



## Changes in DNA content in various tissues of the fish *Cyprinus carpio communis* exposed to sublethal concentration of Machete

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**Abstract:** *Machete is commonly used as a pre-emergent herbicide in agriculture. Deoxyribonucleic acid gets affected and the content of DNA is reduced in the tissues of the fish exposed to sublethal concentration the herbicide, Machete. The changes in the DNA content in the tissues of kidney, brain, brain, gill, liver and muscle indicate the effect of toxicity when the fish is exposed to sublethal concentrations of the herbicide. The DNA is estimated by the methods of Searchy and MacLennis. The DNA content in various tissues of the fish, Cyprinus carpio communis exposed to pesticidal concentrations along with the control is the highest in kidney.*

**Key words:** D[ ] [ ] Herbicide, Machete[ ] [ ] Sublethal concentration, Cyprinus carpio communis

### INTRODUCTION

Machete is the commercial product of Butachlor introduced in India. It is commonly used as pre-emergent herbicide in transplanted rice. Since its launch in 1975, Machete has been the leading rise in application of the herbicide for farmers all over the country. It is essential for farmers to be able to maximize the benefits of using Machete to control weeds in rice fields, leading to increase yields and lower input costs. When the herbicides are persistent in the environment, they may cause problems of mass mortality of many non-target organisms, both terrestrial and aquatic organisms. The persistence of these pesticides in the environment due to the indiscriminate use of the pesticides and biomagnifications of the pesticides cause changes in nucleic acid contents.

Toxicity can be defined as the inherent capacity of a toxicant to effect adversely any biological activity of an organism. The best method for evaluating toxicity of a toxicant is the determination of Lethal Concentration (LC) or Lethal Dose (LD), which represents the amount of poison per unit weight killing 50% (LC50 and LD50) of the particular population of the experimental animals. One of nucleic acid contents known as Deoxyribonucleic acid (DNA) gets effected and the content is reduced in the tissues of the fish with the application sublethal concentration of the herbicide. The DNA content in the tissues of kidney, lungs, brain, gill and muscle show the effect and extent of toxicity when the fish is exposed to the herbicide, 50% emulsifiable concentrate Machete (EC).

### MATERIALS AND METHODS



### Estimation of Deoxyribonucleic Acid (DNA)

The DNA content is estimated by the methods of Searchy and MacLennis [1&2]. The selected and freshly dissected 100 mg of tissue was taken and homogenized in 5 ml of 0.5 N perchloric acid and the contents were boiled for 20 minutes at 90°C in a hot water bath and then cooled to room temperature. After cooling, the tissue homogenates were centrifuged. The supernatant obtained was separated to estimate both the DNA by the following procedures:

Some portion of the supernatant was taken into a clean test tube and 5 ml of Diphenylamine reagent (1g of Diphenylamine dissolved in 100 ml of glacial acetic acid) and 2.5 ml of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added. The contents were boiled for 10 minutes at 90°C in a hot water bath and then incubated at room temperature for 20 h. After 20 h, the optical density (OD) values were taken at 595 nm on spectrophotometer; DNA standard graph was prepared by using calf thymus as standard.

### RESULTS AND DISCUSSION

The DNA content in various tissues of *Cyprinus carpio communis* (Linnaeus) exposed to sublethal

concentration of Machete along with the control is the highest in kidney followed by gill, liver, brain and muscle (Table 1 and Graph1). The lyotropic gradation series in order given below:

Kidney > Gill > Liver > Brain > Muscle

Exposed to sublethal concentrations of Machete (50%), the DNA content was significantly decreased in all the tissues of the test fish, the lyotropic series in terms of decrement DNA content given below:

Kidney > Brain > Liver > Gill > Muscle

The DNA content in all the tissues of *Cyprinus carpio communis* (Linnaeus) showed very significant decrease over the control and the decrease was due to the toxicity of Machete.

Nucleic acids have an important role in all biological activities and also regulates the biological synthesis of proteins which are structural and functional. Any alteration in N acid content leads to variations of protein profile. In the present work, the decreased values were observed in all the tissues that were exposed to toxicants which also agree with the earlier reports [3,4&5].



**Table 1**

Changes in the amount of D.N.A.  $\mu\text{g/g}$  wet weight of different tissues of *Cyprinus carpio communis* (Linnaeus) exposed to sublethal concentration of Machete 50% E.C. (commercial product)

Tissue	Control	Machete
Gill	743.19	597.66
	$\pm 13.82$	$\pm 5.1$ (-19.58%)
Liver	681.18	508.64
	$\pm 9.17$	$\pm 4.43$ (-25.32%)
Kidney	990.48	590.19
	$\pm 9.45$	$\pm 6.94$ (-40.41%)
Brain	664.582	539.01
	$\pm 7.85$	$\pm 6.64$ (-18.89%)
Muscle	585.99	386.356
	$\pm 7.23$	$\pm 4.6116$ (-34.06%)

Values represent mean of five observations  
 + indicates the Standard Deviation  
 \* indicates values are significant at  $p < 0.05$   
 % change (+) indicates increased percentage  
 (-) indicates decreased percentage

Machete induced chromosomal breaking increased with an increase in concentration of the herbicide. Chromosome break is more generally considered to involve DNA molecule responsible for linear continuity of chromosome and may arise due to unfinished synthesis or mass repair of DNA [6]. In freshwater fish, Ateeq *et al.*, [7&8] have reported the micronuclei formation, DNA damage and apoptosis in the erythrocytes of catfish *Clarias butachlor* on three species of catfish, *Heteropneustes fossilis*, *Clarias batrachus* and *Channa punctatus*, silver carp *Hypophthalmichthys molitrix* and rice-field eel *Monopterus albus* [9].

A cytogenetic part of the present study had already shown that 2,4-D at the highest sub-lethal concentration (75 ppm) induced 9.64 mean% micronuclei, while butachlor (2.5 ppm) recorded 7.44 mean% micronuclei [7].

Butachlor is thought to inhibit the synthesis of long chain fatty acids [10]. The level of RNA in liver is higher than brain, gill, muscle and kidney. The RNA levels reflect the intensity to protein synthesis [11] and metabolic activity of tissue [12].

Pollutants alter the behavioural pattern, growth, reproductive potential and resistance to disease in aquatic organisms by altering the biochemical changes [13,14&15].

Chromosomal and mitotic aberrations induced by the three herbicides (Butachlor, Benthocarb and Fluchloralin) tested are similar to aberrations induced by other pesticides. Studies on pentachlorophenol, 2,4-D and Butachlor have already confirmed their genotoxic potential with regard to micronuclei induction and cytotoxic effects on three fish species, *Heteropneustes fossilis*, *Channa*



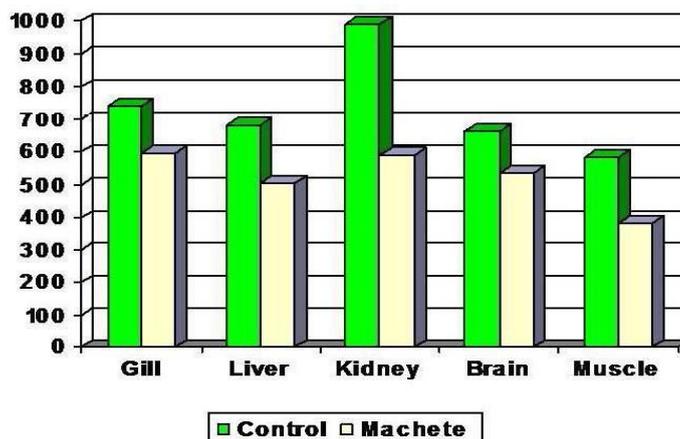
*punctatus*, and *C. batrachus*. Specifically, our earlier study on the genotoxicity of 2,4-D and Butachlor on *C. batrachus* emphasized that these two compounds induce micronuclei significantly [7].

A significant decrease in nucleic acids of gastro intestine tract was

reported. However, the decrease in nucleic acids content after diazinon treatment was not significant. The treatment of endosulfan in mucosa and submucosal tissues shows very little impact on nucleic acid content. However, in diazinon treatment the DNA was completely decreased.

**Graph 1**

Changes in the amount of D.N.A. mg/g wet weight of different tissues of *Cyprinus carpio communis* exposed to sublethal concentration of Machete (E.C)



## CONCLUSION

Toxicity of machete shows its effect on different tissues in the body of the fish, *Cyprinus carpio communis*. The Tissues of gills, liver, kidney, brain and muscle are affected and the DNA content in the tissues is decreased. The DNA content of kidney is much reduced due to the toxic effects of machete

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