

# Sodium Fluoride (NaF) Affects Concentration of Nucleic Acid (DNA) in Fresh Water Fishes *Cirrhina mrigala* and *Labeo rohita*

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**Abstract:** Static bioassay for acute 96h and chronic 30 days were performed using sub-lethal 945 ppm ( $LC_0$ ) and lethal 960 ppm ( $LC_{50}$ ) concentration of Sodium Fluoride on the fresh water fishes *C. mrigala* and *L. rohita*. The behavior and mortality rate were recorded. After acute and chronic concentration of sodium fluoride exposed various fishes and collect the tissue like gill, muscle, liver, and kidney were subjected to biochemical (nucleic acid DNA and RNA) analysis. The result showed marked alternation in experimental fish due to toxicity of Sodium Fluoride compared with control group.

**Keywords:** Sodium fluoride, Biochemical, Nucleic acid DNA

## 1. Introduction

Inorganic Fluorides are introduced into the environment as a result of natural emission (eg. Volcanic activity) and anthropogenic sources. Depending on metrological condition and season, gaseous and particulate inorganic fluorides are transported in air and ultimately are deposited on land or open water bodies. Important anthropogenic sources of fluoride to the aquatic environment include municipal waste and effluents from fertilized producing plants and aluminum refineries. In water mobility and transport of inorganic fluoride are dependent on PH, water hardness, and the presence of ion exchange mineral. In water inorganic fluoride remain dissolved in solution under acidic condition, low hardness, and the presence on ion exchange material (Cuker and Shilts 1979; Sahu and Karim 1989.) As a consequence free fluoride level is generally low (Skjelkvale 1994, Radic and Barlic 1995).

Inorganic fluoride are toxic to aquatic organism and indirectly its impact occur on the human being because we are consume aquatic organism for reach protein resource. NaF is one of the toxicant may caused adverse biological effects on the all living organism such as change in nucleic acid concentration (DNA and RNA), carbohydrate, lipid, and protein metabolism, reproduction, impairment, reduce embryonic and development life stage, and alternation size and growth.

Sodium fluoride (NaF) is the most common inorganic fluoride used in aquatic toxicity studies reported by Sanders and Cope (1966). Toxicity studies with fluoride containing effluent include Woodwiss and Fertwell (1974), Damkaer and Dey (1989), Camargo (1991), Camargo and Tarazona (1991), Samal (1994). Reactions of fluoride have been examined in several studies on aquatic animal, chiefly on fishes. If fishes exposed to poisons amount of sodium fluoride (NaF) become apathetic, loss weight, violent movement, increases secretion and wander aimlessly (Neuhold and Singler 1960). Sodium fluoride (NaF) acts as poisons and interrupting metabolic process such as glycolysis,

lipid and synthesis of protein particularly fishes (Julio A. Camargo, 2003). Significant alternation in protein metabolism on acetylcholinesterase activities and oxygen consumption in fresh water crabs have been described by Reddy and Venugopal (1990) under fluoride toxication effect caused by exposure to inorganic fluoride has been observed in aquatic animals (Kalpana et al. 1964, Sigler and Newhold 1972, Mishra and Mohapatra 1998). Inorganic fluoride toxicity is negatively correlated to water hardness and positively correlated to temperature (Pimentel and Bulkley 1983). The initial phase of acute inorganic fluoride intoxication in fresh water species such as rainbow trout and carp is characterized by apathetic behavior accompanied by Neuhold and Sigler 1960 and Newhold 1972). In many cases, the surviving young fish had curved spines (Singler and Neuhold 1972).

The present studies was under taken to evaluate the toxic effect of sodium fluoride (NaF) on biochemical changes in nucleic acid concentration (DNA and RNA) on different tissue such as gill, liver, kidney and muscle of fresh water carp *C. mrigala* and *L. rohita*

## 2. Material and Method

### Nucleic acid

Nucleic acid is nitrogen containing compound of high molecular weight, found in association with protein in the cell. The nucleic acid -protein complexes are known as nucleo-protein and these can be separated in to components like protein and nucleic acid by chemical treatment. Two main groups of nucleic acids are known, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Hydrolysis of DNA and RNA under controlled condition yield nucleotides which can be regarded as basic unit of nucleic acid.

After acute and chronic exposure to fluoride, the alive fishes were sacrificed and the tissues (gill, liver kidney and muscle) were quickly pooled, weighed and used for estimation of DNA.

**i) Estimation of DNA by the diphenylamine reaction:**

- Prepared separate marked tubes containing 1ml, 2ml, and 3ml aliquots of the isolated DNA dissolved in standard saline and similar aliquots of 0.5 mg DNA/ml.
- Make all sample tube and a separate blank upto 10ml with D.W.
- Add 4ml of diphenylamine reagent to each tube and after mixing, heat the tubes in a boiling water bath.

Read the absorbance of blue solution at 595nm against blank.

**3. Result**

The nucleic acid plays a key role in protein synthesis. It is well known relationship between nucleic acid level and the rate of protein synthesis. Nucleic acid content is considered as an index capacity of an organism for protein synthesis. It is evident that very slight changes in physiological condition of the body it reflects in nucleic acid (DNA). Fishes are aquatic animals and very sensitive to fluctuation of aquatic environment. Levels of total DNA and RNA content in different organs of *Cirrhinus mrigala* and *Labeo rohita* after exposure to the sodium fluoride concentrations as compared to control given in tabled 1,2,3 and 4.

**Control of Acute Test:**

As compared to control, the DNA content in all the experimental organs decreases due to acute exposure to LC<sub>0</sub> and LC<sub>50</sub> concentrations of sodium fluoride. The present depletion was more significant (P< 0.05) kidney (12.82), gill (12.13), liver (11.11), muscle (6.38). In LC<sub>50</sub> DNA depletion was more significant than the LC<sub>0</sub> concentrations there was (P<0.05) kidney (23.07), gill (18.49), liver (16.66) and muscle (12.76).

**Table 1:** Level of total DNA content in different organs of *Cirrhinus mrigala* exposed to acute concentration of sodium fluoride

S. No.	Organs	Control mg/gm wet. Wt.	LC <sub>0</sub> at 96 h mg/gm wet. wt.	LC <sub>50</sub> at 96 h mg/gm wet. wt.
1.	Muscle	0.47 ± 0.03	0.44 ± 0.01	0.41 ± 0.02*
2.	Liver	0.18 ± 0.01	0.16 ± 0.03	0.15 ± 0.01*
3.	Gill	1.73 ± 0.06	1.52 ± 0.01	1.41 ± 0.03**
4.	Kidney	0.39 ± 0.03	0.34 ± 0.04	0.30 ± 0.01**

Value are means I SD of six replicates significant at p < 0.05, \* p < 0.01, \*\*\* p < 0.01.

**Control of Chronic Test:**

**Table 2:** Level of total DNA content in different organs of *Cirrhinus mrigala* exposed to chronic concentration of sodium fluoride

S.No.	Organs	Control mg/gm wet. Wt.	LC <sub>0</sub> at 30 days (48ppm) mg/gm wet. wt.	LC <sub>50</sub> at 30days (96ppm) mg/gm wet. wt.
1.	Muscle	0.49 ± 0.02	0.46 ± 0.03	0.42 ± 0.02*
2.	Liver	0.20 ± 0.01	0.17 ± 0.02	0.16 ± 0.04*
3.	Gill	1.79 ± 0.04	1.47 ± 0.04	1.38 ± 0.01**
4.	Kidney	0.43 ± 0.02	0.31 ± 0.01	0.29 ± 0.03 **

Value are means I SD of six replicates significant at p < 0.05, \* p < 0.01, \*\*\* p < 0.01.

As compared to control, the DNA content in all the organs decreases due to chronic exposure to LC<sub>0</sub> and LC<sub>50</sub> concentrations of sodium fluoride. The present depletion was more significant in LC<sub>0</sub> concentration was (P< 0.05) kidney (27.90), gill (17.87), liver (15), muscle (6.12). In LC<sub>50</sub> DNA depletion was more significant than the LC<sub>0</sub> there was (P<0.05) kidney (32.55), gill(22.90), liver (20) and muscle (14.28).

The DNA content in all issues, muscle, gill, liver, and kidney after exposure to fish LC<sub>0</sub> and LC<sub>50</sub> in sodium fluoride concentrations reduced significantly.

**Control of Acute Test**

As compared to control, the DNA content in all the organs decreases due to acute exposure to LC<sub>0</sub> and LC<sub>50</sub> concentrations of sodium fluoride. The present depletion was more significant (P< 0.05) kidney (15.78), gill (11.37), liver (11.11), and muscle (6.97). In LC<sub>50</sub> concentration of sodium fluoride exposed to experimental showed more significant depletion as compared LC<sub>0</sub> concentration there was (P<0.05) kidney (23.68), gill (17.36), liver (16.66) and (9.30).

**Table 3:** Level of total DNA content in different organs of *Labeo rohita* exposed to acute concentration of sodium fluoride.

S. No.	Organs	Control mg/gm wet. Wt.	LC <sub>0</sub> at 96 h mg/gm wet. wt.	LC <sub>50</sub> at 96 h mg/gm wet. wt.
1.	Muscle	0.43 ± 0.06	0.40 ± 0.01	0.39 ± 0.01*
2.	Liver	0.18 ± 0.01	0.16 ± 0.05	0.15 ± 0.03*
3.	Gill	1.67 ± 0.02	1.48 ± 0.01	1.38 ± 0.03**
4.	Kidney	0.38 ± 0.01	0.32 ± 0.04	0.29 ± 0.01**

Value are means I SD of six replicates significant at p < 0.05, \* p < 0.01, \*\*\* p < 0.01.

**Control of Chronic Test:**

**Table 4:** Level of total DNA content in different organs of *Labeo rohita* exposed to chronic concentration of sodium fluoride

S. No.	Organs	Control mg/gm wet. Wt.	LC <sub>0</sub> at 30 days (48ppm) mg/gm wet. wt.	LC <sub>50</sub> at 30days (96ppm) mg/gm wet. wt.
1.	Muscle	0.47 ± 0.04	0.44 ± 0.01	0.41 ± 0.03*
2.	Liver	0.19 ± 0.02	0.17 ± 0.02	0.16 ± 0.01*
3.	Gill	1.67 ± 0.04	1.34 ± 0.01	1.26 ± 0.02**
4.	Kidney	0.41 ± 0.02	0.31 ± 0.01	0.28 ± 0.03 **

Value are means I SD of six replicates significant at p < 0.05, \* p < 0.01, \*\*\* p < 0.01

As compared to control, the DNA content in all the organs decreases due to chronic exposure to LC<sub>0</sub> and LC<sub>50</sub> concentrations of sodium fluoride. The present depletion was more significant in LC<sub>0</sub> concentration was (P< 0.05) kidney (24.39), gill (19.76), liver (10.52), muscle (6.38). In LC<sub>50</sub> DNA depletion was more significant than the LC<sub>0</sub> there was (P<0.05) kidney (31.79), gill (24.55), liver (15.78) and muscle (12.76).

The DNA content in all issues, muscle, gill, liver, and kidney after exposure to fish LC<sub>0</sub> and LC<sub>50</sub> in sodium fluoride concentrations reduced significantly. The order of decreases of DNA in experimental fish is observed in the following

manner kidney > gill > liver > muscle showed in table- fig- .

#### 4. Discussion

In present investigation significant depletion was observed in the DNA concentration of all the experimental tissues such as muscle, gills, liver and kidney of experimental fish after the exposed in acute and chronic concentrations of sodium fluoride. The depletion of DNA occurs due to the alternation in pentose phosphate pathway and blocking of the metabolism of amino acid thereby preventing cells from synthesizing protein. In fact study has shown that sodium fluoride (NaF) inhibit protein synthesis and interferes with amino acid metabolism. Another possible reason may be depletion of protein for its utilization in conversion to glucose (Sirvastava N, Kaushik N, Gupta P. 2002).

Gilman, 1987; Elsair and Khelfat, 1988; Godfrey and Watson, 1988; Kaminsky et. al., (1990) studied the effect sodium fluoride on metabolic pathway associated with lipid, carbohydrate, bone and energy metabolism, single transduction pathway as well as influence a number and enzymatic activities then the result of sodium fluoride inhibit the synthesis a DNA and protein, inhibit cell proliferation, and is cytotoxic.

M.et.al, 1986, Patil and Bhunya, 1987 observed that sodium fluoride induced cytogenetic damage in bone marrow or sperm cells that is chromosomal aberration, micronuclei, alternation in sperm morphology to rodent by interventional injection. Klein et al., 1974 studied the effect fluoride on means spleen cells and absorbed that fluoride inhibited DNA repair. Chen J. et al., (2002) reported that the sodium fluoride could induce DNA damage and apoptosis in rats brain.

In present work DNA is reduced in all issue in all selected organs of experimental (LC<sub>0</sub> and LC<sub>50</sub>) at 96 hrs in exposure fish. It is indicate that Naf inhibited more enzymes that work in DNA was synthesis.

#### 5. Conclusion

The nucleic acid plays a key role in protein synthesis. There is a well known relationship between nucleic acid level and the rate of protein synthesis. Nucleic acid content is considered as an index for capacity of an organism for protein synthesis. The accumulation of fluoride changes physiological condition of the body and reflect on the structural alteration in DNA. Aquatic animals, especially fishes, are very sensitive to fluctuation of aquatic environment Cole et al., (1986) observed that fluoride has increase the frequency of mutation at the thymidine kinase locus in cultured mouse lymphoma and human lymphoblastoid cells. Crespi et al., (1990) studied that in human lymphoblastoid cells, the mutagenic response at the thymidine kinase locus after 20d exposure to sodium fluoride was no linear and lower than predicted from extrapolation of the 28h exposure, a result suggested that indicative of a threshold for the mutagenicity of sodium fluoride at concentration above 50 mg/liter. The pattern of induced chromosomal aberration, the increased formation of endoreduplicated cells, delay in cell cycle and increased sensitivity of cells in the G<sub>2</sub> phase due to sodium fluoride. It affect consistently with a mechanism of clastogenicity

involving an inhibition of DNA synthesis and repair, and it has been studied that the effect of sodium fluoride is upon the synthesis of protein involved in DNA synthesis and repair rather than involving direct interaction between fluoride and DNA by Aardema et al., (1989 a,b). Tsutsui et al., (1984a, 1984b, 1984c) observed that sodium fluoride increase unscheduled DNA synthesis in Syrian hamster embryo cells, human foreskin fibroblasts and human keratinocytes. The similar result was observed in present investigation that the levels of total DNA content in different organs of *Cirrhinus mrigala* and *Labeo rohita*, after exposure to the sodium fluoride concentrations as compared to control is presented in table. As compared to control, the DNA content in all the organs was found to decrease due to acute and chronic exposure to sodium fluoride may be irregularity in DNA synthesis. The present depletion was more significant in kidney than gill, liver and muscle. In general, the depletion was observed in DNA in all the selected tissues such as muscle, gills, liver and kidney of experimental fish after acute and chronic exposure. The depletion of DNA mostly occurs due to the alteration in pentose phosphate pathways Gilman, (1987); Elsair, and Khelfat, (1988); Godfrey and Watson, (1988). Kaminsky et al. (1990) studied the effect of sodium fluoride on metabolic pathway associated with lipid, carbohydrate, energy metabolism, single transduction pathway, as well as enzymatic activities and observed the inhibition of DNA and protein synthesis, that ultimately inhibit the cell proliferation. Patil and Bhunya, (1987) observed that sodium fluoride induced cytogenetic damage in the bone marrow and sperm cells due to chromosomal aberration, micronucleation, alteration in sperm morphology to rodent by interventional injection. Klein et al., (1974) studied the effect of fluoride on spleen cells and observed that fluoride inhibited DNA replication. Similar kind of DNA damage has been observed in apoptosis of rat brain by Chen et al, (2002). The loss is believed to be due to inhibition of enzymatic activity of those enzymes that work on DNA synthesis.

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