RESEARCH ARTICLE

Effect of Insecticide Formulation of Quinalphos 25 % EC (Flash) on Gross Morphology of Embryo of *Gallus domesticus*

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Abstract

Quinalphos 25 % EC Flash (O, O-diethyl-O-quinoxalin-2-4 phosphorothioate), is an Organophosphate insecticide.Due to its acaricidal and insecticidal properties it is used widely in this country for crop protection on vegetables, cotton, tea, fruits and other cereals.The objective of the present investigation was to determine developmental toxicity of Flash in chick embryo. Fertilized eggs of *Gallus domesticus* (30 eggs per treatment group) on 0 day incubation were immersed in 15.62ppm, 31.25ppm and 62.50ppm dose concentrations of Quinalphos for 60 min at 37°C. All the doses were according to recommended dose (31.25ppm) which is used for field application. Two control groups were used: One group of 30 eggs was immersed in double distilled water (DDW) and a second group of 30 eggs were kept as untreated. All eggs were opened on embryonic day 4 to assess the mortality rate, wet body weight, embryonic growth and gross morphological malformations. Results revealed that Quinalphos (Flash) induced more embryo mortality, significant reduction in body weight, retarded growth and various external abnormalities in the dose dependent manner and observed gross malformations in surviving embryos like runted growth, subcutaneous hemorrhage, hematomas, brain, eye defects and various limb deformities. Therefore, in the view of this study, it can be concluded that commercial formulation of insecticide containing Quinalphos as an active ingredient, was toxic and teratogenic at all the doses in chick embryo.

Key Words

Gallus domesticus, Malformations, Quinalphos, Teratology

Introduction

Agriculture fulfills needs of the masses by providing foods and fibers. But nothing is free in this world. The cost paid is the degradation of our environment. An increase in global food demand has resulted in a significant increase in the use of pesticides in agriculture. The World wide application of pesticides has necessitated an appraisal of their potential hazards to man and animals.

They play a great role in inducing teratogenicity and behavioral toxicity in humans and animals. The deleterious effects of pesticides on individual physiology poses a serious problem, but the situation becomes grave when it is observed that pesticides have a far reaching effect on growing embryos and consequently endangering the generations to come.

Organophosphate pesticides are widely used in agriculture. Their ability to degrade rapidly made them an attractive alternative to organochlorine pesticides. Their toxicity is due to the ability of these compounds to inhibit acetyl cholinergic junctions of the nervous system.

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Organophosphate insecticides are derivatives of phosphoric and phosphonic acid and is readily hydrolyzed in water, thus do not persist for prolonged periods in the environment. Organophosphates react with a serine hydroxyl group within the enzyme active site, phosphorylating the hydroxyl group and yielding a hydroxylated leaving group. This process inactivates the enzyme and blocks the degradation of the neurotransmitter acetycholine. The signs of intoxication include restless, anorexia, convulsions or tetany preceding death (O'Brien, 1967).The phosphorylation of acetylcholinesterase by Organophosphatess is persistent; reactivation of the enzyme can take less or much time.

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The chick, as a warm-blooded higher vertebrate, undergoes true growth during its morphogenesis in which the embryo and its developing organs increase dramatically in size, similar to the situation in the human embryo. Therefore, studies of toxic effects of chemicals in the chick embryo are highly relevant for understanding the abnormalities in human (Dareste, 1877; Ancel, 1950; Karnofsky, 1952) referred studies on chick embryo because of the easy availability, accessibility and cost efficiency. It is one of the popular models for developmental and toxicological study.

Although avian systems are different than mammalian systems but they are useful for screening xenobiotics widely used in the ecosystems. Lastly, we all know that Poultry is a source of nutrition for mankind. Therefore,it is important to study the effects of pesticide exposure in poultry birds. Various studies demonstrated that the inert ingredients in the pesticide formulations enhance the toxicities of active ingredients and suggested that pesticide registration and their environmental monitoring should include full assessment of formulations (Cox and Surgan, 2006).

Material and Methods

Present study was aimed at investigating the toxic effects of an Organophosphate insecticide on the chick embryo purchased from poultry farm at Ajmer, India. Toxicity was evaluated in terms of gross morphological malformations produced at different concentrations of the formulation tested.

To assess the embryotoxicity of the insecticide, a preliminary dose determining experiment was conducted. Six groups of 10 eggs were dosed on day" 0" of incubation with different doses of the insecticide, that is, 15.625, 23.43, 31.25, 46.50, 62.50 and 78.12ppm.The doses were calculated according to the recommended dose (31.25ppm) used for field application. The dilutions were made in distilled water. The eggs were immersed for one hour duration on day "0" of incubation. Based on the percent hatchability and rate of development, the toxicity of the compound was estimated, and three doses of insecticides, which had minimal, median and sub lethal effects, were chosen for further studies. The Quinalphos was administered into the egg through immersion technique. In nature, avian eggs are usually exposed to pollutants externally through the shell; therefore, the experimental protocol of Somer *et al*, (1974) was used in the study of chick embryo development.

Fertilized eggs were administered with three different doses of Quinalphos 25% EC Flash on the day "0" of incubation. The eggs were incubated at 37°C with a relative humidity of 70% with proper ventilation. At day 4 embryos were taken out from the eggs, and toxicity was evaluated in terms of morphological changes in the developing embryo.

Table 1. Toxicity of Quinalphos 25% EC in the Flash chick embryos on day 4 of incubation (Toxicant Exposure=0 day)

Treatment	Number of eggs/ Treatment	Mortality (%)	Number of Surviving embryos	Wet Body weight (gm)
Control I(Untreated)	30	0	30	0.433±0.02
Control II (Vehicle treated)	30	0	30	0.36±0.01
12.5ppm	30	0	30	0.225±0.018**
25ppm	30	6.67	28	0.198±0.005***
50ppm	30	16.6	25	0.126±0.018***

Table 2. Incidence of malformations in surviving chick embryos treated with Quinalphos 25% EC Flash on day 4 of incubation.(Toxicant Exposure=0 day)

*Deformities	Category (10 embryos for each experiment)				
	Control I	Control II	15.62 ppm	31.25 ppm	62.50 ppm
Total number of deformed embryos	0	1 (10%)	2(20%)	3 (30%)	4 (40%)
Haemorages	0	0	0	0	1
Hematomas	0	0	2	2	2
Edema	0	0	1	1	1
Runt	0	1	0	1	2
Acephaly	0	0	0	0	1
Microcephaly	0	1	1	1	2
Macrocephaly	0	0	0	1	1
Micropthalmia	0	1	0	1	2
Macropthalmia	0	0	0	1	1
Limb Size Reduction	0	0	0	1	2
*All the deformities were seen in each experiment however the total incidence of malformations were found to increase with increasing concentrations.					

Results and Discussion

A single short dipping treatment of unincubated eggs into aqueous solutions of Quinalphos 25% EC Flash, was teratogenic for the chick embryo at all concentrations tested (Fig.3). Significant reduction in wet body weight and % mortality rate (Fig.1) was observed in dose dependent manner. The mortality percentage was highest at the highest dose and lower in the lowest dose(Table 1). Increase in embryolethality was Dose dependent. The similar results were observed by Verrett *et al*, 1969, Korhonen *et al*, 1983, Kumar and Devi, 1992, Pourmirza, 2000, Sahu and Ghatak, 2002, Rachid *et al*, 2008, Wagh *et al*, 2011 and Mobarak *et al*, 2011 in chick embryo exposed to various xenobiotics.

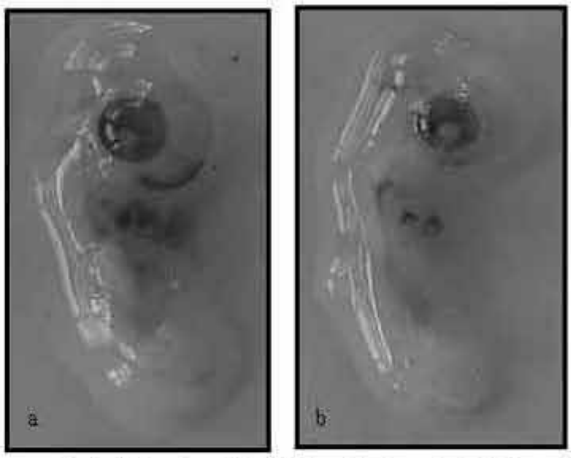

Figure 1. (a)Control I-Untreated and (b) Control II-Treated (DDW)

In the present study the defect most consistently found were Hemorrhages, Hematomas, Runt, Edema, Macrocephaly, Macropthalmia and Reduced size of brain, eye cup and limbs (Table 2). In some treatment group's absence of eye and beak was noted (Fig.2). Mufti and Nasim (1987) also shared the same teratological changes in the 7th day chick embryos with the dose of 2mg/egg of dimicron (an organophosphate insecticide). Furthermore, Misawa *et al*, (1981) also observed same kind of teratological abnormalities in the chick embryo induced with diazinon an Organophosphate insecticide. Ahmad and Asmatullah (2007) observed Xenobiotic induced malformations in fetuses of pregnant mice treated with chlorpyrifos at 18, 36, and 72 mg/kg b.wt., which included head and skeletal abnormalities such as, microcephaly, hydrocephaly, agnathia, anophthalamia, micromelia, hind limb twist, sacral hygroma, drooping twist, and kinky tail. Organophosphates and pyrethroids are known to influence neurotransmission (Rose *et al*, 1999). The vertebral defects are been attributed to decrease in acetylcholinestrase and the associated disruption of cholinergic system (Greenberg and La Ham, 1970; Landauer,1975; Meiniel,1978; Walker,1971).This inhibition during the phase of embryonic development becomes more lethal, because acetylcholine is one of the transmitters that provide neurotrophic input , regulating the proliferation, differentiation, and migraton of its target cells.

Thus, at an early stage of cell development, a given neurotransmitter signal may activate the genes required for replication of the target cell, whereas, at a later stage,

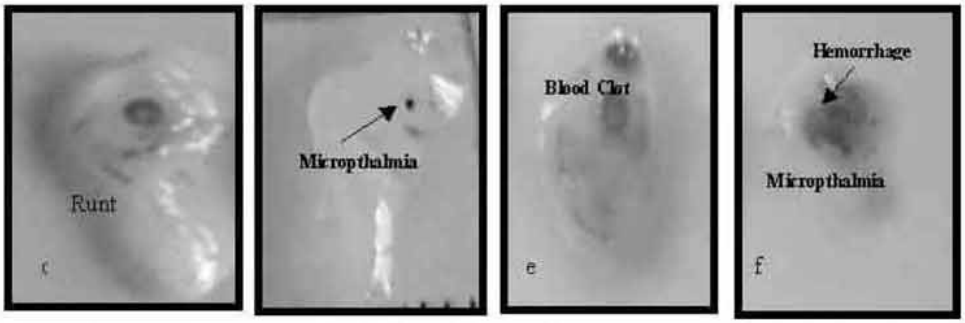

Fig. 2. Malformed embryos on day 4 of incubation (c) 15.62ppm-Low Dose(Runt) (d) 31.25ppm-Moderate Dose(Micropthalmia) and (e,f) 62.50ppm-High Dose(Blood Clot, Micropthalmia, and Hemorrage

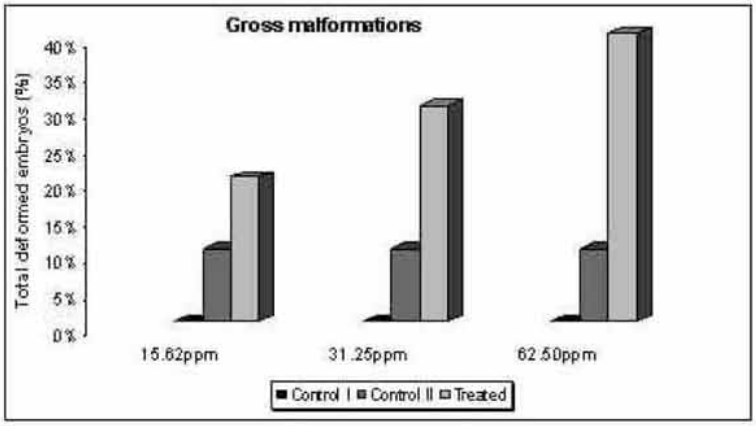

Fig.3. Gross Malformations on day 4 of incubation

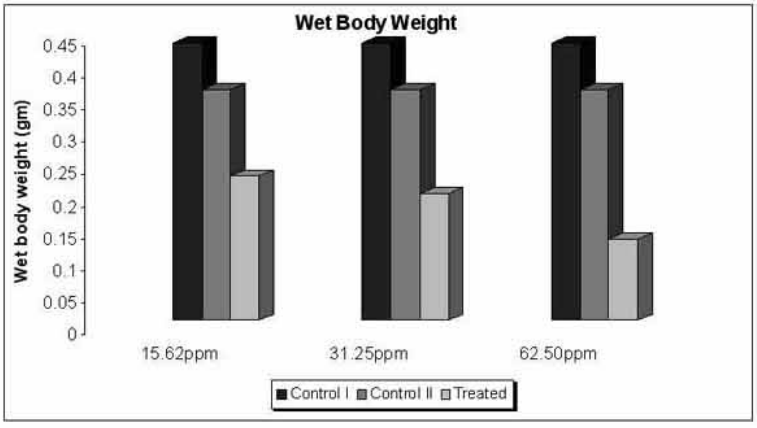

Fig.4. Wet body weight (gm) on day 4 of incubation.

the same transmitter and signal may initiate the transition from replication to (Slotkin, 2005) differentiation. Hence, any hindarance to the functioning of AChE during early embryonic development would mean debilitation much severe than just neurotoxicity. This could be the reason for the presently observed malformations in the quinalphos treated embryos (Uggini *et al*, 2010).

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References

Ahmad, K.R., Asmatullah (2007) Teratological effects of Chloropyrifos in mice. *Iran J Toxicol* **1**:91-99.

Ancel, P. (1950) La chimioteratogenese chez les vertebras. Doin, Paris.

Cox, C, Surgan, M. (2006) Unidentified inert ingredients in pesticides: Implications for human environmental health. *Environ Health Perspect* **114**:1803-1806.

Dareste, C. (1877) Recherches sur la production artificielle des monstruosites ou Essais de teratogenie experimentale. C. Reinwald, Paris. Stain tech.

Greenberg, J.La.Ham.QN. (1970) Reversal of malathion-induced teratisms and its biochemical implications in the developing chick. *Can J Zool* **48**:1047-1053.

Karnofsky, D.A. (1955) The use of the developing chick embryo in pharmacologic research. *Stanford U Bull* **13**:247-259.

Kumar, K.B., Devi, K.S. (1992) Teratogenic effect of methyl parathion in developing chick embryos. *Vet Human Toxicol* **34**:408-410.

Landauer, W. (1975) Cholinomimetic teratogens: Studies with chicken embryos. *Teratology* **12**:125-140.

Mieniel, R. (1978) Neuroactive compounds and vertebral teratogenesis in the bird embryo. *Experientia* **34**:394-396.

Misawa, M., Doull, J., Uyeki, E.M.(1982) Teratogenic effects of cholinergic insecticides in chick embryos. III. Development of cartilage and bone. *J Toxicol Environ Health* **10**:551-563.

Mobarak, Y.M., Al-Asmari, M.A. (2011) Endosulfan impacts on the developing chick embryos: Morphological, Morphometric and Skeletal Changes. *International Journal of Zoological Research* **7**:107-127.

Mufti, S.A., Nasim, R. (1987) Avian embryotoxicity of dimicron. A commonly used insecticide. *Biologia* **33**:109-21.

O'Brien, R.D. (1967) Insecticides: Action and Metabolism. Academic Press, New York : 55-56.

Pormirza, A.A. (2000) Toxic effects of malathion and endosulfan on chick embryo. *J Agr Sci Tech* **2**:161-166.

Rachid, R., Houria, D.B., Mohammad Reda, D. (2008) Impact of flufenoxuron on IGR pesticide on Gallus domesticus embryonic development in ovo. *JCAB* **2(3)**: 87-91.

Rose, R.L., Hodgson, E., Roe, R.M. (1999) Pesticides. In: Marquartdt H, Schafer SG, McCLellan RO, Welsch F, editors. Toxicology. San Diego, CA: 663-669.

Sahu, C.R., Ghatak, S. (2002) Effect of dimicron on developing chick embryo: malformations and other histopathological changes. *Anat Histol Embryol* **31**:15-20.

Slotkin, C.A. (2005) Developmental Neurotoxicity of Organophosphatase:A case study of chloropyrifos. Toxicology of organophosphatase and Carbamate compound. In Gupta, R.C. (Ed.). Toxicity of organophosphate and Carbamate pesticides. *San Diego : Elsevier* : 293-314.

Somer, J., Moran, E.T., Reinhart, B.S., Stephenson, G.R. (1974) Effects of external application of pesticides to the fertile egg on hatching success and early chick performance 1. Pre-incubation spraying with DDT and Commercial Mixtues of 2,4-D : Picloram and 2,4-D: 2,4,5-T. *Bull Environ Contam Toxicol* **11** : 33-38.

Uggini, K.G., Patel, V.P., Balakrishnan, S. (2010) Embryotoxic and Teratogenic effects of pesticides in chick embryos:A Comparative study using two commercial formulations Willey periodicals Inc DOI 10.1002/tox.20627.

Verrett, M.J., Mutchler, M.K., Scott, W.F., Reynaldo, E.F., McLaughlin, J. (1969) Teratogenic effects of captan and related compounds in the developing chicken embryo. *Ann Ny Acad Sci* **160**:334-343.

Wagh, P., Deshpande, S.G., Salokhe, S.G. (2011) Studies on the effect of the insect growth regulator lufenuron on embryogenesis of chick Gallus domesticus (white leghorn strain). *Int J Pharm Bio* **1(2)**:82-88.

Walker, N.E. (1971) The effect of Malathion and Malaxon on esterase and gross development of the chick embryo. *Toxicol Appl Pharmacol* **19**:590-601.