Available online at www.elixirpublishers.com (Elixir International Journal)

Aquaculture

Elixir Aquaculture 43 (2012) 6946-6951

ISSN: 2229-712X

Heamatological responses of *Cyprinus carpio* L. exposed to sub-lethal concentrations of monocrotophos

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ARTICLE INFO

Article history:

Received: 15 January 2012;

Received in revised form:

30 January 2012;

Accepted: 15 February 2012;

Keywords

Monocrotophos,

Blood indices,

Cyprinus carpio,

Sublethal.

ABSTRACT

The blood parameters like red blood cell count, white blood cell count, haemoglobin, packed cell volume, mean cell haemoglobin, mean cell volume and mean cell haemoglobin concentration were estimated in Cyprinus carpio exposed to1/10th (8.64ug/L) and 1/15th(5.67ug/L)of lethal concentrations of monocrotophos for 5.10, 20, 1nd 30 days. The results showed alterations indicating the pesticide effect being severe as RBC count decreased, WBC count increased, Hb decreased, PCV increased, MCV, MCH and MCHC appeared decreased.

Introduction

Blood is a suitable means of indicating and identifying the effects of stress, environment and health status of fish in a given area. In order to undertake the cultural practices the studies of hematology and blood chemistry in different fish species are of comparative physiological interest (Parma DeCroux, 1994). Celik (2004) reported that fish blood is very important to accurately evaluate the health of species. A variety of pollutants affect the water course which receive domestic, industrial and in terms of its ramifications and environment consequence (P. Tawari-Fufeyin, et.al, .2008). Alterations in fish blood are also observed due to the influence of capture and capture methods to perturb the blood parameters (Bouck and Ball, 1966). Chemical pollutants induce either increase or decrease in hematological levels, their effect depends on fish species, age, the sexual cycle of spawners and diseases (Golovina, 1996; Luskova, 1997). The blood reveals conditions within the body of fish long before any outward manifestation of disease. The close contact of environment to fish makes them susceptible to physico-chemical changes reflected in their blood (Wilson and Taylor,1993). Gender has a great influence on haematology of fish (Dacie and Lewis 1991), Gabriel et al.,(2001), Akinrotimi et.al.,(2010). Gobacher and Skaya(1977) observed some chronic effects of organic phosphate insecticide on fish blood. Human destruction influences the aquatic environment in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life. The main source of freshwater pollution can be the discharge of untreated waste, dumping of industrial effluent, and run-off from agricultural fields. In recent years blood physiology is used to clinically diagnose the fish due to the close association between the circulatory system and the external environment. The present study is an effort to assess the fish blood under the effect of Monocrotophos exposed for short and long durations at sub lethal concentrations.

Materials and methods

The live healthy *Cyprinus corpio* were procured from the state fisheries department, Dharwad, India, weighing 80-90 grams (±2g),size 10-12 inches(± 0.2 inches) respectively. The fish were treated with 0.1% KMnO4 to remove any fungal or viral infection, stored in large plastic tubs and fed daily. They were acclimatized to the laboratory conditions for a period of 15 days at 24±.1 ^{o}C. The physicochemical characteristics of water were analyzed following the methods mentioned in APHA (2005), that were found as Temperature: 24±2^{o}C, pH7.2 (±0.2) at 24 ^{o}C, DO: 9.1±0.8 mg/L, CO_2 : 6.2±0.4 mg/L, Total hardness: 22.8±3.4 mg as $CaCO_3$/L, Phosphates: 0.390±.002µg/L , Salinity: nil, Specific gravity: 1.0030 and conductivity: less than 10 uScm-1. Water was renewed daily and 12-12 h of photoperiod was maintained during acclimation and test periods. The fish were divided into batches of 10 each and were exposed to the toxicant for further studies. A batch of fish maintained alongside without monocrotophos, served as control. The experiment was repeated thrice for accuracy. Taking into consideration of the fact that the effect of pesticide on fish becomes consistent within 96 hours of exposure, Lc50 96 hours (86.4ug/L) of monocrotophos was taken as lethal concentration for *Cyprinus carpio* to study the haematological responses (Maniyar R A et al, 2011), 1/10th (8.64µg/L) and 1/15th (5.76µg/L) of Lc50 96 hrs were taken as sub lethal concentrations for further studies. Blood was collected by severing the caudal peduncle/ by puncturing heart taking care that the blood was not hemolysed. It was collected in vials coated with 2% EDTA, as an anticoagulant. A drop of blood was examined under microscope for the presence of parasites if any. Blood free from any infection was used to study the hematological parameters. To avoid variation, the samples were collected at a particular time during early hours of the day. The RBC and WBC count was done by employing the methods of Dacie & Lewis (1984).Hb estimation was done by Sahlis,

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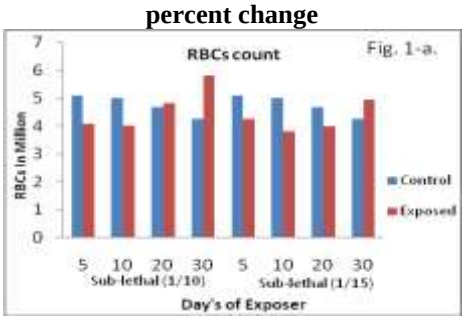
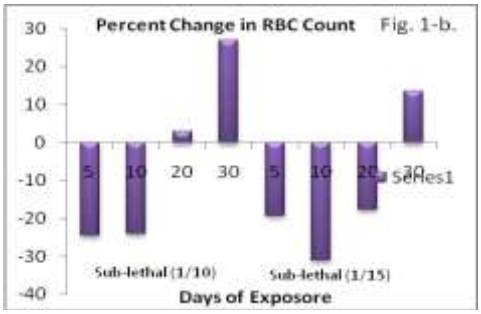
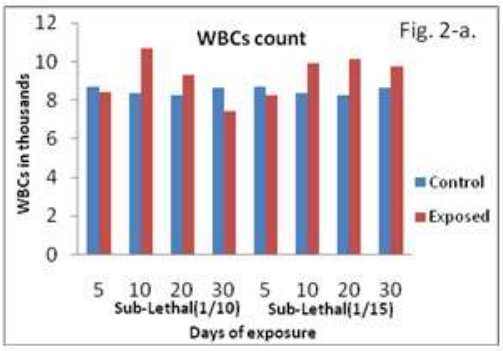
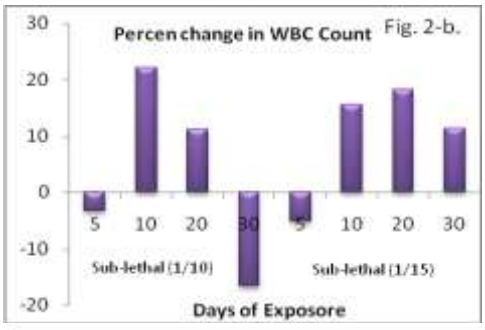
method. Mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell haemoglobin (MCH) were calculated using the following equations: MCHC = (Hb/PCV) 100, MCH= (Hb/RBCs) 10 and MCV= (PCV/RBCs) 100 (Wickham, Costa and Eisner,1990).

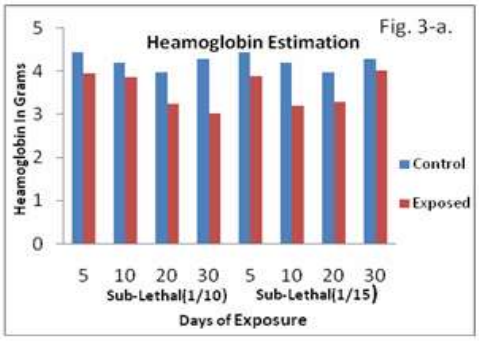
Graphs of heamatological indices of mean values and percent change

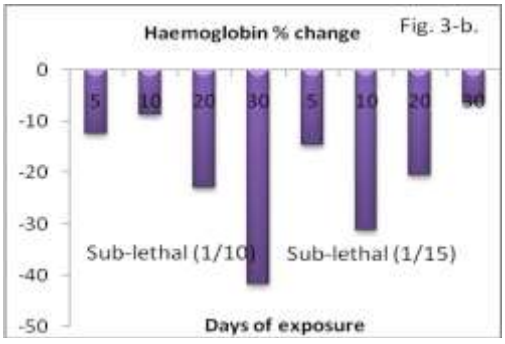


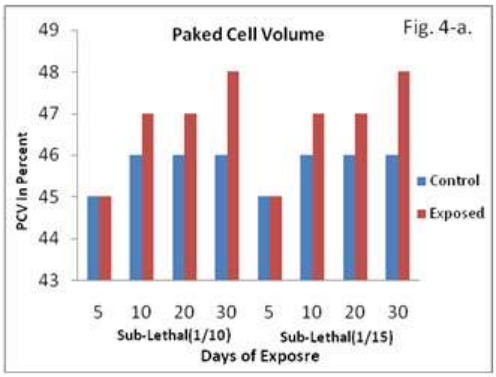


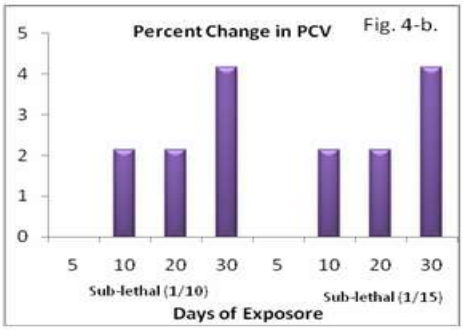


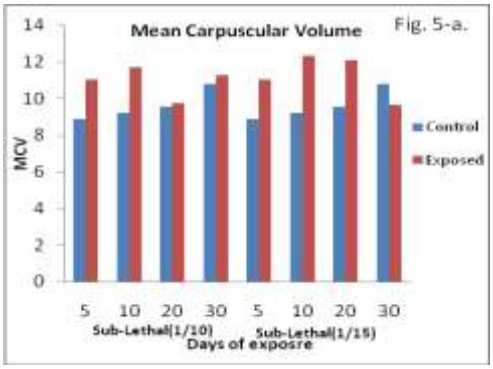


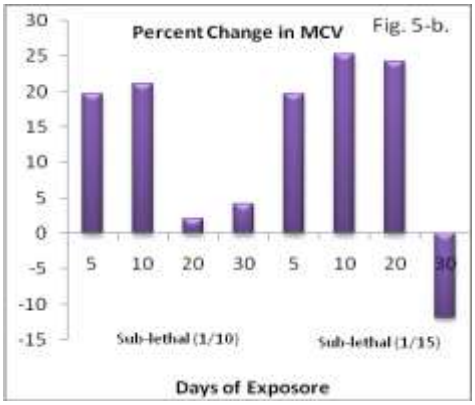


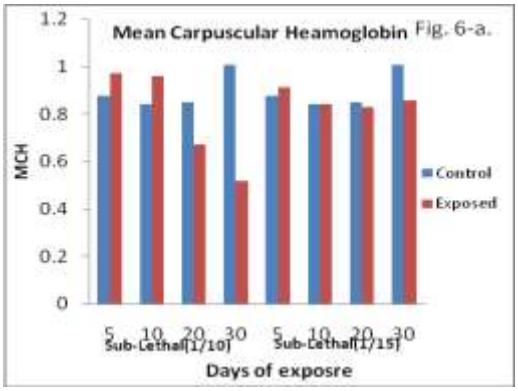


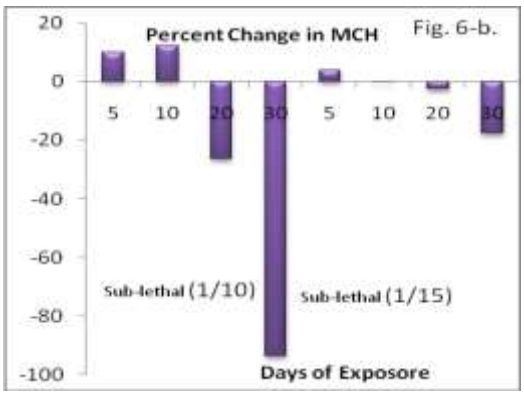


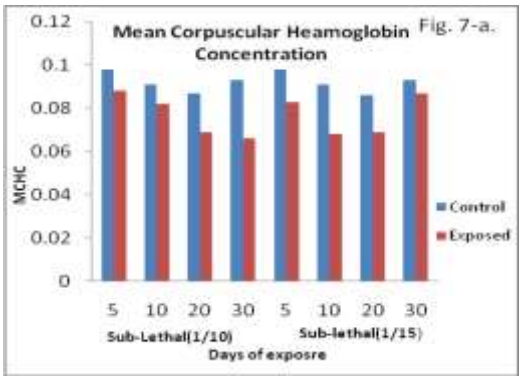


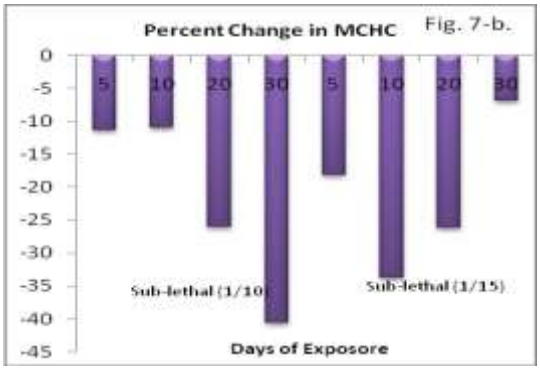












Results

The data in Table- 1 indicates that the fish exposed to two sub-lethal concentrations (8.64 and 5.76 µg/L) of monocrotophos for 5, 10, 20 and 30 days showed considerable variation over control(Table-1,Fig-1a, 1b to 7 a and 7b).

RBC count showed abrupt variation over control in both the concentrations. At $1/10^{th}$ concentration maximum (24.5 %) decrease was recorded on 5^{th} day, (24.13 %) decrease on 10^{th} day. On 20^{th} day it increased (2.91%) above the control values and on 30^{th} day showed (26.8 %) above the control values. At $1/15^{th}$ concentration maximum (30.97%) decrease was recorded on 10th day, (19.3%) decrease on 5^{th} day and (17.6%) decrease on 20^{th} day. On 30^{th} day the value was (13.59 %) above the normal values (Fig-1a and 1b).

WBCs also appeared abruptly varied. At $1/10^{th}$ exposure appeared (3.33%) decreased on 5^{th} day but a sharp (22.14 %) increase than the control values on 10^{th} day, (11.16 %) increase on 20^{th} day, but (16.55%) decrease on 30^{th} day than the control level. At $1/15^{th}$ concentration on 5^{th} day recorded (5.21%) decrease, on 10^{th} day (15.47%) above the control, 20^{th} day showed (18.18%) above and on 30^{th} day (11.36%) above the control values (Fig-2a and 2b).

Hemoglobin was found to be decreased in both concentrations throughout the exposure period. Maximum(41.72%) decrease was recorded on 30^{th} day of $1/10^{th}$ exposure while all the remaining values w ere (12.4%) decreased on 5^{th} day, (8.81%) decreased on 10^{th} day and (22.4%) decreased on 20^{th} day. $1/15^{th}$ exposures showed(14.7%) decrease on 5^{th} day, maximum(31.25%) decrease recorded on 10^{th} day,(20.6%) decreased on 20^{th} day and (6.46%) decrease on 30^{th} day than the control (Fig-3a and 3b).

PCV did not show any alteration on 5^{th} day of $1/10^{th}$ and $1/15^{th}$ concentrations while at $1/10^{th}$ and $1/15^{th}$ exposures on 10^{th} and 20^{th} days the values were similar(2.13%)increase than the control values. (4.17%) increased were the values on 30^{th} day of both $/10^{th}$ and $1/15^{th}$ exposures than the control values (Fig- 4a and 4b).

MCV found (11.83%) decreased on 30^{th} day of $1/15^{th}$ exposure while all the values were above the control values (positive at both the sub-lethal exposures except on 30^{th} day of $1/15^{th}$ exposure (Fig-5a and 5b).

MCH at $1/10^{th}$ exposure recorded (9.89%) increase over the control on 5^{th} day, (12.29%) increase on 10^{th} day. On 20^{th} day (26.49%) decrease than the control and a maximum (93.64%) decrease was recorded on 30^{th} day. At $1/15^{th}$ exposures on 5^{th} day it was (3.95%) above which (0.31%) decreased on10th day. On 20^{th} day the (2.53%) reduced and on 30^{th} day (17.65%) decrease was noted (Fig-6a and 6b).

MCHC values decreased in both the concentrations on all exposure days with variable reduction. At $1/10^{th}$ concentration (11.4%)decrease recorded on 5^{th} day, (10.98%) decrease on 10^{th} day, (26.09%) decrease on 20^{th} day and (40.64%) decrease on 30^{th} day. At $1/15^{th}$ exposures (18.1%) decrease on 5^{th} day, (33.82%) decrease on 10^{th} day, (26.1%) on 20^{th} day and (6.89%) decrease on 30^{th} day than the control values.(Table-1,Fig7a&7b).

Discussions

The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants and toxicants of various types. Sub-lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms. Pollutants can result in several physiological dysfunctions in fish which induce changes in blood parameters as a result of blood water contact. In the present study the exposure of fish to sub-lethal concentrations of monocrotophos for 5, 10, 20 and 30 days caused significant alterations in haematological parameters of Indian fresh water fish *Cyprinus carpio*.

A significant reduction in RBCs, Hb content and HCT in Nile Tilapia (*Oreochromis niloticus*) was found on exposure to Cadmium by Husain A et al.(2011). Erythrocytes and PCV decreased and Hb, WBC count increased in *Clarias gareipinus*

exposed to Cadmium and Lead by Tawari- Fufeyin P et al.(2008). A decrease in RBC count in *Clarias gareipinus* exposed to Tobacco leaf extract was due to haemolysis of blood thereby causing an increase in RBC production to capture the limited Oxygen induced by poison for sustenance of respiration as observed by Kori-Saikpere et al., (2008). Dharamsingh et al (2007) observed significant decrease in RBCs in fish exposed to sub-lethal concentrations of Copper causing anemia and blood cell injury. M.Santhakumar et al, (1999) observed a decrease in RBCs exposed to monocrotophos in *Anabas testudinus* which was due to decreased erythropoesis as is regulated in most vertebrates by the erythropoietin produced in kidneys (Gordon et al., 1967). Erythropoeitin induces stem cells to differentiate in erythroblasts. Erythropoeitin also activates Pyridoxal phosphate in developing RBCs inducing Hb synthesis (Reddy et al., 1992). Hypoxia constitutes the fundamental stimulus for erythropoesis with the kidneys as probable sensing organ for low blood oxygen tension (Jacobson & Krautz, 1968). Monocrotophos reduces the ventilator movements and decreased oxygen intake by impairing neuromuscular transmission through AChE inhibition (Murphy, 1980). A structurally intact and normal functioning kidney is essential for erythropoietin production (Gordon et al., 1967). Kidney damages usually cause a decrease in erythropoietin level that decreases RBC production and Hb synthesis under hypoxic condition (Reddy et al., 1992). M.Ramesh et al (2008) reported similar results in *Cyprinus carpio* exposed to chlorpyrifos with decreased RBCs and Hb. In *Cirrhinus mrigala* Hb decreased on exposure to Bismark brown and acid leather brown by S.Afaq et al. (2010). The reduction in haematological values might be due to anaemia caused by erythropoesis, haemosynthesis and osmoregulatory dysfunctions or due to erythrocyte destruction in haematopoeitic organ (Jenkins et al, 2003, Seth and Saxena, 2003). The results in the present investigation may be attributed to the findings of M.Santhakumar et al., (1999), Kori-Saikpere (2008), Dharamsingh et al.,(2007) and M. Ramesh et al.,(2008).

High WBC count on 10^{th} and 20^{th} day at (8.64µg/L) exposure and 10^{th} ,20^{th} and 30^{th} day at(5.76µg/L) exposure of monocrotophos were observed in *Cyprinus carpio* in the present study. M. Santha kumar et al,(1999) reported a significant increase in leucocytes which may be involved in regulation of immunological functions and prolonged exposure may inflict immunological deficiency. Similar findings are recorded by the other researchers (Siddique et al.1991; Figar et al. 1995). The decrease during first five days and then increase in WBCs in the present study may be attributed to the toxicity of monocrotophos which may have caused the injuries on gills and gut cells where most WBCs must have died leading to lesser WBC count in the early days of exposure. As a result of toxicity the cellular humoral response of the body was triggered by producing more WBCs which migrated to the site. WBCs defend the body against toxic and foreign substances and produce antibodies(Oxford dictionary,2002). The findings of Atamanalp et al. (2002)showed a decrease in WBCs in *Oreochromis mykiss* exposed to mancozeb are partly in agreemaent with our findings.

PCV may show the extent of cell swelling or shrinkage through its decrease or increase. Olanike Kudirat et al.,(2007) found decreased PCV on exposure of experimental fish to Lead and an increase in MCV, MCH and MCHC. MCH and MCHC decreased in Rainbow trout exposed to mancozeb by M.Atamanalp et al.,(2002). Hb decreased MCHC increased in *Cyprinus carpio* exposed to endosulphan as reported by Chandrasekar & N. Jayabalan (1993). Kori Saikpere et al., (2008) observed HCT, Hb ,RBCs, WBCs MCV MCH and MCHC decrease in Heteroclarias species on exposure to sub lethal doses of Zinc. R.Dobsikova, Z.Sobodova et al.,(2009) observed an increase in PCV in Cyprinus carpio during and after transportation due to stress and disturbances in transport conditions. Tilapia zilli showed an increased PCV on exposure to Aluminium by SF Alwan et al.,(2009) which may be on one hand due to increased volume of RBCs caused by osmotic changes due to ion losses from blood plasma and on the other hand by increased number of RBCs as a result of adrenergic-splenic contraction in hypoxic conditions. In the present investigations the increased PCV and MCV may be attributed to the observations made by R. Dobsikova et al,.2009 and S.F.Alwan et al.,2009. The decrease in MCH and MCHC may be due to absence of haemopoesis and haemoglobin synthesis under the influence of monocrotophos.

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Table – I Haematological parameters under the influence of Monocrotophos

	Days	Sub-Lethal(1/10)				Sub-lethal(1/15)			
		5	10	20	30	5	10	20	30
RBC'S	Control	5.08	4.99	4.68	4.26	5.08	4.99	4.68	4.26
	Exposed	4.08	4.02	4.82	5.82	4.26	3.81	3.98	4.93
	S.D	±0.02	±0.07	±0.016	±0.016	±0.11	±0.01	±0.02	±0.012
	% Change	-24.5	-24.13	2.91	26.8	-19.3	-30.97	-17.6	13.59
WBC'S	Control	8.69	8.36	8.28	8.66	8.69	8.36	8.28	8.66
	Exposed	8.41	10.66	9.32	7.43	8.26	9.89	10.12	9.77
	S.D	±0.34	±0.0.3	±0.14	±0.204	±0.20	±0.74	±0.27	±0.76
	% Change	-3.33	22.14	11.16	-16.55	-5.21	15.47	18.18	11.36
S.DHb	Control	4.45	4.20	3.98	4.28	4.45	4.20	3.98	4.28
	Exposed	3.96	3.86	3.24	3.02	3.88	3.2	3.3	4.02
	S.D	±0.17	±0.36	±0.44	±0.037	±0.18	±0.17	±0.46	±0.11
	% Change	-12.4	-8.81	-22.84	-41.72	-14.7	-31.25	-20.6	-6.46
PCV	Control	45	46	46	46	45	46	46	46
	Exposed	45	47	47	48	45	47	47	48
	S.D	±4.89	±3.68	±2.06	±3.68	±2.52	±1.53	±4.58	±3.12
	% Change	00	2.13	2.13	4.17	00	2.13	2.13	4.17
MCV	Control	8.858	9.22	9.544	10.798	8.858	9.22	9.544	10.798
	Exposed	11.03	11.69	9.751	11.26	11.03	12.33	12.06	9.655
	S.D	±0.25	±0.58	±0.67	±0.57	±0.06	±0.49	±.47	±0.24
	% Change	19.69	21.13	2.12	4.1	19.69	25.22	24.25	-11.83
MCH	Control	0.875	0.842	0.850	1.005	0.875	0.842	0.850	1.005
	Exposed	0.971	0.96	0.672	0.519	0.911	0.84	0.829	0.857
	S.D	±0.02	±0.05	±0.043	±0.018	±0.08	±0.07	±0.83	±0.06
	% Change	9.89	12.29	-26.49	-93.64	3.95	-0.31	-2.53	-17.65
MCHC	Control	0.098	0.091	0.087	0.0930	0.098	0.091	0.086	0.093
	Exposed	0.088	0.082	0.069	0.066	0.083	0.068	0.069	0.087
	S.D	±0.01	0±.09	±0.009	±0.003	±0.01	±0.01	±.006	±.214
	% Change	-11.4	-10.98	-26.09	-40.64	-18.1	-33.82	-26.1	-6.89

Data are expressed as mean ± S.D. and % change.