Pesticides have long been in use in the agricultural fields, plantation farms, and also for household purposes. However, there are a considerable number of studies which prove the detrimental effects of the pesticides that include biochemical, histopathological, and genetic effects. The aim of this article is to present a review on the effects of pesticides on leukocytes which have been analyzed through various assays including chromosome analysis, cytokinesis-block micronuclei assay, comet assay, semen, and sperm analysis. The studies have shown organophosphates and carbamates to be the most potential sources of genotoxicity and the individuals exposed to these groups of pesticides are relatively more prone to genotoxicity. Further investigation on molecular mechanism by which the pesticides affect the genome of cells needs to be carried out.

**Keywords:** Pesticides, Genotoxicity, Chromosome analysis, Cytokinesis-block micronucleus cytome assay, Semen analysis.

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**INTRODUCTION**

Pesticides, herbicides, fungicides, and insecticides have been long in use in agricultural practices as they are effective and quick [1]. However, the detrimental effects of these chemicals on human health are prominent as seen on the immediate workers who get extensively exposed. In general, the agricultural workers of the developing countries are more exposed prolonged because these countries rely on manual spraying. There are also long-term effects of oral ingestion along with the dermal exposure or respiratory inhalation. Studies reveal that the chemical pesticides are mostly responsible for causing mutagenic and carcinogenic effects. The broad classes of pesticides and insecticides used mostly include organophosphate, carbamate, pyrethroid, organochloride, and sulfonylurea. With the increase in research conducted on these chemicals, there are a host of pesticides that are getting banned in various countries. This article is a review on the genotoxic effects of all such classes of pesticides and their formulations derivatives on human peripheral leukocytes. There are a host of assays and experiments done to analyze the cytotoxic and genotoxic aspects of chemicals on the human lymphocytes which include chromosomal aberration frequency analysis, sister chromatid exchange, cell cycle kinetics, comet assay, and micronuclei assay [2].

**Organophosphates**

These are the most commonly used group of pesticides and have been broadly studied for genotoxicity [3]. There were studies done with a number of organophosphates which comprised azodrin, diazinon, dichlorodien, dimehotox, durban, ethion, fenthion, malathion, methylparathion, paraenthion, phosdrin, R-1303, and viozene [2]. Cell line proliferation, sister chromatid exchange, metabolic activation assays were performed using different concentration range of the chemical which was 0.02, 0.2, 2, and 20 μg/μl. Low doses did not have significant effects on the cell proliferative rate. The cultures treated with 2 μg/μl showed prominent inhibition of cell proliferation ranging from 31% of growth in cultures treated with azodrin to 67% of growth in cultures treated with diazinon. The inhibition increased further with an increase in concentration to 20 μg/μl which showed 11% of growth for azodrin treated cultures, having the lowest growth and 53% of growth in cells treated with malathion having the highest. These data were supported strongly by the mitotic index calculation done on the cultures. Azodrin (at high concentrations of 2 and 20 μg/μl) showed the most pronounced inhibitory effect on mitosis with 2±11 cells in mitotic stage out of 100 cells scored against 20±4 in the control condition. Phosdrin had also shown a similarly significant effect at 0.2 μg/μl with 5±1. Sister chromatid exchange analysis showed marked increase in its frequency for malathion, parathion, and R-1303 treated cells for 0.2 μg/μl concentration whereas for 2 and 20 μg/μl dose treated cells all treatments showed elevation excepting ethion, fenthion, and diazinon.

Acephate, a widely used pesticide in India, has lethal concentration 50 (LC50) found to be 45 μm as determined by trypan blue dye exclusion method. Peripheral human leukocytes [3] treatments with sub-LCs of these pesticides have shown to induce satellite associations, chromatid breaks, and chromatid gaps as the frequent types of chromosomal aberrations. Comet assay performed supported these results and revealed an elevated number of DNA single strand breaks in the cells treated with the same sub-LCs. The results fall into the statistically significant area carried out by t-test with p<0.05. Cell viability was seen to be decreasing with increasing dose of the pesticide indicating dose-dependent genotoxicity as it showed 100% mortality at 70 μm concentration of the compound. Chromosomal aberration analysis showed 8% increase in satellite association, 7% increase in chromatid breaks, and 9% increase in chromatid gap against the normal controls. The comet assay results showed an increase in the tail length from 0.1 to 0.95 μm with the increase in acpeth concentration level from 0 to 7 μm.

Chromosomal aberration, sister chromatid exchange, mitotic index analysis, and glucose-6-phosphate dehydrogenase enzyme activity were analyzed for four widely used organophosphate pesticides which are glyphosate, vinclozolin, atrazine, and DPX-E9636 [4]. Each of the compounds showed dose-dependent increase in cytotoxicity as suggested by the increase in the percentage of aberrant cell and sister chromatid exchange. Furthermore, there was a significant reduction in mitotic index and cell proliferative index at the highest concentration level of the four compounds. Profenos another extensively used organophosphate has LC50 as 3.5 μm and has shown increase in satellite association (12-14%), chromatid breaks (8-15%), and chromatid gaps (4-9%) with increase in concentrations [5].

**Organochlorines**

Dicamba is an organochloride, a methoxybenzoic acid which is commercially used in various formulations such as Oracle, Vanquis, and Benvel. Dicamba and its derivative Banvel have been tested on human leukocyte cells from a dose range of 10-500 μg/μ1[6]. At 200 and
Novozir Mn80 (fungicide 2,4,5-T, 2,4-D 10-25
Pesticide mixture of Organophosphates
Sister chromatid
Chromosomal
3-24
rate index at those respective doses. Aldicarb and cytophosphane have
and corresponding decrease in cell cycle progression and proliferative
fungicide, there were significant increase in sister chromatid exchange
and cell cycle progression assays was conducted on human
Analysis of the frequency of chromosomal aberration, sister chromatid
have been banned in the United States [10].
administered orally given as a single dose. Thyroid iodine uptake was
blood cultures of workers exposed to the compound. In a consented
pesticide have shown frequent chromosomal aberration in peripheral
Amitrole or aminotriazole, belonging to the carbamate category of
Pyrethroid
These classes of insecticides comprise mainly of the household
insecticides, and they are synthetic version of the compound present
in the extract of chrysanthemum flowers. Cypermethrin, one of the
most commonly used pyrethroid have not been categorized as highly
dangerous as suggested by some of the studies where they have not
been seen to induce chromosomal aberrations and sister chromatid
exchange [15,16]. Fenvalerate has been seen to increase both. In
addition, both of them have been found to reduce cell cycle kinetics
by decreasing cell proliferative index and cell cycle progression at the
concentration level of 10 µg/ml.

A comparative study was performed using five pyrethroid pesticides,
namely, cypermethrin, deltamethrin, fenpropathrin, fenvalerate,
and permethrin, and they were assessed on the basis of micronuclei

Table 1: Data on workers working in chemical plants are compiled

| Chemical plant workers-exposed population/control population | Exposure to the pesticide | Duration of exposure (in years) | Cytogenetic biomarker assessed | Result |
|-------------------------------------------------------------|---------------------------|--------------------------------|-------------------------------|--------|
| 45/31 [24]                                                  | Novozir Mn80 (fungicide containing mancozeb) | Up to 2                         | Chromosomal aberration sister chromatin exchange | Positive (+1.82) |
| 15/50,10 formulators, five packers [25]                     | Azynphos methyl, methyl parathion, malathion, dimethoate 2,4,5-T2,4-D | Not available                   | Sister chromatin exchange      | Positive (+1.22) |
| 19/36 [26]                                                  | Pesticide mixture of cyanazine, malathion, 2,4-D, alachlor, atrazine Organophosphates | 10-25                           | Chromosomal aberration         | Positive (+2.06) |
| 20/20 [27]                                                  | Pesticide mixture of cyanazine, malathion, 2,4-D, alachlor, atrazine Organophosphates | 6-30 (sample collected 8 months after subjects were exposed) | Chromosomal aberration         | Positive (+6.11) |
| 135/111 [28]                                                | Sister chromatid exchange | 3-24                            | Sister chromatid exchange      | Positive (+1.86 smokers) +1.70 non-smokers |
assays on whole blood as well as human leukocytes [18]. All of the five pesticides showed dose-dependent cytotoxicity with fenpropatrin proving to be the most cytotoxic of them all. In this study, nuclear division index and cytokinesis-block proliferation index were determined which supported the results yielded by the micronuclei assays and also included the trinucleated and tetranucleated cells. Out of all, cypermethrin and fenpropathrin showed the maximum number of micronuclei formation in the whole blood cells, and deltamethrin showed an elevation in the same in isolated human leukocytes. Trypan blue dye exclusion assay conducted to check for cell viability showed very low exclusion of micronuclei. These results were then compared with the control results of the study conducted by Mitra and Abilash (2016) which showed not available.

There were studies examining 12 workers exposed to fenvalerate in situ hybridization technique was performed on the sperm samples provided by the volunteers, the results of which revealed significant increase in aneuploidy in the exposed workers and also a rise in the number of sex chromosome trisomies found to be 0.742±0.131% against the control group that showed 0.386±0.140%. The frequency of chromosomes 18 trisomies was higher 0.326±0.069% against the control reading which was 0.124±0.068%.

### COMPARATIVE ANALYSIS

A comparative study on organophosphates such as dimethoate, chlorpyrifos, monocrotophos, and organochlorine like endosulfan was carried out in one of the studies which revealed endosulfan and monocrotophos to be highly cytotoxic whereas dimethoate is least toxic comparatively [20]. Monocrotophos being an acetylcholinesterase inhibitor has lethal dose 50 value of 14 mg/kg body weight for oral ingestion and 112 mg/kg body weight for dermal contact in mammals. Endosulfan is a benzodioxathiepin is a proved endocrine toxic and also causes deformation in male and female genitalia. Dimethoate is an acetylcholinesterase which has a rapid metabolic half-life, and 76-100% is excreted within 24 hrs of ingestion. Trypan blue dye exclusion assay conducted to check for cell viability showed very low exclusion of micronuclei. These results were then compared with the control results of the study conducted by Mitra and Abilash (2016) which showed not available.

### Table 2: Data on workers involved in pesticide spraying and exposed to single pesticide are compiled

| Pesticide sprayers-exposed population/control population | Exposure to the pesticide | Duration of exposure (in years) | Cytogenetic biomarker assessed | Result |
|--------------------------------------------------------|---------------------------|---------------------------------|--------------------------------|--------|
| 36/15 [29]                                             | Workers in forestry: 2,4-D,MCPA | Not available                   | Sister chromatid exchange     | Negative |
| 61/42 [30]                                             | Workers in papaya plantations exposed to ethylene dibromide | 5                               | Chromosomal aberration        | Negative |
| 20/17 [31]                                             | Fumigators (in open field): Phosphine, other pesticides | 6-28 days                       | Chromosomal aberration        | Negative |
| 25/25 [32]                                             | Fumigators (in open field): Phosphine | Discontinuous use in 8 months   | Chromosomal aberration        | Positive (+3.60) |
| 20/26 [33]                                             | Fumigators: Phosphine | Not available                   | Micronuclei                    | Negative |
| 35/21 [34]                                             | Fumigators: Phosphine | 2-months                        | Chromosomal aberration        | Negative |
| 38/16 [35]                                             | Sprayers of 2,4-D | Discontinuous in exposure       | Micronuclei                    | Negative |
| 31/31 [36]                                             | Fungicide sprayers spraying (dithiocarbamate) ethylenebis malathion | Not available                   | Chromosomal aberration        | Positive (+1.33) |
| 13/30 [36]                                             | Farmers growing tomatoes | 3 months                       | Chromosomal aberration        | Negative |
| 31/30 [36]                                             | Sprayers of fungicides | 0.3-22                          | Micronuclei                    | Negative |
| 32/27 [37]                                             | Fumigators applying methylbromide | Discontinuous use in exposure   | Micronuclei                    | Negative |
| 12/10 [38]                                             | Sprayers of 2,4-D | Not available                   | Micronuclei                    | Negative |

MCPA: 2-methyl-4-chlorophenoxyacetic acid

### Table 3: Data on workers involved in pesticide spraying and exposed to a mixture of pesticide are compiled

| Pesticide sprayers-exposed population/control population | Exposure to the pesticide | Duration of exposure (in years) | Cytogenetic biomarker assessed | Result |
|--------------------------------------------------------|---------------------------|---------------------------------|--------------------------------|--------|
| 80/25 [39]                                             | Mixture of the following pesticides: Carbamates, organochlorines, heterocyclic compounds, dithiocarbamates, phenoxy-acetic acids, nitro-compounds, pyrethroids, phthalimides, copper and sulphur containing chemicals | 1 to <15                        | Chromosomal aberration        | Positive (+2.67 to+3.91) |
| 15/15 [40]                                             | Workers in vineyards: DDT, copper sulfate, dieldrin, metasystox dithane, lindane, quinalfos dichlorvos | 6-12                            | Chromosomal aberration        | Positive (+2.72 to+3.92) |
| 55/61 [41]                                             | Workers of greenhouses: Insecticides (organophosphates, carbamates,); fungicides, acaricides, pyrethroid | 3-15                            | Chromosomal aberration        | Positive (+1.19 to 1.55) |
| 27/30 [30]                                             | Workers of vegetable garden: BHC, parathion, urea, dime thoate, malathion, gormor, fenithrothion, DDT | 5-40                            | Chromosomal aberration        | Positive (+1.73-2.04) |

BHC: Benzene hexachloride, DDT: Dichlorip diphenyl-trichloroethane
Table 4: Data on agricultural workers involved in agriculture works are compiled

| Pesticide sprayers-exposed population/control population | Exposure to the pesticide | Duration of exposure (in years) | Cytogenetic biomarker assessed | Result |
|--------------------------------------------------------|--------------------------|--------------------------------|-------------------------------|--------|
| 10/9 [42]                                              | 2,4-D, MCPP, MCPP, diquat, dithiocarbamates | 2-30                           | Chromosomal aberration         | Negative |
| 94/77 [43]                                             | Carbamates, ureas, triazines, organophosphates, organochlorines, thioacarbamates | 1-35                           | Sister chromatid exchange      | Negative |
| 71/30 [44]                                             | Benzimidazoles, organochlorines, pyrethroids, carbamates, nitroorganics, dithiocarbamates, phthalimides, morpholines, organophosphates, thioacarbamates | 2-50                           | Micronuclei                    | Negative |
| 10/20 [45]                                             | Carbamates, organophosphates dithiocarbamates | 6                               | Chromosomal aberration         | Negative |
| 18/20 [46]                                             | Captan, diazinon, endosulfan, malathion, carbofuran | 2-25                           | Micronuclei                    | Negative |
| 20/20 [47]                                             | Chlorpyrifos, terbufos, fenamiphos, thialbenzole imalizatable, gramoxone, dibromochloropropene | Not available                   | Chromosomal aberration         | Positive (+1.32) |
| 23/25 [48]                                             | Organophosphates, carbamates | 0-16                           | Chromosomal aberration         | Positive (+3.26) |
| 20/15 [49]                                             | Agrimycin, curzate, benlate, cercozin, folicur, dacostar, lannate, endosulfan, manzate, recop, microsheld, outhene, nivacon, pyrimicin, sencor, roundup | 10-45                          | Chromosomal aberration         | Negative |

MCPP: 2-methyl-4-chlorophenoxyacetic acid

Comet assay evaluating DNA single strand breaks which showed longest tail length for monocrotrophos 2.18±0.75 µm followed by chlorpyriphos with 1.89±0.63 µm whereas dimethoate produced 1.76±0.54 µm length tail on the addition of 10 times higher concentrations.

There have been studies made by comparing different chemical classes of pesticides, dimethoate, and methyl parathion belonging to the organophosphates, propoxur, and pirimicarb from carbamates, and cypermethrin and permethrin from pyrethroids [21]. These chemicals were added in the concentration gradient of 10, 50, 100, and 200 µg/ml. Comet assay performed in the experiments showed that there was a significant increase in tail length with the increase in concentration. Dimethoate showed the highest values at 100 and 200 µg/ml while methyl parathion showed long tails at 10, 50, and 200 µg/ml and its tail intensity was maximum at 100 and 200 µg/ml. Propoxur also had shown increased tail length and intensity at 50, 100, and 200 µg/ml. Pirimicarb showed the maximum tail length at 10 and 200 µg/ml while permethrin and cypermethrin showed an increase at 50 and 200 µg/ml. In these studies, however, discrepancies were indicated as the tail length seemed to be independent of the compound and dependent on the concentration whereas for cypermethrin and permethrin this increase was rather dose dependent.

A comparative study comprising of five pyrethroids showed weak genotoxic effects of Cypermethrin, Deltamethrin, Fenarimol and Zineb in peripheral leukocytes treated with these pesticides [22].

In another study, assessing the DNA damage of various pesticides comprising of fungicides like Chlorothalonil, Carbendazim, Fenarimol and Zineb, insecticides like Deltamethrin, Malathion, Malaoxon, Isomalothon, Permethrin, N,N-diethyl-m-toluamide and Diazinon herbicide as Terbutryn, soil fungint like Methyl thiocarbamoylamine through comet assay showed positive results for all the cases except Carbendazim and Malathion [23]. The concentration of Chlorothalonil was 10 µM, that of Deltamethrin was >100 lg/ml, those of Malaoxon and Isomalothon were 25, 75, 200 µM, Terbutryn was of 100–150 µg/ml concentration, Methyl thiocarbamoylate was of <5 µg/ml, those of Permethrin, N,N-diethyl-m-toluamide and Diazinon was 0.5–1.0 mM, Fenarimol was of 36 nM and Zineb was used in 50, 100 µg/ml of concentration.

OCCUPATIONAL EXPOSURE AND ITS ANALYSIS

The individuals who are involved with the formulations, manufacturing, and spraying of the pesticides are exposed to a mixture of pesticides, their active ingredients as well as the different by-products.

The data on workers working in chemical plants are given in Table 1. In Table 2, data on workers involved in pesticide spraying and exposed to single pesticide are compiled. Data on workers involved in pesticide spraying and exposed to a mixture of pesticide are compiled in Table 3. Data on agricultural workers involved in agriculture works are compiled in Table 4.

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