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# A Study on Genotoxic Potential of Acephate in *Clarias batrachus*

**Subhashree Jagyanseni[1], Saswati Mishra[2] and Satya Narayan Sahoo[3]**

[1]Research Scholar, Department of Life Sciences, GIET University, Gunupur, Odisha, INDIA.

[2]Assistant Professor, Department of Bio-Tech/Life Sciences, GIET University, Gunupur, Odisha, INDIA.

[3]HOD Zoology, Niali College, Niali, Cuttack, Odisha, INDIA.

[1]Corresponding Author: subhashree1995mishra@gmail.com

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**ABSTRACT**

**Acephate is an insecticide made up of organophosphates. It is applied to food crops, citrus trees, on golf courses, in commercial or institutional buildings, and as a seed treatment. Products containing acephate can be purchased as tablets, liquids, granules, powders, and water-soluble packs. Acephate 75% brand name-Asataf insecticide manufactured by TATA RALLIS was used for the test. The solvent used was glass double distilled (g.d.d.) water. Fresh water catfish *Clarias batrachus* were collected from local water bodies of Cuttack district. All the fishes were acclimatized for fifteen days in laboratory aquaria containing 30L dechlorinated tap water prior to the initiation of the experiment. The peripheral blood smear slides were prepared from the blood collected by caudal incision in accordance with Al-Sabti (1986) and Das and Nanda (1986) with some modifications which were prepared animals were sacrificed after 24, 48 and 72 hours of Exposure and were used for each treatment group in both types of administrations (IP and dermal). The increased concentration of acephate directly affects our biological fish sample i.e. *Clarias batrachus.* Acephate is causing serious problems in fish as per our genotoxicity study of acephate on *Clarias batrachus. Clarias batrachus* is a commonly found fish species in fresh water habitat which includes ponds, ditches, wetlands and rice fields of India specially in Odisha.The irrational use of pesticides containing acephate in agriculture cause harmful effects on *Clarias batrachus*, which is a most important species of fish for maintaining the aquatic diversity.**

***Keywords-*** Acephate, *Clarias batrachus*, peripheral blood smear, IP, Dermal, genotoxic potential.

## I. INTRODUCTION

Acephate is an insecticide made up of organophosphates. It is applied to food crops, citrus trees, on golf courses, in commercial or institutional buildings, and as a seed treatment. Products containing acephate can be purchased as tablets, liquids, granules, powders, and water-soluble packs. Acephate is categorised by the EPA as a "possible human carcinogen." After receiving acephate as part of their diets for two years, more animals developed liver or adrenal gland tumours. Mice that were fed high doses of acephate all at once had DNA damage in blood cells, although the damage was repaired four days after the exposure.

Fish and amphibians are only minimally harmful to acephate. Salamander hatchlings exposed to high concentration of Acephate experienced decreased development, activity, and eating as well as an increase in muscle and spinal column abnormalities. Acute toxic effects of acephate on freshwater fish *Puntius sophore* are the subject of a 2016 study by Gavit and Patil [1].

*Channa punctata*'s haematological changes were covered by Satish et al. in 2018. due to acephate[2].

According to Shahi& Singh, (2014) pesticides decrease a number of haematological parameters in *Clarias batrachus* fish, including Hb, RBC, and WBC [3].

Several insecticides have been shown to have genotoxic potential on different freshwater fish by Campana et al. in 1999, Cavas et al. in 2003, and Ismail et al. in 2018 [4,5,6]. The genotoxic potential of acephate in *Clarias batrachus* was attempted to be shown in this paper.

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## II. MATERIALS AND METHODS

***Test Chemical:*** Acephate 75% brand name-Asataf insecticide manufactured by TATA RALLIS was used for the test. The solvent used was glass double distilled (g.d.d.) water.

***Dose:*** Three different doses 20, 35, and 50 mg/kg body weight were administered intraperitoneally (i.p.) to *Clarias batrachus*. In another set of experiment, another set of same fishes were exposed to 20, 35 and 50 ppm of acephate in laboratory aquaria. Live fish weighing (100 to 150 g) were collected from non contaminated ponds of domestic use. Prior to chemical treatment fishes were kept in the laboratory aquaria and acclimatized.

***Experimental Animal*** -

Fresh water catfish *Clarias batrachus* were collected from local water bodies of Cuttack district. All the fishes were acclimatized for fifteen days in laboratory aquaria containing 30L dechlorinated tap water prior to the initiation of the experiment. The aquarium water was aerated continuously and changed every day. The fishes were fed twice daily on commercial fish food. The feed remains, excretory waste and dead animals were removed from aquaria to avoid any stress and contamination. The water quality parameters were checked daily.

***Time:*** The blood smear slides were prepared after 24, 48 and 72 hours of Exposure.

***Experimental Protocol:*** In the present investigation four fish individuals were used for each treatment group in both types of administrations (ip, and dermal).

***Micronucleus test (MNT)***

Following the administration of insecticide, animals were sacrificed after 24, 48 and 72 hours. The peripheral blood smear slides were prepared from the blood collected by caudal incision in accordance with Al-Sabti (1986) and Das and Nanda (1986) with some modifications [7&8]. The details of the procedure is as follows:

1. Thin smears of peripheral blood obtained from caudal region were made on grease-free clean slide and allowed to dry in air
2. Slides were fixed in absolute methanol for 10-15 minutes and dried in air.
3. The slides were stained in 15-20% Giemsa solution at pH 7.0 for 1 to 1.30 hours.
4. The slides were gently washed in tap water and dried in air.

All slides were coded before screening. From each specimen 4000 erythrocytes (1000 per slide) were scored under oil immersion. The nonrefractile particles, which resembled nuclei in all respects, except size were considered to be micronuclei. The size of micronuclei varied from 1/5 to 1/28th of the main nucleus, in case of *Clarias batrachus.*

## III. RESULTS AND DISCUSSION

**Table 1: Incidence of micronucleated peripheral blood cells of fish (*C. batrachus* exposed to Acephate)**

| Dose (PPM) | Time (hr.) | No. of cells studied | Route | No. of micro-nucleus | No. of nuclear abnormalities | Route | No. of micro-nucleus | No. of nuclear abnormalities |
|---|---|---|---|---|---|---|---|---|
| Control | 24 | 16000 | Dermal | 2 | 1 | IP | 2 | 2 |
| | 48 | 16000 | Dermal | 3 | 2 | IP | 2 | 3 |
| | 72 | 16000 | Dermal | 3 | 2 | IP | 3 | 3 |
| 20 | 24 | 16000 | Dermal | 5 | 3 | IP | 8 | 3 |
| | 48 | 16000 | Dermal | 7 | 3 | IP | 9 | 4 |
| | 72 | 16000 | Dermal | 10 | 4 | IP | 11 | 5 |
| 35 | 24 | 16000 | Dermal | 8 | 3 | IP | 9 | 5 |
| | 48 | 16000 | Dermal | 9 | 4 | IP | 10 | 5 |
| | 72 | 16000 | Dermal | 10 | 5 | IP | 12 | 6 |
| 50 | 24 | 16000 | Dermal | 9 | 4 | IP | 9 | 8 |
| | 48 | 16000 | Dermal | 10 | 5 | IP | 12 | 8 |
| | 72 | 16000 | Dermal | 12 | 7 | IP | 13 | 9 |

In dermal exposure, the incidence of micronucleus and nuclear abnormalities of peripheral blood cells of fish (*C. batrachus* exposed to Acephate) was significantly increased from control to application of higher doses of Acephate in PPM (i.e. 20,35 and 50 PPM) and the time (24, 48 and 72 hour) of exposure was also played a significant role in rise in the number of micronucleus as well as number of nuclear abnormalities observed under the compound microscope. The intraperitoneal application of acephate resulted in the increase in both number of micronuclei found and the occurrence of more number of nuclear abnormalities

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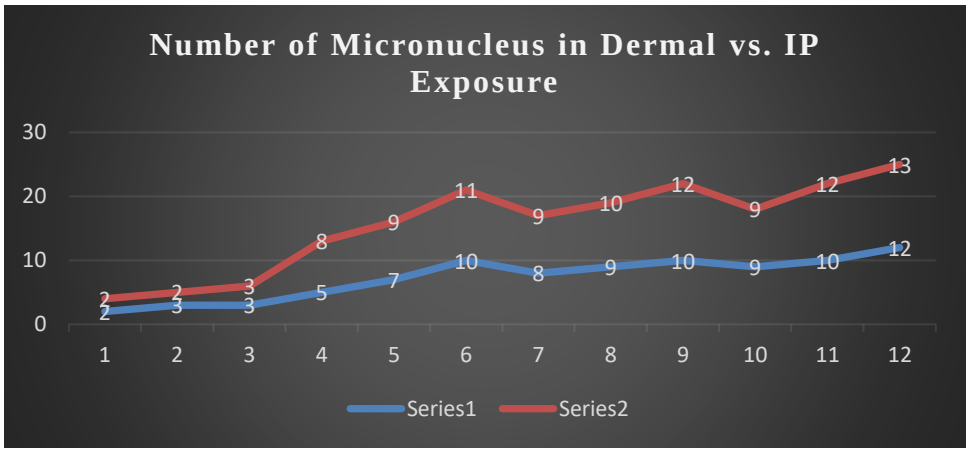
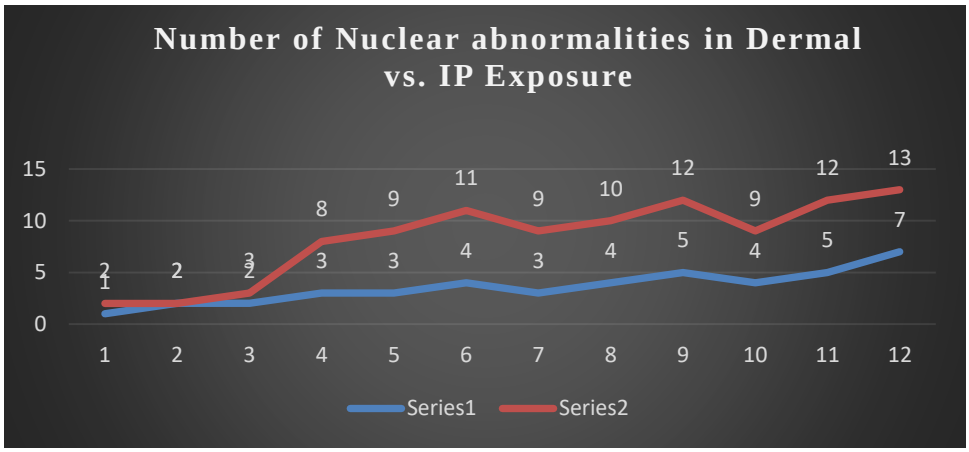
while applying higher PPM of acephate (i.e. 20,35 and 50PPM). The calculated t value for the data i.e. number of micronucleus in dermal versus intraperitoneal is 3.19 [$p<0.05$; $p<0.01$ (Student's t-test)] which shows the significant variation of number of micronucleus found in dermal exposure to that of number micronucleus found in intraperitoneal exposure. The calculated t value for the data i.e. number of nuclear abnormalities in dermal versus intraperitoneal is 4.93 [$p<0.05$; $p<0.01$; $p<0.001$ (Student's t-test)] which shows the significant variation of number of nuclear abnormalities during dermal exposure to that of number of nuclear abnormalities during intraperitoneal exposure to acephate.




**Graph 1: Number of Micronucleus in Dermal exposure versus Number of Micronucleus in Intraperitoneal (IP) exposure to acephate (Series1-Dermal, Series-2-Intraperitoneal/IP)**




**Graph 2: Number of nuclear abnormalities in Dermal (Series1) exposure versus Number of nuclear abnormalities in Intraperitoneal/IP(Series2) exposure to acephate**

**Fig 1: Fish *C. batrachus* before treatment of Acephate**

**Fig 2: Fish *C. batrachus* after intraperitoneal exposure to acephate**

**Fig 3: Fish *C. batrachus after* dermal exposure to acephate**

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## IV. CONCLUSION

The chemical compound i.e. Acephate that is present in the widely used insecticides nowadays has the severe toxic property on various aquatic animals specially fish. The increased concentration of acephate directly affects our biological fish sample i.e. *Clarias batrachus.* Acephate is causing serious problems in fish as per our genotoxicity study of acephate on *Clarias batrachus. Clarias batrachus* is a commonly found fish species in fresh water habitat which includes ponds, ditches, wetlands and rice fields of India specially in Odisha.The irrational use of pesticides containing acephate in agriculture cause harmful effects on *Clarias batrachus*, which is a most important species of fish for maintaining the aquatic diversity.

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