Int J Pharm Bio Sci 2013 Jan; 4(1): (B) 267 - 274

Research Article **NeuroBiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INSECTICIDE MARSHAL® EC INDUCES TERATOLOGICAL CHANGES IN THE EMBRYOS OF *GALLUS domesticus.*

P. MATHUR, *N. TAPARIA, K. NITU AND L .SHAHANI

Department of Zoology, The IIS University, Jaipur, Rajasthan-302020, India

ABSTRACT

Present study was designed to investigate the toxic effects of Carbosulfan 25% EC Marshal on the developing chick embryo at critical period of its developmental stage. Fertilized eggs of pure breed of chick (BV 300) were immersed in low, medium and sub lethal concentrations of the toxicant for 60 minutes on day 4 of incubation. The gross morphological and skeletal features were studied on day 10 and day 15 of the incubation period. The pesticide caused musculoskeletal deformities in skin, limbs, head, neck, skull, lower body and overall reduction in ossification of skeleton. The embryolethality percentage was highest at the highest dose and lower in the lowest dose. However, incidence of malformations was found to be significant and showed increase with the increasing concentrations of the toxicant. Thus, insecticide Marshal EC with active ingredient Carbosulfan is a potent teratogen with reference to avian systems.

KEYWORDS : **Carbosulfan. Chick embryo .Malformations.Skeleton. Teratology**

N. TAPARIA
Department of Zoology, The IIS University, Jaipur, Rajasthan-302020, India

INTRODUCTION

Toxic chemicals like pesticides are both ubiquitous and unique. They play dual role; they are important in controlling harmful pests but at the same time may also pose great threats to non target species. They are designed to kill, repel, or harm living organisms [3, 23]. The ill effects caused by pesticides affect populations of economically important organisms [20]. In the present investigation, the Carbamate insecticide was used as a toxicant to study its potential in causing damage to the chick embryo. Carbosulfan 25% EC Marshal due to its acaricidal and insecticidal properties is used extensively in agriculture but their residues often reach the non target organisms. This formulation is widely used for the control of caterpillar and sucking pests of rice and chilies. Carbamates act like Organophosphates by inhibiting acetyl cholinesterase (AchE).However, the inhibition of AchE is reversible and therefore the lethality is lower in Carbamates as compared to Organophosphates [1]. Some Carbamates have been reported for its mutagenic, embryotoxic, teratogenic, and carcinogenic effects. Poultry is the rich source of nutrition and is consumed worldwide. Due to increasing use of pesticides in poultry feed and management practices, transport of its metabolites is quite probable [22]. Moreover, protective mechanisms found in adults are not fully functional in the fetus and therefore the concern is for the developing embryo [12]. The chick embryo is good model for determining the effects of xenobiotics on growth and development [10] .Chick model eliminates the biotransformation of xenobiotics and hence the toxicity found is due to the toxicant used and not from its metabolites [19]. It is popularly used because it is found to be inexpensive and sensitive that provides information on embryonic lethality, teratogenicity, growth retardation, metabolism as well as systemic toxicity and imunopathological effects of xenobiotic agents [11]. The teratogenic and embryotoxic potential of Carbosulfan 25% EC Marshal has not been studied so far with the avian embryonic model. The present study was undertaken to understand the teratogenic effect of Carbosulfan 25% EC Marshal, commercially available pesticide on the chick embryos.

MATERIALS AND METHODS

The toxicant Carbosulfan 25% EC Marshal® (Carbamate) 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 embryotoxicity and teratogenicity of the insecticide was determined by performing preliminary dose determining experiment. The doses were calculated according to the recommended dose (31.25 mg/l) used for field application. The dilutions were made in distilled water (DW). The eggs were dipped for 60 minutes on day 0 of incubation. The toxicity of the compound was estimated on the basis of the viability and development of the chicks. Three doses of insecticides, which had low, moderate and highly toxic effects, were chosen for further studies

Five groups (30 fertile eggs, each) were randomly selected. On day 4 of incubation the eggs of first three groups were immersed individually, for 60 minutes, in Low-15,62mg/l, Median-32.50mg/l and High- 62.50mg/l doses, respectively. The fourth group was treated with distilled water for the same period and served as control group and the fifth group, was left untreated. Dipping of the eggs on day 4 of incubation has many advantageous. Egg viability can be determined, as the growth of vasculature is clearly visible. Secondly, the embryos get targeted towards survival after passing the first critical period of organogenesis [4]. After immersion, the eggs were dried and kept in an incubator at 37.5˚C with relative humidity 65-70%. Eggs were candled before treatment and the unfertilized eggs were removed from the experiments. The eggs were opened on day 10 of incubation to study gross morphology and on day 15 to study the skeletal development. The

weight of wet body and morphological abnormalities were noted and the abnormalities observed were captured using digital camera.

Skeletal staining

On embryonic day 15 embryos were processed for staining by technique described by Inouye [9].

Statistical Tests

Statistical analysis were performed to calculate wet body weight using student's t-test, significance of differences were attributed at $P<0.05$, $P<0.01$ and $P<0.001$. The teratological observations were analyzed by using Mann-Whitney U-test. The calculations were done using SPSS and significance was calculated at ά=0.05 levels.

RESULTS

The dipping of eggs for short time into aqueous solutions of Carbosulfan 25% EC Marshal, was teratogenic for the chick embryo at all concentrations tested. In the present study, the defects mostly found on day 10 in all the treated embryos were-Hemorrhages (bleeding under skin), Hematomas (blood patches), Edema (swelling due to accumulation of fluids beneath the skin), macrocephaly, abnormal bulge on the brain, Macropthalmia and gartrochisis (herniating organs) (Fig.1). In some treatment group's absence of eye and beak was noted. Treated embryos on day 15 exhibited one or more types of malformations. Most consistently following deformities were recorded in all the embryos irrespective of the concentration of the dose; reduced ossification of ribs, cervical vertebrae, metacarpus and digits, short kinked caudal vertebrae and reduced pygostyle, flexed digits, shortness of humerus and scapula, small sized skull, short beak and abnormally formed frontals and parietals(Fig.2). The mortality percentage was found to increase in dose dependent manner on day 10 (Table 1). However, no significant difference was recorded in the body weight of the embryos autopsied on day 10 and 15 of incubation period (Table2). Significant difference in the malformations were found at Moderate (31.25mg/l) and High (62.50mg/l) dose levels on day 10 (Table 3) and at Low (15.62mg/l), Moderate (31.25mg/l) and High (62.50mg/l) concentrations of the toxicant used on day 15 of the embryo development (Table 4).

Table 1
Toxicity of Marshal EC in the chick embryos on 10th day of incubation (Toxicant exposure-"4th"day)

Treatment	Number of eggs/ Treatment	Mortality (%)	Number of Surviving embryos	Surviving embryos with Malformations (%)	(N)	Wet Body weight (gm)
Control I(Untreated)	30	6.6	28	7.1	2	2.77±0.28
Control II (DW)	30	13.3	26	19.23	5	2.03±0.28
15.62ppm	30	20	24	25	6	1.60±0.51
31.25ppm	30	26.6	22	36.3	8	2.19±0.35
62.50ppm	30	33.3	20	55	11	1.60±0.49
*The values are expressed as mean±standard error (S.E.) for wet body weight.						

Table 2
Toxicity of Marshal EC in the chick embryos on 15th day of incubation (Toxicant exposure-"4th"day)

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	15.78	3	13.92±0.37
Control II (DW)	20	15	17	23.52	4	13.44±1.02
15.62ppm	20	20	16	31.25	5	13.94±0.27
31.25ppm	20	35	13	69.23	9	12.74±0.30
62.50ppm	20	25	15	46.66	7	13.45±0.49

**The values are expressed as mean±standard error (S.E.) for wet body weight.*

Table 3
Gross Malformations in the chick embryos exposed on 4th day with different concentration of the Marshal® EC

Treatment (30 embryos in each group)	Gross-Malformations (Mean Ranks + Z Values)				
	Skin	Head	Neck	Limbs	Lower Body
Control I	26.46	27.00	26.50	27.50	27.96
Control II	28.62	28.04	28.58	27.50	27.00
Z Value	-1.107	-1.038	-1.482	.000	-.964
Control I	25.43	25.00	26.50	25.00	24.93
15.62ppm	27.75	28.25	26.50	28.25	28.33
Z Value	-1.193	-1.909	.000	-1.909	-1.581
Control I	23.39	23.50	24.50	24.50	23.89
31.25ppm	28.18	28.05	26.77	26.77	27.55
Z Value	-2.048*	-2.329*	-1.612	-1.612	-1.692
Control I	21.36	21.50	23.50	22.50	21.86
62.50ppm	28.90	28.70	25.90	27.30	28.20
Z Value	-2.850*	-3.066*	-1.691	-2.446*	-2.531*

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

Table 4
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
Control I	17.89	18.00	18.95	18.95	17.95
Control II	19.18	19.06	18.00	18.00	19.12

Z Value	-.608	-1.057	-.946	-.946	-.695
Control I	16.34	16.00	17.42	17.42	15.92
15.62ppm	19.97	20.38	18.69	18.69	20.47
Z Value	-1.505	-2.282*	-.751	-.751	-2.003*
Control I	12.68	12.50	15.34	15.34	13.84
31.25ppm	22.08	22.35	18.19	18.19	20.38
Z Value	-3.380*	-3.886*	-1.473	-1.473	-2.705*
Control I	14.79	15.00	15.39	16.39	15.39
62.50ppm	20.93	20.67	20.17	18.90	20.17
Z Value	-2.337*	-2.685*	-2.100*	-1.305	-2.100*

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

Figure 1
Photographs of 10 day old chick embryos

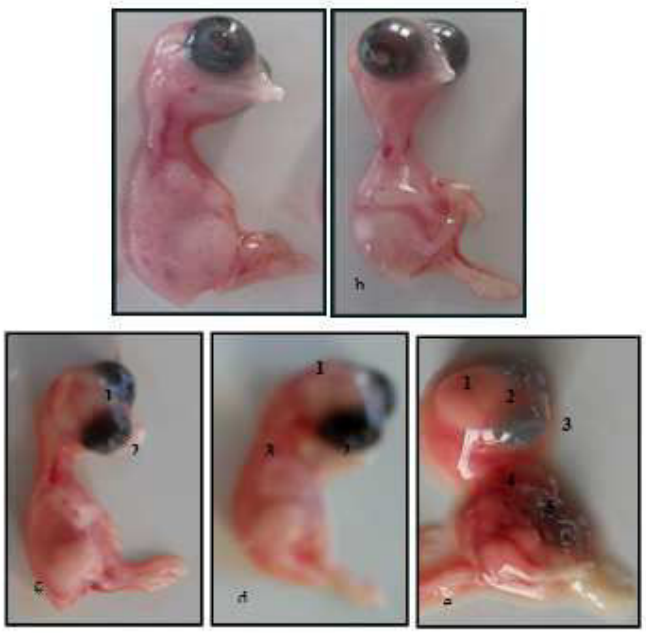

Figure 1 Photographs of 10 day old chick embryos, Untreated, Treated(DW) and carbosulfan treated.(a) **Untreated**, (b) **Treated** (DW), (c) **15.62ppm**-(1)Macrocephaly and (2) Macropthalmia, (d) **31.25ppm**-(1) Macrocephaly, (2) Macropthalmia and (3) Hematomas, (e) **62.50ppm**-(1) bulge in brain, (2) Macrocephaly, (3) absence of beak, (4) severe Haemorages and(5) gastrochisis.
*Malformed embryos exhibited one type or 2-4 types of malformations.

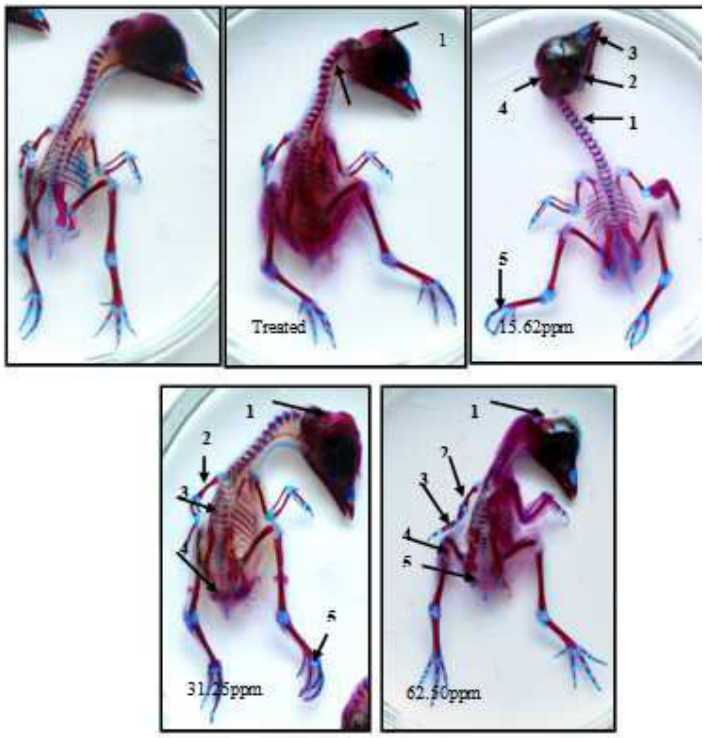

Figure 2 Photographs showing dorsal views of 15 day old chick embryos –untreated, treated (DW) and treated (Carbosulfan). **Untreated embryo** an untreated embryo has following parts: Premaxilla; Lower jaw; Skull; Cervical vertebrae; Wing; Ribs; Femur; Tibia; Metatarsus; Pelvic girdle; Cauda; vertebrae; Pygostyle; Digits. **Treated embryo** an embryo treated with DW shows (1)unossified hind parts of frontals and pterygoid, and (2)shortness and fusion of atlas and axis vertebrae.**15.62ppm** (1)reduced ossifications of ribs and cervical vertebrae, (2)small sized skull and (3)beak,(4) incomplete ossification of frontals, parietals and palatine, and (5)flexed digits.**31.25ppm** (1) incomplete ossification of frontals, parietals and palatine,(2) short humerus (3) reduced ossifications of thoracic vertebrae, (4) short kinked caudal vertebrae and reduced pygostyle, (5) flexed digits.**62.50ppm** (1) incomplete ossification of frontals, parietals and palatine,(2) short humerus,(3) radius,ulna and (4)femur on the right side,and (5) reduced pygostyle.
*Malformed embryos exhibited one type or 2-4 types of malformations.

DISCUSSION

The occurrence of embryonic death after dipping of the eggs into toxicant was found to show inverse relationship with the concentration of the dose tested. This trend may simply be observed because the amount of toxicant used is an important factor in regulating the viability and development of an organism [5]. Similar reporting was given with chick embryos and various other pesticides used for treatment [6, 14, 15, 24, 12, 26]. Reduced respiration during the critical period i.e. 4th day of the embryonic development might have caused increase in the levels of the toxic compounds like CO_2, lactic acid and ammonia. Accumulation of these toxic substances in the embryo due to absence of its elimination from the shell might have caused embryonic death [17]. Apart from all the probable answers to most of the teratogenicity observed, liver plays a very crucial role in the transformation of the xenobiotics; but liver develops and functions after 4th-5th day in the chick embryo and it is quite possible that the accumulation of the toxicant which must have taken place in first few days of the development resulted in observed teratogenic response [18]. Hemorrages, hematomas and edema formation with the use of Marshal might be due to generalized vascular damage and poor utilization of the yolk. This observation is in view with the pericardial and peritoneal edema recorded in fish eating birds, exposed to organochlorine insecticides [6]. The beak and limb defects might be due to the reaction of the insecticide with cation ionophores which modulate their movement across lipid membranes. Influx of calcium across the cell membranes might have been decreased with the binding of toxicant with the calcium binding protein calmodulin that might

affect the bone development [2,8,16]. Many of the above observed morphological and skeletal defects in chick embryo and with various classes of insecticides widely applied in the agricultural fields have been reported [2, 15,21,24,12]. Few commonly observed muscular and skeletal malformations are Macropthalmia, Macrocephaly, hemorrages, hematomas, reduced ossifications of the skeleton, limb deformities, malformed beaks, crooked digits, and incomplete formation of the skull. The vertebral defects are linked with Acetyl-Cholinesterase inhibition [7, 22, 25]. The influence of carbamates is reversible and therefore many significant malformations were observed only at increasing concentrations. Toxic effects at high doses and less or no effect at lower doses indicate that Carbosulfan might have been metabolized to its toxic metabolites which at lower doses could be detoxified with glutathione but at higher concentrations the level of the glutathione must have dropped in the liver. Thus damage caused by reactive metabolites could also lead to the development of teratological anomalies in the growing embryo [2]

CONCLUSION

We conclude that Marshal disrupts embryonic development in the chick embryo and causes teratogenic and embryotroxic effects, which coincides with other avian teratogenic studies. In this study it was observed that induction of the pesticide during the “critical period” in the development of the chick played an important role in inducing more pronounced teratogenicity. Moreover, incapability of the Carbamate insecticide to reverse the mode of action of AchE at high dose levels might have caused the observed musculoskeletal deformations. Hence, it is strictly recommended that limited and recommended dose levels should be used to avoid the harmful effects of the pesticides to the non-target organisms including humans. The harmful effects of the Marshal could also be tested with the mammalian models including humans to find out its possible role as a mutagen or teratogen and the limit of exposure up to which the pesticide could capably reverse the harmful effects produced during exposure.

ACKNOWLEDGEMENT

We like to thank The IIS University, Jaipur in providing suitable facilities to carry forward our experimentation work. We also thank the University Grants Commission, India, for providing assistance in the form of minor project to The Department of Zoology, The IIS University, Jaipur

REFERENCES

1. Alvares AP, Pharmacology and toxicology of carbamates. In: Ballentyne B, Marrs TC (eds) Clinical and experimental toxicology of organophosphates and carbamates. Butterworth and Heinemann, Oxford. 40–46, (1989).
2. Anwar K, Cypermethrin, a Pyrethroid induces teratological and biochemical changes in young chick embrys. Pakistan J Biol Sci, **(**6**)**: 1698-1705, (2003).
3. Cox C and Surgan M, Unidentified Inert Ingredients in Pesticides: Implications for Human and EnvironmentalHealth. Environmental Health Perspectives, 114(12**)**:1803-1806, (2006).
4. DeWitt JC, Meyer EB and Henshel DS, Environmental toxicity studies using chickens as surrogates for wildlife: effects of injection day. Arch Environ Contam Toxicol. (*48)*: 270-277, (2005).
5. Edward J G, Scientific Considerations in Monitoring and Evaluating Toxicological Research. Hemisphere Publishing Corporation, New York 221(1981).
6. Gilbertson M, Kublak T, Ludwig J and G. Fox , Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarity to chick-edema disease. J Toxicol Environ Health (33**)**: 455-520, (1991).

7. Greenberg and J. La. Ham QN, Reversal of malathion-induced teratisms and its biochemical implications in the developing chick. Can J Zool. (48**)**: 1047-1053, (1970).
8. Imamura L, Hasegawa H, Kurashina K, Hamanishi A, Tabuchi A and Tsuda M,Repression of activity-dependent c-fos and brain-derived neurotrophic factor m RNA expression by pyrethroid insecticides accompanying a decrease of ca $^{2+}$ influx into neurons. J Pharmacol Exp Ther (295):1175-82, (2000).
9. Inouye M, Differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red. S. Cong. Anom. (16):171-173, (1976).
10. Karnofsky D A, The chick embryo in drug screening: survey of teratological effects observed in the 4-day chick embryo. In Teratology: Principles and Techniques. University of Chicago Press. 194-213, (1965).
11. Kemper FH and Luepke NP, Toxicity testing by hen's egg test (HET). Food Chem Toxicol *(24)*: 647-648, (1986).
12. Mobarak Y M and Al-Asmari M A , Endosulfan Impacts on the Developing Chick Embryos: Morphological, Morphometric and Skeletal Changes. International Journal of Zoological Research. (7)*: 107-127, (2011).*
13. Newbold R R, Padilla-Banks E, Synder R J, Philips T M and Jeferson W N, Developmental exposure to endocrine disruptors and the obesity. Reproductive Toxicology. (23**)**:290-296, (2007).
14. Pourmirza A A, Toxic effects of malathion and endosulfan on chick embryo. J Agri Sci Tech (2**)**: 161-166, (2000).
15. Rao J V, Swamy A N, Yamin S, Rao S H and Rahaman M F, Teratism induced in the developing chick by RPR-V, an organophosphate. Fd Chem Toxic. 30(11**)**:945-951, (1992).
16. Rashatwar S S, Matsumura F, Interaction of DDT and Pyrethroids with calmodulin and its significance in the expression of enzyme activities of phosphodiesterase. Biochem Pharmacol. (34**)**: 1689-1694, (1985).
17. Romanoff A L, Effect of composition of air on the growth and mortality of the chick embryo. Journ Morph and Physiol. **(**50**)**: 517-525, (1930).
18. Romanoff A L, The avian embryo. The Macmillan Comp. New York 11, (1960).
19. Repetto M, Guillen A and Rodriguez-Consuegra A, Embryotoxicity of toxic oils and anilides. Analytical Chemistry, Symp. Ser. (80): 19-26, (1984).
20. Tripathi S and Srivastav A K, Alterations in the Profile of Blood Cells of Wistar Rats Induced by Long Term Ingestion of Chlorpyrifos. Int J Pharm Bio Sci. 1 (4), (2010).
21. Uggini K G, Patel P V and Balakrishnan S, Embryotoxic and Teratogenic Effects of Pesticides in Chick Embryos: A Comparative Study Using Two Commercial Formulations. Wiley Periodicals. Inc. DOI 10.1002/tox.20627, (2010).
22. Upshall D G, Roger J C and Casida J E, Biochemical studies on the teratogenic action of bidrin and other neuroactive agents in developing hen eggs. Biochemical Pharmacology (17**)**: 1529-1542, (1968).
23. U.S. EPA, U.S. Environmental Protection Agency, What Is a Pesticide? Available: http://www.epa.gov/pesticides/ about/index.htm. accessed 12 April 2006, (2005 c)
24. Wagh P, Deshpande S G and Salokhe S G, 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, (2011).
25. Walker N E, The effect of malathion and malaoxon on esterases and gross development of the chick embryo. Toxicol Appl Pharmacol. (19): 590-601, (1971).
26. Zhao F, Mayura K, Kocurek N, Edwards J F, Kubena L F , Safe S H and Phillips T D, Inhibition of 3,3′4,4′,5-pentachlorobiphenyl-induced chicken embryotoxicity by 2,2′4,4′,5,5′-hexachlorobiphenyl. Fundamental and applied toxicology. (35):1-8, (1997).