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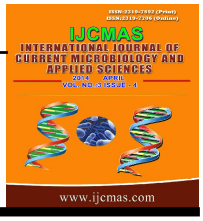

Original Research Article

Effect of an Environmental Pollutant-Marshal, Carbamate Insecticide on the Development of Avian Embryo

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A B S T R A C T

The present study was undertaken to evaluate the effect of an environmental pollutant, Carbosulfan 25% EC (Marshal®) on avian development using embryos of Gallus *domesticus* as an experimental model. Fertilized pure breed (BV 300) eggs were immersed in Marshal for one hour on day 7 of incubation at 15.62ppm (low), 31.25ppm (moderate) and 62.50ppm (high) doses of the toxicant. The embryos were recovered on day 15 of incubation and growth retardation, embryolethality and induction of teratogenesis was observed. Significant reduction in wet body weight and high incidence of mortality rate was recorded at moderate (31.25ppm) and at high (62.50ppm) doses. The skeletal malformations noted were mainly overall reduction in ossification, reduced- limbs, skull, caudal, thoracic and cervical vertebrate. However, skeletal malformations were noted in all the embryos irrespective of the concentrations of the dose; with significant malformation at high dose (62.50ppm) in thoracic vertebrae.

Introduction

The greatest attention has been paid to the pesticides among the many potential environmental pollutants as the inducers of developmental neurotoxicity. Pesticides have found widespread use in the home and in agriculture (U.S. EPA 2006; Slotkin *et al* 2007).Pesticides are intentionally released into the environment to enhance food production by eradication of harmful pest species. However, the indiscriminate use of pesticides in agriculture and public health has caused serious environmental and health problems to the humans and cattle (Yaduvanshi *et al* 2010).

Carbamate pesticides play role in agriculture by causing toxicity to inhibition of acetylcholinesterase (AchE) and have roles in neurologic development (Brimijoin and Koenigsberger, 1999). Carbosulfan is active against caterpillars, green leaf hopper, brown plant hopper, gall midge, stem borer and leaf folder of paddy and white aphids of chillies. The neurotransmitter acetylcholinesterase is potentially inhibited by carbosulfan in rats.

Sign of toxicity were generally observed when acetylcholinesterase activity was inhibited by more than 35% and tremors occurred at inhibition by more than 70% (Renzi *et al* 1986).Carbosulfan is in the priority list of compounds along with dimethoate and malathion for toxicological evaluation by Joint FAO/ WHO meeting on pesticide residues.

Human exposure to insecticides can occur during storage, transport, mixing, loading and application. The major routes of insecticide exposure to agricultural workers include dermal and respiratory (Ksheerasagar *et al* 2011).

In the present investigation, the carbamate insecticide was used as an environmental pollutant to study its potency in causing damage to the chick embryo.

Chick embryo model has being used in recent work for studies of developmental neurotoxicity of organophosphates as well as other neurotoxicants (Yanai *et al* 2004, 2008).Studies in mammalian models incorporate both direct and indirect neurodevelopment effects. With chick embryo we can administer pesticides directly to the medium surrounding the embryo without maternal mediation (Alhifi, 2011).

The teratogenic and embryotoxic potential of an environmental pollutant- Marshal has not been studied with the avian embryonic model. The present study was undertaken to understand the embryogenesis of Carbosulfan 25% EC on *Gallus domesticus*..

Materials and Methods

The toxicant Carbosulfan 25% EC Marshal® is a commercially available insecticide and was purchased from the registered trader of pesticides from Jaipur, Rajasthan, India. The pure lines of fertilized BV 300 eggs were obtained from the poultry farm at Ajmer, Rajasthan, India.

The doses were calculated according to the recommended dose (31.25ppm) used for field application. Three doses of insecticides, half of the recommended, recommended and double of the recommended were chosen for further studies. Five batches (30 fertile eggs, each) were selected. On day 7 of incubation the eggs of first three batches of eggs were immersed individually, for one hour, in low-15.62ppm, moderate-32.50ppm and high-62.50ppm doses, respectively. The fourth batch was treated with distilled water and served as control group and fifth batch was left untreated to study background toxicity. Moreover, after passing the first critical period of organogenesis, immersion of eggs on day 7 of incubation made the embryo targeted for its survival. Immersion method has been preferred over the injection method as it reciprocates field exposure resulting into environmental contamination. Moreover, embryo gets exposed to the contaminant for a longer period of time with immersion technique (Varga *et al* 2002).

After immersion, the eggs were dried and kept at 37.5˚C with relative humidity 65-70%, and embryonic survival was monitored via candling.

Skeletal staining procedures for 15 day embryos were performed using the protocol described by Inouye 1976.

Statistical analysis was performed by using Mann-Whitney U test for

teratological observations with the aid of SPSS and significance calculated at ά=0.05 levels and wet body weight was determined using student's t-test; significance attributed at $p<0.05$, $p<0.01$ and $p<0.001$.

Results and Discussion

The immersion of eggs for short time into aqueous solutions of Marshal was teratogenic for the chick embryo at all concentrations tested. Various deformations were recorded in all the embryos irrespective of the dose tested. With increase in dose concentration reduction in body weight, increase mortality rate and increase in various skeletal malformations were noted. Significant and highly significant reduction in wet body weight and high incidence of mortality rate 25% and 40% was computed at moderate (31.25ppm) and at high (62.50ppm) doses respectively (Table-1). All the defects were consistently found in all embryos at all the doses. Each animal was recorded with one or more than one defect among the following malformations- reduced ossification of ribs, cervical vertebrae, metacarpus and digits, short kinky caudal vertebrae and reduced pygostyle, flexed digits, shortness of humerus and scapula, small sized skull, short beak and abnormally formed frontals and parietals (Figure-1). However, significant results were seen only at high dose (62.50ppm). Among the skeletal embryogenesis; the significant defects were seen in thoracic vertebrae (Table-2).

The incubation period from immersion to occurrence of embryo death was inversely related to the dosage of insecticides. A large body of evidences explains the susceptibility of increase in the embryolethality with increasing concentration of the toxicant (Pourmirza, 2000; Uggini *et al* 2010; Beldomenico *et al* 2007; Mathur *et al* 2013; Gilbertson *et al* 1991).The high level of embryonic mortality might have occurred by the intervention in metabolic process (Rouabhi *et al* 2008).

The present results revealed statistically more embryonic deaths using the high dose (62.50ppm) of Marshal with highly significant reductions of wet body weight. This result is similar to the findings reported by Mobarak and Al-Asmari (2011) with 21mg Endosulfan per egg of developing chick embryos and Gilbertson *et al* (1991) with organochlorine contaminants in fish eating birds.

The skeletal defects were observed in all the treatment groups among the various observable malformations none was seen specifically in any one category. All the reported defects were found across the groups and one animal was seen with one or more than one defect. However, significant results were obtained in the high dose (62.50ppm) and in the thoracic vertebrae. Role of acetyl-cholinesterase inhibition in disruption of cholinergic system and skeletal defects have been reported by many (Slotkin 2005; Upshall *et al* 1968; Landauer 1975).The skeletal system is one of the potential targets of pesticide toxicity (Compston *et al*1999; Garg *et al* 2004).

Carbamate insecticide has the same mechanism of action as phosphoorganic insecticides, but are easily absorbed and quickly metabolized and excreted. Therefore, acute poisoning with carbosulfan was not seen in the present finding. Moreover, the immersion of eggs on day 7 of incubation made the

Table.1 Toxicity of Marshal in the chick embryos on 15th day of incubation (Toxicant exposure-7thday)

Treatment	Number of eggs/ Treatment	Mortality (%)	Number of Surviving embryos	Surviving embryos with Malformations (%)	(N)	Wet Body weight (gm)
Control I(Untreated)	20	5	19	10.52	2	13.921± 0.375
Control II (Treated)	20	15	17	17.64	3	13.22 ±0.620
15.62mg/l	20	15	17	23.52	4	13.37±0.33
31.25mg/l	20	25	15	40	6	12.41±0.35*
62.50mg/l	20	40	12	41.6	5	11.17±0.23**

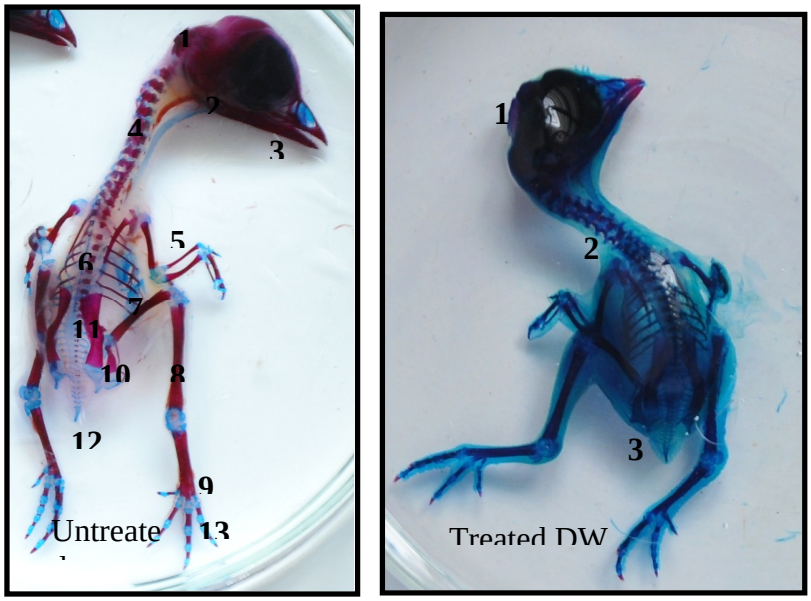
Each value in body weight (gm) - represents Mean±Standard error. *= p≤0.05 (Significant), **=p≤0.01 (Highly Significant), ***=p≤0.001 (Very Highly Significant).

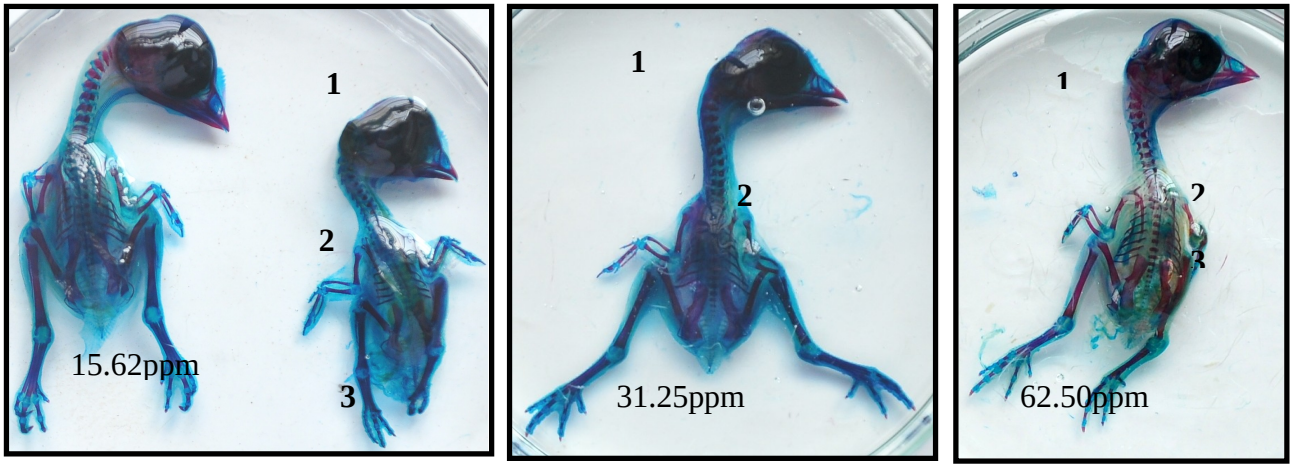
Table.2 Skeletal malformations in chick embryo on 15th day of incubation

Treatment (20 embryos in each group)	Skeletal Malformations (Mean Ranks + Z Values)				
	Skull	Cervical Vertebrae	Thoracic Vertebrae	Caudal Vertebrae	Limbs
Untreated	18.89	18.00	18.95	17.95	18.39
Treated	18.06	19.06	18.00	19.12	18.62
Z Value	-.496	-1.057	-.946	-.695	-.116
Untreated	18.39	18.00	18.45	17.45	18.39
15.62mg/l	18.62	19.06	18.56	19.68	18.62
Z Value	-.116	-1.057	-.080	-1.164	-.116
Untreated	17.79	16.50	16.39	16.89	16.79
31.25mg/l	17.13	18.77	18.90	18.27	18.40
Z Value	-.388	-1.616	-1.305	-.812	-.763
Untreated	15.13	15.50	14.32	15.32	14.63
62.50mg/l	17.38	16.79	18.67	17.08	18.17
Z Value	-1.050	-1.258	-2.036*	-1.029	-1.540

Mean Ranks and Z Values computed by **Mann-Whitney Test** using **SPSS**
*Significance at 0.05 level of significance.

Figure 1 Photographs showing dorsal views of 15 day old chick embryos –untreated, treated (DW) and treated (Flash). **Untreated embryo** an untreated embryo has following parts: (1) Premaxilla (2) Lower jaw, (3) Skull (4) Cervical vertebrae (5) Wing, (6) Ribs, (7) Femur,(8) Tibia, (9) Metatarsus ,(10) Pelvic girdle, (11) Caudal vertebrae, (12) Pygostyle, (13) Digits. **Treated embryo** an embryo treated with DW shows (1) unossified hind parts of frontals and pterygoid, and (2) Reduced ossification of vertebrae (3) Kinky caudal vertebrae. **Low dose-** (1) reduced ossifications of the frontals and paritals, (2) short limbs (3) crooked toes. **Moderate dose-** (1) incomplete ossification of frontals, parietals and palatine, (2) short humerus. **high dose-** (1) incomplete ossification of frontals, parietals and palatine, (2 short humerus, (3) reduced ossifications of thoracic vertebrae.





*Malformed embryos exhibited one type or 2-4 types of malformations.

possibility for liver to start functioning and hence the toxicant at lower doses might have conjugated with glutathione whereas drop in the glutathione level at high dose might have shown incapability to detoxify the toxic compound (Anwar, 2003). On comparing bird embryos to mammals it is found that bird embryos are exposed to the toxicant during the whole incubation period as metabolic products are unable to get out of the egg (Fry, 1995). Apart from this the immersion method added to the teratogenicity by keeping the pesticide in association with the embryo for a longer time period (Varga *et al* 2002). Hence a very negative effect of the toxicant during the incubation period must have resulted in above mentioned teratogenesis induced by an environmental pollutant Marshal.

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