IN VITRO GENOTOXIC EFFECT OF TWO ORGANOPHOSPHORUS AND TWO PYRETHROID INSECTICIDES ON HUMAN LYMPHOCYTES

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ABSTRACT

The widely used insecticides, chlorpyrifos, chlorpyrifos-methyl, cypermethrin and deltamethrin, were evaluated in vitro for their potential genotoxicity effect on peripheral blood lymphocytes of three groups of volunteers: smoker pesticide exposed volunteers (SPEV); non-smoker pesticide-exposed volunteers (NSPEV) and non-smoker non-pesticide exposed volunteers (NSNPEV). Chromosomal aberrations (CA) were scored as genetic endpoint. Results revealed that the tested insecticides induced a significant increase in total chromosomal aberrations (CA) in pesticide exposed volunteer groups (SPEV and NSPEV) as compared with control groups in a concentration dependent manner. The highest genotoxic effect was obtained by chlorpyrifos and the lowest genotoxic effect was obtained by chlorpyrifos-methyl. Overall results revealed that smoking and previously exposure of volunteers to pesticides increased chromosome aberrations of their lymphocyte cells.

Key words: human lymphocytes, smokers, volunteers, organophosphorus and pyrethroid insecticides, chromosomal aberration, genotoxic effect.

INTRODUCTION

Large amounts of pesticides are released daily into the environment and, hence, they represent a potential hazard not only to

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the human genetic material, but also to other living species, since their residues and derivatives are known to contaminante field crops. Several reports were demonstrated that many commonly used pesticides had genotoxic properties (IARC, 1991).

The organophosphorus (OP) and pyrethroid pesticides are known to possess high activity against abroad spectrum of insect pests and lack persistence in the environment (Vijveberg and Van den Bervken, 1990). The impairment effects on public health of these pesticides cannot be denied. Genotoxic effects are considered among the most serious of the possible potential side effects of agriculture pesticides. Data of genotoxicity and carcinogenicity of OP and pyrethroid insecticides are rather controversial, depending on the genetic system or the assay used (Moretti et al., 1997). Therefore, this study aimed to further evaluate the potential genotoxic effects of sublethal doses of two OPs, chlorpyrifos & chlorpyrifos-methyl and two pyrethroids, cypermethrin & deltamethrin on humans lymphocytes.

MATERIALS AND METHODS

Test chemicals:

Two pyrethroid insecticides: Cypermethrin (25% EC), (RS) - α- cyano-3- phenoxybenzyl (1RS, 3RS; 1RS, 3RS) - 3- (2, 2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate; and Deltamethrin (2.5% WP), (S) - α- cyano-3- phenoxybenzyl (1R, 3R)-3- (2, 2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate.

Two organophosphorus insecticides: Chlorpyrifos (48% EC), O, O-Diethyl- O- (3, 5, 6-trichloro-2- pyridinyl) phoshorothioate and Chlorpyrifos-methyl (50% EC), O, O-Dimethyl- O- (3, 5, 6-trichloro-2-pyridinyl) phosphorothioate. Also, standard carcinogenic compound: Benzidine (98% a.i.), 1, 1'-biphenyl-4, 4'-diamine was used in this study.

Serial water dilutions for each compound were prepared and 100µl of each dilution were added to each culture as a concentrations equal to; (300µg a.i./ml and 120µg a.i./ml) from chlorpyrifos-methyl, (9.6µg a.i./ml and 3.84µg a.i./ml) from chlorpyrifos, (25µg a.i./ml and 10µg a.i./ml) from cypermethrin, (13.5µg a.i./ml and 5.4µg a.i./ml)
from deltamethrin, and (30.9µg a.i./ml and 12.36µg a.i./ml) from benzidine as positive control and 100µl D.W. from negative control.

**Sample collection:**
Blood samples were collected from three different groups of volunteers (10 donors per group) of normal healthy male volunteers. The individuals of the 1st group were smoker pesticide-exposed volunteers (SPEV). The individuals of the 2nd group were non-smoker pesticide-exposed volunteers (NSPEV) while the individuals of the 3rd group were non-smoker and non-pesticide exposed volunteers.

**Cytogenetic methods:**
The standard leucocytes cultures used in this investigation consisted of minimum essential medium (Eagle) with L-Glutamine (Sigma), supplemented with 10% fetal calf serum (Sigma) and 1% penicillin-streptomycin (1000 units 1000 µg/ml). For each 10 ml of this medium, 0.5 ml of whole blood, 0.25 ml phytohemaglutinin (Gibco) were added. The culture was incubated in tightly sealed tubes at 37ºC for 72 hrs. The proper concentrations of tested compounds (three replicate per concentrate) were added to the cultures 10 hrs. before harvesting. At 70 hrs. 20µl of colchicine, 10µg/ml (GIBCO), was added to each culture and the cultures were reincubated for 2 hours (Buckton and Evans, 1973). The culture was centrifuged for 8 min. at 1200 rpm, the supernatant was discarded and was suspended in a pre-warmed hypotonic solution (0.057 M KCl) for 10 min. at 30 ºC and centrifuged for 8 min. at 1200 rpm, then the cell pellet was fixative (methanol: glacial acetic acid, 3:1) for 20 min at room temperature. Samples for microscopic observation were obtained by carefully dropping cell suspension from a Pasteur pipette onto clean wet slides. They were air-dried, stained with 10% Giemsa (Goto et al., 1978). The cells were photographed and chromosomes were investigated for deletion, stickiness, exchange, fragment …etc.

**RESULTS AND DISCUSSION**

Cultures derived from human peripheral blood samples have been used for a number of years to detect chromosome aberrations. The preparations are used diagnostically in medical studies, to identify
numerical changes and structural rearrangements in individual patients, and as a screening system for groups of workers exposed to potentially harmful agents (Garry, et al., 1990).

The in vitro effect of the tested insecticides on human lymphocytes revealed that these compounds caused significant increase in the total chromosome aberrations (CA) of the three different groups of human volunteers in a dose dependent manner. The highest increase in the percentage was observed in the first group (SPEV) and the lowest increase was found in the 3rd groups (NSNPEV), interaction-dependent manner as shown in tables (1, 2 & 3). The genotoxic effect of chlorpyrifos was more pronounced than that of chlorpyrifos methyl. Also, chlorpyrifos was most toxic (acute oral LD$_{50}$ for rats 135-163 mg/kg) than chlorpyrifos-methyl (acute oral LD$_{50}$ for rats >3000 mg/kg) as recorded in Pesticide Manual (2003). The two OP insecticides contain the same structure except the substitution of ethyl groups in chlorpyrifos by methyl groups in chlorpyrifos-methyl which was less in its mammalian toxicity and genotoxicity than chlorpyrifos. This substitution may affect the intrinsic toxicity and genotoxicity and/or sensitivity of the structure to the metabolic systems in the treated rats. These genotoxic effects are in agreement with the findings of Lieberman et al (1998) that chlorpyrifos and diazinon have genotoxic effects even at domestically sprayed levels. Also tests using cells from human lymph nodes showed that chlorpyrifos causes an increase in the frequency of sister chromatid exchanges, SCEs, (Sobti et al., 1982). A test using human white blood cells showed a similar increase in, SCEs (Nelson et al., 1990). Chlorpyrifos caused genetic damage in human blood and lymph cells, mice spleen cells and hamster bone marrow cell (Cox, 1994). On the other hand, there is no available literature about the genotoxicity of chlorpyrifos-methyl.

Cypermethrin and deltamethrin are very active pyrethroid insecticides and are used to control pests of variety crops. Although they considered to be safe for mammals (Perry et al., 1998), literature on in vivo genotoxicity of cypermethrin and deltamethrin are limited and rather controversial, depending on the genetic system or the assay used. Our results revealed that these insecticides caused significant increase in the percentages of total aberrations in blood lymphocytes
of three different groups of human volunteers, (SPEV), (NSPEV) and (NSNPEV) as shown in tables (1, 2 & 3). Deltamethrin was more genotoxic than cypermethrin. It is well established that the two pyrethroids contain the same alcohol moiety (α-cyano-3-phenoxybenzyl), but substitution of 3(2, 2-dichlorvinyl)-2, 2-dimethyl cyclopropane carboxylic acid in structure of cypermethrin by 3-(2, 2-dibromoviny)-2, 2-dimethyl cyclopropane carboxylic acid gives deltamethrin which was more genotoxic than cypermethrin. The above mentioned substitution may affect the intrinsic toxicity; genotoxicity and/or induced sensitivity of the structure to the metabolic systems in the treated rats (Abbassy et al., 2005).

These results in agreement and disagreement in some cases with those obtained by many authors. Surralles et al. (1995b), reported that cypermethrin has a weak genotoxic activity in vitro. In contrast, the same research group (Surralles et al., 1995a) reported that cypermethrin did not increase the ratio of excision repairable DNA lesion converted to micronuclei. Nehez et al. (1998) reported that cypermethrin caused no significant increase in the chromosomal aberrations after rat treatments. Nehez et al. (2000) showed that treatment of rats with cypermethrin caused an increase in the number of aberration cells. Abdel Wahab (2002) reported that cypermethrin caused significant changes in mitotic activity of mice bone marrow cells. Also, data on the genotoxicity of deltamethrin are rather controversial, depending on the genetic system or the assay used (Villarini et al., 1995). Beilshmidt, (1990) reported that fenvalerate and deltamethrin have shown increases in various kinds of cancers. The in vitro genotoxicity of deltamethrin has been evaluated by assessing the ability of the insecticide to damage DNA (as evaluated using the single-cell microgel-electrophoresis or comet assay) and micronuclei (MN) in human peripheral blood leucocytes (Villarini et al., 1998). They found that in the presence of metabolic activation (+S9 mix), deltamethrin is able to induce DNA damage. The frequency of SCE and MN were not statistically increased in deltamethrin-treated cells as compared to controls with and without S9 mix.

Finally, data in Table (4) summarizes the results of total chromosomal aberration percentages of human lymphocytes obtained from our study. It was noticed that control group in the first group of
volunteers (SPEV) have the highest percentage of lymphocytes chromosomal aberration (12%) followed by the 2nd group (NSEP) (5%) and the 3rd group (NSNEP) (3%). Also, all formulated insecticides and reference carcinogenic compound increased total chromosomal aberration percentages of human leucocytes compared to control groups. The highest percentage of total aberration percentage was observed in first volunteers group (smokers and pesticide-exposed volunteers and the lowest was observed in third group of volunteers (non-smokers and non-exposed to pesticides).

The genetic effect of smoking (tobacco) and exposure to pesticides were described in studies using other test systems. An additive effect of smoking in inducing a chromosomal damage was also demonstrated, a significant increase in CA (Rupa et al., 1988 & 1989) and SCE (Padmavathi et al., 2000 was observed in smokers compared with non-smokers from pesticide-exposed groups. A high frequency of chromosomal damage was detected in smoking greenhouse workers who had not used protective gloves (Lander et al., 2000).

In conclusion, our in vitro studies revealed that tested pesticides have definite genotoxic effect by using human lymphocyte cells. Smoking and previously exposure of volunteer persons to pesticides increased the aberrations of their lymphocyte cells. These findings support previous findings of Dulout et al (1985); Rupa et al (1989) and Scarpato et al (1996a, b).

The importance of study the genotoxic effects of pesticides is their association with increased cancer risk. Since, Hagmar et al. (1995) stated that an increase in the level of chromosome breakage appears to be relevant biomarker of future cancer risk.
Table (1): Effect of formulated insecticides and benzidine on chromosomal aberration in human lymphocytes of blood samples from smoker-pesticide exposed volunteers (SPEV).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration (µg a.i./ml)</th>
<th>Chromosomal aberration (%)</th>
<th>(1) Total aberration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>F</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>120</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>300</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>3.84</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>25</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>5.4</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>13.5</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Benzidine</td>
<td>12.36</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Benzidine</td>
<td>30.9</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

(1) A number of 100 metaphases were counted; D: Deletion; F: Fragment; RCF: Robertsonian Centric Fusion; and S: Stickiness; statistical difference from the control: significant at P ≤0.05 & **highly significant at P ≤0.01.

Table (2): Effect of formulated insecticides and benzidine on chromosomal aberration in human lymphocytes of blood samples from non-smoker pesticide-exposed volunteers (NSPEV).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration (µg a.i./ml)</th>
<th>Chromosomal aberration (%)</th>
<th>(1) Total aberration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>F</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>120</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>300</td>
<td>6</td>
<td>8</td>
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</tr>
<tr>
<td>Cypermethrin</td>
<td>10</td>
<td>13</td>
<td>0</td>
</tr>
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<td>Cypermethrin</td>
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<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>5.4</td>
<td>10</td>
<td>0</td>
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<tr>
<td>Deltamethrin</td>
<td>13.5</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Benzidine</td>
<td>12.36</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Benzidine</td>
<td>30.9</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

(1) A number of 100 metaphases were counted; D: Deletion; F: Fragment; RCF: Robertsonian Centric Fusion; and S: Stickiness; statistical difference from the control significant at P ≤0.05 & **highly significant at P ≤0.01.
Table (3): Effect of formulated insecticides and benzidine on chromosomal aberration in human lymphocytes of blood samples from non-smoker-pesticide non exposed volunteers (NSNPEV).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration (µg a.i./ml)</th>
<th>Chromosomal aberration (%)</th>
<th>Total aberration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>F</td>
</tr>
<tr>
<td>Control</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>120</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
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<td>3</td>
<td>8</td>
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</tr>
<tr>
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<td>0</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>5.4</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>13.5</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Benzidine</td>
<td>12.36</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>30.9</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

A number of 100 metaphases were counted; D: Deletion; F: Fragment; RCF: Robertsonian Centric Fusion; and S: Stickiness; statistical difference from the control significant at P ≤0.05 &**highly significant at P ≤0.01.

Table (4): Effect of formulated insecticides and benzidine on chromosomal aberration in human lymphocytes of blood samples from different volunteers (SPEV), (NSPEV) and (NSNPEV).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration (µg a.i./ml)</th>
<th>SPEV Total aberration (%)</th>
<th>NSPEV Total aberration (%)</th>
<th>NSNPEV Total aberration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>12</td>
<td>5</td>
<td>3</td>
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<tr>
<td>Chlorpyrifos-methyl</td>
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<td>28*</td>
<td>24*</td>
<td>20*</td>
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<tr>
<td>Chlorpyrifos</td>
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<td>33*</td>
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<tr>
<td></td>
<td>9.6</td>
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<td>35**</td>
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<tr>
<td>Cypermethrin</td>
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<td>32*</td>
<td>30*</td>
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<td></td>
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<td>50**</td>
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<td>31*</td>
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<tr>
<td>Deltamethrin</td>
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<td>34*</td>
<td>31*</td>
<td>30*</td>
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<td></td>
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<td>Benzidine</td>
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<td>47**</td>
<td>38*</td>
<td>35**</td>
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<td></td>
<td>30.9</td>
<td>53**</td>
<td>43**</td>
<td>40**</td>
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</table>

Statistical difference from the control significant at P<0.05 &**highly significant at P ≤0.01.
REFERENCES


تأثير السمية الجينية (خارجيا in vitro) لاثنين من المبيدات الفوسفورية واثنين من المبيدات البيروثرويدية على ليمفوسيل الدم الإنسان.

مصطفى عبد اللطيف عباسي 1 – عبد السلام حلمي بلال 2 – عبد الباعث الصيحي 3 – عبد التواب حليم موسى 4

قسم مكافحة الآف كلية الزراعة بدمنهور
قسم الوراثة – كلية الزراعة – جامعة الإسكندرية
المركز القومي للبحوث الزراعية – الدقي - الجيزة

يهدف البحث إلى دراسة تأثيرات المبيدات الفوسفورية العضوية "كلوربيريفوس"، "كلوربيريفوس ميثيل"، المبيدات البيروثرويدية "سيبرمثرين"، "دلتامثرين" خارجيا in vitro على الليمفوسيل lymphocytes من دم ثلاث مجموعات من المتطوعين: مدخنين ويتعرضوا للمبيدات، غير مدخنين ويتعرضوا للمبيدات، وغير مدخنين ولا يتعرضوا للمبيدات. وأوضحت النتائج ما يلي:

1. أن الشذوذات الجينية في حالة المقاورة في المجموعة الأولى (SPEV) كانت نسبة مرتفعة (12%) عن المجموعة الثانية (NSPEV) (5%) عن المجموعة الثالثة (NSNPEV) (3%).
2. كانت هناك زيادة معنوية في الشذوذات الكروموسومية في المجموعة الأولى (SPEV) عن المجموعة الثانية (NSPEV) عن المجموعة الثالثة (NSNPEV).
3. كان مبيد الكلوربيريفوس أشد تأثيراً في إحداث الشذوذات الكروموسومية بينما كان مبيد الكلوربيريفوس ميثيل هو أقل تأثيراً لهذه الشذوذات.
4. التدخين يزيد من حدوث الشذوذات الكروموسومية التي تحدثها المبيدات في خلايا الليمفوسيل الخاصة بدم المدخنين.