Research Journal of

Environmental Toxicology

ISSN 1819-3420

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Toxicology 3 (3): 124-131, 2009
ISSN 1819-3420

Acute Toxic Effects of Endosulfan 35 EC (Endocel) upon Oral Gavage and Dietary Admixture in Japanese Quails

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Abstract: A study was conducted to determine the acute toxic effects of Endosulfan 35 EC (Endocel) in Japanese quails by oral gavage and through dietary admixture. The acute oral LD_{50} value of Endosulfan 35 EC (Endocel) by gavage was determined to be 141 mg kg^{-1} b.wt. (equivalent to 46 mg kg^{-1} b.wt. of Endosulfan active ingredient) with fiducial limits of 56 and 171. The Acute Dietary LC_{50} value of Endosulfan 35 EC (Endocel) was 3590 mg kg^{-1} (which is equivalent to 1197 mg kg^{-1} b.wt. of Endosulfan active ingredient) with fiducial limits of 2563-6925. Endosulfan was relatively more toxic (LD_{50} 41 mg kg^{-1}) by the oral gavage administration when compared to the dietary route (LC_{50} 3569 mg kg^{-1}).

Key words: Acute oral toxicity, acute dietary toxicity, LD_{50}, LC_{50}, Endosulfan 35 EC, Japanese quails

INTRODUCTION

Endosulfan was first described in 1956, as a neurotoxic organochlorine insecticide of the cyclodiene family of pesticides and was introduced as an experimental insecticide in the same year. It was first registered in the USA in 1960. It was formulated as emulsifiable concentrate, water wettable powder, dust and granules. Endosulfan has been used against a wide variety of agricultural pests but not against stored products or household pests. Endosulfan is widely used as an insecticide and acaricide in India, Brazil, Australia and other countries (Extonet, 1996). Endosulfan is highly to moderately toxic to bird species, with reported oral LD_{50} values in mallards ranging from 31 to 243 mg kg^{-1} and in pheasants ranging from 80 to greater than 320 mg kg^{-1}. The reported 5 day dietary LC_{50} is 2906 ppm in Japanese quail. Male mallards from 3 to 4 months old exhibited wings crossed high over their back, tremors, falling and other symptoms as soon as 10 min after an acute, oral dose. The symptoms persisted for up to a month in a few animals (Extonet, 1996). The pesticides, which are more or less structurally related to DDT, were very low toxicity to most non-target species, including insects, fish, birds and mammals (March, 1976). Residues of DDT and DDD, but not of DDE, were much lower in survivors 112 days after dosage. The relative importance of DDT and DDD in brains could not be determined, but DDE appeared not to be critical (Lucille *et al.*,

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1996). Twenty five insecticides were tested for their toxicity to hen embryos at various concentration using an egg injection technique. Of the two major groups, the organophosphorus compounds are much toxic than the organochlorine (Dunachie and Fletcher, 2008). Certain pesticides tend to be more acutely toxic to birds than to mammals. This trend is marked with carbamates, less marked with organophosphates (Walker, 1983). There is also some evidence that birds have a greater capacity for bioaccumulation of persistent pesticides such as dieldrin and DDE than do mammals of similar size (Walker, 1983). The stored DDT residues present a hazard to birds, which utilize stored fat during periods of stress due to reproduction, cold weather, disease, injury, limited food supply, or migration (Aldeen *et al*., 1972). Dietary concentrations of DDT dissolved in vegetable oils were usually more toxic than diets containing comparable amounts of powdered DDT (Blus, 1977). A greater percentage of PCB treatment was retained in brain, liver, carcass and feathers than the percentage of DDT + DDD + DDE treatment (Greichus *et al*., 1974). The DDE at environmental levels does not affect osmoregulation or nasal gland Na-K-ATPase either in ducks or in two species of oceanic birds (Miller *et al*., 1976). Investigations of the mortality of the fish eating birds at Tule lake in California indicated that birds died as a result of their exposure to toxaphene, which was applied to adjoining agricultural areas (James, 1966). 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). 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). There is no information on the acute toxic effects of Endosulfan 35% EC by oral gavage or dietary route in birds. Also, the findings of this study formed the basis for selecting the doses for 20 week reproductive toxicity (dietary route) study in Japanese Quails. Hence, the present study was undertaken.

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 (W and V), 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 Test Guideline 205 (OECD, 1984) and the Indian Guideline (Gaitonde, 1978).

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-benzadioxathiepin 3-oxide [CAS No. is 115-29-7 (alpha-isomer,959-98-8; beta-isomer, 33213-65-9]. A market sample 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 coloured liquid and it was stored at ambient temperature.

Birds

Acute Oral Toxicity (LD_{50})

Three male and three female Japanese quails (*coturnix coturnix japonica*) were allotted randomly into four treatment groups, the birds were procured from a registered breeder. The birds were individually housed and identified by cage card and leg band number. The cages had facilities for *ad libitum* feed and drinking water.

Acute Dietary Toxicity (LC_{50})

Fifty Japanese quail (*coturnix coturnix japonica*) chicks aged 14 days were used to conduct the study with Endosulfan 35 EC (Endocel). The chicks were randomly allotted as follows-twenty chicks to control group, ten chicks each for the three treatment groups. The chicks were housed in groups of ten and identified by cage card and leg band number. The cages had facilities for feed *ad libitum* and drinking water *ad libitum.*

Administration of the Test Material

Acute Oral Toxicity (LD_{50})

Endosulfan 35 EC (Endocel) was suspended in de-ionised water and administered to quails at four dose levels 100, 120, 180 and 200 mg kg^{-1} b.wt. A single dose of the test material was administered as oral gavage to birds fasted overnight.

Acute Dietary Toxicity (LC_{50})

Endosulfan 35 EC (Endocel) was mixed with the quail feed to attain the concentrations of G2: 1000, G3: 2000 and G4: 4000 mg kg^{-1} Endosulfan 35 EC (Endocel) in diet. The control group birds were fed the control feed without the test material. The feed was offered to three different groups daily for five days followed by 3 days of normal feed.

Observations

Acute Oral Toxicity

The quails were observed for toxic signs four times on day 1 (the day of test material administration) at hourly intervals and once daily for a further 14 days. The body weights were measured before treatment and on days 8 and 15.

Acute Dietary Toxicity

The birds were observed daily for toxic signs, feed consumption was measured daily and the bodyweights were recorded before treatment and on days 6, 9 and 14, respectively. The bodyweight and feed consumption in the control group was considered 100% and the other groups were compared to this and represented as percentage of the control values.

Stability of the Test Material in Feed

Endosulfan 35% EC was stable in quail feed for 7 days. The active ingredient in feed was analyzed using High Performance Liquid Chromatography (HPLC). The active ingredient concentrations were 91.8 and 9902 as compared to nominal concentration of 100 and 1000 ppm, respectively.

Statistical Analysis

The dose and mortality were considered for calculation of LD_{50} for oral study and the LC_{50} for the dietary study. The LD_{50} and the LC_{50} were calculated using the validated SYSTAT® 12 Statistical software version 12.02.00, Chicago, USA.

Test Conditions (Acute Oral Toxicity)

Typically, acute toxicity is determined using at least three doses spaced appropriately to produce test groups with a range of toxic effects and mortality rates. These data are used to produce a dose response curve and, where possible, permit an acceptable determination of the LD_{50}, the Lethal Dose to produce 50% mortality.

The above conditions as set forth were fulfilled in the present study.

Conditions for the Validity of the Test (Acute Dietary Toxicity)

- The mortality in the controls should not exceed 10% at the end of the study
- There must be evidence that the concentrations of the material being tested has been maintained at a minimum of 80% of the nominal concentration in the diet during the first 5 days of the study
- The lowest treatment level should not result in compound-related mortality or observable toxic effect

In the present study there was no mortality in the control group. The active ingredient concentration in the diet was within 90% of the nominal concentration. There was no test material related mortality or observable effects in the lowest treatment dose. These facts fulfill the conditions set forth in the current study.

RESULTS AND DISCUSSION

Acute Oral Toxicity

Following gavage administration of the test item, the birds elicited dose related toxic signs and mortality in the treated groups (Table 1). The treatment did not have effect on the body weight of surviving birds (Fig. 1). The body weights of the surviving birds was 121-124 g at start of treatment

Table 1: The results of the acute oral toxicity

Group and dose (mg kg^{-1})	No. of tested	Toxic/clinical signs	No. of dead	Mortality (%)
G1 100	6	Diarrhea at 3 h post treatment and the birds were normal after 24 h post treatment	0	0
G2 120	6	Diarrhea at 2 h post treatment, two birds died within 4 h post treatment and the other four birds were normal 24 h post treatment	2	33
G3 180	6	Diarrhea at 2 h post treatment, four birds died within 3 h post treatment and the other two birds were normal 24 h post treatment	4	66
G4 200	6	Diarrhea at 1 h post treatment and all the birds died within 2 h post treatment	6	100

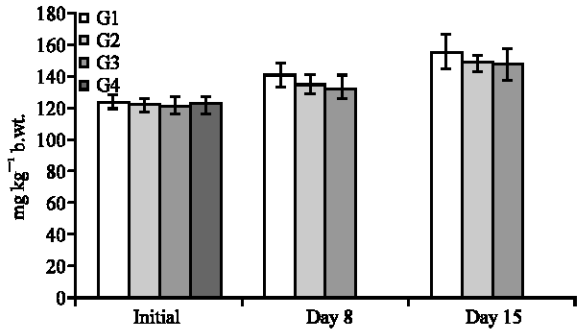

Fig. 1: Body weight changes in Quails administered with a single dose to different groups 100 (G1), 120 (G2), 180 (G3) and 2000 (G4) mg kg^{-1} b.wt. of Endosulfan

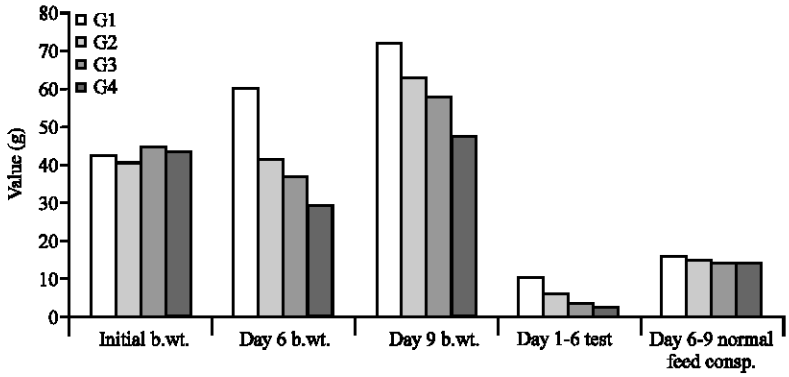

Fig. 2: Bodyweight changes and food consumption in quails treated with endosulfan as dietary admixture at the concentrations of 0- control (G1), 1000 (G2), 2000 (G3) and 4000 (G4) mg kg^{-1} in diet

and 147-155 g at termination of the treatment (Fig. 1). Based on the above findings, the Acute Oral LD_{50} value of Endosulfan 35 EC (Endocel) in Japanese quails was determined to be 141 mg kg^{-1} b.wt. (equivalent to 46 mg kg^{-1} b.wt. of Endosulfan active ingredient) with fiducial limits of 56 and 171.

Acute Dietary Toxicity

Table 2 clearly indicates that Endosulfan treatment as a dietary feed admixture for 5 days elicited dose related toxic signs and mortality. Based on these data, the Acute Dietary LC_{50} value of Endosulfan 35 EC (Endocel) was determined to be 3590 mg kg^{-1} (which is equivalent to 1197 mg kg^{-1} b.wt. of Endosulfan active ingredient) with fiducual limits of 2563-6925. Body weight and food consumption data are presented in Fig. 2.

The data (Fig. 2) clearly suggests that administration of Endosulfan induced a remarkable dose-related decrease in body weight at day 6 compared to the concurrent controls. A similar trend in decreased in feed consumption was apparent in the first 6-days in the treated groups. However, the body weight and food consumptions showed reversal trend in all treated groups by day-9 (Fig. 2).

The test material intake was calculated based on the concentration of the test material in the diet and the total amount of food consumption for each group (Fig. 3).

Table 2: The results of the acute dietary toxicity

Group and dose	No. of tested	Toxic/clinical signs	No. of dead	Mortality (%)
G1 0	20	Nil	0	0
G2 1000	10	Lethargy, weakness and diarrhea in two birds on day 1 and 2 and both died, the remaining birds were normal	2	20
G3 2000	10	Lethargy, weakness and diarrhea in four birds, in addition one bird had ataxia. The four birds died on day 2	4	40
G4 4000	10	Lethargy, weakness and diarrhea in all birds, tremors and ataxia in six birds. The six birds died on day 2 and the remaining birds were normal after day 4	6	60

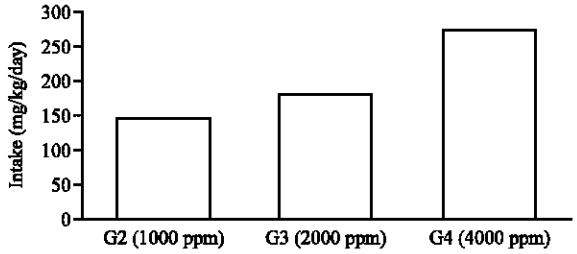

Fig. 3: Test material intake (mg/kg/day) in quails treated with endosulfan as dietary admixture at the concentrations of 0-control (G1), 1000 (G2), 2000 (G3) and 4000 (G4) mg kg^{-1} in diet

There is no information on the acute toxic effects of Endosulfan 35% EC by oral gavage or dietary route in birds. Therefore the discussion is limited to comparison with the other compounds of the cylodiene family.

A single dose oral gavage administration of the compound resulted in diarrhea in all treated birds between 1 to 3 h following Endosulfan administration. As expected, a dose related increase in mortality was apparent. Using the mortality data, the calculated Acute oral LD_{50} value of endosulfan 35% EC was determined to be 141 mg kg^{-1} b.wt. The observed LD_{50} is comparable to the reported value of 31-243 mg kg^{-1} (Endosulfan Technical) in mallard ducks (Hudson *et al*., 1984). Based on the above results, Endosulfan 35% EC is classified as Highly Toxic by the oral route.

Adminsitration of Endosulfan by the dietary route resulted in lethargy, weakness and diarrhea in most treated birds. Dietary administration of Endosulfan resulted in dose related decrease in body weight. The food consumption was transiently decreased which returned to normal by Day 9 of the study. Based on mortality data, the calculated Acute dietary LC_{50} value of endosulfan 35% EC was determined to be 3569 mg kg^{-1} of diet. The observed LC_{50} is comparable to the reported value of 2906 mg kg^{-1} (Endosulfan Technical) for Japanese quails (Hill and Camardere, 1986). Based on the above results, Endosulfan 35% EC is classified as Moderately Toxic by the dietary route. Difference in toxicity elicitation was seen in the oral versus dietary route. This is because in the acute oral study, the test article dose is directly placed as a bolus dose into the stomach of fasted birds at once, thereby the uptake of the test item is swift and the reactions are seen rapidly. However, when Edosulfan was administered admixed in the feed to birds, the relative exposure of the test compound is not as rapid as by the gavage mode of administration. Hence, the peak exposure of the test item

is comparatively less which resulted in reduced toxicity. The feed consumption pattern is also dependent on the palatability of the feed which is a function of the concentration of the test material in the feed. In the dietary mode of administration, it is quite possible that compound may be adsorbed to the food /feed ingredients (food-chemical interaction) which may contribute to reduced exposure of the compound to birds. This has a bearing on reduced toxicity of the compound by the dietary route. These factors may account for variability in the toxicity of the chemical administered by oral and dietary routes.

The toxic signs observed in the acute oral gavage mode are limited to diarrhea, whereas in the dietary mode, lethargy, weakness, ataxia and tremors were observed. Acute oral studies conducted in 3-4 month old male mallards treated with endosulfan resulted in birds exhibiting wings crossed high over their back, tremors, falling and other symptoms as soon as ten minutes after an oral gavage dose administration. The symptoms persisted for up to a month in few birds. The diarrhea and the nervous signs produced by Endosulfan are attributed to stimulation of the Central Nervous System which is the major characteristic of Endosulfan poisoning (Hudson *et al.*, 1984).

In the current study, there was a significant dose related reduction in the feed consumption during the treatment period (5 days) and the feed intake was comparable to the control group at the end of the 3 day recovery period indicating reversal. The decrease in feed consumption may be attributed to either the biological effect of endosulfan or due to the palatability of the feed.

There was a significant dose related bodyweight reduction at the end of the 5 day treatment period and the body weight gain was comparable to the control group at the end of the 3 day recovery period indicating the recovery. The decrease in body weight is due to the decreased feed consumption as indicated earlier.

In conclusion, Endosulfan was relatively more toxic (LD_{50}: 41 mg kg^{-1}) by the oral gavage administration compared to the dietary route (LC_{50} : 3569 mg kg^{-1}).

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