrate the detrimental effects on the development of chick embryo.

## **JUSTIFICATION**

As the study of teratogenicity or developmental toxicity cannot be conducted during embryogenesis of humans, a wide variety of laboratory animals have been employed in detecting important toxic and teratogenic properties of chemical substances and, for estimating risk to human and environmental health.

The present study will help to know the possible adverse effects of dicofol and deltamethrin containing insecticide formulations on developing embryos of *Gallus domesticus*, as a model, which could lend support to the idea of the embryotoxicity and teratogenicity of these pesticides on developing foetus of other animals and also the generalized harmfulness of these insecticides in the environment. And this knowledge could later be used in making a judicious or harmonious use of these popular insecticides. Findings from this work will provide additional information and knowledge in the field of developmental toxicology.

## **OBJECTIVES**

The present teratological study has been planned on developing chick embryo with the following objectives-

1. To observe the congenital anomalies (morphological and skeletal), if any, in developing chick embryo exposed with commercial formulations of insecticide dicofol and deltamethrin.
2. To assess the effect of these insecticides on certain biochemical parameters of chick embryo.
3. To determine the effect on activity of brain acetylcholinesterase enzyme of chick embryo.
4. To know the hepatotoxic (pathological and biochemical) changes in the liver of developing chick embryo exposed to above mentioned insecticides.

## **MATERIALS AND METHODS**

### **Insecticides**

For the present study, dicofol (18.5 % EC) with commercial name COLONEL-S, manufactured by Indofil Chemicals Company, Mumbai, India and deltamethrin (2.8% EC) with commercial name Decis<sup>®</sup>, manufactured by Bayer CropScience Limited, Gujarat, India were used.

### **Experimental Subject**

Fertilized eggs of BV 300 breed were collected, cleaned and kept in an incubator with capabilities of maintaining and monitoring temperature, humidity and turning the eggs periodically. The temperature in the incubator was maintained at  $38 \pm 0.5^{\circ}\text{C}$  and the relative humidity was kept between 70-80%.

### **Experimental design**

The eggs were exposed to different doses of each insecticide or vehicle by immersion technique (dipping for 1 hour at  $37^{\circ}\text{C}$  temperature). The used dose concentrations of insecticides were 250, 500 and  $1000 \text{ mg L}^{-1}$  for dicofol and 12.5, 25 and  $50 \text{ mg L}^{-1}$  for deltamethrin which were based on the recommended dose ( $25 \text{ mg L}^{-1}$  of deltamethrin and  $500 \text{ mg L}^{-1}$  of dicofol) of each insecticide used for crop protection. There were two control groups, control group I (untreated eggs) and control groups II (eggs which were immersed in vehicle .i.e. distilled water). Thirty eggs were assigned for each treatment group. All the eggs were kept for incubation until the time of their sampling.

### **Experimental plans**

There were three sets of experiment based on exposure of eggs on different critical periods of chick embryogenesis -

1. A predefined number of unincubated fertilized eggs were obtained and exposed on day "0" of incubation with low, medium and high doses of each insecticide. All the eggs were kept for

incubation and candled daily. Infertile eggs were discarded. Chick embryos were sacrificed on embryonic day (ED) 4, 7 and 16 for their teratological study. On embryonic day 4 and 7, surviving chick embryos were examined for morphological malformations and biochemical estimations of their whole body, while examination of external teratological malformations, histopathological study (liver), biochemical studies (brain and liver) and skeleton preparations were performed on embryo taken out on 16<sup>th</sup> day of incubation.

2. Prior to dosage, fertilized eggs were placed in an incubator to initiate embryonic development. On 4<sup>th</sup> day of incubation, all the eggs were immersed in different suspensions of each insecticide. All eggs were kept for reincubation. Chick embryos were sacrificed on embryonic day 7, 10 and 16 for their teratological study. On embryonic day 7 and 10, surviving chick embryos were examined for morphological malformations and biochemical estimations of their whole body. Evaluation of external teratological malformations, histopathological study (liver), biochemical studies (brain and liver) and skeleton preparations were carried out on 16 day old embryos.

3. Fertilized eggs were incubated until embryonic day 7. On embryonic day 7, eggs were exposed to different doses of each insecticide; dicofol and deltamethrin. The eggs were returned to incubator until the time of sampling. Chick embryos were taken out from the eggs on 16<sup>th</sup> day of incubation for examination of external malformations, histopathological study (liver), biochemical studies (brain and liver) and skeleton preparations.

The two groups; control I and vehicle control (control II) with same number of fertilized eggs were kept for each experimental plan. Each plan was repeated in triplets.

### **Parameters**

Following parameters were studied for each experiment-

- **Teratological parameter**
  - ❖ Survival rate

- ❖ Number of malformed embryo
- ❖ Incidence of morphological malformation (head, beak, eye, neck, limb and lower body)
- ❖ Incidence of skeletal malformation (skull, vertebrae, ribs, sternum, upper limb and lower limb)
- **Biochemical parameters**
  - ❖ Protein content- Lowry *et al.* (1951)
  - ❖ Glycogen- Montgomery (1957).
  - ❖ Cholesterol- Liebermann-Burchard reaction (Henry and Henry, 1974).
  - ❖ DNA and RNA contents by the methods of Schneider (1957) using diphenylamine and orcinol reagents, respectively.
  - ❖ Reduced Glutathione (GSH) Moron *et al.* (1979).

#### **Activities of enzymes**

- ❖ Alkaline Phosphatase (ALP) and Acid phosphatase (ACP) by method of Kind and King (1954).
- ❖ Glutamate oxaloacetate transaminase and Glutamate pyruvate transaminase method of King (1965).
- ❖ Acetylcholinesterase (AChE) Ellman *et al.* (1961).
- Histopathological study of liver.
- Skeletal preparation of 16 day old chick embryo by double staining (Alizarin Red S and Alcian blue) method of McLeod (1980) for examination of skeletal abnormalities.

## RESULTS

### Experimental plan I

Exposure of fertilized eggs to dicofol and deltamethrin on “0” day of incubation resulted in dose dependent decrease of surviving embryos on both the embryonic day 4 and 7. But significant decrease in survivability rate was observed only on ED 4 in the group treated with high dose of deltamethrin. Similarly, mean body weight of embryos on ED 4 was decreased significantly at medium dose of dicofol and high dose of deltamethrin treatment, respectively. No effect was observed on mean body weight of 7 day old chick embryo. The significant number of abnormal survivors was obtained on embryonic day 4 from the group of eggs treated with medium and high dose of deltamethrin, while on ED 7 the percentage of abnormal surviving embryos were significant in the group treated with only high dose of deltamethrin. No significant effect was found on survivability of dicofol treated animals on either of embryonic days (4 and 7). On both the embryonic day 4 and 7, abnormal survivors exhibited number of external malformations such as general growth retardation, subcutaneous hemorrhage, microcephaly, exencephaly, anencephaly, microphthalmia, anophthalmia, exophthalmia, defects in beak and neck. The numbers of embryo with these anomalies were increased with increasing concentration of each of the insecticide.

On embryonic day 4, total embryonic glycogen content showed significant decrease at 1000 mg L<sup>-1</sup> of dicofol and 25 and 50 mg L<sup>-1</sup> of deltamethrin treatment. There was no significant effect on total protein, cholesterol, and DNA and RNA contents of whole embryo after exposure to different concentrations of these insecticides. Among enzymes, only the activity of ALP showed highly significant elevation in embryos treated with high dose of dicofol. There was no effect on ACP, GPT and GOT activities. On embryonic day 7, only high dose of dicofol treatment resulted in significant depletion of total protein content, while embryonic glycogen content was decreased markedly in the embryos treated with 500 mg L<sup>-1</sup> of dicofol and with 25 and 50 mg L<sup>-1</sup> of deltamethrin concentrations. Total RNA content of 7 day old embryo showed



significant decrease at all the three doses of deltamethrin treatments. Also, medium dose of dicofol treatment resulted in significant elevation of embryonic ALP activity. There was no effect on other enzyme activities.

The embryotoxic effects of insecticides; dicofol and deltamethrin on ED 16 resulted in significant decrease of surviving embryos at 250 and 500 mg L<sup>-1</sup> of dicofol and 25 and 50 mg L<sup>-1</sup> of deltamethrin treatment. Mean body weight of embryos decreased significantly at all the three doses of deltamethrin treatment. No effect on embryonic body weight was observed with dicofol treatment. The groups treated with medium and high dose of deltamethrin had significant number of abnormal survivors displaying lower body malformations (general growth retardation, ectopia viscera, subcutaneous hemorrhage and hematoma) and eye anomalies (anophthalmia and exophthalmia) at maximum rates. Double stained skeletal elements of embryo treated with these insecticides showed various skeletal malformations such as poor ossification of bones, scoliosis, lordiosis, CRS (Caudal Regression Syndrome), defects in skull, ribs and lower limb.

Estimation of biochemical constituents of liver of 16 day old embryo showed that total glycogen content decreased significantly with medium dose of dicofol and with high dose of both the insecticides. No effect on total protein and cholesterol contents were found. The GSH content showed marked decrease in liver of animals treated with high dose of dicofol and all doses of deltamethrin. Liver ALP activity was elevated only in the embryos treated with medium and high dose of deltamethrin. Treatment with medium and high doses of dicofol showed marked depletion in the liver GPT activity. No effects were found on ACP and GOT activities. The activity of brain AChE of insecticide treated animals also remained unaffected.

The examination of liver sections of insecticide treated embryos showed dose dependent increase of pathological lesions such as degeneration and necrosis of hepatocytes with darkly stained pycnotic nuclei, vacuolization, enlarged blood sinusoids, dense leukocyte infiltrations and congestion and/or dilation of central vein in common which ultimately resulted in loss of

radial arrangement of hepatic cord. Activation of Kupffer cells was also observed in few animals treated with medium dose of deltamethrin.

## **Experimental plan II**

The viabilities of embryos were severely affected by treating group of eggs with different doses of each insecticide on 4<sup>th</sup> day of incubation. Decrease in number of surviving embryos was observed on ED 7 and 10 after treating the eggs with low dose of dicofol and medium dose of both the insecticides. Surviving success of embryo was also affected severely with high dose of deltamethrin treatment. On embryonic day 7, mean body weight of embryos was decreased only at high dose of deltamethrin treatment. No effect was observed on body weight of 10 day old insecticide treated embryo. A remarkable dose dependent increase in percentage of abnormal survivors was observed on both the embryonic day (7 and 10) after dicofol treatment. On ED 7, deltamethrin treatment showed marked number of abnormal survivors at its medium and high dose, whereas on ED 10, increase in number of abnormal survivors was highly significant at all of its three dose levels. Most of external malformations observed in 7 and 10 day old embryos consisted of lower body, eye and head anomalies.

Biochemical estimation of 7 day old whole embryo showed that only deltamethrin treatment (medium and high dose) resulted in significant depletion of total embryonic protein content whereas, no effect was observed with dicofol treatment. Total cholesterol and glycogen contents of embryos remained unchanged with either of these insecticide treatments. Marked decrease in total DNA content was observed in dicofol treated animals, whereas a marked elevation in total RNA content was observed in those embryos treated with both dicofol (medium dose) as well as deltamethrin (medium and high dose). The embryonic ALP activity was increased significantly with 1000 mg L<sup>-1</sup> of dicofol and 25 and 50 mg L<sup>-1</sup> of deltamethrin treatment, whereas ACP activity of embryos was decreased only with deltamethrin (medium and high dose) treatment. Further, only dicofol exposure at its medium dose resulted in marked increase of GPT activity while, no effect was found on GOT activity of either of insecticide

treated animals. On embryonic day 10, both the dicofol and deltamethrin treatments resulted in highly significant depletion of total protein and glycogen content of embryos at their higher doses. Total cholesterol content and RNA contents were increased with high dose of each insecticide treatment, whereas DNA content remained unchanged. The embryonic ALP activity was increased significantly at high dose of each insecticide treatment, whereas the GPT activity showed marked elevation only at high dose of deltamethrin treatment. No effects on ACP and GOT activities were observed.

Sampling on embryonic day 16 showed that significant decrease in survivability was exhibited by the embryos treated with high dose of deltamethrin, whereas no effect was found with dicofol treatment. Similarly, mean body weights of embryo were decreased only with medium and high dose of deltamethrin treatments. Dicofol (medium dose) and deltamethrin (medium and high dose) treatment revealed significant percentage of abnormal living embryos which showed various external malformations such as general growth retardation, subcutaneous hemorrhage, ectopia viscera, exencephaly, anophthalmia, beak, neck and limb anomalies. Common skeleton malformations observed in double stained skeletal element of these embryos were of vertebrae and ribs such as poor ossification, displaced and fused bones, lordiosis and scoliosis of spine.

Biochemical studies on the liver of embryos showed that protein content was decreased with both dicofol (medium and high dose) and deltamethrin (high dose) treatment whereas, no effect was found on total cholesterol and glycogen content. The total GSH content got depleted at all the three doses of each insecticide. Among enzymes, ALP activity was increased only at high dose of both the insecticides, whereas GPT activity was increased at 500 and 1000 mg L<sup>-1</sup> of dicofol and 50 mg L<sup>-1</sup> of deltamethrin treatment. No effects were found on ACP and GOT activities of the liver of these insecticide treated embryos. Brain AChE activity also did not get altered after either of the insecticide treatment.

Hepatic tissues of chick embryo treated with dicofol and deltamethrin showed considerable changes such as degeneration of hepatocytes characterized by vacuoles and enlarged blood sinusoids around congested and/or dilated central vein, fatty changes and dense leucocyte infiltration which leads to disturbance in hepatic architecture. These pathological lesions were observed in a non-consistent manner in liver sections of dicofol treated animal, whereas in case of deltamethrin these changes were seen in a dose dependent manner.

### **Experimental plan III**

Eggs exposed on 7<sup>th</sup> day of incubation to different concentrations of dicofol and deltamethrin were opened on embryonic day 16 for their teratological study. The application of only high dose of dicofol resulted in significant decrease of number of surviving embryos and their mean body weight. There was no effect on surviving success and body weight after deltamethrin treatment when compared with control values. The eggs treated with all the three doses of dicofol showed marked increase in number of abnormal survivors. Whereas, deltamethrin exposed at only medium and high dose levels showed significant number of abnormal survivors. Most of the abnormal survivors from insecticide treated group exhibited lower body malformations such as ectopia viscera and hematomas, followed by limb and eye defects. Further, double stained skeletal elements of insecticide treated embryos showed certain abnormalities in their axial and appendicular skeletons such as poor ossification, synostosis, lordiosis, CRS, ribs and skull malformations which were found as insignificant.

Estimation of biochemical contents showed that there were no effects on total protein, cholesterol, glycogen and GSH contents of liver of deltamethrin treated embryos. Total protein content was decreased significantly at medium and high dose of dicofol exposure, while total GSH content was depleted only in those embryos treated with medium dose of dicofol. Total cholesterol and glycogen contents were remained unaffected with dicofol treatment. The liver ALP activity was increased in those embryos treated with 1000 mg L<sup>-1</sup> of dicofol and 25 and 50 mg L<sup>-1</sup> of deltamethrin. Elevations in GPT activity were observed in the liver of embryos treated

with high dose of each insecticide. Activities of ACP and GOT remained unchanged. Dicofol and deltamethrin did not cause any significant change in brain AChE activity of chick embryo.

Sections through the liver of chick embryo treated with insecticides showed degenerative changes in hepatocytes with pycnotic nuclei, fatty infiltrations, congestion and dilation of central vein, cytoplasmic vacuolations and destructed blood sinusoids filled with large number of leucocyte cells. These lesions were severe in embryo treated with medium and high dose of deltamethrin. Mild activations of Kupffer cell was also observed in the liver of embryos treated with medium and high dose of dicofol and only with medium dose of deltamethrin.

## **CONCLUSION**

Following conclusions can be drawn from results of the present study:

- Both the insecticide formulations were found to be embryotoxic as their exposure resulted in decrease of survivability success and increase of various congenital malformations in developing embryos with marked depletion in their mean body weight.
- On the basis of exposure on critical periods of embryogenesis, the relative embryotoxicity of these insecticides were more when developing chick embryo were exposed on 4<sup>th</sup> day of incubation than on “0” or 7<sup>th</sup> day of incubation.
- The potent hazards caused by the dicofol treatment were more at the recommended dose. In case of deltamethrin, recommended dose and high dose were found to be toxic.
- The highly significant increase in the number of abnormal surviving embryos obtained from eggs treated on 4<sup>th</sup> day of incubation (critical period in which organogenesis starts) indicated teratogenic susceptibility of developing embryos toward these insecticide formulations.
- Abnormal survivors exhibited various types of external and skeletal malformations which may be directly proportional to dose concentrations of each insecticide.

- The teratogenic propensity of these insecticides were also confirmed by observing dose dependent alterations in biochemical constituents of whole embryo at earlier stages of their development and/or in liver of 16 day old chick embryo.
- The prominent dose dependent pathological lesions observed in liver of 16 day old chick embryo due to toxic action of insecticides were degeneration and necrosis of hepatocytes with vacuolization and enlarged blood sinusoids, leucocyte infiltrations, hepatocytes with darkly stained pycnotic nuclei and congestion of central vein. All these changes resulted in severe damage to architecture of liver.
- The present study further support the concept that dicofol and deltamethrin are non acetylcholinesterase inhibitors as the activity of brain AChE enzyme of embryo remained unaffected with their commercial formulations treated on three different critical periods.

## **LIST OF PUBLICATIONS**

### **International Journal**

1. Nitu, K., Shahani, L., Taparia N. and Bhatnagar P. (2012) Teratogenic and biochemical effects of a formulation containing Dicofol in the chick embryo. *Toxicological & Environmental Chemistry* **94**: 1411-1421.
2. Bhaskar, N., Shahani, L., Taparia N. and Bhatnagar P. (2012) Effect of deltamethrin containing formulation on developing chick embryo: morphological and skeletal changes. *International Journal of Toxicological and Pharmacological Research* **4(4)**: 81-87.
3. Bhaskar, N. and Shahani L. (2013) Effects of commercial formulations of dicofol (Colonel-S) and deltamethrin (Decis<sup>®</sup>) on chick embryo survivability and acetylcholinesterase activity of brain. *International Journal of Zoology and Research* **3(2)**:19-26.
4. Bhaskar, N., Shahani, L., Taparia N. and Bhatnagar P. (2012) Toxicological implications of a commercial formulation of deltamethrin (Decis<sup>®</sup>) in developing chick embryo. *Toxicology and Industrial Health* (In communication).

## **Book Chapters**

1. Nitu, K., Shahani, L., Taparia N. and Bhatnagar P. (2012) Teratological study of formulations of two insecticides; deltamethrin and dicofol in the chick embryo, In *Microbial, Plant & Animal Research* (Edited by Gaur, R.K., K.P. Sharma and R.S. Chundawat), pp. 97-104, Nova Science Publishers Hauppauge, New York.
2. Shahani, L., Nitu, K., Taparia N. and Bhatnagar P. (2012) Effect of insecticide formulation of deltamethrin (decis<sup>®</sup>) on gross morphology of embryo of *Gallus domesticus*, In *Microbial, Plant & Animal Research* (Edited by Gaur, R.K., K.P. Sharma and R.S. Chundawat), pp. 125-132, Nova Science Publishers Hauppauge, New York.

## **International conference articles**

1. Nitu, K., Shahani, L. and Bhatnagar P. (2011) Teratogenic effects of deltamethrin in chick embryo. In: *International Conference on Futuristic Science and Technology in Frontier Areas and 2<sup>nd</sup> Annual Conference of Indian JSPS Alumini Association*. Aug 5-6; Trivandrum. Kerela. pp-83.
2. Nitu, K., Shahani, L., Taparia N. and Bhatnagar P. (2012) Teratogenicity study of two insecticide formulations; Decis and Colonel-S in the chick embryo. In: *International conference on Microbial, Plant & Animal Research*. March 29-31; Lakshmangarh, Sikar (Rajasthan). New Delhi. pp-86.
3. Shahani, L., Nitu, K., Taparia N. and Bhatnagar P. (2012) Effect of insecticide formulation of deltamethrin (Decis<sup>®</sup>) on gross morphology of embryo of *Gallus domesticus*. In: *International conference on Microbial, Plant & Animal Research*. March 29-31; Lakshmangarh, Sikar (Rajasthan). New Delhi. pp-40.
4. Shahani, L., Nitu, K., Taparia N. and Mathur, P. (2011) Toxicity of synthetic pyrethroid deltamethrin using chick as a model. In: *6<sup>th</sup> Biyani International Conference on Innovations in the Latest Healthcare issues*. Sep 19-21, Jaipur. Rajasthan. pp-80.

## **National Conference articles**

- Nitu, K., Shahani, L., Bhatnagar, P., Mathur, P. and Taparia N. (2011) Toxic effect of dicofol in chick embryo. In: *National Conference on Contemporary trends in Biological and Pharmaceutical Research*. March 12-13 Pilani, Rajasthan. pp-28.
- Nitu, K., Shahani, L., Taparia N. and Bhatnagar P. (2011) Dicofol, an organochlorine pesticide induces teratological and biochemical changes in embryos of *Gallus domesticus*. In: *XXXI Annual Conference of Society of Toxicology (STOX), India & International*

- Symposium on Current Trends in Environmental Toxicology*, Dec 22-24. Jaipur, Rajasthan. pp-155.
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  - Nitu, K., Shahani, L., Taparia N. and Bhatnagar P. (2012) The chick embryo: a non-mammalian animal model for screening of nanoparticles toxicity test. In: *National Conference on Materials for Advanced Technologies*. Feb 27-19. Gwalior, Madhya Pradesh. pp-86.
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## **CONTENT OF THESIS**

### **Chapter 1 Introduction**

A detailed description of the selected insecticides; dicofol and deltamethrin and justification of taking chick embryo as an experimental model is given in this chapter.

### **Chapter 2 Review of Literature**

Information regarding toxic effect of selected insecticides on different animals and a review on teratological studies done by other workers on various xenobiotics using chick embryo is detailed in this chapter.

### **Chapter 3 Materials and Methods**

Experimental design, plans, procedure and techniques employed for obtaining appropriate results are described in present chapter.

### **Chapter 4 Observation and Results**

This chapter deals with observed teratological effects (structural, functional and biochemical abnormalities) in developing chick embryo exposed with different dose concentrations of each insecticide on three “critical periods” of embryogenesis.

### **Chapter 5 Discussion**

Present obtained results are discussed and compared with concurrent results reported by other authors.

### **Chapter 6 Summary and Conclusion**

A brief and summarized description of all the previous chapters and important conclusions drawn from present observations are given in this chapter.

### **Chapter 7 Bibliography**

All the references which are followed in the text are alphabetized here.