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Determination of 96 h LC₅₀ Value and Acute Toxicity of Marshal (Carbosulfan 25%EC) on Thyroid and Reproductive Hormones of *Labeo rohita* (Hamilton)

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Abstract: Contamination of water bodies by pesticides can lead to fish death and reduced fish productivity etc. which can affect humans consuming these fishes as a source of protein. The present study was designed to determine the 96h LC₅₀ value of a carbamate pesticide, Marshal (Carbosulfan 25% EC) and to investigate effects of this pesticide on the thyroid and reproductive hormones profile of Indian major carp *Labeo rohita* (Hamilton). The present study indicates that Marshal has potential hormonal stress on *Labeo rohita*. The mortality data obtained were analyzed by SPSS 16.0 based on Finney's Probit Analysis Statistical method. The 96h LC₅₀ value for Marshal was found to be 10 µl/L. Effects of this carbamate pesticide on serum T₃, T₄ and TSH, testosterone and estradiol were investigated at sub-lethal concentration. Serum T₃, T₄ and TSH were studied for 96 h and testosterone and estradiol for 4 days and 15 days. These parameters (except TSH) decrease after exposure of the fish to pesticide.

Keywords: Marshal (Carbosulfan 20%EC), *Labeo rohita*, 96h LC₅₀ value, Thyroid hormone, Testosterone, Estradiol

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Introduction

Many pesticides have been shown to affect the human endocrine system. Some of them including carbamate pesticides are strongly resistant to biodegradation and accumulate in the food chain (Silberman and Taylor, 2020). These endocrine disrupting chemicals can

mediate their activity by binding with hormone receptors and cause reproductive disorders. Intentionally or unintentionally farmers use these pesticides to make the situation from bad to worse not only in India but also throughout the world as well.

The number and quantities of carbamate pesticides used in agriculture continue to increase day by day, replacing other pesticides such as organochlorine and organophosphorous pesticides. One of the major agricultural pesticide is carbosulfan which play important role in increasing agricultural productivity through controlling pests; on the other hand they cause damage to both terrestrial and aquatic non-target organisms. Marshal (25% EC formulation of Carbosulfan) is a systemic, broad-spectrum carbamate insecticide registered in more than 80 countries. Marshal helps the farmer to rescue many crops from most of the sucking and lepidopterous insect pests like aphids, thrips, hoppers, borers, mites and nematodes. It is extremely toxic to mammals including human by inhibition of acetylcholinesterase activity (Capkin *et al.*, 2014).

Marshal (Carbosulfan 25%EC) which is viscous brown liquid in colour is a thiocarbamate. Flammable gases are generated by the combination of thiocarbamates and dithiocarbamates with aldehydes, nitrides and hydrides. Thiocarbamates and dithiocarbamates are incompatible with acids, peroxides and acid halides. Fish is directly affected by these pesticides when leaches out from agricultural area into water bodies.

The present study is an effort to determine the 96 h LC₅₀ value of a carbamate pesticide, Marshal (Carbosulfan 25%EC) and assess the toxic impacts of Marshal on hormonal profiles of Indian major carp *Labeo rohita* which is the prime cultured species in India.

Materials and Methods

Experiments were conducted from November to February, 2020. Healthy *Labeo rohita*

(Hamilton) fish (20 ± 1.4 g; 12.7 ± 0.75 cm) were brought from local market to laboratory carefully in oxygen filled plastic bags to avoid injury and disinfected for five minutes in 0.05% KMnO₄ solution. Then they were transferred to glass aquarium containing about 80 L dechlorinated tap water and provided sufficient oxygen supply. Fish were acclimatized to laboratory conditions for at least 14 days under 12:12 (light:dark) photoperiod. During acclimatization fish were fed daily with commercial food pellet daily about 3% of their body weight. Fish were maintained in static renewal conditions, where water and pesticide were completely replaced every 24 h, transferring fish to freshly prepared toxicant solutions to maintain dissolved oxygen concentration and pesticide concentration constant. Dead fishes were removed immediately to avoid fouling of water. Fishes were starved for 24 h prior to toxicity testing and were not fed during the experiment.

Water characteristics were measured by using guidelines of APHA (2005). Chlorine-free tap water was used having water qualities -- temperature 26 ± 2 °C; pH 7.2 ± 0.4 ; dissolved oxygen 7.3 ± 0.2 ppm and total hardness 233 ± 1.78 ppm.

96 h LC₅₀ Determination:

Pilot experiments were run to determine the LC₅₀ value for the pesticide through 0-100% mortality range test under laboratory conditions as described by Finney (1971). Control and five concentrations were taken for each test and 10 fish were introduced to aquarium of 80 L capacity. Each experiment was repeated three times to obtain constant results during 96 h acute toxicity testing. The calculated 96 h LC₅₀ values was 10 µl/L

(Fig. 1) for Marshal. The sub-lethal concentrations taken for experiments were 0.5 µl/L, 1 µl/L, 2 µl/L, 4 µl/L and 8 µl/L of Marshal. Serum T₃, T₄ and TSH were studied for 96 h and Testosterone and Estradiol for 4 days and 15 days.

Fig. 1: Probit log graph for determination of 96 h LC₅₀ of Marshal 25% EC to *Labeo rohita*. Dose= Log₁₀ scale.

Serum preparation and analysis:

At the end of duration (96 h, 4 days and 15 days), fish of control and experimental groups were anesthetized with MS222 (Ethyl 3-aminobenzene methanesulfonate salt, Sigma) for T₃, T₄, TSH, testosterone and estradiol level measurement. Blood samples were taken from cardiac puncture and collected in anticoagulant-free centrifuge tubes. Serum was obtained by centrifuging blood at 4000 rpm for 10 min. Serum samples were then stored at -80 C until analysis.

Estimation of serum T₃ (Triiodothyronin), T₄ (Thyroxin), TSH (Thyroid Stimulating Hormone), Testosterone and Estradiol:

The determination of serum level of T₃, T₄, TSH, Testosterone and Estradiol was done by using ELISA kit (RFCL Limited, Mumbai) according to manufacturer's instruction. The

optical density of control and experimental samples were read within 10 min using microplate reader (BIO-RAD) with a 450 nm filter. Serum concentration of T₃, T₄, TSH, Testosterone and Estradiol were expressed in ng/ml, µg/dl, µIU/ml and ng/ml, respectively.

The data collected from control and treated groups were represented as Mean± S.E for each group. The data were tested for statistical differences using a One-Way ANOVA followed by Duncan's Multiple Range Test (DMRT) in SPSS 16.0. Statistical differences were determined at P <0.05 levels for all analysis.

Results

The sub-lethal concentrations taken for experiments were 2 µl/L, 4 µl/L and 8 µl/L of Marshal for 4 days and 15 days. Fish mortality was increased significantly when the concentration and the time of exposure were increased (Table 1). No mortality was observed in the control group during the experiment. From the present study it was found that Marshal was toxic to *L. rohita*. Tables 2, 3 and 4 depict the parameter estimates of the Probit analysis and chi square test for the acute toxicity of Marshal to *Labeo rohita*.

After exposure of the pesticide, *Labeo rohita* showed altered behaviour. In low concentrations of pesticide, the responses were of a lesser degree. Control fish behaved normally, fish aggregated in the bottom of aquarium. Irregular, erratic and darting swimming movements, loss of balance, drowning and change in body pigmentation became more apparent with increase in duration of exposure at all test concentrations of pesticide. They slowly became lethargic, hyperactive and secreted copious amount of

Table 1: Mortality of *Labeo rohita* after Marshal 25% EC exposure

CONC. (µl/L)	Log10 CONC.	TOTAL NO.	NO. DEAD	% MORTALITY	PROBIT
CONTROL	-	10	0	0	-
1.25	0.096	10	1	10	3.72
2.5	0.397	10	2	20	4.16
5	0.698	10	4	40	4.75
10	1	10	5	50	5.00
20	1.30	10	6	60	5.25
40	1.60	10	8	80	5.84
80	1.90	10	10	100	---

Table 2: Parameter estimates of 96 h LC₅₀ Probit Analysis for Marshal 25% EC

'Parameter'	'Estimate'	'Std. Error'	'Z'	'Sig.'	'95% Confidence Interval'	
					'Lower Bound'	'Upper Bound'
PROBIT ^a Conc	1.534	.330	4.645	0.000	0.886	2.181
Intercept	-1.474	.364	-4.050	0.000	-1.838	-1.110

a. "PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)"

Table 3: Chi-Square Tests of 96 h LC₅₀ Probit Analysis for Marshal 25% EC

	'Chi-Square'	'df ^a '	'Sig.'
'PROBIT Pearson Goodness-of-Fit Test'	1.540	5	0.908 ^b

^a. Statistics based on individual cases differ from statistics based on aggregated cases.

^bSince the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits.

Table 4: 96 h LC₅₀ Value for freshwater teleost *L. rohita* after exposure to Marshal

Pesticide	96 h LC ₅₀ Value (µl/L)	Regression Equation
Marshal 25%EC	10	Y= -1.5+1.25*X

mucus all over their body. With increasing exposure of pesticide their opercular movements became least and died with their mouth opened.

One-Way ANOVA results of acute exposure to Marshal indicated significant decrease in serum T₃ ($P = 0.001$; $F = 29.155$) (Fig. 2) and T₄ ($P = 0.024$; $F = 6.046$) (Fig. 3) with increasing dose exposure when compared to control. Unlike, T₃ and T₄, significant increase in TSH ($P = 0.018$; $F = 6.836$) (Fig. 4) with increasing dose exposure was noticed. Serum T₃ reduction was more pronounced than T₄. Reduction in body weight of fish was also found with increasing concentration of the pesticides.

Fig. 2: Estimation of serum T₃ in *L. rohita* exposed to Marshal 25% EC for 96 h. '0' indicates 'control' fish. Data are represented as Mean $\pm$ S.E. ($n=6$). Values are significantly different ($P < 0.05$) from each other according to Duncan's multiple range tests. 'F' value= 29.155.

At 15th day of the experiment testosterone ($P = 0.04$ and $F = 3.801$) and estradiol levels ($P = 0.001$; $F = 21.065$) in pesticide exposed fish were decreased drastically (Figs. 5, 6). Both hormone levels decreased on exposure to sub-lethal concentrations at 4 days also with increasing dose (Figs. 5, 6). Maximum decrease was observed at 15 days exposure to the toxicant.

Fig. 3: Estimation of serum T₄ in *L. rohita* exposed to Marshal 25% EC for 96 h. '0' indicates 'control' fish. Data are represented as Mean $\pm$ S.E. ($n=6$). Values are significantly different ($P < 0.05$) from each other according to Duncan's multiple range tests. 'F' value= 6.046.

Fig. 4: Estimation of serum TSH in *L. rohita* exposed to Marshal 25% EC for 96 h. '0' indicates 'control' fish. Data are represented as Mean $\pm$ S.E. ($n=6$). Values are significantly different ($P < 0.05$) from each other according to Duncan's multiple range tests. 'F'- value=6.836.

Discussion

Few studies reported the effects of EDC (Environmental Disrupting Chemicals) on reproduction, sex steroids (Waring and Brown, 1997) and thyroid hormones (Griffin *et al.*, 2000). Suppressed level of thyroid hormones have been reported in salmonoid fishes which depicts physiological stress responses (Pickering, 1993; Schreck *et al.*,

Fig. 5: Serum testosterone level in *L. rohita* exposed to Marshal for 4 and 15 days. 0' indicates 'control' fish. Data are represented as Mean $\pm$ S.E (n=6). Values are significantly different $P=0.04$ according to Duncan's multiple range tests and 'F' value=3.801.

Fig. 6: Serum estradiol level in *L. rohita* exposed to Marshal for 4 and 15 days. 0' indicates 'control' fish. Data are represented as Mean $\pm$ S.E (n=6). Values are significantly different from each other according to Duncan's multiple range tests and 'F' value=21.065.

1997; Tyler *et al.*, 1998; Veeraiah *et al.*, 2013). Significant reduction in serum T_3 level was recorded in case of *Sarotherodon mossambicus* after Dimecron exposure (Pankhurst and Van, 1997). Environmental contaminants affect plasma thyroid hormone levels and cause thyroid dysfunction in fish (Dey and Saha, 2014). Both T_3 and T_4 regulate growth,

development and metabolism of fish and also involved in many of the biological functions (McDonald and Milligan, 1997). T_4 is the predominantly circulating hormone, though T_3 is metabolically more active and changed according to metabolism. Presence of T_3 in lesser amount may indicate a rapid turnover from T_4 to T_3 before usage (Steenland *et al.*, 1997).

In this study certain behavioural alterations have been noticed after Marshal exposure to *Labeo rohita*. Alterations in behaviour of dimethoate exposed *Heteropneustes fossilis* have been reported in past (Thangavel *et al.*, 2005; Pandey *et al.*, 2009). Similar abnormal behavioural pattern in *Channa orientalis* were noticed by Saxena *et al.* (1997) after exposure of nuvan and dimecron. Dey and Saha (2014) have observed alterations in behaviour of dimethoate and Lambda-cyhalothrin exposed *Labeo rohita*.

It may be stated that the sluggish behaviour of fish was to cope up with pesticide exposure and thereby reducing toxic impact. On the other hand causes behind the mild changes in T_4 level is still unclear. The decreased secretion of thyroid hormones could bring physiological alterations in their potential roles in regulating osmoregulation, growth and metabolism (Waring and Brown, 1997) which was also supported by sluggishness of the fish noticed in present study. Serum TSH levels with increasing dose exposure may be due to positive feedback of low amount of circulating T_3 and T_4 at the peripheral level to hypothalamo-pituitary-thyroid (HPT) axis.

Exposure to 10 µg/L of DE-71 significantly reduced thyroxine (T_4) levels and significantly

upregulated the transcription thyroid-stimulating hormone (TSH) genes in a concentration-dependent manner. The findings of our study suggest that the elevation in the TSH levels can be attributed to the reduced negative feedback from the hypothalamus and pituitary due to decreased levels of circulating T₄ (Liqin *et al.*, 2010).

Pesticides are reported to cause degenerative changes in gonads and arrest gametogenic processes either by acting directly on the gonads or by interfering with the secretory activity of the hypothalamo-hypophyseal-gonadal/thyroid axis that regulates various reproductive events. Decrease in levels of testosterone after pesticide exposure in this study may be related with testicular degeneration of leydig cells and alteration in the testis gene expression. Similarly decrease in estradiol may be due to gonadal follicle cells degeneration.

In the present study it was observed that, short-term Marshal exposure (4 days) did not exert appreciable changes in estradiol and testosterone levels. On the contrary, prolonged Marshal exposure (15 days) significantly decreased testosterone and estradiol levels in dose- and time-dependent manner. The decrease in hormone action may be due to the interference of pesticide with the synthesis, transport, metabolism and elimination of hormones, thereby decreasing the circulating concentration of hormones. Similar results were found after exposure of sub-lethal concentration of dicofol (18.5%EC) to *Channa punctatus* (Veeraiah *et al.*, 2013).

Pesticides cause degenerative changes in gonads and arrest gametogenic processes either directly on the gonads or indirectly by

interfering with the secretory activity of the hypothalamo - hypophyseal-gonadal/thyroid axis that regulates various reproductive events. Thyroid hormones after pesticide treatment decrease in this study. Secretion of hormones such as testosterone, estradiol and thyroid hormones are in general lowered, leading to cessation of gametogenesis, vitellogenesis, oocyte maturation, ovulation, spermiation, etc. Adverse effects of pesticides have also been demonstrated on fecundity, fertilization, hatching, and postembryonic development.

Conclusion

The present investigation demonstrated that Marshal is toxic to *L. rohita*. Marshal had profound impact on behaviour, thyroid and reproductive hormone levels of *L. rohita* at sub-lethal concentrations and also had adverse effects on fish physiology due to impaired metabolism and pesticide induced stress.

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References

- APHA. (2005) Standard methods for the examination of water and wastewater. 21st Ed. Washington, DC. USA.
- Capkin E, Boran H and Altinok I. (2014) Response of acetylcholinesterase (AChE) in the erythrocyte and liver of rainbow trout exposed to carbosulfan. Turkish J Fish Aquat Sci. 14: 643-650.
- Dey C and Saha SK. (2014) A comparative study on the acute toxicity bioassay of dimethoate and lambda-cyhalothrin and effects on thyroid hormones of

- freshwater teleost fish *Labeo rohita* (Hamilton). Int J Env Res. 8: 1085-1092.
- Griffin JE, Griffin JE and Ojeda SJ. (2000) Text Book of Endocrine Physiology, 4th ed., Oxford University Press, UK. pp 303-327.
- Liqin Yu, Deng Jun, Xiongjie Shi, Chunsheng Liu, Ke Yu and Bingsheng. Z. (2010) Exposure to DE-71 alters thyroid hormone levels and gene transcription in the hypothalamic–pituitary–thyroid axis of zebrafish larvae. Aquat Toxicol. 97: 226-233.
- McDonald G and Milligan L. (1997) Ionic, osmotic and acid-base regulation in stress. In: Fish Stress and Health in Aquaculture. Cambridge University Press, pp 119-144.
- Pandey RK, Singh RN, Singh S, Singh NN and Das VK. (2009) Acute toxicity bioassay of dimethoate on freshwater air breathing catfish *Heteropneustes fossilis* (Bloch). J Env Biol. 30: 437-440.
- Pankhurst NW and Van Der Kraak G. (1997) Effects of stress on reproduction and growth of fish. In: Fish Stress and Health in Aquaculture. pp 73-93.
- Pickering AD. (1993) Growth and stress in fish production. Aquacul. 11: 51-63.
- Saxena V, Trivedi S and Saxena DN. (1997) Acute toxicity of two organophosphorus pesticides nuvan and dimecron to a freshwater murrel, *Channa orientalis* (Schndider). J Environ Poll., 4: 79-83.
- Schreck CB, Olla BL and Davis MW. (1997) Behavioral responses to stress. In: Fish Stress and Health in Aquaculture, Cambridge University Press, pp 145-170.
- Silberman J and Taylor A. (2020) Carbamate Toxicity. In: StatPearls. Treasure Island (FL): StatPearls Publishing; <https://www.ncbi.nlm.nih.gov/books/NBK482183/>
- Steenland K, Cedillo L, Tucker J, Hines C, Sorenson K, Deddens J and Cruz V. (1997) Thyroid hormones and cytogenic outcomes in backpack sprayers using ethylenebis (dithiocarbamate) (EBDC) fungicides in Mexico. Environ Hlth Perspect. 105: 1126-1130.
- Thangavel P, Sumathirai K, Karthikeyan S and Ramaswamy M. (2005) Endocrine response of the freshwater teleost, *Sarotherodon mossambicus* (Peters) to dimecron exposure. Chemosphere 61: 1083-1092.
- Tyler CR, Jobling S and Sumpter JP. (1998) Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol. 28: 319-361.
- Veeraiah K, Dhilleswa Rao H, Hyma Ranjani G, Venkatrao G and Vivek C. (2013) Changes in hormonal activities of the fish *Channa punctatus* (Bloch) under exposure To difocol 18.5% (EC) sub-lethal concentration. Int J Rec Sci Res. 4: 1631-1637.
- Waring CP and Brown JA. (1997) Plasma and tissue thyroxine and triiodothyronine contents in sublethally stressed, aluminum-exposed brown trout (*Salmo trutta*). Gen Comp Endocr. 106: 120-126.