# Genetic DNA Damage in Agricultural Workers Exposed to Pesticides in the Dabaliapara area of Barpeta District Assam, India

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## ABSTRACT

**Introduction:** In agricultural field pesticides are used to protect the crops but they poses a potential hazard to the farmer and the environment. In the present investigation occupational exposure of different types of pesticides and DNA damage of individual spraying the pesticides in the agricultural field.

**Material and methods:** Blood samples of 30 exposed workers (after daylong spraying) and 30 control workers belong to Dabaliapara area, of Barpeta districts of Assam were evaluated using Comet Assay.

**Result:** Significant differences were found in DNA damage between freshly exposed workers and controls workers. In the exposed individual comet parameters are increased significantly viz. tail length of the comet increased and cell migration of exposed workers increased as compared to control group (16.50 ± 4.32 vs. 7.68 ± 5.55). Confounding factors during pesticides exposure such as age, smoking habits, alcoholic drinking and dietary habit were expected to modulate the damage.

**Conclusion:** The evidence of genetic hazard related to exposure of pesticides which results a serious threat to the environment as well as to the human health, is a matter of concern which needs educational awareness to reduce the toxic effects of the pesticides.


**Keywords:** Agricultural workers, Comet assay, DNA damage, Genotoxicity, Pesticide

## INTRODUCTION

The pesticides has been using rapidly in the recent years. These pesticide are released in the environment daily as a large scale and many of them have drastic effect on non-target species as well as represent a potential hazard to the human health.

The pesticides induced to breaking DNA[1] and thus affect the DNA replication and its ability to carry information.[2] DNA damage together with cellular response can establish instability through multiple pathways[3] and can be consider as strategy for risk assessment and it is a reliable biomarker. Molecular epidemiological study showed that pesticides exposed farmers are at the risk of tumour like leukemia[4], non Hodgkin's lymphoma[5], soft tissue sarcoma.[6]

Single cell gel electrophoresis (SCGE) or comet assay has been used increasingly in human biomonitoring studies. The assay is a rapid sensitive tool to demonstrate damazing effect of different compound on DNA at the exposed individual body cells. In the exposed cells with damaged DNA fragments increases level of migration from nucleus, generating a comet shape[7] Despite the fact that Comet assay is a technique used in epidemiological health study by molecular epidemiologist, yet a few number of investigators applied this technique to evaluate genotoxic effect of pesticides in human population.[8] The present study reports the relation between pesticides and DNA damage of individual spraying the pesticides in the agricultural field of Dabaliapara area of Barpeta district.

## MATERIAL AND METHODS

The study involved 60 subjects – 30 sample from exposed and 30 sample from control groups in Dabaliapara area of Barpeta district. The control groups were selected from the general population with no history of occupational exposure to pesticides or any environmental agents. All the subjects were asked to complete a face to face questionnaires which included standard demographic data (such as age, gender etc.) as well as medical occupational questionnaires (Hours per day working, year of exposure, use of protective measures, environment at the time of spraying, wind direction, temperature etc.). They have to sign a written consent form before taking blood sample.

### Study design

The alkaline single cell gel electrophoresis assay was carried out according to technique given by Singh et al (1988)[9] with slight modification of original technique. To avoid possible bias blood samples were coded. For study DNA damage, blood samples were collected from exposed and controlled groups. A total of 60 blood sample were taken from veins of the objects into a EDTA vial, put on ice and brought to the laboratory for the Comet assay.

Slides were prepared in duplicate in per person. Fully frosted microscopic slides were converted with 140µl of 0.75% normal melting point agarose (40º-42º C).After application of a cover slip slides were allowed to gel at 4ºC for 10 minutes. A sample of 20µl of whole blood was mixed to 0.5% of 110µl of low melting point agarose (37ºC). After carefully removing the cover slips and a second layer of 110µl of sample mixtures was pipette onto the pre-coated slides and allowed to solidify at 4º C for 10 minutes, with the coverslip in place. Again the coverslip was removed and a third layer of 110µl of low melting point agarose (LMPA) was pipette onto the slides and allowed to gel at 4ºC for 10

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mints. From the slides, cover slips were removed and were dipped in freshly prepared lysing solution (2.5 M NaCl, 100 mM $Na_2EDTA$, 10 mM Tris-HCL, pH10, 1% of sodium N-lauroyl sarcosinate, 1of % Triton X-100 and 10% of DMSO (Dimethyl Sulfoxide) and refrigerated whole night. After that the slides were transferred in the prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 13) for about 20 minutes. Electrophoresis was conducted for 30 minutes at 24 V and adjusted the current to 300 mA by increasing or decreasing the level in the electrophoresis tank.

To remove the excess buffer, the slides were drained and kept on a tray and washed for 3 times for 5 minutes duration each of neutralization buffer (0.4 M Tris HCL, pH 7.5). Next for 10 minutes slides were dehydrated with absolute methanol and kept at room temperature to dry. To minimize the artefactual DNA damage the procedure was carried out in the dim light. Slides were stained with Silver staining method. All the slides were scored by one person to avoid the inter scorer variability. The slides were examined at 100 X magnification using 10 X eye piece and 10 X objectives. A total of 50 individual cells were screened per subjects (25 cells from each slide). In the undamaged cells, the nucleus was intake without tail and in damaged cells there is a comet like structure showing damaged DNA fragment with tail of the comet. The damaged cells have the comet appearance. The comet tail length denoted the length of the DNA migration. The damage of DNA can be estimated measuring the length of DNA migration which appeared as comet tail length. The DNA migration and DNA damage of each cells was measured using the specific software CASP 1:1.

## STATISTICAL ANALYSIS

Mean and standard deviation (Mean) were measured for each of the studied parameter. The DNA damage were measured from Comet Assay. The differences of DNA damage were measured using t-test. Multifactor analysis of variance (ANOVA) were used to check the significance difference. P-value of 5% was measured in this analysis. All the analysis were performed by software package SPSS 10.0 version.

## RESULTS

The effect of occupational exposure to pesticides on the levels of DNA damage in leukocytes of pesticides used workers and control subjects were assessed by the Comet assay. Table 1 represents the distribution of subjects with respect to sex, age, smoking and exposure in years. The distribution of gender and smoking habit. was similar (Table 1 and 2). The mean comet tail lengths in leukocytes of exposed workers and the control subjects using the Comet assay are summarized in Table 1 and Table 2.

The exposed workers had a significantly greater mean DNA tail length in comparison to the control groups (17.69±5.25 vs 10.72±7.89) where p value is 0.0034. Among the smokers the exposed workers had significantly greater tail length than control groups. There is a slight difference between the workers of less than 10 years of exposure and more than 10 years of exposure (15.50±3.12versus 17.68±5.48,

| Parameter | No. of subjects N=30 | Comet tail length (Mean±SD) (µm) | t and p value |
|---|---|---|---|
| Smoking:<br>Smokers<br>Non –smokers | <br>23<br>7 | <br>5.60 ±1.71<br>5.20±0.70 | <br>t= 4.1773<br>p= 0.0006 |
| Year of exposure:<br><10<br>≥10 | <br>9<br>21 | <br>5.18 ±1.40<br>6.24±1.71 | <br>t= 1.9368<br>p= 0.0706 |
| Age:<br><35<br>≥35 | <br>14<br>16 | <br>6.24±1.27<br>4.87 ±1.50 | <br>t= 0.4177<br>p= 0.6835 |
| Gender:<br>Male<br>Female | <br>30<br>0 | <br>5.51±1.52<br>0 | <br>t= 13.0684<br>p= 0.0001 |

**Table-1:** Mean comet tail length in pesticides workers (Exposed) according to smoking, exposure, age and gender.

| Parameters | No. of subjects N=30 | Comet tail length (Mean±SD) (µm) | t and p value |
|---|---|---|---|
| Smoking<br>Smokers<br>Non -smokers | <br>20<br>10 | <br>12.95±10.53<br>12.11 ±4.92 | <br>t= 1.0420<br>p= 0.3376 |
| Age<br><35<br>≥ 35 | <br>0<br>30 | <br>0<br>12.38± 6.07 | <br>t= 4.9937<br>p= 0.0005 |
| Gender<br>Male<br>Female | <br>30<br>0 | <br>12.38±6.07<br>0 | <br>t= 4.9937<br>p=0.0005 |

**Table-2:** Mean comet tail length in controlled persons according to smoking, age and gender

p=0.142417) in (table 3). Age has showed significant effects on DNA in (Table 3). The results of ANCOVA when age was included as a covariate are summarized in table 3. The effects of occupational pesticides exposure on DNA damage were quite significant (P<0.05).

## DISCUSSION

The investigation was conducted to evaluate genetic damage in the workers employed in pesticides spraying operation in the agricultural field, utilizing the Comet assay. The result shows that occupational exposure of mixture of pesticides induces an increase in the level of DNA damage exposed to mixture of pesticides in the area of Dabaliapara of Assam.

In the Dabaliapara area of Barpeta district DNA damage of 30 nos. of pesticides exposed workers compared with the 30 nos. of workers who are not using the pesticides and are taken as control. The investigation genotoxic of potential of workers handling pesticides using comet assay are few. The comet assay was used to quantify the level of DNA damage in mononuclear leucocytes of blood sample of pesticides exposed workers in agricultural field. The farmers of Franch who were occupationally exposed to combined action of pesticides, showed significantly high amount of genetic damage.[10] In another study by the same authors, the comet

| Parameters | No.of Subjects (%) | Frequency of cells showing migration Mean±SD | Anova P=value | Mean tail length (Mean±SD | Anova P=value |
|---|---|---|---|---|---|
| Duration (Years)<br>≤10<br>>10 | <br>16(53.33%)<br>14(46.66%) | <br>15.50±3.12<br>17.68±5.48 | <br>F=0.02238<br>P=0.883561<br>Not Significant at <0.05 | <br>5.00±1.53<br>5.94±1.47 | <br>F=2.46461<br>P=0.142417<br>Significant at <0.05 |
| Age(Years)<br>≤30<br>>30 | <br>12(40%)<br>18(60%) | <br>14.12±3.69<br>18.00±4.20 | <br>F=8.54228<br>P=0.11131<br>Significant at <0.05 | <br>6.21±1.41<br>5.07±1.49 | <br>F=0.81996<br>P=0.380513<br>Not Significant at <0.05 |
| Smokers<br>Non-smokers | 23(76.66%)<br>7(23.33%) | 18.65±3.22<br>11.04±1`02 | F=49.00247<br>P<0.00001<br>Significant at <0.05 | 5.60±1.71<br><br>5.20±0.70 | F=17.44996<br>P=0.000566<br>Significant at <0.05 |
| Alcoholics<br>Non- alcoholics | 4(13.33%)<br>26(86.66%) | 15.16±4.67<br>5.40±1.59 | F=36.11518<br>P<0.00001<br>Significant at <0.05 | 6.10±1.26<br>5.40±1.59 | F=23.05498<br>P=0.000109<br>Significant at <0.05 |
| Vegetarian<br>Non-vegetarian | 9(30%)<br>21(70%) | 19.69±4.49<br>4.95±2.30 | F=2.81237<br>P=0.112965<br>Not significant at <0.05 | 15.09±3.62<br>5.9±1.17 | F=2.66839<br>P=0.133408<br>Not significant at <0.05 |
| With protective measure<br>Without protective measure | <br>7(23.33%)<br>23(76.66%) | <br>15.94±3.56<br>16.67±4.68 | <br>F=0.22786<br>P=0.638863<br>Not Significant at <0.05 | <br>5.47±1.41<br>5.52±1.62 | <br>F=8.293<br>P=0.012114<br>Significant at <0.05 |

**Table-3:** Comet parameters in 30 freshly exposed cases showing DNA damage in relation to duration of exposure, smoking, drinking and dietary habits.

assay was used to assess DNA damage in the farmers. The result showed increase in genetic DNA damage level after one day spraying with pesticides mixture.[11] There are few studies in which no significant increase in DNA damage in exposed workers in comparison to control was found, which could be due to the difference in work condition like use of varied quality of protective equipments and variable duration of exposure etc. In another study it was found that lack of protective measure taken by the workers increased the amount of DNA damage or genotoxic damage. Since DNA damage is an important step in the events of exposure of carcinogenic pesticides leading to cancer. Application of comet assay in the peripheral lymphocytes is a risk assessment process in the monitoring of the potential of genotoxic effect may be due to commulative effect of all or some of the pesticides, it is not possible to attribute damage to any particular agent. The result of such type of study on the subjects occupationally exposed to pesticides using a variety of genotoxic assays, suggest that the mixture of pesticides in long term occupational exposure effect on the DNA of somatic cells. The detected DNA damage due to cytotoxic and/or genotoxic effects. The genetic damage demonstrated in the current study and evaluated as an increase in comet tail length could possibly originate from DNA single –strand breaks, repair of DNA double strand breaks, DNA adduct formation or DNA-DNA and DNA-protein crosslinks. No significant increase in DNA damage with some factors like age, smoking, drinking dietary habit were also analyze in our result. Similarly there were no significant relationship between the DNA damage was found in the workers and smoking habit, drinking habit, similar observation were reported by some other workers.

It can be concluded that pesticides did cause DNA damage long with duration of exposure. The confounding factor including age, smoking, diet were expected to modulate the genotoxic effect of xenobiotic, but not significantly in the absence of any positive correlation between these factors, the comet parameter suggested that the DNA damage probably caused by carcinogenic pesticides which were spraying in the agricultural field by the farmers of Barpeta District Assam.

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