International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 4, p. 111-120, 2018

INNSPUB

RESEARCH PAPER OPEN ACCESS

# Evaluation of acute toxicity and behavioral response of herbicide pendimethalin to freshwater fish *Channa punctata* (Bloch)

**Pramathesh Kalita[1*], Kamal Choudhury[2]**

[1]*Department of Zoology, Gauhati University, Guwahati, Assam, Pin-781014, India*

[2]*Department of Zoology, B. Borooah College, Guwahati, Assam, Pin-781007, India*

**Key words:** Pendimethalin, *Channa punctata*, $LC_{50}$, Safe level, Behavioral changes.

http://dx.doi.org/10.12692/ijb/13.4.111-120 Article published on October 14, 2018

## Abstract

The aquatic animals specially the fishes are highly sensitive to the changes in their surrounding environment, including increasing pollution of water bodies that are directly exposed to the toxic chemicals like pesticides and herbicides. Though these are beneficial in agricultural fields for insect-pest and weed management, because of their uncontrolled spraying these chemicals may cause severe damage to the aquatic fauna specially fishes and human being through food chain. Herbicide pendimethalin is one of the broadly used chemicals in agricultural field to control weeds. In the present investigation, acute toxicity test (96 h) was conducted in semi static system to evaluate the lethal toxicity of pendimethalin EC (98.8%) by "probit analysis" method, as well as its safe level to freshwater fish, *Channa punctata* (Bloch) and the behavioural response of exposed fishes. The 96 h $LC_{50}$ value was calculated and found to be 2.20 mg/L indicating that pendimethalin is toxic to the fish species under study. The safe levels estimated by different methods for pendimethalin showed large variation (0.110-0.000220). In addition to dose and dose-time dependant increase in mortality rate, behavioral changes like hyperactivity, increased swimming, faster opercular activity etc. were also observed in response to the test chemical. On the basis of the present study we should restrict indiscriminate use of this herbicide in near aquatic bodies.

* **Corresponding Author:** Pramathesh Kalita ✉ pramatheshkalita@gmail.com

## Introduction

Identification and determination of toxicants and its ecological effects on natural communities and ecosystem is one of the fundamental goals of ecotoxicology and hazard assessment. Due to urbanization, industrialization and agricultural activities; freshwater bodies are highly polluted with different kinds of chemicals as well as pesticides, herbicides etc. released from various industries and agricultural fields that impair water quality directly or indirectly and become unsuitable for aquatic organisms due to their persistence, bioaccumulation, toxicity and bio magnifications in the food chain (Palaniappan *et al.,* 2009), which is a key concern for the health of aquatic organisms (Wagenhoff *et al.,* 2011). Herbicides are the most widely used chemicals in agricultural fields (National Academy of Sciences, 1993) for controlling unwanted grasses and broad leaf weeds. Chronic exposure and accumulation of these xenobiotics by aquatic biota can result in biochemical alterations that produce adverse effects not only in the exposed organisms but also in human beings via food chain (IARC 1993).

Pendimethalin, [N-(1-ethylpropyl)- 2,6-dinitro-3,4-xylidine] is a herbicide belongs to dinitroaniline family widely used in Agricultural fields for broad spectrum control of annual grasses and certain broadleaf weeds in commercial crops (Engebretson *et al.,* 2001**;** EI-Sharkawy *et al.,* 2011). Pendimethalin has been classified as moderately persistent bio accumulative toxic (PBT) compound (Roca *et al.,* 2009) and a group C carcinogen "possible human carcinogen" **(**USEPA, 1997)**,** that has the ability to bio magnify, and can bio concentrate up to 70,000 times their original concentrations (Ritter *et al.,* 2007). It has been considered as highly toxic chemical to fish and aquatic invertebrates (Meister, 1992) reported to causes negative mutagenic effects in mammalian and bacterial cells. In the year 2006, Dimitrov *et al.* showed that pendimethalin causes induction of chromosomal aberrations and micronuclei in bone marrow cells in mice. Studies on the toxicity effects of pendimethalin on fish and other aquatic organisms are very limited (Ahmed and Moustafa, 2010; Abd-Algadir *et al.,* 2011 and El- Sharkawy *et al.,* 2011). A few earlier findings clearly warned of the genotoxic potential of analytical grade pendimethalin in wide range of organisms including fish species.

To study the effects of pollution as well as for the early detection of aquatic environmental contamination, fishes have been used as a biomarker (Van der Oost *et al.,* 2003) because of their ecological and economical relevance (Jiraungkoorskul *et al.,* 2002; Moustafa *et al.,* 2016) as well as they are very sensitive to a wide variety of toxicants leading to change in aquatic environment**.** Similarly behavioral study is one of the important parameters of toxicology as it gives direct responses of the fish to herbicides and related chemicals. Therefore in view of the ecological impact it seems essential to study the adverse effect of such chemicals so as to formulate proper strategies regarding their use for safe guarding fishes along with other aquatic organisms.

Median lethal concentration ($LC_{50}$) of a particular test chemical is the most widely accepted basis for acute toxicity test at which 50% of the test organism undergoes death in a particular length of exposure usually 96 h. Therefore, the present investigation is aimed to determine the acute toxicity of herbicide pendimethalin to freshwater fish *Channa punctata* by determining the $LC_{50}$ value as well as analysing behavioral response due to its toxic effect. This species was selected for the bioassay because of its easy availability in North east India throughout the year as well as can be easily raised under laboratory conditions and fulfils most of the requirements of a model species.

## Materials and methods

*Test animal*

The live healthy specimens of freshwater fish *Channa punctata*, belonging to the family Channidae and order Perciformes with average body size of 18±3.2 cm and body weight of 80±5.0 g were procured from local sources and were treated with 0.5% $KMnO_4$ solution for two minutes to avoid any dermal infection (Pandey *et al.,* 2005) and then acclimated in laboratory conditions for 20 days to in large (100 litre capacity) glass aquarium prior to exposure to semi static system. During the acclimatization period fish were fed daily with commercial dry food pellets (Tokyo pellets). Fishes were kept under normal day and night condition by maintaining photoperiod and every effort was made to provide optimal condition for fish as far possible in the laboratory. No mortality observed during this period.

*Test chemical*

Before exposure, physiochemical properties of experimental water were tested according to APHA / AWWA / WEF (1998). Analytical standard pendimethalin (98.8% EC) was selected for the present study. The chemical structure and specifications of the test chemical are summarized in Fig. 1 and Table 1. DMSO (Dimethyl sulfoxide) was used as a solvent.

*Acute toxicity bioassay*

Definite acute toxicity bioassay was conducted in semi static system to determine the $LC_{50}$ value of pendimethalin by exposing the fishes to eleven different concentrations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4 mg/L) along with one control (without test chemical) and one DMSO (solvent) exposed, keeping 10 numbers of mature fish specimens selected randomly in each unit.

The experiment was set in triplicate following the recommendations of APHA, AWWA, WPCF (1998). Stock solution of pendimethalin was prepared by diluting 10 mg of analytical-standard pendimethalin in 1 mL of DMSO to make the strength of 10 mg/mL. Different test concentrations for the acute toxicity bioassay were prepared by diluting the solution with appropriate tap water in semi static unit 4 hours before the addition of fish samples. No feed was provided to fish during the experimentation period as recommended by Ward, Parrish (1982) and Reish, Oshida (1987). Frequent monitoring was made to observe mortality upto 96 hours after which mean mortality from a particular dose and its replicate was calculated.

The 96 h $LC_{50}$ value of pendimethalin was calculated from the data obtained in acute toxicity bioassay by Finney's method (1971) of "probit analysis" and was calculated at 95% confidence limits using the formula of Mohapatra and Rengarajan (1995).

The safe level for the chemical at 96 hour exposure was estimated based on Sprague (1971), Committee on Water Quality Criteria (CWQC, 1972), National Academy of Sciences/ National Academy of Engineering (NAS/NAE, 1973) and International Joint Commission (IJC, 1977); all are based on an "application factor (AF)".

*Behavioral study*

Fish behaviour was observed under sub-lethal concentrations of pendimethalin exposure for 96 hours as suggested by Kumari *et al.* (1997).

*Data analysis*

The data obtained were statistically analyzed by SPSS computer statistical software (version 21). The one way ANOVA along with Duncan's multiple range test (DMRT) was used to determine significant difference between the means at $P < 0.05$ level.

**Results**

Physiochemical parameters of aquarium water were measured and maintained during experimentation. The overall fluctuation ranges of water quality are listed in Table 2. Water temperature ranged from 26.90 to 28.60°C during experimentation. $p^H$ of water was recorded as slightly alkaline which ranged from 7.40 to 7.90. Likewise dissolved oxygen and total alkalinity were also recorded during experimental period which ranged from 7.2 to 7.8 and 90.45 to 98.60 mg/L respectively.

*Acute toxicity*

Total mortality of fish observed against different concentration groups at 96 hours of exposures is presented in Table 3.Table clearly showed that mortality rate increased with the concentration of pendimethalin, indicating a direct proportional relationship between mortality and concentration of test chemical. No mortality was recorded in control and DMSO exposed groups and were observed to be healthy during the experimental period. Among pendimethalin treated groups, there was no mortality recorded at 0.4 mg/L concentration after 96 h exposure.

**Table 1.** Specification of the test chemical.

| Common name | CAS R. No. | EC No. | Grade | Supplier | Alternate names |
|---|---|---|---|---|---|
| Pendimethalin | 40487-42-1 | 254-938-2 | Analytical standard | Sigma Aldrich | Prowl, Stomp |

Table 4 showed an extensive study of lethal concentrations ($LC_{10-90}$) of pendimethalin for *Channa punctata*. The 96 h $LC_{50}$ value was calculated from the regression equation y= 4.43x+3.48 and found to be 2.20 mg/L (2.00-2.40). The safe levels of pendimethalin by different methods at 96 h exposure on *Channa punctata* are listed in Table 5, which showed a large variation.

**Table 2.** Physiochemical properties of test water.

| Parameters | Unit | Mean | Range |
|---|---|---|---|
| Air temperature | °C | 27.80 | 26.90-28.60 |
| Water temperature | °C | 24.90 | 24.40-25.50 |
| $p^H$ | - | 7.60 | 7.40-7.90 |
| Dissolved oxygen (DO) | mg/L | 7.30 | 7.20-7.80 |
| Free carbon dioxide ($FCO_2$) | mg/L | 0.75 | 0.72-0.81 |
| Total alkalinity | mg/L | 95.00 | 90.45-98.60 |
| Total hardness (as $CaCO_3$) | mg/L | 78.00 | 75.00-81.00 |

*Behavioral effects*

Fish exposed to different concentrations of pendimethalin exhibited a number of abnormal behaviour as compared to control ones (Table 6). During the initial period of exposure, fishes became alert, stopped moving and remained static in position in response to sudden change in surrounding environment. Afterwards fishes tried to avoid the toxic water for sometime which was manifested by increased swimming, jumping, restlessness, hyperactivity etc. Faster opercular activity was observed initially but later decreased with increase of exposure period. In higher concentration groups fishes exhibited erratic swimming suggesting loss of equilibrium followed by hanging vertically in water.

**Table 3.** Data on probit value against test concentrations.

| Concentration (mg/L) | $Log_{10}$ of Concentration | Total exposed | Mortality at 96 hrs. | Percentage (%) Mortality | Probit value |
|---|---|---|---|---|---|
| 0.0 | Control | 10 | 0 | 0 | 0 |
| 0.0 | DMSO | 10 | 0 | 0 | 0 |
| 0.4 | -0.398 | 10 | 0 | 0 | 1.91 |
| 0.8 | -0.097 | 10 | 1 | 10 | 3.12 |
| 1.2 | 0.079 | 10 | 2 | 20 | 3.27 |
| 1.6 | 0.204 | 10 | 2 | 20 | 4.43 |
| 2.0 | 0.301 | 10 | 3 | 30 | 4.86 |
| 2.4 | 0.380 | 10 | 5 | 50 | 5.21 |
| 2.8 | 0.447 | 10 | 6 | 60 | 5.50 |
| 3.2 | 0.505 | 10 | 8 | 80 | 5.75 |
| 3.6 | 0.556 | 10 | 9 | 90 | 5.98 |
| 4.0 | 0.602 | 10 | 9 | 90 | 6.18 |
| 4.4 | 0.643 | 10 | 10 | 100 | 6.36 |

Under such conditions, breathing rate and surfacing activity of exposed fishes increased as efficiency of oxygen uptake decreased considerably. Copious amount of mucus was secreted from whole body of fish continuously and a thick layer of mucus was deposited in the buccal cavity and gills that increases with the increasing of concentration of toxicant. Body pigmentation was decreased as well as discolouration of skin was also viewed even in lower concentration groups.

**Table 4.** Lethal concentrations ($LC_{10\text{-}90}$) of pendimethalin at 96 h for *Channa punctata* (Average length 18±3.2 cm and weight 80±5.0 g, n=10).

| | | 95% confidence limits | |
|---|---|---|---|
| Lethal concentrations | Pendimethalin (mg/L) | Lower | Upper |
| $LC_{10}$ | 1.11 | 0.89 | 1.29 |
| $LC_{20}$ | 1.40 | 1.19 | 1.58 |
| $LC_{30}$ | 1.66 | 1.46 | 1.84 |
| $LC_{40}$ | 1.92 | 1.72 | 2.10 |
| $LC_{50}$ | 2.20 | 2.00 | 2.40 |
| $LC_{60}$ | 2.51 | 2.30 | 2.76 |
| $LC_{70}$ | 2.91 | 2.65 | 3.23 |
| $LC_{80}$ | 3.44 | 3.10 | 3.94 |
| $LC_{90}$ | 4.36 | 3.82 | 5.22 |
| Slope ± SEM | 4.43 ± 0.18 | | |
| Intercept ± SEM | 3.48 ± 0.08 | | |
| $\chi^2$ value | 0.99 | | |
| $p$ | $< 0.05$ | | |

*Control group (theoretical spontaneous response rate) = 0.000.

**Discussion**

The present investigation explicated the acute toxicity of herbicide pendimethalin to freshwater fish, *Channa punctata*. The result of 96 h $LC_{50}$ value of pendimethalin (98.8 % EC) was determined to be 2.20 mg/L, which can be considered as in moderately toxic level when compared to the values worked out by earlier workers so far. For commercially formulated pendimethalin product Stomp 50% EC, the 96 h $LC_{50}$ value for monosex *O. niloticus*, calculated using a static bioassay test was 4.92 mg/L, which was considered to be moderately toxic (Louis *et al.,* 1996). Ahmed and Moustafa (2010) determined the 96 h $LC_{50}$ of the same product in Nile tilapia as 3.55 mg/L. Moreover, the 96 h $LC_{50}$ values for other formulated products containing 45% pendimethalin are 0.52, 0.92 and 1.9 mg/L for rainbow trout, bluegill sunfish and channel catfish, respectively (Munn *et al.,* 2006; USEPA, 1997).

**Table 5.** Estimate of safe levels of pendimethalin at 96 h exposure time.

| Test chemical | 96 h $LC_{50}$ (mg/L) | Method | AF | Safe level |
|---|---|---|---|---|
| Pendimethalin | 2.20 | Sprague (1971) | 0.1 | 0.220 |
| | | CWQC (1972) | 0.01 | 0.0220 |
| | | NAS/NAE (1973) | 0.1-0.0001 | 0.220-0.000220 |
| | | IJC (1977) | 5% of 96 h $LC_{50}$ | 0.110 |

These acute toxicity data have been used to derive water quality guidelines for regulatory measures (Sunderam *et al.,* 1994).

The 96 h $LC_{50}$ value reported in the present investigation was higher than 7.75 μg/L, 0.268 mg/L and 0.4 mg/L as recorded by Pandey *et al.* (2006), Nwani *et al.* (2010), Kumar *et al.* (2007) for the same species exposed to endosulfan, carbosulfan and cypermethrin respectively. Result also showed that herbicide pendimethalin is more toxic in *Channa* *punctata,* in comparison to various chemicals used in agricultural fields, such as $LC_{50}$ of glyphosate at 32.54 mg/L (Nwani *et al.,* 2010) and carbamate at 17.41 mg/L (Singh *et al.,* 2007).

It appears that the differences in $LC_{50}$ values reported earlier with those obtained in the present study are with dissimilarities. In this regard, as reported by Gupta *et al.* (1981), it can be assumed that various physiochemical factors like temperature, variations in $p^H$ of water, total hardness of water, dissolved oxygen etc. influence the $LC_{50}$ values of toxic chemicals. Even the age, size and health of fish species (Abdul Farah *et al.,* 2004) may also influence bioassay techniques. Sprague (1969) also observed variations in acute toxicity even in case of a same species and same toxicant depending on the size, age and conditions of the test species along with the experimental factors.

**Table 6.** Impact of pendimethalin on the behavioral pattern of *Channa punctata* (Average length 18±3.2 cm and weight 80±5.0 g, n=10) exposed to the herbicide up to 96 h.

| | | Pendimethalin (mg/L) | | |
|---|---|---|---|---|
| Parameters | Control | 0.220 | 0.440 | 0.660 |
| Hyperactivity | - | + | ++ | +++ |
| Rate of swimming | + | + | ++ | ++ |
| Loss of balance | - | + | + | +++ |
| Rate of opercular activity | + | + | ++ | ++ |
| Surfacing activity | + | + | ++ | +++ |
| Colour of skin | - | + | + | ++ |
| Convulsions | - | - | + | ++ |

*The increase or decrease in the level of behavioral parameters is shown by numbers of (+) sign. The (-) sign indicates normal behavioral conditions.

The estimated safe levels determined by various methods obtained in the present study showed a large variation which has resulted in controversy over its acceptability (Buikema *et al.,* 1982, Pandey *et al.,* 2005). Mount and Stephan (1967) underscored the fact that extrapolation of laboratory data to the field is not always meaningful, and therefore it is difficult to accept a particular concentration based on the laboratory experiments that may be considered 'safe' in the field. Kennega (1979) also emphasized that the major weakness in calculation of accumulation factor (AF) is its dependence on $LC_{50}$ value.

The most meaningful scientific information obtained from acute toxicity bioassay is derived from behavioural changes, clinical observations and post-mortem examinations of animals rather than from the specific value of the $LC_{50}$ (Eaton and Gilbert, 2008). One of the observed results of the present study on behavioural responses is the rapid swimming activity of the herbicide exposed fishes during the exposure period which was possibly due to the effect of the chemical on the nervous system. This behaviour appeared to be directly related to the concentration of the chemical.

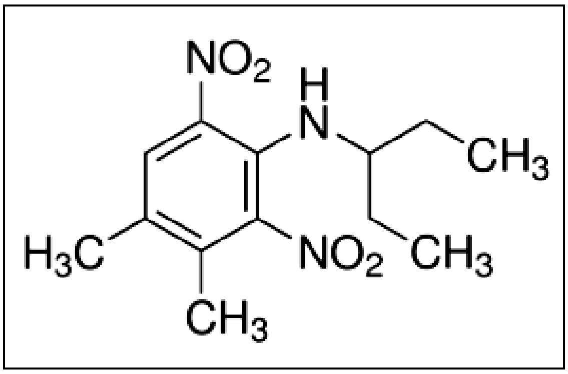


**Fig. 1.** Chemical structure of pendimethalin.

The stressful and erratic behaviour of *Channa punctata* also tend to indicate respiratory impairment probably due to the effect of the chemical on gills. The abnormal behaviour of the fish and subsequent death imply that the toxic effect is mediated through the disturbed nervous/cellular enzyme system affecting the normal respiratory functions and nervous system, which involves control of almost all vital activities (Desaiah and Koch, 1975a, 1975b; Bansal *et al.,* 1985; Pandey *et al.,* 2005). The abnormal movements shown by the exposed fishes may have resulted from hypercontractions of the muscles due to AchE (cholinesterase) inhibition at the highest pendimethalin concentration (EI-Sharkawy *et al.,* 2011).

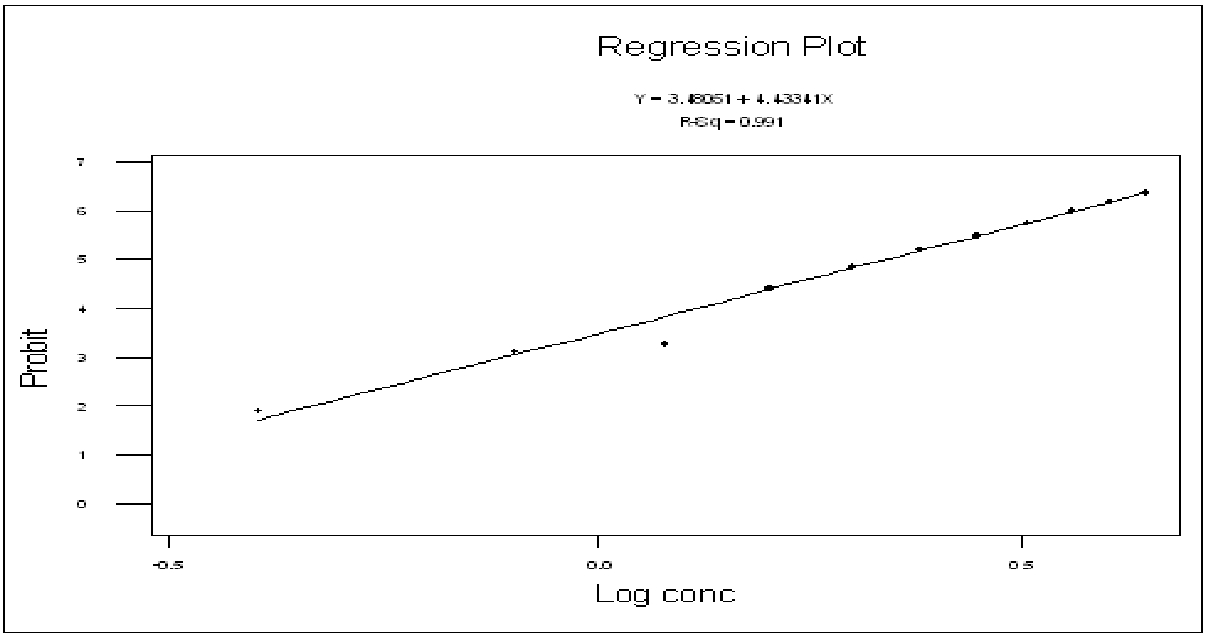


**Fig. 2.** Plot of adjusted probits and predicted regression line for pendimethalin to freshwater fish *Channa punctate.*

In addition, excess mucus secretions occurred may be due to respiratory manifestations forming thick coatings on gill tissue (Ferguson, 1989 ; El-Sayed *et al.,* 2015). As reported by (Pandey *et al.,* 1990) hyper secretion of mucus and decreased body pigmentation is due to the dysfunction of endocrine/pituitary gland under toxic stress environment causing changes in the number and area of mucus gland**.**

Similar behavioural responses like restlessness, abnormal swimming and loss of balance were also observed in *Channa punctata* while exposed to mercuric chloride and malathion (Pandey *et al.,* 2005), hexavalent chromium (Mishra and Mohanty, 2008), and cypermethrin and λ-cyhalothrin (Kumar *et al.,* 2007).

Since no abnormal behaviour was observed from the fishes of control group, so it is clear that the altered behaviour and mortality observed in the treated groups is mainly due to pendimethalin toxicity.

**Conclusion**

The result of the present study showed that herbicide pendimethalin is toxic to *Channa punctata* reflecting a high risk assessment. This study also showed the significance of behavioural parameters in assessing the hazards of the herbicide to fish even at lower concentrations. Thus, fish can effectively employed as a key indicator of environmental toxicity. Since chemical determination of any persistent toxicant at sublethal levels may not provide information on the severity of contamination so, biological monitoring using a series of assays in a 'key species' could allow a sensitive as well as predictive approach regarding the potential risk of persistent contaminants like herbicides and pesticides, which is helpful in formulating the safe levels of such bioaccumulative chemicals having genotoxic potential. Therefore, it is important to demonstrate the sensitivity of genotoxic effects of pesticides and herbicides in aquatic organisms; particularly in fish as it may cause some risk to similar species in natural environment.

**Acknowledgments**

The authors express their deep sense of gratitude to Institutional Biotech Hub, B. Borooah College, and Department of Zoology, B. Borooah Collage under Gauhati University, Assam; India for encouragement and providing necessary laboratory facilities.

**References**

**Abd-Algadir MI, Idris OF, Elkhier MKS.** 2011. Effect of pendimethalin herbicide on fish (Tilapia nilotica) skeletal muscles, gills and its influence on human. World Journal of Life Sciences and Medical Research **1,** 5–10.

**Ahmed EG, Moustafa GG.** 2010. Evaluation of the toxic effects of herbicide Stomp 50% EC (pendimethalin) on health of Oreochromis niloticus. Zagazig Veterinary Journal **38,** 8-17.

**APHA/AWWA/WPCF.** 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 20th ed. New York.

**Bansal SK, Murthy RC, Chandra SV.** 1985. The effects of some divalent metals on cardiac and branchial $Ca^{2+}$-ATPase in a freshwater fish Saccobranchus fossilis. Ecotoxicology and environmental safety **9(3),** 373-377.

**Buikema Jr. AL, Niederlehner BR, Cairns Jr. J.** 1982. Biological monitoring part IV-toxicity testing. Water research **16(3),** 239-262.

**Committee on Water Quality Criteria (CWQC).** 1972. Areport of the committee on water quality criteria. Ecological Research Series EPA-R3-73-003.

**Dimitrov BD, Gadeva PG, Benova DK, Bineva MV.** 2006. Comparative genotoxicity of the herbicides roundup, stomp and reglone in plant and mammalian test systems. Mutagenesis **21(6),** 375–382.

**Desaiah D, Koch RB.** 1975. Inhibition of ATPases activity in channel catfish brain by Ketone and its reduction product. Bulletin of environmental contamination and toxicology **13(2),** 153-158.

**Eaton LD, Gilbert SG.** 2008. Principles of toxicology In: Klassen CD, editor. Casarett and Doull's toxicology: The basic science of Poisons, 7th ed. New York: McGraw-Hill. p 11-44.

**EI-Sayed YS, Samak DH, Abou-Ghanema IY, Soliman MK.** 2015. Physiological and oxidative stress biomarkers in the freshwater monosex Nile tilapia, Oreochromis niloticus L. exposed to pendimethalin-based herbicide. Environmental toxicology **30(4),** 430-438.

**El-Sharkawy NI, Reda RM, El-Araby IE.** 2011. Assessment of Stomp® (Pendimethalin) toxicity on Oreochromis niloticus. Journal of American Science **7(10),** 568-576.

**Engebretson J, Hall G, Hengel M, Shibamoto T.** 2001. Analysis of pendimethalin residues in fruit, nuts, vegetables, grass, and mint by gas chromatography. Journal of Agricultural and Food Chemistry **49(5),** 2198–2206.

**Farah MA, Ateeq B, Ali MN, Sabir R, Ahmad W.** 2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere **55(2),** 257-265.

**Ferguson HW.** 1989. Textbook of Systemic Pathology of Fish. Iowa, Canada: Iowa State University Press.

**Finney DJ.** 1971. Probit analysis. Cambridge University Pres., London, England, p.333.

**Gupta PK, Khangarot BS, Durve VS.** 1981. The temperature dependence of the acute toxicity of copper to a freshwater pond snail, Viviparus bengalensis L. Hydrobiologia **83(3),** 461-464.

**IARC.** 1993. Monographs on the evaluation of Carcinogenic Risks to Humans: Beryllium, Cadmium, Mercury and exposures in the glass manufacturing Industry, IARC, Lyon. **58**.

**IJC.** 1977. New and Revised Great Lakes Water Quality Objectives. Windsor, Ontario, p. 1.

**Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P.** 2002. Histopathological effects of Roundup, a glyphosate herbicide on Nile tilapia (Oreochromis niloticus). Science Asia **28,** 121-127.

**Kenega EF.** 1979. Aquatic test organism and methods useful for assessment of chronic toxicity of chemicals. In: Dickson KL, Maki AW, Cairns Jr. J. (Eds.), Analysing the Hazard Evaluation Process. American Fisheries Society, Washington DC, p. 101.

**Kumar A, Sharma B, Pandey RS.** 2007. Preliminary evaluation of the acute toxicity of cypermethrin and λ-cyhalothrin to Channa punctatus. Bulletin of environmental contamination and toxicology **79(6),** 613-616.

**Kumari R, Singh RK, Khanna YP, Sharma B.** 1997. Carbofuran induced stress mediated syndromes in Clarius batrachus. In: Proceedings of international conference on industrial pollution control technology, p 113-119.

**Louis AH, Diana LW, Patricia H, Elizabeth RS.** 1996. Pesticides and aquatic animals: A guide to reducing impacts on aquatic systems. Virginia Cooperative Extension, Virginia State University, Virginia **24.**

**Mishra AK, Mohanty B.** 2008. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, Channa punctatus (Bloch). Environmental Toxicology and Pharmacology **26(2),** 136-141.

**Meister RT.** 1992. Farm Chemicals Handbook. Meister Publishing Company, Willoughby, OH.

**Mohapatra BC, Rengarajan K.** 1995. Manual on bioassays in the laboratory and their techniques. Central Marine Fisheries Research Institute, Special Publication **64,** 1-75.

**Mount DI, Stephan CE.** 1967. A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2, 4-D. Transactions of the American Fisheries Society **96(2),** 185-193.

**Moustafa GG, Shaaban FE, Hadeed AH, Elhady WM.** 2016. Immunotoxicological, biochemical, and histopathological studies on Roundup and Stomp herbicides in Nile catfish (Clarias gariepinus). Veterinary world **9(6),** 638–647.

**Munn MD, Gilliom RJ, Moran PW, Nowell LH.** 2006. Pesticide toxicity index for freshwater aquatic organisms. US Geological Survey Scientific Investigations Report **5148, 81**.

**NAS/NAE.** 1973. Water Quality Criteria. US Government Printing Office, Washington DC, WQC 1972, EPA-R3-033.

**Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS.** 2010. Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch). International Journal of Aquatic Research **2,** 105-111.

**Palaniappan PR, Karthikeyan S.** 2009. Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish Cirrhinus mrigala individually and in binary solutions with nickel. Journal of Environmental Sciences **21(2),** 229-236.

**Pandey A, Kunwar GK, Datta Munshi JS.** 1990. Integumentary chromatophores and mucous glands of fish as indicator of heavy metal pollution: A simple scale method for evaluation of quality of freshwater. Journal of Freshwater Biology **2(2),** 117-122.

**Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Verma MS.** 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish Channa punctatus (Bloch). Ecotoxicology and environmental safety **61(1),** 114-120.

**Pandey S, Nagpure NS, Kumar R, Sharma S, Srivastava SK, Verma MS.** 2006. Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost Channa punctatus (Bloch) by alkaline single-cell gel electrophoresis. Ecotoxicology and environmental safety **65(1),** 56-61.

**Reish DL, Oshida PS.** 1987. Short term bioassay. In: Manual of methods in Aquatic Environment Research. Part 6. FAO Fish. Tech. pap **247,** 1-62.

**Ritter L, Solomon KR, Forget J, Stemeroff M, O'Leary C.** 2007. Persistent organic pollutants: An Assessment Report on: DDT, Aldrin, Dieldrin, Endrin, Chlordane, Heptachlor, Hexachlorobenzene, Mirex, Toxaphene, Polychlorinated Biphenyls, Dioxins and Furans. Prepared for The International Programme on Chemical Safety (IPCS).

**Roca E, D'Errico E, Izzo A, Strumia S, Esposito A, Fiorentino A.** 2009. In vitro saprotrophic basidiomycetes tolerance to pendimethalin. International Biodeterioration and Biodegradation **63(2)**, 182–186. http://dx.doi.org/10.1016/j.ibiod.2008.08.004.

**Singh SP, Chaudhary R, Gaur KK.** 2007. A study of toxicity and behaviour of freshwater fish, Channa punctatus (Bloch) after intoxication of carbamate and synthetic pyrethroid fenvalerate. Journal of Ecophysiology and Occupational Health **7(1/2),** 39.

**Sprague JB.** 1969. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. Water Research **3(11),** 793-821.

**Sprague JB.** 1971. Measurement of pollutant toxicity to fish: 111. Sublethal effects and "SAFE" concentrations. Water Resource **5**, 245.

**Sunderam RIM, Thompson GB, Chapman JC, Cheng DMH.** 1994. Acute and chronic toxicity of endosulfan to two australian cladocerans and their applicability in deriving water quality criteria. Archives of Environmental Contamination and Toxicology **27(4)**, 541–545.

**USEPA.** 1997. Reregistration Eligibility Decision (RED): Pendimethalin. EPA 738-R97-007-Office of Prevention, Pesticides, and Toxic Substances. Washington, DC: US EPA.

**Van der Oost R, Beyer J, Vermeulen NP.** 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental toxicology and pharmacology **13(2),** 57-149.

**Wagenhoff A, Townsend CR, Phillips N, Matthaei CD. 2011.** fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshwater Biology **56(9),** 1916-1936.

**Ward GS, Parrish PR.** 1982. Toxicology tests. In: Manuals of methods in aquatic environment Research, Part 6. FAO Fish. Tech. Pap **185,** 1-23.