Int J Pharm Bio Sci 2014 July ; 5 (3) : (B) 543 - 552

Research Article **Ecotoxicology**

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

CYTOTOXIC AND CHROMOTOXIC EFFECTS OF HERBICIDE STOMP XTRA ON AIR BREATHING TELEOST *CHANNA PUNCTATUS* (BLOCH)

AMRITA MULLICK, ANAND M JHA* AND OM PRAKASH SHARMA

Department of Zoology, R. N. A. R. College, Samastipur- 848101, India

ABSTRACT

The *in vivo* genotoxic potential of Stomp Xtra herbicides was evaluated in a freshwater fish *Channa punctatus* by assessing mitotic index in the kidney cells and micronuclei in the peripheral blood cells. Fish were exposed to five sub-lethal concentrations of Stomp Xtra containing a.i. pendemethalin at 0.293 (10% of LC50), 0.587 (20% of LC50), 0.881 (30% of LC50), 1.174 (40% of LC50) and 1.468 (50% of LC50) mg/L. For genotoxicity assay, hematopoietic cells from the kidneys and caudal vein of exposed fishes were examined after 24, 48, 72 and 96 h of exposure. Decrease in mitotic index in the kidney cells and increase in frequency of micronucleated erythrocytes in the peripheral blood cells with an increase in concentration of the herbicide were observed in treated fish. Two-way analysis of variance test (ANOVA test) revealed statistically highly significant effect of concentration of the herbicide as well as duration of treatment on mitotic index and frequency of micronucleated erythrocytes.

KEYWORDS: Stomp Xtra, Pendemethalin, mitotic index, micronuclei, kidney cells, peripheral blood cells

ANAND M JHA
Department of Zoology, R. N. A. R. College, Samastipur- 848101, India

***Corresponding author**

INTRODUCTION

Among all the chemical pollutants, pesticides are the major aquatic pollutants in many parts of the world. These chemicals are very toxic and enter into aquatic ecosystems through soil surface run-off or from area where they are applied[1]. Pesticides not only deteriorate the life sustaining quality of water but also produce toxic effects on non-target organisms. The pollutants, especially herbicides, produce deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes[2-11]. The last three decades have witnessed increased interest among the scientific community in detecting the genotoxicity of pesticides used in agriculture. This interest has resulted in identification of a large number of pesticides as genotoxins. Stomp is a group D herbicide used for the control of annual ryegrass and wireweed in wheat, barley and peas and annual grasses and certain broadleaf weeds in various crops. The active constituent: of this herbicide is Pendimethalin (N-(1-ethylprophyl)- 3, 4-dimethyl -2,6- dinitrobenzenamine). The United States Environmental Protection Agency[12] has classified Pendimethalin as persistent bioaccumulative toxic and a group C carcinogen "possible human carcinogen". Dimitrov et al.[13] showed induction of chromosomal aberrations and micronuclei in bone marrow cells of pendimethalin treated mice. However, negative mutagenic effects were shown in mammalian and bacterial cells exposed to pendimethalin. Pendimethalin caused mitotic disturbances (c-metaphases, anaphasal and telophasal chromosome bridges, multipolar anaphases) and interphase abnormalities (micronuclei, multinuclear cells). Mitotic disturbances were caused by abnormalities in the organization of the tubulin cytoskeleton[14]. In the present study, an attempt was made to study the cytotoxic and chromotoxic effects of Stomp in freshwater fish *Channa punctatus*. The cytotoxic and chromotoxic effects of this herbicide were studied by assessing the mitotic depression in the rapidly dividing hematopoietic cells of the kidney and frequency of micronucleated erythrocytes in the peripheral blood.

MATERIALS AND METHODS

Healthy specimens (12-16 cm length and 20-30g weight) of the common fresh water fish *Channa punctatus* were obtained from a single population from local fish market and were treated with 0.05% $KMnO_4$ solution for 2 minutes to avoid any dermal infection. Fish were acclimatized for 15 days in the laboratory in glass aquaria containing 30 L non-chlorinated tap water (Physico-chemical properties, pH 7.4 ± 0.2; Temperature 26 ± 2^0C; Alkalinity 63 ± 4.8 mg/L; Total Hardness 275 ± 2.5 mg/L; D. O. 7.0 ± 0.2 mg/L). The fish were fed *ad libitum* with minced boiled chicken eggs. Proper aeration was maintained in test as well as control aquaria by air pumps and stone diffusers throughout the experiments. Feeding was stopped 24 h prior to the commencement of the experiment and the fish were kept starved throughout the period of the experiment. Water of the aquaria was changed after every 24 h, leaving no fecal matter, unconsumed food or dead fish, if any. Stock solution of Stomp Xtra (BASF India Ltd., Mumbai, India) was prepared by dissolving measured amount of the herbicide in distilled water. Cyclophosphamide (Endoxan), a known clastogenic agent, was used as positive control chemical. Prior to the commencement of the experiment median lethal concentration (LC50) was determined by employing short-term static toxicity assay. For toxicity assay 8 concentrations of Stomp Xtra containing a.i. pendemethalin at 1.35, 1.80, 2.25, 2.70, 3.15, 3.60, 4.05 and 4.55 mg/L were prepared. 30 adult fishes of almost similar size and weight (average length 15±1.5 cm and weight 26.5±2.0 g) were exposed to each test concentration. Mortality was recorded after 24 h interval each day at the same time up to 96 h. Experiment was carried out in three replicates. The LC50 values of various intervals were calculated by using Trimmed Spearman-Karber (TSK) Program Version 1.5 program downloaded from website of Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, U. S.

Environmental Protection Agency, Cincinnati, Ohio 45268. Five concentrations of Stomp Xtra, which were below the determined LC 50 value for 96 h (2.937 mg/L) were selected for treatment of fishes. Acclimated fishes were divided into seven groups and treated for 24, 48, 72 and 96 h as follows:

Group I: control fishes kept in aquarium containing tap water,

Group II: positive control fishes kept in aquarium containing 5 mg/l cyclophosphamide.

Group III-VII: were kept in separate aquaria containing one of the five concentrations of Stomp Xtra which are 0.293 (10% LC50), 0.587 (20% LC50), 0.881 (30% LC50), 1.174 (40% LC50) and 1.468 (50% LC50) mg/L**.**

Kidney cells were used for assessment of effect on cell division. Slides were prepared following the method suggested by Nagpure and Barat[15]. After each of the exposure periods of 24 h, 48 h, 72 h and 96 h, five fishes each from the respective experimental as well as control aquaria were sacrificed. The fishes were injected 0.05 % colchicine, dissolved in glass distilled water, intramuscularly (at 1 mL/100 gm of body weight) one and half hour prior to sacrifice. The kidneys were taken out by dissecting the fish. The kidneys were cut into small pieces and homogenized in 10 mL hypotonic solution (0.56% KCl) in glass tissue homogenizer to prepare cell suspension. The cell suspension was poured in a 15 mL centrifuge tube and incubated for about 25-35 minutes at room temperature for swelling of the cells. The hypotonic treatment was stopped by adding 1 mL of freshly prepared, chilled Carnoy's fixative (methanol: acetic acid in 3 : 1 ratio). The fixative was mixed gently with Pasteur pipette. The cell suspension thus obtained was centrifuged at 1200 rpm for 10 min. The supernatant was discarded and the cell pellet was re-suspended in 7-8 mL of chilled fixative and again centrifuged for 10 min at 1200 rpm. The process of washing of the cell pellet with fixative was repeated thrice to get clear whitish pellet. Slides were prepared by air drying technique and stained with 10% Giemsa in phosphate buffer (pH 6.8). 5000 cells were counted at a magnification of 100 X for number of mitotically dividing cells.

At the end of treatment period i.e. 24, 48, 72 and 96 h five fishes from control and each treatment group were sacrificed and blood was drawn from the heart regions by cardiac puncture using the cold hypodermic micro syringes pre-rinsed with heparin (anticoagulant). The collected blood from the control and experimental groups was expelled on clean glass slides and thin smears were prepared. The slides were air-dried for 24 h, fixed in methanol for 10 minutes, and stained in 10% Giemsa (v/v). Five slides were prepared for each fish. Slides were coded and scored blind. To detect micronuclei in erythrocytes, the slides were analyzed using 100 X oil-immersion lens. 1000 cells were scored from each slide for the presence or absence of micronuclei in their cytoplasm. Micronuclei were identified as small (diameter less than one-third of the main nucleus) non-refractive, circular or ovoid chromatin bodies separated from the main nucleus and have similar staining as the main nucleus[16]. Data were subjected to two-way analysis of variance (ANOVA) to determine the significance of the effect of treatment as well as period of treatment on mitotic index and micronuclei, if any. Dunnet multiple comparison test was conducted to test the significance of difference between control and treated animals. p values < 0.05 were considered statistically significant. All statistical analyses were performed by using Graphpad prism 3 and 5 software.

RESULTS

The LC 50 values and their confidence limits of Stomp Xtra (Pendimethalin) are shown in Table I. The 24, 48, 72 and 96 h LC 50 values of Stomp Xtra were 3.867, 3.650, 3.298 and 2.937 mg/L, respectively. Just after introduction to test solution behavioral changes in experimental fishes were noticed. Fishes showed increased swimming, surfacing and hyperactivity and jerky movements. Restlessness, rapid surfacing and colour fading were prominent after 24 h exposure. After 48 h exposure, the fishes showed slightly reduced activity, gradual increase in color fading and a thin film of mucous was noticed on body surface. After 72 h

exposure, the fishes showed reduced swimming activity. After 96 h a thick film of mucous on whole body was observed in almost all fishes. Treated fishes also lost natural coloration. The obtained results of mitotic activity in kidney cells of fish *(C. punctatus)* treated with five different concentrations of Stomp Xtra (Pendimethalin) for four exposure times are shown in Table 1. The mitotic index evaluated as percentage of dividing cells in control fishes (group I) was 7.31 ± 0.31. The cytotoxicity of cyclophosphamide (group II) to the fish was evident by significant decrease in mitotic index when compared to control fish of group I. Treatment of fish with Stomp Xtra (Pendimethalin) resulted in concentration related decrease in the mitotic index, as compared to control. However, prolongation of exposure period resulted in gradual increase in mitotic index. Statistically highly significant ($p<0.01$) reduction in mitotic index in the fish treated with Stomp Xtra were noticed in Dunnett multiple comparison test, except for the lowest concentration 0.293 mg/l at 72 and 96 h exposure periods (Table 2). It is also apparent from the Fig. 1 that inhibitory effect of herbicide treatment on mitotic activity of cells gradually became weak. Two-way analysis of variance test (ANOVA test) revealed highly significant ($p<0.001$) differences between the treatments as well as periods of treatment (Table 3).

Frequencies of micronucleated erythrocytes in fishes exposed to 0.293, 0.587, 0.881, 1.174 and 1.468 mg/L. of Stomp Xtra and parallel negative and positive controls are presented in Table 4. A total of 5000 cells was scored for each group to examine the micronuclei. It is evident from the table that treatment of fish with Stomp Xtra (Pendimethalin) resulted in concentration and period of treatment related increase in the frequency of micronucleated erythrocytes in the peripheral blood when compared to untreated control fish. The differences between mean number of micronucleated erythrocytes in the peripheral blood of treated fish and control fish were statistically significant at all the concentrations and exposure durations except at 0.293 mg/L after 24 and 48 h and at 0.587 mg/L after 24 h of treatment. Two-way analysis of variance test (ANOVA test) revealed highly significant ($p<0.001$) difference between the concentrations of Stomp Xtra as well as periods of treatment (Table 5).

Table 1
LC50 values of Stomp Xtra and their 95% confidence limits at different exposure periods in Channa punctatus

Duration (h)	LC 50 (mg/l)	Lower confidence limit (mg/l)	Upper confidence limit (mg/l)
24	3.867	3.756	3.99
48	3.65	3.576	3.798
72	3.298	3.112	3.338
96	2.937	2.831	3.058

Table 2

Effect of Stomp Xtra treatments on the cell division in the kidney cells of Channa punctatus

Chemical/ Concentration	Mitotic Index (%)			
	24 h	48 h	72 h	96 h
Control	7.38±0.44	7.48 ± 0.44	7.24 ± 0.26	7.16 ± 0.12
CP	3.80 ± 0.23**	4.42 ± 0.25**	4.74 ± 0.48**	5.60 ± 0.55**
0.293 mg/L	5.14 ± 0.18**	5.48 ± 0.15**	6.44 ± 0.25	6.85 ± 0.22
0.587 mg/L	4.90 ± 0.30**	5.50 ± 0.10**	5.85 ± 0.24*	6.20 ± 0.19*
0.881 mg/L	4.02 ± 0.25**	4.64 ± 0.28**	5.75 ± 0.27**	5.96 ± 0.27**
1.174 mg/L	3.75 ± 0.37**	4.22 ± 0.22**	4.58 ± 0.39**	5.26 ± 0.11**
1.468 mg/L	3.25 ± 0.21**	3.40 ± 0.34**	4.38 ± 0.19**	4.80 ± 0.18**

CP, Cyclophosphamide used as positive control
1000 cells per fish and total 5000 cells have been scored in each case
Mitotic index (MI %) = Number of dividing cells / total No. of cells scored X 100
**(p<0.05) and ** (p <0.01) differ significantly from the control in Dunnet multiple comparision test.*

Table 3

Two-way analysis of variance (ANOVA) of mitotic index showing significant variation between treatments as well as periods of treatment

Sources of Variation	df	Mean Square	F-value
Between periods	3	5.6951975	37.6429***
Between treatment	5	2.031015278	13.4241***
Residual	15	0.151295278	

**** significant at p<0.001*

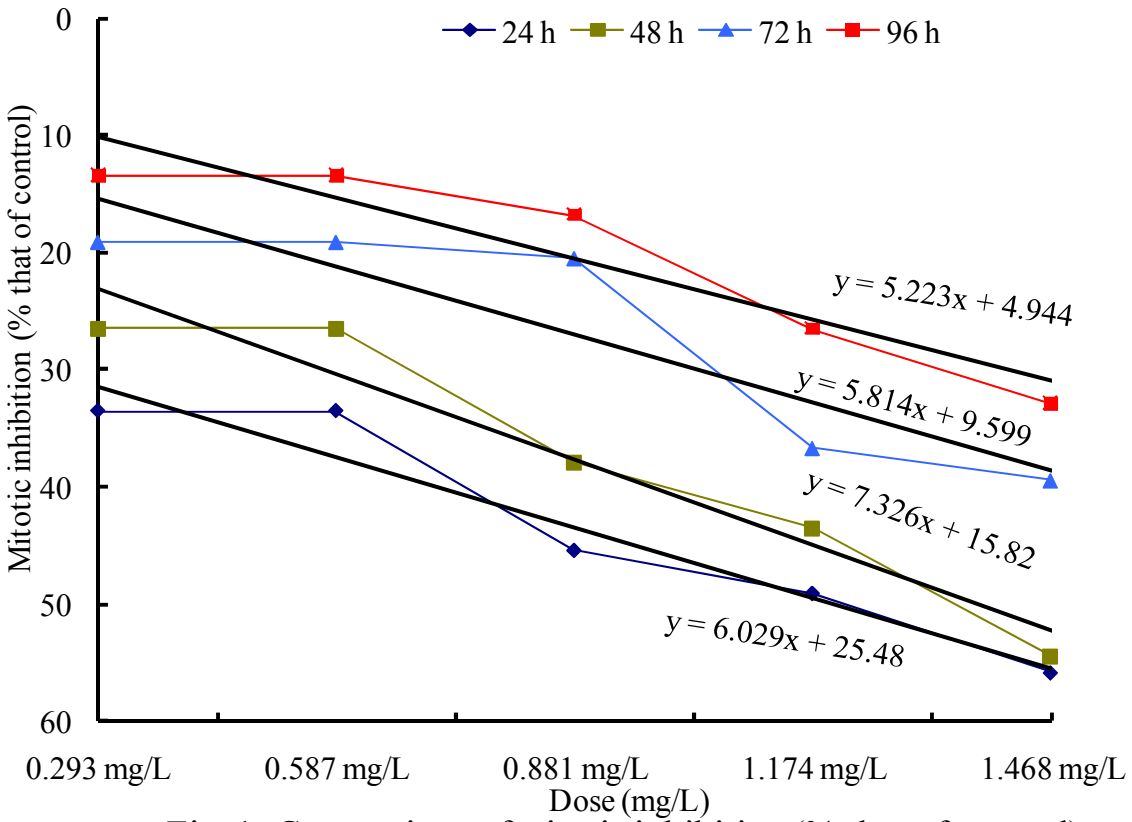

Fig. 1: Comparison of mitotic inhibition (% that of control) observed in the kidney cells of fish after treatment with Stomp Xtra herbicide at various sampling regimens

Table 4

Frequency of micronucleated erythrocytes in blood of freshwater fish Channa punctatus treated with different concentration of Stomp Xtra herbicide

Chemical / Dose	Frequency (%) of micronucleated erythrocytes*			
	24 h	48 h	72 h	96 h
Control	0.024 ± 0.016	0.022 ± 0.030	0.030 ± 0.020	0.022 ± 0.011
CP	0.36 ± 0.092**	0.48 ± 0.135**	0.59 ± 0.135**	0.68 ± 0.082**
0.293 mg/L	0.060 ± 0.016	0.102 ± 0.024	0.116 ± 0.036*	0.142 ± 0.044**
0.587 mg/L	0.078 ± 0.028	0.114 ± 0.018*	0.140 ± 0.020*	0.180 ± 0.030**
0.881 mg/L	0.114 ± 0.030**	0.154 ± 0.028**	0.175 ± 0.028*	0.22 ± 0.026**
1.174 mg/L	0.145 ± 0.025**	0.198 ± 0.032**	0.212 ± 0.034**	0.29 ± 0.042**
1.468 mg/L	0.174 ± 0.028**	0.22 ± 0.042**	0.25 ± 0.048**	0.38 ± 0.038**

1000 cells per fish and total 5000 cells have been scored in each case
** (p<0.05) and ** (p <0.01) differ significantly from the control in Dunnet multiple comparision test*

Table 5
Two-way analysis of variance (ANOVA) of micronucleated erythocytes showing significant variation between treatments as well as periods of treatment

Sources of Variation	df	Mean Square	F-value
Between periods	3	0.0267839	29.36***
Between treatment	5	0.0118249	12.96***
Residual	15	0.0009120	

*** significant at p<0.01 and <0.001 respectively*

DISCUSSION

Behavioral alterations in fish are sensitive indicators of stress caused after exposure to toxic chemicals[17-19]. Many workers have reported behavioral changes such as erratic jumping movements, occasional somersaults and lateral swimming on one side of the body, covering of body with secretion of mucus etc. in different fishes after exposure to sub-lethal concentrations of toxic chemicals[20-22]. Behavioral changes like erratic swimming, restlessness and surfacing, as observed in present study may be an avoiding reaction to the herbicide present in the medium. Loss of balance during swimming might be due to some neurological impairment in central nervous system as evident by inhibition of ACHE by pollutants[23-26]. In the recent past, cytogenetic investigations for genotoxicity monitoring of water contaminants by employing fish has gained enhanced interest[27-40]. These studies have clearly demonstrated the utility of fishes as indicators for the monitoring of environmental carcinogens, teratogens and mutagens. Stomp was negative in mouse bone marrow micronucleus induction in either male or female animals[41]. It was also negative for human lymphocytes sister chromatid exchange (SCE) induction[39]. Stomp induced significant increases in micronucleus frequency in both plant cells and mouse PCEs[13]. The active ingredient in Stomp is pendimethaline, which belongs to the nitroaniline class of herbicides, some of which (e.g.oryzalin) have well documented antimicrotubule effects[42]. There are very few published reports on the potential *in vivo* genotoxic effects of Stomp (Pendimethalin) on fish[43]. In the present study all the tested concentrations of Stomp produced inhibitory effect on the mitotic activity of kidney cells as is evident from the reduction in the mitotic index. Maximum inhibition in the mitotic activity was recorded at 24 h of exposure and, there after, mitotic activity gradually increased with the lapse of exposure period. This is probably due to sudden exposure to the herbicide might have caused severe stress on the exposed animals, but gradually with the lapse of time the animals became adapted to such situation. It has been suggested that a family of highly conserved cellular proteins referred to as the heat shock proteins (HSPs) characterizes the stress response in fish at the cellular level[44-45]. Extensive studies have revealed that HSPs assist the folding of nascent polypeptide chains, act as a molecular chaperone, and mediate the repair and degradation of altered or denatured proteins and are also active in supporting various components of cell signaling, including the cytoskeleton, enzymes, and steroid hormone receptors[46]. HSPs are also involved in the most basic cellular processes, such as cell division and growth. Inhibition of mitotic activity as observed in the present study may be due to suppressive effects of Stomp on the activity of HSPs. In addition, arrest of mitosis due to repair of genetic damage may have contributed to decreased values of mitotic index.

The micronucleus assay in the hematopoietic cells is one of the most sensitive tools to evaluate the genotoxic property of water contaminants. The test has been applied for both *ex situ* and *in situ* monitoring of genotoxic effects due to exposure to environmental pollution[39]. The results of the present study indicate that exposure of fishes to Stomp resulted in concentration and period of treatment related increase in the frequency of micronuclei in the erythrocytes of the peripheral blood. Micronuclei are formed due to condensation of acentric chromosome fragments or lagging chromosomes that fail to incorporate into daughter cell nuclei during cell division. Therefore, cytogenetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation and thus the incidence of micronuclei serves as an index of genotoxic effect. The obtained results on the micronucleated erythrocytes in the peripheral blood demonstrate that Stomp induced clastogenic effect and spindle dysfunction in blood cells of exposed fishes. The obtained results of mitotic activity of kidney cells and micronucleated erythrocytes in the peripheral blood of fish *(C. punctatus)* revealed that the stress caused by exposure to herbicide is capable of causing inhibition of cell proliferation and clastogenic effect leading to chromosome break and spindle dysfunction in kidney and blood cells of exposed fishes.

Conflict of Interest

Conflict of interest declared none.

REFERENCES

1. Tanguy A, Boutet I, Laroche J, and Moraga D, Molecular identification and expression study of differentially regulated genes in the pacific oyster *Crassostrea gigas* in response to pesticide exposure, FEBS J, 272, 390–403, (2005).
2. Canna-Michaelidou S, and Nicolaou A, Evaluation of the genotoxicity potential (by Mutatox TM test) of ten pesticides found as pollutants in Cyprus, Sci Tot Environ, 193, 27 – 35, (1996).
3. Das P, and John G, Induction of sister chromatid exchanges and chromosome aberrations *in vivo* in *Etroplus suratensis* (Bloch) following exposure to organophosphorus pesticides, Toxicol Lett, 104, 111 – 116, (1999).
4. Devaux A, Pesonen M, and Monod G, Alkaline comet assay in rainbow trout hepatocytes, Toxicol In vitro, 11, 771 – 779, (1999).
5. Garaj-Vrhovac V, and Zeljezic D, Evaluation of DNA damage in workers occupationally exposed to pesticides using singlecell gel electorphoresis (SCGE) assay. Mutation Res, 469, 277-285, (2000).
6. Giri A, Sharma GD, Prasad SB. Mutagenic effects of Carbhosulfan, a carbamate pesticide, Mutation Res, 519, 75-82, (2002).
7. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, and Pokethitiyook P, Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*), Environ Toxicol 18: 260-267, (2003)..
8. Kumar R, Nagpure NS, Kushwaha B, Srivastara SK, and Lakra WS. Investigation of the genotoxicity of malathion to fresh water teleost fish *Channa punctatus* (Bloch) using the micronucleus test and connect assay, Arch Environ Contain Toxicol, 58: 123-130, (2009).
9. Nwani CD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK. Mutagenic and Genotoxic effects of Carbosulfan in fresh water fish *Channa Punctatus* (Bloch) using micronucleus assay and alkaline single – cell gel electorphoresis, Food and Chem Toxicol, 48: 202-208, (2010).
10. Mohamed H. Mona Reda M, Gaafar Ibrahim B, Helal Nahla E, Omran Wesam,

and M Salama, Evaluation of cytotoxic effects of atrazine and glyphosate herbicides on *Biomphalaria glabrata* snails, The Journal of Basic & Applied Zoology, 66: 68–75, (2013).
11. Fournier M, Cyr D, Blakley B, Boermans H, and Brousseau P, Phagocytosis as a biomarker of immunotoxicity in wildlife species exposed to environmental xenobiotics, Am Zool, 40: 412–420, (2000).
12. US Environmental Protection Agency. R.E.D Facts: Pendimethalin. Washington, DC: USEPA; (1997).
13. Dimitrov B D, Gadeva P G, Benova D K, and Bineva M V. Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems, Mutagenesis, 21 (6): 375-382, (2006).
14. Tylicki Adam, El|bieta Kowalska-Wochna, Anna Ocilowicz, Renata Ilasz, Mitodepressive and Cytotoxic Effects of Short-Term Exposure to Relatively Small Doses of Pendimethalin Evaluated by *Allium* Test, Polish J of Environ Stud,19(1): 193-199, (2010).
15. Nagpure N S, and Barat, A, (1997). A simplified method of fish chromosome preparation by *in vitro* colchicine treatment, *Ind J Exp Biol* 35: 915-916, (1997).
16. Al-Sabti K, and Metcalfe, C D, Fish micronuclei for assessing genotoxicity in water, Mutation Res 343: 121–135, (1995).
17. Olla B L, Pearson W H, and Studholme A L, Applicantly of behavioral measures in environmental stress assessment, Rapp PV Renn Cons Int Explor Mer, 179: 162-179, (1980).
18. Sharma U D, and Shukla S, Behavioural dysfunction of fresh water prawn, *Macrobrachium lamarrei (Crustacea ecapoda)* following exposure to synthetic detergent, linearalkyl benzene sulphonate. Biol. Mem. 16 (12): 58-61, (1990),
19. Agarwal S K, Bioassy evaluation of acute toxicity levels of mercuric chloride to an airbreathing fish *Channa punctatus* (Bloch): Mortality and behaviour study, J Environ Biol, 12: 99-106, (1991).
20. Hussein SY, El-Nasser MA, Ahmed SM. Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthys auratus* at Assiut Egypt, Bull Environ Cont Toxicol 57: 503-510, (1996).
21. Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Acute toxicity bioassays of mecuric chloride and malathion on air breathing fish *Channa punctatus* (Bloch). Ecitoxicol. Environ. Saf 61: 114-120, (2005).
22. Chandra S, Toxic effects of Malathion on acetlcholinesterase activity of liver, brain and gills of freshwater catfish *Heteropneutes fossilis*. Environ Conserv 9: 45-52, (2008).
23. Ferguson H W, Textbook of Systemic Pathology of fish, 1st ed., Iowa State Univ. Press, Amer.Iowa 50010, Canada, (1989).
24. Devi M, and Fingerman M, Inhibition of acetylcholinesterase activity in the central nervous system of the red swamp crayfish, *Procambarus clarkii,* by mercury, cadmium and lead, Bull Environ Contam Toxicol, 55: 746-750, (1995).
25. Lata S, Gopal K, and Singh N N, Toxicological evaluations and morphological studies in a cat fish *Clarias batrachus* exposed to carbaryl and carbofuran, J Ecophysiol Occup Hlth 1: 121-130, (2001).
26. Patro L, Toxicological effects of cadmium chloride on Acetyl cholinesterase activity of freshwater fish, *Oreochromis mossambicus* Peters, Asian J Exp Sci 20: 171-180, (2006).
27. Anitha B, Chandra N, Gopinath P M, and Durairaj G, Genotoxicity evaluation of heat shock in gold fish *(Carassius auratus),* Mutation Res, 467: 1-8, (2000).
28. Al-Sabti K, Chlorotriazine reactive azo red 120 textile dye induces micronuclei in fish, Ecotoxicol Environ Saf, 47: 149-155, (2000).
29. Banu B S, Danadevi K, Rahman M F, Ahuja Y R, and Kaiser J, Genotoxic effect of monocrotophos to sentinel species using comet assay, Food Chem Toxicol, 39: 361 – 366, (2001).

30. De Lemos C T, Rodel P M, Terra N R, and Erdtmann B, Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes, Environ Toxicol Chem 20: 1320-1324, (2001).
31. Ateeq B, Abul Farah M, Niyamat Ali M, and Ahmad W, Induction of micronuclei and erythrocyte alterations in the catfish *Clarias batrachus* by 2,4-dichlorophenoxyacetic acid and butachlor, Mutation Res, 518: 135-144, (2002).
32. Abul Farah A, Ateeq B, Niyamat Ali M, and Ahmad W, Evaluation of genotoxicity of PCP and 2,4-D by micronucleus test in freshwater fish *Channa punctatus,* Ecotoxicol Environ Saf, 54: 25-29, (2003)..
33. Klobucar I V G, Pavlica M, Erben R, Papes D, Application of the micronucleus and comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments, Aquatic Toxicol, 64: 15-23, (2003).
34. Buschini A, Martino A, Gustavino B, Monfrinotti M, Poli P, Rossi C, Santaro M, Porr A J M and Rizzoni M, Comet assay and micronucleus in circulating erythrocytes of *Cyprinus carpio* specimens exposed in situ to lake waters treated with disinfectants for potabolization, Mutation Res, 557: 119-129. (2004).
35. Yadav K K, and Trivedi S P, Evaluation of genotoxic potential of chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations, Asian Pacific Journal of Cancer Prevention, 7: 472-476, (2006).
36. Çavaş T, *In vivo* genotoxicity of mercury chloride and lead acetate: Micronucleus test on acridine orange stained fish cells, Food and Chemical Toxicology 46: 352-358, (2008).
37. Mohamed M M, El-Fiky S A, Soheir Y M, and Abeer A I, Cytogenetic studies on the effect of copper sulfate and lead acetate pollution on *Oreochromis niloticus* fish, Asian Journal of Cell Biology 3: 51-60, (2008).
38. Choudhary Jaya, Singh R, and Jha Anand M, Heavy metal induced genotoxic response in an air breathing teleost *Channa punctatus* (Bloch), Int J Env Sci, I (4): 355-361, (2010).
39. Choudhary Jaya, Abha, and Jha Anand M, Cytogenetic effect of chromium trioxide in an air breathing teleost *Channa punctatus* (Bloch), Int J Pharm Bio Sci, 2(1): 246-253, (2012)
40. Gebel T, Kevekordes S, Pav K, Edenharder R, and Dunkelberg H, In vivo genotoxicity of selected herbicides in the mouse bone marrow micronucleus test, Arch Toxicol, 71: 193–197, (1997).
41. Dunkelberg H, Fuchs J, Hengstler JG, Klein E, Oesch F and Struder K, Genotoxic effects of the herbicides alachlor, atrazine, pendimethaline and simazine in mammalian cells, Bull Environ Contam Toxicol, 52, 498–504, (1994).
42. Morejohn LC, Bureau TE, Mole`-Bajer J, Bajer AS, and Fosket DE, Oryzalin, a dinitroaniline herbicide, binds to plant tubulin and inhibits microtubule polymerization in vitro, Planta 172: 252–264, (1987).
43. Nabela I. El-Sharkawy Rasha M Reda, and Iman E El-Araby, Assessment of Stomp® (Pendimethalin) toxicity on *Oreochromis niloticus,* Journal of American Science, 7(10): 568-576, (2011).
44. Iwama GK, Thomas T Philip, Forsyth Robert B, and Vijayan M M, Heat shock protein expression in fish, Reviews in Fish Biology and Fisheries 8: 35-56, (1998).
45. Iwama G K, Vijayan M M, Forsyth R, and Ackerman P, Heat shock proteins and physiological stress in fish, Am Zool, 39: 901–909 (1999).
46. Iwama G K, Afonso Luis O B, and Vijayan M M, Stress in Fish. AquaNet Workshop on Fish Welfare., Campbell River, B.C., Canada, pp. 1-6: (2004).