# Micronucleus Assay in Fishes

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## *Abstract*

The in vitro micronucleus assay is a mutagenicity test system which becomes one of the popular methods to assess the chemicals that induce the formation of small membrane bound DNA fragments such as micronuclei in the cytoplasm of interphase cells. The micronuclei accurse due to genetic damage and originate from acentric fragments or whole chromosomes that are unable to migrate with the rest of the chromosomes during the anaphase of cell division. Fish are the model animal for genotoxic studies which alarms the environmental alteration and degradation. In this article, the micronucleus assay in fishes is reviewed.

***Key Word –*** Biomarker,DNA damage, Micronucleus, Fresh water fishes, Polution, Micronucleus.

## *Introdouction*

The micronucleus test is a simple and sensitive assay for in vivo evaluation of genotoxic properties of various agents. Micronuclei can be defined, as "the micronuclei are small particles consisting of acentric fragments of chromosomes or entire chromosome which lag behind at anaphase stage of cell division. After telo-phase these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm" (OECD, 1997). Micronucleus test (MNT) is a widely used cytogenetic method used to assess chromosomal damage induced by various genotoxicants. Mutagensis and xenotoxcity employed this methodology to analyze possible xenotoxic risk due to exposition to hazarads xenobiotics, in different organisms including aquatic sensitive organisms **Machado Da Rocha et.al., 2009**. **Schroder (1966)** studied first the formation of micronuclei (MNi) in mammalian bone marrow cells, subsequently this assay was developed by W. Schmid (1975) in mammalian systems, since then it is used as quickest methodology for screening the genotoxic agents in various mammalian models (Schmid 1976). The Mni are also known as Howell-Jolly bodies in mammals. Like mammalian species, MNT has also been adopted to study genotoxicity in fishes. Fish provide a suitable model for monitoring aquatic genotoxicity and waste water quality because of their ability to metabolize xenobiotics and accumulate pollutants (Grisolia and Corderio, 2000). Micronuclei in fish could be smaller in size than that suggested by **Schmid (1975)**, because most fish chromosome are much smaller (e.g. 1/10 to 1/30 of the size of principal nucleus). The formation of micronuclei dependent on proliferation of cells. MN assay in fish species is sensitive, fast and an important biomarker of mutogenic exposure in the environment. The aim of this article is togather the published data on the use of fish MN assay in bio-monitoring and genotoxicity.

## *Principal of the MN assay :*

Micronuclei (MN) arise in the mitotic cells from chromosomal fragments or chromosomes that lag behind in anaphase and are not integrated in to the daughter nuclei. Micronuclei harbouring chromosomal fragments result, e.g. from (A) direct DNA breakage (B) replication on a damaged DNA template and (C) inhibition of DNA synthesis. MN harbouring whole chromosomes are primarily formed from failure of the mitotic spindle, kinetochore, or other parts of the mitotic apparatus or by damage to chromosomal sub-structures, alterations in cellular physiology, and mechanical disruption. Thus, an increased frequency of micronucleated cells is a biomarker of genotoxic effects that can reflect exposure to agents with clastogenic (chromosome breaking; DNA as target) or aneugenic (aneuploidogenic; effect on chromosome number; mostly non-DNA target) modes of action.

## *Micronuclei Formation :*

Micronuclei are expressed in dividing cell that either contain chromosome breaks lacking centromere or whole chromosomes that are unable to travel to the spindle poles during mitosis. At telophase, a nuclear envelope forms around the lagging chromosome and fragments which uncoil and gradually, assume morphology of an interphase nucleus with exception that they are smaller than the main nuclei in the cell, hence the term micronuclei. As per **Schmid (1975)** micronuclei are formed in cytoplasm through following process.

(a) In anaphase, acentric chromatid and chromosomal fragments lag behind when the centric elements moves towards the spindle poles. Micronuclei arise from chromosomal fragments or acentric chromosomes that are not incorporated into daughter nuclei at mitosis because they lack a centromere.

(b) The lagging element may be included in the nuclei of the daughter cells, but a proportion from one or several secondary nuclei that are much smaller than the principal nucleus (1/5 to 1/20) and are called micronuclei.

## *Micronuclei assay in fishes-*

Al **Sabti and Metcalfe** (1995) hve reviewed the literature on clastogenic effects of many chemicals and physical agents on fish cells, with emphasis on the induction of the micronuclei in teleost species. **Svobodova et.al.** (1998) studied the effect of Malachite green on Cyprinus caprio by using MNT. Castano et.al., (1998) studied the effect of cadmium on rainbow trout and reported that the frequency of micronuclei in treated animals was slight higher than that of control group. Hayashi et.al. (1998) reported that micronuclei test could be used to assess genotoxicity of polluted water. Campana et.al. (1999) also used MNT as indicator in fish Cheriodon Interruptus interruptus for assessment of genotoxic effect of pyrethroid lambda-cyhalothrin. Gustavino et.al. (2001) have reported dose dependent increase in the micronucleus frequency in fish Cyprinus carpio when expose to x-rays and colchicines.

**Williams and Metcalfe** (1992) developed an in vivo hepatic MN assay with rainbow trout (Oncorhynchus mykiss) exposed to a chemical hepatic necrogen, allyl formate Poongothai et.al., (1996) studies the effect of polluted sewage water in five different species of fishes by using MNT as too. The frequency of micronuclei was statistically significant in both the groups.

The measurement of nuclear anomalies (NA), such as the presence of micronuclei (MN) and 'lobes', has been successfully utilized in many field studies of genotoxic effects of contaminated sediments in butterfish, *Pholis gunnelus* by **Bombail et.al. (2001).** The induction of micronuclei and other nuclear abnormalities in renal erythrocytes of rainbow trout *Oncorhynchus mykiss* by six genotixic compounds namely colchicines, mitomycin, cyclophosphamide, acrylamide, methyl-methanesulfonate, and n-ethyl-N-nitrosourea was evaluated and the results showed that cyclophosphamide induces the formation of micronuclei and also the other nuclear abnormalities; n-ethyl-N-nitrosourea, acrylamide, and colchicines induce only micronuclei; mitomycin-C induces only other nuclear abnormalities but not micronuclei whereas, methyl-methanesulfonate does not induce nuclear abnormalities in rainbow trout at the does assayed in the work done by **Ayllon nd Gareia-Vazquez, (2001).** Micronucleus test was performed in situ on eels (*Anguilla Anguilla)* from river sites with different levels of heavy metal pollution (cadmium and mercury) by **Sanchez-Galan et.al. (2001),** both cadmium and mercury induced micronuclei expression in eels when injected, and it was concluded tht these heavy metals are genotoxic for European eel. Fish micronuclei tests (MN) were used by **Grisolia and Starling (2001)** to evaluate the ability of wastewater from two municipal sewage treatment plants that empty into lake Paranoa to cause genetic damage.

An increases in micronuclei frequencies were observed by **Llorente et.al. (2002)** in fish living in supposedly polluted areas vs. control area when a field study was conducted to investigate the appearance of alterations in the peripheral blood cells of wild populations of fish *(Cyprinus corpio).* **Gravato and Santos (2002)** exposed sea bass to the chemicals benzo [a] pyrene, naphthalene nd beta-naphthofla vone and their results indicated tht benzo [a] pyrence as the most genotoxic compound, followed by naphthalene and beta-naphthoflavone (B (a) P>NAPH>BNF) measured by MNT. Ayllon et.al. (2000) analysed micronuclei in brown trout *Salmo trutta* specimens sampled in the Trubia River, upstream and downstream of the emissions from a Spanish military factory to assess genotoxicity risks derived from military wastes.

**Al-Sabti (2000)** has used micronucleus induction in fish erythrocytes to study the risk to aquatic ecosystems due to the genotoxicity of Chlorotriazine Reactive Azo Red 120 textile dye. It was found that micronuclei increased not only in a dose-dependent maner but also in a time-dependent way, compared with negative (tap water) and positive (10 ppm benzene) control groups. **Ayllon and Garcia-Vazquez (2000)** measured both micronuclei and other nuclear abnormalities in renal erythrocytes from European minnow *Phoxinus phoxinu* and mollie *Poecilia latipinna*, with the aim to contribute to the standardization of the micronucleus test for fish species.

**Sanchez et.al. (2000)** have micronuclei (MN) estimations by means of flow cytometry, and tested the capability of flow cytometry to evaluate MN induction and cell cycle alterations in an established fish cell line (RTG-2) and showed that it can improve the assay by means of its speed and objectivity, which makes the assay very useful for genotoxicity assessment of aquatic chemicals. **Hayashi et.al. (1998)** have shown that the micronucleus assay is applicable to freshwater and marine fishes and that gill cells are more sensitive than hematopoietic cells to micronucleus-inducing agents. **Sanchez-Galan et.al. (1988)** have also demonstrated the sensitivity of the micronucleus test in kidney erythrocytes to biomonitor fresh water ecosystems using brown trout (*Salmo trutta*) and showed a positive association between micronuclei average and fluctuating asymmetry at the populational level suggested that fluctuating asymmetry tests could be potential indicators of environmental threat. **Zakhidov et.al. (1996)** reported very high frequencies of erythrocytes with micronuclei in fish inhabiting highly polluted water bodies.

MNT was designed to study dose and time yield effects of mercury after indirect exposure in vivo to fish *cyprinus carpio* and significant increase in MN frequencies was observed at higher concentrations (20.0 and 200.0 mg. Hg 0/1) after 31 days of exposure, followed by slight stabilization and gradual decrease **(Nepomuceno et.al., 1997).**

**Al-Sabti** (1995) was developed a novel technique of binucleated method that depends on the cytokinesis blocking of hepatic cells in fish to enable the scoring of micronuclei in cells that have completed only one division after genotoxic insult. **Al-Sahti et.al.,** (1994) have also investigated the cytogenetic effects of chromium (Cr(VI) nd Cr(III)) under laboratory and field conditions in Prussian carp (*Carassus auratus gibelio*) using micronuclei induction in erythrocyte cells and reported n increase in the frequency of micronuclei compared with the control groups.

Micronucleus formation in fish erythrocytes, as an indicator of chromosomal damage, has been increasingly used to detect the genotoxic potential of environmental contaminants. Nucleolar organizer regions (NORs) stained with colloidal silver techniques indicate sites of active RNA transcription. The number and size of NORs in interphase nuclei reflect cellular activities such as proliferation and differentiation of cells. **Cavas & Gozukara (2003)** have used nuclear (micronucleus frequency) and nucelolar (chnges in quantitative characteristics of nucleoli) biomarkers to evaluate the functional and structural genotoxic effects of the pyrethroid insecticide lambda-cyhalothrin on Garra rufa (pisces: Cyprinidae).

**Rodriguez-Cea et.al.,** (2003) have used three fish species Brown trout, *Salmo trutto,* European eel, Anguilla Anguilla, and European minnow, *Phoxinus phoxinus*, inhabiting European freshwater ecosystems, as in situ pollution biomarkes by using the micronucleus test in renal erythrocytes. Experimental exposure (by immersion) to different concentrations of

cyclophosphamide, colchicines, and cadmium showed that brown trout are more sensitive to the three compounds than minnows and eels.

**Gravato and Santos, (2003)** have studies genotoxic potential of various chemical agents on *Dicentrarchus labrax* (sea bass) during different exposure periods to benzo(a)pyrene (B(a)P), a envioronmental pollutant, and measured a number of assays including erythrocytic micronuclei (EMN) and erythrocytic nuclear abnormalities (ENA) as genotoxicity biomarkers.

## *Micronuclei Assay in India-*

In Indi, studies on micronuclei test have been initiated by **Manna et.al. (1985)** who reported occurrence of micronuclei in erythrocytes and chromosomal aberrations in *Oreochromis mossambicus* treated with aldrin, cadmium chloride and x-rays. The percentage of micronuclei was low but chromosomal aberrations were higher in x-rays treated specimens. **Sadhukhan and Manna (1986)** observed micronuclei when fishes (*Oreochromis mossombicus*) were exposed to five chemicals (anisole, cobalt chloride, lithium chloride, rogar 30E and zinc sulphate). They developed a method of detecting micro nucleated cells from the gills of tilapia, treated with x-rays and two chemicals and emphasized that there was no need to stimulate cell division in this rapidly proliferating target organ. **Das and Nanda (1986)** noted an increase in micronuclei frequency in the erythrocytes of *Heteropneustes fossilis* exposed to mitomycin C and to paper mill effluent. **Barat et.al. (1998)** studied the effect of organophosphate pesticide malathion in *Channa punctatus.* Recently, MNT have been reported to be sensitive for detecting genotoxicity in two fish species namely *Mystus vittatus* and *C. punctatus,* **(Kushwaha etlal., 1999, 2000)** at very low level of malathion pesticide in aquatic medium and there was dose dependent increase in micronuclei formation. The study of **Ateeq, (2002)** showed a positive dose-response relationship in fishes exposed to two herbicides namely 2,4-D, and butachlor for genotoxic and cytotoxic endpoints. Herbicides used were found to be genotoxic as well as cytotoxic in this fish. An *in vivo* study of the effect of pentachlorophenol was carried out by **Ahmad et.al. (2002)** with a pre-acclimatized fish species, *Heteropneustes fossilis* using micronucleus test, and the results showed all micronuclei frequencies were significantly different from contro ($P<0.05$) with a linear relationship between the percentage of micronucleated erythrocytes and dose at all levels.

**Ali et.al. (2008-09)** studied the genotoxic effects of chlorpyritos in Channa punctatus using MN assay 7 comet assay. They reported that MN induction was highest on day 14 of 203.0 microg/1 of CPE. **Kushwaha et.al., (2012)** evaluated the genotoxic potential of polluted water of river Gomati in two fishes Channa punctatus and Mystus vittatus. The fishes were kept in sites in nylon cages to the polluted water of river Gomati. The induction of DNA damage and micronuclic were determined in blood erythrocytes using comet assay and micronucleus test. Their results revealed that C. punctatus is more sensitive to aquatic pollutant.

**Kumar et.al. (2013)** studied the xenotoxicity of Arsenic on Channa punctatus and carrasius auratus. In this study the micronucleus essay was used to assess the xenotoxic potential of arsenic at its various exposure level. The significant increase in the frequency of micronucleated erythrocyte were observed in both fishes. **Bhatnagar et.al. (2016)** have studied the incidence of nuclear abnormalities in the blood with of fresh water fish Cirrihinus mrigala using micronucleus (MN) assay as potential tool for assessment of genotoxicity.

## *Conclusion-*

MN assay has become a very popular tool for assessment of genotoxic potential of various chemical agents by using fish as a model. MN test is proved to be very authentic in the genotoxic analysis in natural water as well as in experimental condition. Genotoxic effect in fish is a matter of great concern due to their potential effects on human health after consumption. The detection of micro nucleur (MN) help up us to understand the condition of water quality as well as the health of particular species.

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