ISSN 2321–340X

Journal of Aquatic Biology & Fisheries | Vol. 4 | 2016 | pp. 64-71

STRESS RESPONSE OF BIOMOLECULES (PROTEIN, LIPIDS, CARBOHYDRATES AND GLYCOGEN) IN A FRESH WATER FISH *RASBORA DANDIA* EXPOSED TO PERCHLORATE

Divya, P.S.[1*] and Benno Pereira, F.G.[2]

[1]*Department of Aquatic biology and Fisheries, University of Kerala, Thiruvananthapuram-695581*
[2] *Department of Zoology, University of Kerala, Thiruvananthapuram-695581*
Email: divyanu111@gmail.com

Abstract: Perchlorate (ClO_4^-) is an emerging pollutant affecting aquatic organisms as well as human health and widely reporting from ground and surface waters near military sites and manufacturing units. In the present study we observed various biochemical changes in *Rasbora dandia* exposed to perchlorate at environmentally relevant concentration (6 to 14 mg/L). Biochemical factors such as total lipids, proteins, carbohydrates and glycogen in liver and muscles were analyzed in a*Rasbora dandia* after 90 days of perchlorate exposure. Significant changes were observed in the biochemical composition of the exposed fishes compared to control. Total lipids and glycogen level were significantly ($p<0.05$) decreased in treated fishes compared to control. Whereas carbohydrates level was significantly ($p<0.05$) increased in exposed fishes. This study underlines the need for more rigorous observation of flora and fauna at perchlorate contaminated places.

Key words: Carbohydrates, Glycogen, Lipids, perchlorate, *Rasbora dandia*

INTRODUCTION

Perchlorate ($ClO4^-$) is a persistent pollutant has been widely reporting from various part of the world. The environmental release of perchlorate has mainly associated with the widely used areas such as aerospace programs and military installation (Trumpolt *et al*.,2005; Morrison *et al*.,2006). Perchlorate contamination has been continuously reporting from various countries such as USA, Japan, Canada, England, Korea, China and India (Dasgupta *et al*.,2005; Munster *et al*., 2009; Kosaka *et al.,* 2007; Her *et al*., 2011; Quin *et al.,* 2014; Kannan *et al*., 2009; Isobe *et al*., 2013 and Nadaraja *et al.,* 2015). Perchlorate contamination was reported from different food materials such as rice, fruits, leafy vegetables, breast milk and from various water sources such as drinking water, ground water, surface water, sea water, snow and rainwater also(Kirk *et al*., 2005; Shi *et al.,* 2007; Murray *et al*., 2008;Smith *et al*., 2001; Snyder *et al*., 2005; Ye *et al*., 2013 and Qin *et al.,* 2014).

As an endocrine disruptor, perchlorate contamination generated considerable concern for human health**.** The main mechanism of perchlorate toxicity is related to the thyroid gland and thyroid hormone production (Crane *et al*. 2005; Liu *et al*. 2006).Perchlorate can act as a strong inhibitor of the sodium iodide symporter (NIS) and it competitively inhibit with iodide and blocks iodide uptake across the basolateral membrane of thyroid follicles which reduce thyroid hormones production (Wolff, 1998; Clark, 2000; Yu *et al.,* 2002). The current health advisory level for ClO_4^-, based on the reference dose recommended by National Academy of Sciences is 15µg/L (USEPA, 2008). According to the report of World Health Organization (WHO) the established provisional maximum tolerable daily intake (PMTDI) of perchlorate is 0.01 mg/kg body weight (WHO, 2010).The Office of Environmental Health Hazard Assessment (OEHHA) proposed Public Health Goal (PHG) of 1 µg/L for ClO_4^-in drinking water (OEHHA, 2012). However many other countries including India are not to set up a drinking water standard for perchlorate so far.

Perchlorate salts are water soluble and mobile in aquatic ecosystem which may persist for many decades under typical ground and surface water

conditions (Urbasky, 1998; Merrill *et al.,* 2003). Due to this nature aquatic organism mainly fishes inhabiting in the ClO_4^-contaminated areas were greatly exposed and the major route of exposure of perchlorate in fish body is through gills (Smith *et al*., 2001; Bradford, 2002; Park, 2003). Fish act as a bio indicator organism and play an important role to monitor water pollution (Santhananm *et al.,* 1987).Previous studies reported that perchlorate causes various toxic effect on fishes such as morphological deformities, reproductive abnormalities, developmental abnormalities, disruption of thyroid follicles and reduce primordial germ cell number (Mukhi *et al*., 2005; Park *et al.,* 2006; Bernhardt *et al.,*2011; Crane *et al*., 2005; Bradford *et al.,*2005; Mukhi and Patino, 2007; Capps *et al.,* 2011 and Petersen *et al*., 2016). In addition, perchlorate causes oxidative stress in DNA and glutathione level in Zebra fish (Liu *et al*., 2006). However the data on effect of perchlorate on biomolecules of fish was lacking.

In the present study we aim to analyze the effect of perchlorate on biomolecules such as protein, lipids, glycogen and carbohydrate in liver and muscles of *Rasbora dandia* exposed to sub lethal concentration of perchlorate.

MATERIALS AND METHODS

Fish and experimental design

Healthy and adult fresh water fish *Rasbora dandia* (Valenciennes, 1844) (6.1 ± 2.4 cm of total length, 4.2±1.6 g of weight, as mean ± S.D), were collected from a local vendor for toxicity studies. The fish were transported to lab in live condition with care to avoid any mechanical injury. They were kept in a glass aquarium tank having 2000 L capacity filled with de chlorinated tap water and provided continues aeration. Prior to experiment, fishes were acclimatized in laboratory conditions for a period of two weeks. Fishes free of any deformities, disease or lesions and showed good health conditions was selected for the experiments. Potassium perchlorate was used to prepare for stock solution. Prior to experiment 48 hrs LC 50 was calculated. The aquaria were labeled from T_1 to T_5 and filled with different sub lethal concentration of ClO_4^- solution (6, 8, 10, 12 and 14 mg/L respectively) along with a control. The study concentration was selected based on the average perchlorate concentration reported from the contaminated pond (studysite) (unpublished data). The acclimatized fishes were randomly divided into 6 groups each containing 10 fishes was introduced into five rectangular glass aquaria having 120 L capacity filled with 90 L ClO_4^- solution for sub lethal toxicity studies. All the experiment was made in triplicates. Fishes were fed with commercial food (Aquamix pellet) during the exposure period. During the exposure period, 90 % of the media were replaced and filled with fresh media twice per week. Water samples from each aquarium were collected at regular intervals for perchlorate and physicochemical analysis. After 90 days of exposure, five fishes from each exposure group were collected for biochemical assay.

Perchlorate analysis of water

Instrumental analysis

Perchlorate in the water samples were analyzed using Ion Chromatography system (IC-1100, Dionex) with a self-regenerating anion suppressor (ASRS 300) and a conductivity detector. The Ion Pac AS 16 column specific for ClO_4^- ion with a lower detection limit of 2 ppb (µg /L) is used in this study in combination with AG 16 guard column (USEPA methods 314.0 and 314.1). The eluent used was 50 mM Sodium hydroxide (NaOH) at a flow rate 1.5 mL /min. The injection volume was 1000 µL. All reagents were purchased from Sigma Aldrich and standards were prepared in ultra-pure milliQ water (Millipore). Three sets of calibration curves were generated ranging from 5-30, 50"100 µg/L and 100"500 µg/L. Laboratory reagent blank and fortified samples were also analyzed for QC**.** The mean recovery of $ClO_4^-$with the AS16 column and analytical condition was 100±10%.

The physico-chemical characteristic of the water samples were analyzed according to standard procedure (APHA,2005).

Biochemical analysis

The biochemical constituents such as protein, lipids, glycogen and carbohydrates were analysed from muscle and liver of the exposed fish by using standard procedures. Total proteins, total lipids, glycogen and carbohydrates were analysed according to the methods of Lowry *et al*.,1951; Folch *et al*., 1957 and Van der Vier, 1954 and Roe,

1967 respectively.The results were expressed in percentage of wet weight of the tissue.

Statistical Analysis

The data obtained were statistically analyzed by using statistical package SPSS (version 20). The data were subjected to one way ANOVA. The significant level was taken as 0.05 ($p < 0.05$).

RESULT AND DISCUSSION

Perchlorate concentration of water

The detected perchlorate concentration of water samples collected from the each experiment tank was close to the nominal concentrations were represented in table.1. At the end of the exposure period the actual concentration of perchlorate from each tank was 4.6±0.51mg/L, 6.2±0.45mg/L, 7.8±0.62mg/L, 9.92±0.52mg/L and 11.02±0.49mg/L (mean ± SD) respectively for 6, 8, 10, 12 and 14 mg/L nominal concentration. The percentage of perchlorate removal from each tank was ranged from 17.33% to 23% and the average perchlorate removal was 21.22± 0.52. The physicochemical characteristics of water samples used for toxicity study were given in table 2.

Biochemical analysis

The biochemical analysis showed that total lipids and Glycogen concentration in liver and muscles were significantly ($p < 0.05$) decreased in exposed fishes compared to control.

The glycogen level in liver and muscles of the exposed and control fish were shown in Fig. 1A and Fig. 1B. In the present study the glycogen concentrations (mean ± SD) in liver were reduced from 0.813 ± 0.94 mg/g (control) to 0.712 ± 0.54 mg/g at low concentration (6mg/g) and 0.511 ± 0.78 mg/g at high concentration (14mg/L) respectively. However in muscles, the glycogen level was reduced from 0.251 ± 0.72 mg/g (control) to 0.21 ± 0.55 mg/g at low concentration (6 mg/L) and 0.181 ± 0.64 mg/g at high concentration (14mg/L) respectively. In the present study it was observed that the glycogen concentration in liver of the exposed fish was reduced to 12.42% at low concentration (6 mg/L) and 37.26 % at high concentration (14 mg/L) compared to control. Whereas in muscles, the glycogen concentration at low concentration (6 mg/L) and high (14 mg/L) concentration were decreased up to 16.33% and 27.88 % respectively compared to control.

The total lipids concentration in liver and muscle of the exposed fish and control were shown in Fig. 2A and Fig. 2B.The result showed that the lipid concentration in liver and muscles were significantly reduced in treated fish compared to control ($p<0.05$). Total lipid concentration in liver (mean ± SD) of the exposed fishes were reduced from 15.8 ± 0.47 mg/g (control) to 15.2 ± 0.34 mg/g at low concentration (6 mg/L) and 13.1 ± 0.54 mg/g at high concentration (14mg/L). Similarly in muscles the total lipid concentration (mean ± SD) of the exposed fish were reduced from 9.6 ±0.51 mg/g (control) to 9.4 ± 0.34 mg/g at low concentration and 7.76 ± 0.54 mg/g at high concentration. From the present study it was observed that the lipid concentration in muscles and liver of the exposed fishes treated at low concentration (6mg/L) were not significantly reduced. However at high concentration (14mg/L) it was decreased up to 19.1 % in muscles and 17.08 % in liver compared to control.

Carbohydrates level of the exposed fishes showed a significant increase($p<0.05$) compared to control fish (Fig.3A and Fig. 3B). The carbohydrates level in liver was increased from 5.25 ± 0.25 mg/g (control) to 6.47 ± 0.45 mg/g at low concentration (6mg/L) and 7.51 ± 0.66 mg/g at high concentration (14mg/L). From the present study it was observed that the carbohydrate concentration in liver of the exposed fishes at low concentration (6 mg/L) were increased to 23.23 % and at higher concentration (14mg/L) it was increased up to 43.04 % compared to control. Similarly in muscles the carbohydrate concentration of the exposed fish was increased from 7.2 ± 0.51 mg/g (control) to 7.94 ± 0.34 mg/g at low concentration and 8.9 ± 0.54 mg/g at high concentration. From this study it was observed that carbohydrates concentration in muscle of the exposed fishes at low concentration (6 mg/L) was increased to 10.2 % and at higher concentration (14mg/L) it was increased up to 23.6 % compared to control.

The total protein concentrations (mean ± SD) in liver was reduced from 18.93 ± 0.48 mg/g (control) to 18.52 ± 0.54 mg/g at low concentration (6mg/L) and 17.84 ± 0.52 mg/g at high concentration (14mg/L) and in muscles it was reduced from 65.31 ± 0.52 mg/g (control) to 64.21 ± 0.55 at low

Table.1. Perchlorate concentration (mg/L) measured from water samples collected from the experiment tank during the exposure period.

Sl No.	Nominal concentration (mg/L)	Actual concentration (Mean ± S.D)		
		30Days	60 days	90Days
1	6	5.8±0.78	5.2±0.24	4.6±0.51
2	8	7.3±0.65	6.9±0.64	6.2±0.45
3	10	9.49±0.32	8.8±0.28	7.8±0.62
4	12	11.08±0.45	10.22±0.48	9.92±0.52
5	14	13.61±0.14	12.91±0.69	11.02±0.49

Table.2. Water quality parameters measured from water samples collected from the experiment tank during the exposure period.

Sl. No.	Parameters	mean ± S.D
1	Temperature (°C)	27.6 ± 1.3
2	PH	7.3 ± 0.4
3	Dissolved oxygen(mg/L)	4.63 ± 0.38
4	Free Co_2 (mg/L)	1.24 ± 0.12
5	Total alkalinity (mg/L)	5.92 ± 1.4
6	Total hardness (mg/L)	24 ± 1.8

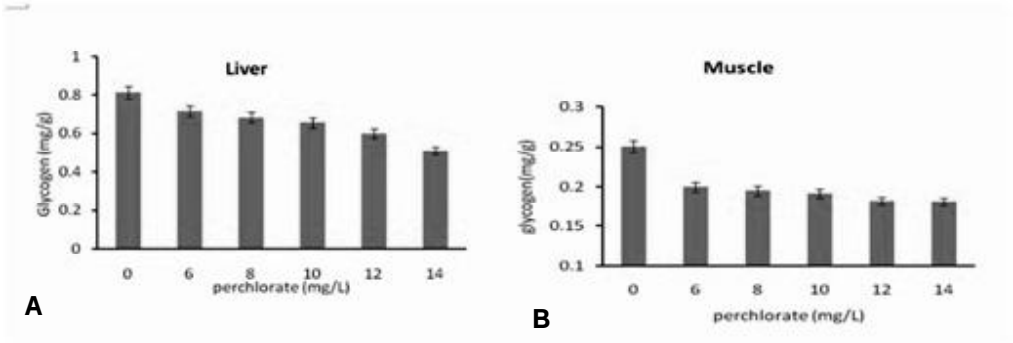

Fig. 1(A-B). Total glycogen content in liver and muscle tissues of *Rasbora dandia* exposed to different concentration of potassium perchlorate.Each bar diagram represent mean ± S.D.

concentration (6 mg/L) and 63.3 ± 0.51 mg/g at high concentration (14mg/L respectively). From the present study a non-significant changes were observed in protein concentration in liver and muscle of the exposed fishes compared to control.

Fishes are responding to various stressors by a series of biochemical and physiological stress reactions called secondary stress response (Mazeaud and Mazeaud, 1981). Changes in biochemical composition are an indicative of long term exposure to stressors (Mayer *et al.*,1992). In the present study the biomolecules such as carbohydrates, lipids and glycogen level in muscles and liver were significantly varied in response to perchlorate concentration. Glycogen is mainly stored in liver and liver glycogen act as a readily available source of energy through glycolysis. Similarly a fall in liver and muscle glycogen may be due to the utilization to meet demand of energy of fish due to the

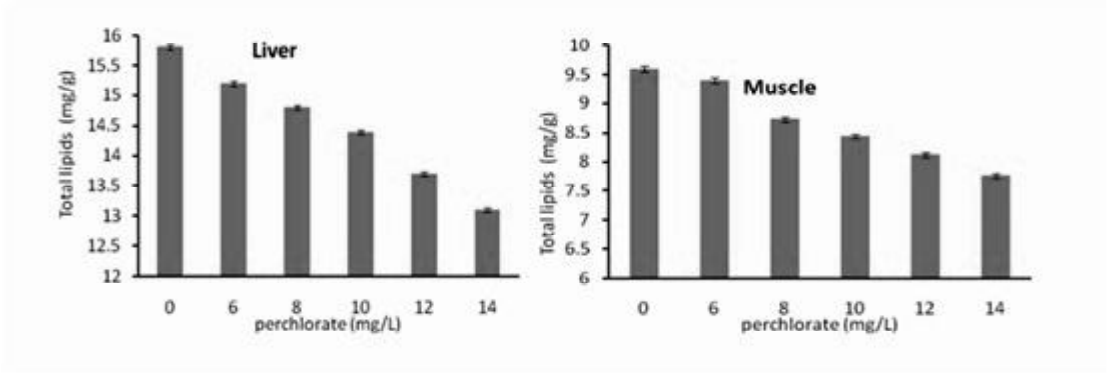

Fig. 2(A-B). Total lipid content in liver and muscle tissues of *Rasbora dandia* exposed to different concentration of potassium perchlorate. Each bar diagram represents mean ± S.D.

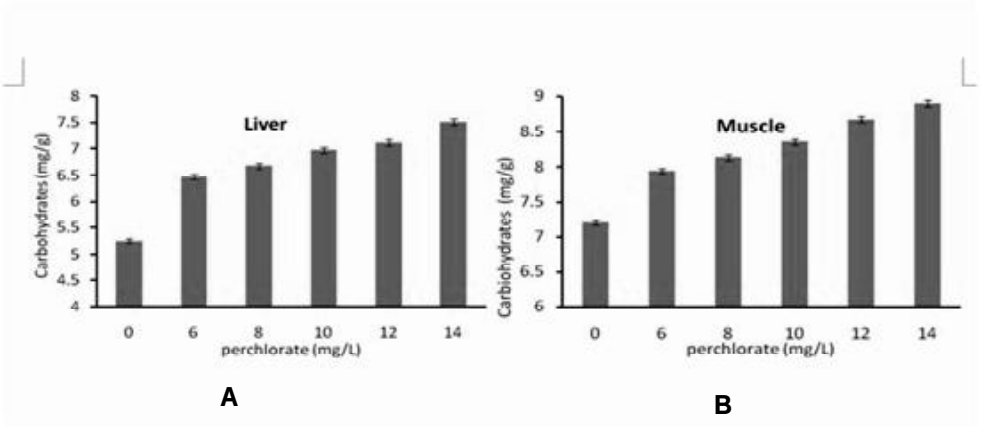

Fig. 3(A-B). Total Carbohydrate concentration in liver and muscle tissues of *Rasbora dandia* exposed to different concentration of potassium perchlorate. Each bar diagram represents mean ± S.D.

toxicants.There are some previous studies which reported biochemical variation in fishes due to the exposure of different toxicants. Sobha *et al.,* 2007 reported that when *catlacatla* exposed to cadmium chloride a decrease in biochemical constituent such as glycogen,total proteins, lipids were observed. Several previous studies reported that perchlorate cause oxidative stress in plants and animals.

Thyroid hormone have major role in regulating carbohydrate, protein and lipid metabolism and affect growth and cell differentiation in vertebrates (Clark, 2000). Perchlorate competitively inhibits iodide uptake through the NIS and therefore affect thyroid hormone synthesis and significantly reduced serum thyroxine (T_4) and triiodothyronine (T_3)(Yu *et al*., 2002; Strawson *et al*., 2004). In our another study it was observed that at environmentally relevant concentration of perchlorate (6 – 14 mg/L) can impair thyroid hormone synthesis and causes significant changes in thyroid hormone concentration in *Rasbora dandia* (unpublished). Similarly the variations observed in bimoleculesof the perchlorate treated fishes may be due to the changes in thyroid hormone caused by perchlorate. Sudarshan and Kulkarni, 2013 reported that muscle protein, glycogen, lipid and cholestrol content were low in *Notopterus notopterus* treatedwith thyroxine. Similarly Ramesh,(2013) reported that protein, carbohydrates and lipid content were varied in *Anabas testudineus* exposed to L- thyroxine. These results also support our observation.

CONCLUSION

From this study it can be concluded that the variations of total lipid, glycogen and carbohydrate level in *Rasbora dandia* in response to perchlorate is due to the increased use of the molecules to meet energy requirement for detoxification process. Perchlorate at environmentally relevant concentration was found to induce biochemical changes in *Rasbora dandia* and affecting normal health condition. This study therefore highlights the need for more rigorous observation of flora and fauna at perchlorate contaminated places.

ACKNOWLEDGEMENTS

The present study was conducted with the financial assistance from the University of Kerala. The infrastructural support from Department of Aquatic Biology and Fisheries and sampling analysis from CSIR- NIIST were also acknowledged in this study.

REFERENCES

Anupama,V.N. Prajeesh, G.P. and Krishnakumar, B. 2015**.** Surveillance of perchlorate in groundwater, surface water and bottled water in Kerala, India. *J. Environ. Health Sci. Eng*. 13 (2015) 1.

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Ed, American Public Health Association, Washington, DC.

Bernhardt, R.R. Von Hippel, F.A.and Hara**,** T.M.O. 2011. Chronic perchlorate exposure causes morphological abnormalities in developing stickleback. *Environ.Toxicol. Chem*. 30: 1468-1478.

Bradford, C. 2002.Perchlorate uptake and effect on thyroid function in fish. Master's thesis Texas Tech university, Lubbock, Texas.

Bradford, C.M. Rinchard Carr, J.A. and Theodorakis, C. 2005.Perchlorate affects thyroid function in Eastern mosquitofish (*Gambusia holbrooki*) at environmentally relevant Concentrations. *Environ Sci Technol* 39(14): 5190-5195.

Capps,T. Mukhi,S. Rinchard,J.J. Theodorakis,C.W. Blazer,V.S. and Patino, R. 2011. Exposure to Perchlorate induces the formation of macrophage aggregates in the trunk kidney of Zebra fish and Mosquito fish. *Journal of Aquatic Animal Health* 16 (3): 145-151.

Clark, J.J.2000.Toxicology of perchlorate .In:,E.T.(Ed.),Perchlorate in the Environment. Kluwer academic / Plenum Publishers, New York, USA, pp.15-29.

Crane, H.M. Pickford, D.B. Hutchinson, T.H. and Brown, J.A. 2005. Effects of ammonium perchlorate on thyroid function in developing fathead minnows, *Pimephales promelas*. *Environ Health Perspect* 113(4): 396-401.

Dasgupta, P.K. Nartubekabgim, P.J. Jackson, W.A. Anderson, T.A. Tian, K. Tock, R.W. and Rajagopalan, S. 2005. The origin of naturally occurring perchlorate: Role of atmospheric processes. *Environ. Sci. Technol.* 39(6): 1569-1575.

Fisher**,** J.W. 2003. PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radio iodide in male rat. *Toxicol. Sci*. 73: 256-269.

Folch, J. Lees, M. and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. boil. chem*. 226 (1): 497 - 509.

Her, N. and Jeong, H. 2011. Occurrence of perchlorate in drinking water and seawater in South Korea. *Environ. Contam. Toxicol.* 61 (2):166-172.

Isobe,T. Ogawa, S.P. Sugimoto, R. Ramu, K. Sudaryanto, A. Malarvannan, G. Devanathan, G. Ramaswamy, R. Munuswamy, N. Ganesh, D.S. Sivakumar, J. Sethuraman, .Parthasarathy, V. Subramaniam, A. Field, J. and Tanabe, S. 2013. Perchlorate contamination of groundwater from fireworks manufacturing area in South India. *Environ. Monit. Asses*. 185 (2013) 5627-5637.

Kannan, K. Prammsma, M.L. Oldi, J.F. Kunisue, T. and Sinha, R.K. 2009. Occurrence of perchlorate in drinking water, ground water, surface water and human saliva from India *Chemosphere* 76: 22-26.

Kirk, A.B. Martinelango, P.K. Tian, K. Dutta, A. Smith, E.E. and Dasgupta, P.K. 2005. Perchlorate and iodide in dairy and breast milk. *Environ. Sci. Technol.* 39(7): 2011-2017.

Kosaka, K. Asami, M. Matsuoka, Y. Kamoshita, M. and Kunikane**,** S. 2007. Occurrence of perchlorate in drinking water sources of metropolitan area in Japan. *Water Research* 41(15): 3474-3482.

Liu, F.J, Cobb, G.P. Anderson, T.A. Cheng, Q.Q. and Theodorakis, C.W. 2006. Uptake, accumulation and depuration of sodium perchlorate and sodium arsenate in zebrafish *(Danio rerio). Chemosphere* 65: 1679-1689.

Liu,F. J., Wang, J.S., Theodorakis, C.W.2006 Thyrotoxicity of sodium arsenate, sodium perchlorate, and their mixture in zebrafish *Danio rerio, Environ.Sci.Technol.2006,Vol 40 (3429-3436)*.

Lowry, O.M. Rosenbrough, N.J. Farr, A.C. and Randall**,** R.F. 1951. Protein estimation with Folin Phenol reagent. *J. Biol. Chem*., 193(1): 265-275.

Mayer, F.L., Versteeg, D.J and Rattner, B.A.1992.Biomarkers – Physiological and histological markers of anthropogenic stress. Proceedings.

Viii Pelston Workshop, Lewis Publication. U.S.A.

Mazeaud, M and Mazeaud, F. 1981. Adrenergic responses to stress in fish In: Stress and Fish, (Ed. A. D. Pickring). Academic Press, New York, P. 49-57.

Morrison, R.D., Vavricka, E.A and Duncan, P.B.2006. Perchlorate. In: Morrison RD, Murphy BL, editors. Environmental Forensics: Contaminant Specific Guide. *Elsevier Inc.; Burlington*, MA, USA: pp. 167–185.

Mukhi, S., Carr, J.A., Anderson, T.A and Patino, R. 2005. Novel biomarkers of perchlorate exposure in zebrafish. *Environ. Toxicol. Chem*. 24: 1107-1115.

Mukhi, S and Patino, R. 2007. Effects of prolonged exposure to perchlorate on thyroid and reproductive function in zebra fish. *Toxicol Sci*. 96: 246-254.

Munster, J., Hanson, N.G., Jackson, A.W and Rajagopalan, S. 2009. The fall out offireworks: Perchlorate in total deposition. *Water, air and soil pollution*. 198(1): 149-153

Murray, C.W. Egan, S.K. Kim, H. Beru, N. and Bolger, P.M. 2008. U.S. Food and Drug Administration's total diet study: Dietary intake of perchlorate and iodine. *Journal of exposure science and environmental epidemiology*.18 (6): 571- 580.

Office of Environmental Health Hazard Assessment (OEHHA).2012. "Public health goal for perchlorate in drinking water" Pesticide and Environmental Toxicology Branch, ,California Environmental Protection Agency.

Park, J.W.2003. Toxicological effect and kinetics of perchlorate on fishes. Master's hesis.Texas Tech University, Lubbock, Texas.

Park, J.W., Rinchard, J., Liu, F., Anderson, T.A., Kendall, R.J and Theodorakis, C.W.2006. The thyroid endocrine disruptor perchlorate affects reproduction, growth and survival of mosquito fish. *Ecotox. Environ. Saf*. 63: 343-352.

Petersen, A.M., Dillon, D., Bernhardt, R.A., Torunsky. R., Postlethwait, J.H., Von Hippel, F. A., Buck, C.L., and Cresko, W.A. 2015. Perchlorate disrupts embryonic androgen synthesis and reproductive development in three spine stickleback without changing whole-body levels of thyroid hormone. *General and Comparative Endocrinology*. 210: 130–144.

Qin, X., Zhang, T., Gan, Z and Sun, H. 2014. Spatial distribution of perchlorate, iodide and thiocyanate in the aquatic environment of Tianjin, China: Environmental source analysis *Chemosphere* 111:201-208.

Ramesh,G.K. 2013. Effect of hormones on some biochemical composition of *Anabas testudineus. International Journal of Advancements in Research & Technology* 2(9) 74-81.

Roe, J.H .1967. Methods of Biochemical analysis Vol. 5, (Ed. By Glick Inter. science, New York). p. 44.

Santhananm,R., Sukumaran,N and Natarajan,P.A.1987. Manual of fresh water aquaculture. Oxford and FBH publishing co PVT Ltd, New Delhi-2.

Shi, Y., Zhang,P., Wang,Y., Shi, J., Cai,Y., Mou,S and Jiang,G. 2007. Perchlorate in sewage sludge, rice, bottled water and milk collected from different areas in China. *Environment international* 33(7): 955-962.

Smith, P.N., Theodorakis, C.W., Anderson, T.A and Kendall,R.J . 2001. Preliminary assessment of perchlorate in ecological receptors at the longhorn army ammunition plant (LHAAP), Karnack, Texas. *Ecotoxicology* 10: 305-313.

Snyder, S.A., Vander ford, B.J and Rexing, D.J . 2005. Trace analysis of bromate, chlorate, iodate and perchlorate in natural and bottled waters .*Environ Sci Technol* 39(12): 4586-4593.

Sobha, K., Poornima, A., Harin, P and Veeraiah, K. 2007. A study on biochemical changes in the freshwater fish *Catla catla* (Hamilton), exposed to the Heavy metal Toxicant cadmium Chloride. Kathmandu University *J. Sci. Engineering and Tech* 1(4): 1-11.

Strawson, J., Zhao, Q and Dourson, M. 2004. Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. *Regul. Toxicol. Pharmacol*. 39: 44–65.

Sudarshan, S and Kulkarni, R.S. 2014. Effect of some hormones on the muscle tissue biochemical contents of the fresh water fish *Notopterus notopterus*. *Int J Res Biosci* 3:7- 12.

Trumpolt, C.W., Crain. M., Cullison, G.D., Flanagan, S.J.P., Siegel, L and Lathrop, S.2005 Perchlorate: sources, uses, and occurrences in the environment Remediation 16(1): 65-89.

Urbasky, E.T.1998. Perchlorate chemistry:implications for analysis and remediation. *Bioremediation Journal*.2:81-95

USEPA (U.S. Environmental Protection agency). 2008. Interim drinking water health advisory for perchlorate Rep.No. EPR-08-025, United States Environmental Protection Agency, Washington D.C.

Van der Vier, J.1954. Two methods for determination of glycogen in liver. Biochemical Journal, 57 (3): 410-416.

WHO (World Health Organization). 2010. Joint FAO/ WHO expert committee on food additives seventy-second meeting, Rome.

Wolff,J.1998 .Perchlorate and the thyroid gland. *Pharmacol Rev* 50:89-105.

Ye, L., You, H., Yao, J., Kang, X and Tang, L. 2013. Seasonal variation and factors influencing perchlor

ate in water,Snow, Soil and Corns in Northeastern China,*Chemosphere* 90(10): 2493-2498.

Yu, K.O; .Narayanan,L ; Mattie, D.R . 2002.The pharmacokinetics of perchlorate and its effect on the hypothalamius- pituitary- thyroid axis in the male rat. *Toxicol Appl Pharmacol.* 182: 148-159.

Yu, K.O., Narayanan, L., Mattie, D.R., Godfrey, R.J., Todd, P.N., Sterner, T.R., Mahle, A.,Lumpkin, M.H., Fisher, J.W. 2002. The pharmacokinetics of perchlorate and its effect on the hypothalamus–pituitary–thyroid axis in the male rat. *Toxicol Appl Pharmacol* 182:148-159.