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Effect of Endosulfan 35% Ec on the Egg Laying and Egg Shell Thickness in Japanese Quails

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Abstract: A study was conducted to determine the effect of endosulfan 35% EC on the egg laying and egg shell thickness in Japanese quails. The birds were fed a diet containing the test article at concentrations of 100, 400 and 1000 ppm in diet for a period of 20 weeks. The egg production, cracked eggs and egg shell thickness were measured and then compared with corresponding parameters in the naïve controls. The feed fortified with 100 and 1000 ppm of Endosulfan 35 EC was stable for a period of 7 days with a loss of 8.2 and 8.0%, respectively. The results of the study indicated that Endosulfan 35% EC at the concentration of 100-1000 (ppm) mg kg⁻¹ feed did not affect egg production and egg quality and thereby there appears to be no adverse effects on reproduction in birds.

Key words: Egg production, cracked eggs, dietary route, endosulfan 35 EC, Japanese quails

INTRODUCTION

Endosulfan is widely used as an insecticide and acaricide in India, Brazil, Australia and other countries (Anonymous, 1996). Endosulfan is one of the more toxic pesticides on the market today, responsible for many fatal pesticide poisoning incidents around the world (Pesticide Action Network North America, 2006). Environmental contaminants known collectively as Endocrine Disruptors (EDs) interfere with the mechanisms that govern reproductive development and function in species as diverse as snails, alligators, rodents and human beings (Stoker *et al.*, 2003). Endosulfan is also a xenoestrogen a synthetic substance that imitates or enhances the effect of estrogens and it can act as an endocrine disruptor, causing reproductive and developmental damage in both animals and humans (Varayoud *et al.*, 2008). Endocrine-disrupting chemicals released into the environment can disturb development of the endocrine system and of the organs that respond to endocrine signals in wildlife and humans indirectly exposed during prenatal and/or early postnatal life; effects of exposure during development are permanent and irreversible (Colborn *et al.*, 1993).

The best documents and notorious effect of environmental pollutants on birds in egg shell thinning caused by DDE resulting in crushed eggs and breeding failure of many sensitive raptorial and fish eating birds (Cooke, 1973). Egg shell thinning is correlated with

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inhibition of shell gland calcium ATPase (Kolaja and Hinton *et al.*, 1979; Fox *et al.*, 1978). Organochlorine pollutants and organophosphate pesticides may also influence the breeding behavior of exposed birds. Herring gulls breeding on Ontario, showed decreased incubation attentiveness and decreased defense of territories correlated with a mix of organochlorines (Fox *et al.*, 1978; McArthur *et al.*, 1983). Parathion has been correlated with altered incubation behavior in experimentally exposed mallards and laughing gulls (Bennet *et al.*, 1991; White *et al.*, 1983).

Due to widespread use in the plantation areas of Kerala and Karnataka States in India, several incidences of toxicity in animal and humans were reported. A epidemiological study was conducted in the place where endosulfan had been aerially sprayed two to three times a year for more than 20 years on cashew nut plantations situated in some villages of Kerala to examine the relationship between environmental endosulfan exposure and reproductive development in male children and adolescents, it was found that endosulfan exposure in the male children may delay sexual maturity and interfere with sex hormone synthesis (Saiyed *et al.*, 2003). However, no information is available on the effects of endosulfan in birds. Hence, it was felt the need to study the effect of Endosulfan on reproduction in quails, a representative model of wild life birds. The aim of this study was to determine whether endosulfan exposure induce effects on egg-laying and reproduction in japanese quails. The findings of this study may contribute to the understanding the dose response of endosulfan in controlled conditions which may be extrapolated to field exposure levels.

MATERIALS AND METHODS

Experimental Period

The experiment was conducted during April-May 2007, at Department of Toxicology, Rallis Research Centre (Presently Advinus Therapeutics Pvt. Ltd.) Bangalore, Karnataka, INDIA. The research facility is a Good Laboratory Practice (GLP) accredited by German GLP (BfR), Dutch GLP (WandV), Indian GLP Monitoring Authority, Government of India and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) of the USA. The experimental project was approved by Institutional Animal Ethics committee (IAEC). The experiment was designed on the lines of OECD (1984) Test Guideline 205.

Test Material

Endosulfan 35 EC (Endocel) is a Chlorinated hydrocarbon, with alpha and beta isomers. The chemical name (IUPAC) is 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide. The CAS No. is 115-29-7 (alpha-isomer, 959-98-8; beta-isomer, 33213-65-9). A market sample of Endosulfan 35 EC (Endocel) manufactured by Excel Crop Care Limited, Mumbai, INDIA, with a purity of 35% m/m, Batch No. C-168, Batch Produced on October 2006 and the Expiry date September 2008 was procured for the present study. The test material was a light yellow colored liquid and stored at ambient temperature.

Stability of the Test Item in the Feed

The active ingredient of Endosulfan 35% EC in quail feed was determined by High Performance Liquid Chromatography (HPLC), the analysed concentrations were 91.8 and 990.2 ppm as compared to nominal concentration of 100 and 1000 ppm, respectively. Endosulfan 35% EC was found to be stable in quail feed for 7 days at room temperature. The feed was analysed for endosulfan concentration at the start of treatment and at week 10 and during week 20 of treatment. The analysed concentrations were within the nominal concentrations of 90-110% for 100, 400 and 1000 ppm in the prepared diet.

Table 1: Detail of experimental layout and treatment

Group No.	Dose (ppm)	No. of quails per group		Treatment period (weeks)
		Males	Females	
G1	0	12	24	20
G2	100	12	24	20
G3	400	12	24	20
G4	1000	12	24	20

Birds

Twelve male and 24 female Japanese quails (*Coturnix coturnix japonica*) were randomly allotted into four treatment groups (total No of birds: 48 males and 96 females). The birds were housed in groups of one male and two female per cage and identified by cage card and leg band number. The cages had facilities for *ad libitum* feed and drinking water. Details of experimental layout and treatment in Table 1.

Treatment

Endosulfan 35 EC (Endocel) was mixed with the quail feed to attain the concentrations of G2: 100, G3: 400 and G4: 1000 ppm Endosulfan 35 EC (Endocel) in diet. The control group birds were fed the control feed without the test material. The feed was offered to the different groups daily for seven days a week for 20 weeks. The dose selection was based on prior acute dietary study where in mortality was observed by feeding Endosulfan at a concentration of 1000 mg kg⁻¹ diet concentration.

Observations

The birds were observed for toxic signs and mortality once daily and the food consumption was measured daily. The body weights were recorded before start of treatment and at weekly intervals for 20 weeks.

The number of eggs produced and the quality of eggs were observed during the treatment period of 20 weeks. The egg shell and membrane thickness was determined using micrometer (vernier calipers) for the eggs laid on days 5, 20 and 35.

Statistical Analysis

The hatchability and survival data were analysed by Chi-Square test. The 'Z' (proportion) test was employed for computing hatchability (Finney, 1978).

RESULTS AND DISCUSSION

Endosulfan treatment as a dietary feed admixture at the doses of 100, 400 and 1000 ppm to Japanese quails for 20 weeks did not affect the egg laying and egg shell thickness. Endosulfan treatment did not elicit any toxic signs and mortality at tested doses 100, 400 and 1000 ppm. Endosulfan treatment caused a marginal non-significant decrease in food consumption in all the groups (Fig. 5).

Endosulfan treatment caused a marginal non-significant decrease in body weight, the decrease in bodyweight could possibly be attributed the decreased food consumption (Fig. 3, 4). Endosulfan treatment caused a slight non-significant decrease in egg production (1969 eggs) in the high dose (1000 ppm) when compared to the control group 2198 eggs (Fig. 1).

The egg shell and membrane thickness ranged from 202-236 microns in the control and the treatment groups. Endosulfan treatment did not affect the egg shell and membrane thickness (Fig. 2).

There is no information available in the literature of any study performed to assess the effect of endosulfan on the egg laying or/its effect on the egg quality in Japanese quails or other wild type birds. Hence, the present discussion is in relation to other pesticides of the same class or other avian species. In the present study, there were no effects of Endosulfan 35% EC on the egg production and egg shell thickness of Japanese quails.

The recommended field use concentration of Endosulfan 35% EC is 0.053% a.i. The tested concentration (0.035% a.i.) is equivalent to 66% of the recommended use concentration (0.053% a.i.). Several organochlorine pesticides have been shown to induce reproductive effects in birds.

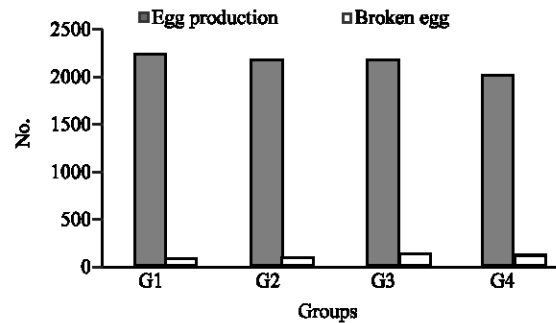


Fig. 1: Summary of weekly egg laying and no. of cracked eggs in quails treated with endosulfan as dietary admixture for 20 weeks at the concentrations of 0-control (G1), 100 (G2), 400 (G3) and 1000 (G4) mg kg⁻¹ in diet

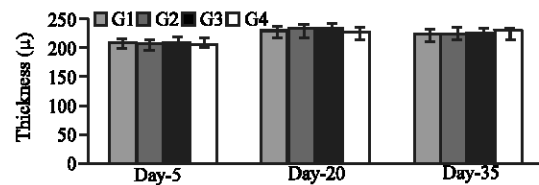


Fig. 2: Summary of egg shell and membrane thickness date (Microns) in quails treated with endosulfan as dietary admixture for 20 weeks at the concentrations of 0-control (G1), 100 (G2), 400 (G3) and 1000 (G4) mg kg⁻¹ in diet

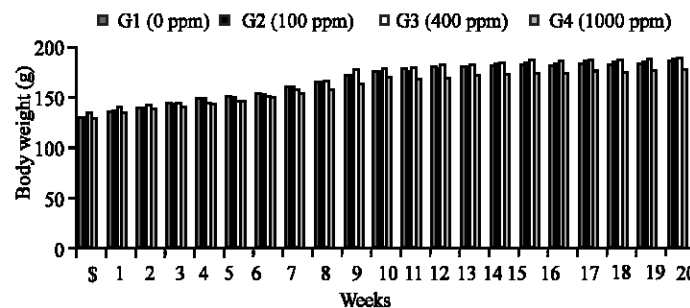


Fig. 3: Summary of body weight changes in quails (Males) treated with endosulfan as dietary admixture for 20 weeks at the concentrations of 0-control (G1), 100 (G2), 400 (G3) and 1000 (G4) mg kg⁻¹ in diet

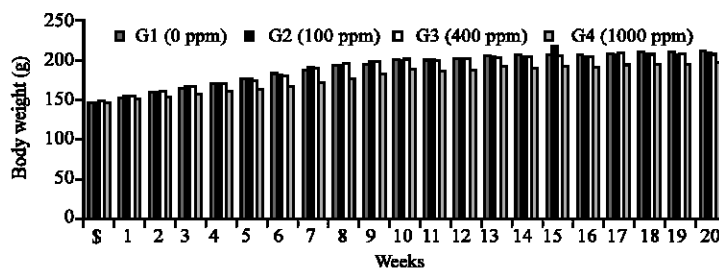


Fig. 4: Summary of body weight changes in quails (Females) treated with endosulfan as dietary admixture for 20 weeks at the concentrations of 0-control (G1), 100 (G2), 400 (G3) and 1000 (G4) mg kg⁻¹ in diet

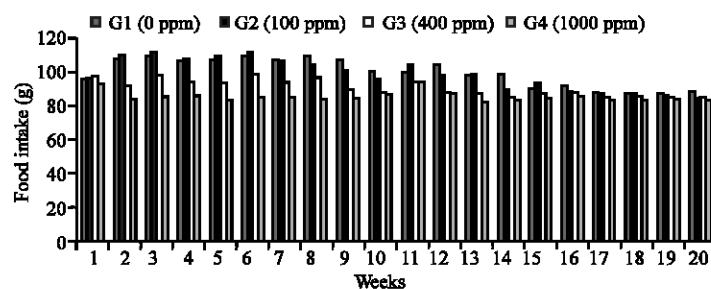


Fig. 5: Summary of feed consumption in quails treated with endosulfan as dietary admixture for 20 weeks at the concentrations of 0- control (G1), 100 (G2), 400 (G3) and 1000 (G4) mg kg⁻¹ in diet

The brown pelican eggs from Anacapa Island, California exhibited thinning of 34% over that of normal and reproduction completely collapsed and residues of collapsed eggs had high levels of DDT and its metabolites (Keith *et al.*, 1970). In another laboratory study it was reported American kestrels were maintained on a diet containing 2.8 ppm p,p'-DDE for 2 years, the egg shells are 10% thinner than those of controls (Weimeyer and Porter, 1970). The American kestrels were maintained on diets containing 0.28 ppm dieldrin and 0.84 ppm DDT, reproductive failure followed the same pattern among these birds, increased egg disappearance and breakage and eating of eggs by adults (Wiemeyer and Porter, 1970). Aroclor 1242, at 10 ppm caused reduced egg production and hatchability and caused thinning of egg shells when fed to White Leghorn Chicken, whereas Aroclor 1254 at 10 ppm did not induce measurable changes in eggshells or reproductive success. At 100 ppm, Aroclor 1254 did cause adverse effects but Aroclor 1260 at 100 ppm did not cause any adverse effect (Heath *et al.*, 1972). Field studies on birds related to endosulfan sprays for tsetse fly control had a severe and possibly prolonged impact on the bird population. Avian exposure to pesticide compounds may be more prevalent in species with affinity for field edges and can also occur by direct consumption of insects, or seeds containing residues, pesticide drift and contact with pesticide coated vegetation (Gard *et al.*, 1995). The eggshell thinning with ducks treated with p,p'-DDE, was observed and this was due to the reduction of the levels of calcium, bicarbonate, chloride, sodium and potassium in the lumen of the eggshell gland during shell formation and also due to the inhibition of prostaglandin PGE₂ synthesis in eggshell gland mucosa (Lundholm, 1997).

In the environment, the birds may be exposed to some pesticides by sprays/drifts either by inhalation or deposition on the feathers and while pruning the birds may ingest the chemical. The birds may also eat the seeds/vegetation/insects exposed to pesticides which act as a source of the toxicant. Additionally, with low level exposures General Adaptability Syndrome or tolerance to insecticides may also be a factor instrumental in nullifying the effect of pesticides, whereas in the laboratory studies the birds have no choice except for the consumption of the pesticide mixed feed or to consume lower quantity of feed.

Under our experimental design, feeding birds with Endosulfan at levels up to 1000 ppm in the diet for 20 weeks had no effect on hatchability of eggs or thickness of egg shells. The results demonstrate that endosulfan exposure at 1000 ppm in diet does not effect the egg-laying and reproduction in Japanese quails.

In the light of these reports it may be inferred that the chlorodiene class of compounds (to which DDE and DDT also belong) have variable responses in the egg production and quality which may also be due to the difference in exposure levels. The findings of the present study is not in agreement with other reported studies in other birds rather than Japanese quails. The differences may be due to the effects of pollutants on reproduction which are mediated at many different physiological levels, the diversity and extent of effects that are difficult to predict because many of the biochemical mechanisms of the side effects of agricultural chemicals are unrelated to the specific mechanisms of action of the designed use. However, it is clear that not all organochlorine compounds produce consistent effects on bird reproduction.

Based on the results of the acute oral toxicity and dietary toxicity of Japanese quails, the highest dose selected was 1000 ppm, which has not caused any reproductive toxicity in Japanese quails, the limitation in this study is the highest dose which is tested, if further higher doses were included it may have resulted in mortality and the birds may not have survived the experimental period of 20 weeks.

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