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Review Article

GENOTOXIC EFFECT OF PESTICIDES ON HUMAN LEUKOCYTE CULTURE: A REVIEW

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ABSTRACT

Pesticides have long been in use in the agricultural fields, plantation firms, 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 much 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 long been 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 hosts 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 genotoxocity [3]. There were studies done with a number of organophosphates which comprised azodrin, diazinon, dichlofenthion, dimethoate, dursban, ethion, fenthion, malathion, methylparathion, parathion, phorate, 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 diazonin. 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 (LC_{50}) 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 fell 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 acephate 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. Profenofos another extensively used organophosphate has LC_{50} 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, Vanquish, and Benvel. Dicamba and its derivative Banvel have been tested on human leukocyte cells from a dose range of 10-500 μg/μl [6]. At 200 and

500 µg/µl, respectively for Dicamba and Banvel, there was significant increase in sister chromatid exchange and reduction in cell kinetics, measured through cell proliferative index and mitotic activity.

Another study carried out with two organochlorides namely alachlor, and maleic hydrazides showed elevation in the chromosomal aberration frequency, sister chromatid exchange as well as micronuclei [7]. However, the optimum concentrations of these two compounds were found to be different. The optimum concentration beyond which there was significant increase in the genotoxicity was found to be 1 µg/ml for alachlor, and that for maleic hydrazide was found to 100 µg/ml. In addition, however, alachlor was found to be clastogenic only at the highest concentration 20 µg/ml based on the number of chromosomal aberrations and micronuclei obtained from the respective assays performed.

A study involving 41 workers exposed to carbon tetrachloride, perchloroethylene, and hexachlorobenzene (HCB) clearly indicated a prominent rise in the frequency of micronuclei in the human lymphocyte cell cultures of the workers assessed by cytokinesis-block micronuclei assay [8].

Micronuclei tests performed on human peripheral leukocyte cells with dichlorobischlorophenylethylene a metabolite of dichlorobiphenyltrichloroetane and HCB indicated that 80 mM is the optimum concentration to cause micronuclei formation at genotoxic level [9]. The compound was tested in a concentration gradient ranging from 10 to 80 mM. However, HCB did not produce much of a significant genotoxic result which was carried out in the concentration range from 0.005 to 0.1 mM.

Carbamates

Amitrole or aminotriazole, belonging to the carbamate category of pesticide have shown frequent chromosomal aberration in peripheral blood cultures of workers exposed to the compound. In a consented experiment conducted on volunteers, a 100 mg of amitrole was administered orally given as a single dose. Thyroid iodine uptake was seen to be inhibited in the volunteers after 24 hrs of ingestion. Deaths due to cancer have been reported of workers exposed to amitrole and chlorophenoxy herbicides for 45 days or more. Subsequently, it has been banned in the United States [10].

There have been similar studies conducted with zineb, zinc containing dithiocarbamate, and its commercial formulation named azzurro. Analysis of the frequency of chromosomal aberration, sister chromatid exchange, and cell cycle progression assays was conducted on human peripheral lymphocytes [11]. At 50 and 100 µg/µl concentrations of this fungicide, there were significant increase in sister chromatid exchange and corresponding decrease in cell cycle progression and proliferative rate index at those respective doses. Aldicarb and cytophosphane have been seen to increase sister chromatid exchange and decrease mitotic progression of the human lymphocyte cells [12,13].

A collective study done to compare the genotoxic effect of a mixture of carbamates that involved carbosulfan, ethyl methanesulfonate, and ethyl carbamate showed that a mixture of carbosulfan and ethyl carbamate each administered at 2 mM concentrations did not bring about much of aberrations in chromosome analysis neither the frequency of abnormal cells were high in the human leukocytes [14]. However, when the concentration of the mixture was raised to 4 mM and 8 mM subsequently, the percentage of abnormal cells and chromosomal aberration increased with 24 hrs of incubation in all the cases. However, there were not any synergism seen in the different concentration mixtures of carbosulfon and ethyl carbamate excepting the mixture at 0.1 mM concentration of carbosulfan and 8 mM concentration of ethyl carbamate. Furthermore, there was a synergistic increase in Mitotic Index seen in the human leukocyte cultures at a mixture of 0.025 mM and 0.5 µm carbosulfan with 200 mM of ethyl carbamate. Whereas Mitotic Index decreased when a mixture of 0.025 and 0.1 mM of carbosulfan was given with 4 mM of ethyl carbamate. In addition, there was a synergism seen in the cultures treated with a combination of carbamate and ethyl methanesulfonate at a concentration mixture of 0.5 µm of each of the compound which increased both the frequency of aberrations in chromosomes and abnormal cells. However, no synergism was observed in the Mitotic Index of the leukocytes at such concentration mixtures. Chromatid breaks, isochromatid breaks, and chromatid exchange were the most frequent aberrations seen in these treatments.

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.

Permethrin, another pyrethroid which is a known neurotoxin have been tested for clastogenic effect. A range of concentration from 50 to 200 µg/ml were used, and the compound showed clastogenic effects as the frequency of chromosomal aberrations increased with the increase in its concentration [17].

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 chromatid exchange	Positive (+1.82) Positive (+1.16)
15/50,10 formulators, five packers [25]	Azynphos methyl, methyl parathion, malathion, dimethoate	Not available	Sister chromatid exchange	Positive (+1.22)
19/36 [26]	2,4,5-T,2,4-D	10-25	Chromosomal aberration	Positive (+2.06)
20/20 [27]	Pesticide mixture of cyanazine, malathion, 2,4-D, alachlor, atrazine	6-30 (sample collected 8 months after subjects were exposed)	Chromosomal aberration	Positive (+6.11)
135/111 [28]	Organophosp-hates	3-24	Sister chromatid exchange	Positive (+1.86 smokers) (+1.70 non-smokers)

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 Sister chromatid exchange	Negative Negative
20/17 [31]	Workers in forestry: 2,4-D,MCPA	6-28 days	Chromosomal aberration	Negative
25/25 [32]	Fumigators (in open field): Phosphine, other pesticides	Discontinuous use in 8 months	Chromosomal aberration	Positive (+3.60)
20/26 [33]	Fumigators (in open field): Phosphine	Discontinuous use in 8 months	Chromosomal aberration	Positive (+3.32)
35/21 [34]	Fumigators: Phosphine	Not available	Micronuclei	Negative
38/16 [35]	Eradication of Medfly program: malathion	Not available	Micronuclei	Negative
31/31 [36]	Fungicide sprayers spraying (dithiocarbamate) ethylenbis	Not available	Chromosomal aberration	Positive (+1.33)
13/30 [36]	Farmers growing tomatoes	2	Chromosomal aberration	Negative
31/30 [36]	Sprayers of fungicides	3 months	Sister chromatid exchange	Positive (+1.13)
32/27 [37]	Fumigators applying methylbromide	0.3-22	Micronuclei	Negative
12/10 [38]	Sprayers of 2,4-D	Discontinuity in exposure	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, dimethoate, malathion, gromor, fenitrothion, DDT	5-40	Chromosomal aberration Sister chromatid exchange	Positive (+1.73–2.04) Positive (+1.44–1.53)

BHC: Benzene hexachloride, DDT: Dichlora diphenyl-trychloroethane

assays on whole blood as well as human leukocytes [18]. All of the five pesticides showed dose-dependent cytotoxicity with fenpropathrin 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 fenpropathr in showed the maximum number of micronuclei formation in the whole blood cells, and deltamethrin showed an elevation in the same in isolated human leukocytes. Permethrin and fenvalerate gave the least toxic results out of all due to the least number of micronuclei produced. This study suggested that pyrethroid pesticides have less cytotoxic and genotoxic effects on human lymphocytes.

There were studies examining 12 workers exposed to fenvalerate and 30 donor control groups [19]. Fluorescence *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 chromosome 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 organochloride 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 LC_{50} value of monocrotophos of 0.72±0.01 mg/ml and endosulfan with 0.73±0.001 indicating their high toxicity. Compared to that, dimethoate is less toxic with LC_{50} value of 6.92±0.3 mg/ml and chlorpyriphos had a moderate vale of 1.74±0.04 mg/ml. The results were consolidated with

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, MCPA, MCPP, diquat, dithiocarbamates	2-30	Chromosomal aberration	Negative
94/77 [43]	Carbamates, ureics, triazines, organophosphates, organochlorines, thiocarbamates	1-35	Sister chromatid exchange	Negative
71/30 [44]	Benzimidazoles, organochlorines, pyrethroids, carbamates, nitroorganics, dithiocarbamates, phthalimides, morpholines, organophosphates	2-50	Micronuclei	Negative
30/31 [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, thiabendazole imalzabile, 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, cercobin, folicur, dacostar, lannate, endosulfan, manzate, recop, microshield, orthene, nuvacron, pyrimicin, sencor, roundup	10-45	Chromosomal aberration	Negative

MCPA: 2-methyl-4-chlorophenoxyacetic acid

comet assay evaluating DNA single strand breaks which showed longest tail length for monocrotophos 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 showed 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 and Fenpropathrin and no genotoxic effects of Fenvalerate and Permethrin. In-vitro cytokinesis-block micronuclei Assay was conducted on human peripheral leukocytes treated with these pesticides. [22]

In another study, assessing the DNA damage of various pesticides comprising of fungicides like Chlorthalonil, Carbendazim, Fenarimol and Zineb, insecticides like Deltamethrin, Malathion, Malaoxon, Isomalathion, Permethrin, N,N-diethyl-m-toluamide and Diazinon herbicide as Terbutryn, soil funginant like Methyl isothiocyanate through comet assay showed positive results for all the cases except Carbendazim and Malathion [23]. The concentration of Chlorthalonil was 10 µM , that of Deltamethrin was >100 lg/ml, those of Malaoxon and Isomalathion were 25, 75, 200 µM, Terbutryn was of 100–150 µg/ml concentration, Methyl isothiocyanate 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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