The Effect of Subacute Doses of Organophosphorus Pesticide, Nuvacron, on the Biochemical and Cytogenetic Parameters of Mice and Their Embryos

M.M. Zahran, K.B. Abdel-Aziz, A. Abdel-Raof and E.M. Nahas

Department of Cell Biology, National Research Centre, Dokki, Giza, Egypt.

Abstract: In the present work, male mice, pregnant females and their embryos were used. The animals were orally administered with three pesticide doses (0.25, 0.3 and 0.65 mg/kg) of Nuvacron daily (for four days). The induction of somatic and germ cells mutation as well as the changes in biochemical parameters (DNA, RNA, protein and enzymes) were evaluated. The results showed that treated animals have high incidence of structural and numerical chromosomal aberrations with various doses. The effect was dose dependent and the rate of mitotic index was decreased by the same manner. The contents of tissue proteins, DNA and RNA as well as choline esterase activity were reduced, while the activity of gamma glutamyl transferase (GGT) was increased. These changes are represented as indicators for genetic damages and biochemical abnormalities, which may result in either genetic ill health or cancer. Therefore, the study recommends that this pesticide and/or its analogues should be prevented or at least used cautiously.

Key words: Organophosphorus, genotoxicity, nucleic acids, enzymes, mice, embryo

INTRODUCTION

Organophosphorous (OP) compounds are among the pesticides which are widely used in agriculture. Their application and usage have increased astronomically in the last decade and will likely increase further in future. Some of these which are used at present in Egypt, are dangerous when mishandled or wrongly used. Monocrotophos (MCP) is commonly known as Nuvacron or Azodrin and is widely used for control of agriculture pests in India, Egypt and the other countries as well[1]. Some studies were conducted on the toxicity of MCP[2,3]. These studies showed that MCP or Furadan administration induced bone marrow depression and splenic hyperplasia in mice. The treatment caused significant decreases in hemoglobin content, total count of RBCs and platelets, erythrocyte sedimentation rate and hematocrit value. In addition, acetylcholine esterase(AchE) activity was decreased while acetylcholine, p-amino buteric acid, epinephrine, norepinephrine, dopamine and 5-hydroxy tryptamine concentrations were significantly increased in mice following treatment with Nuvacron and Furadan. An increase in white blood cell-counts and mutagenicity was observed in rats and birds by MCP treatment which showed that this pesticide was more toxic than its analogues, RPR-II and RPR-V[4-5]. In detailed studies on MCP and its analogues some studies[6,7] reported that microsomal cytochrome P_{450} in different tissues, hepatic glutathione content and the activity of glutathione-S-transferase, brain acetylcholine esterase and Ca^{2+} ATPase were greatly reduced. While the leucocyte count was increased in treated animals by MCP and its 2 analogues.

MCP and other OP pesticides greatly affect genetic materials in the organs of treated animals. In this concern, a study[8] detected structural aberrations: stickiness', laggard chromosomes, disturbed metaphase and anaphase as well as multipolar cells in Gossypium barbendense. This pesticide induced chromosome breaks, chromosome aberration and micronuclei in formation of bone marrow cells of mice following i.p. administration[9]. The pesticide showed high genotoxicity[10], and had a high incidence of chromosomal aberrations, sister chromatide exchanges and cell cycle delay[11-14].

As mentioned before, the MCP has mitotic and genotoxic effects on treated animals as well as in vitro mammalian test systems[15]. However, some negative results were given by some investigators[16-19]. They showed that MCP had no skeletal changes in fetuses and no toxic effect on mice and goat. These contradictions indicate that more investigations must be carried out to fulfill this area of study and to give clear cut about the effects of this pesticide since, the complete impact of the OP compounds on health of agriculture workers and
consumers is still largely unknown. Therefore, the aim of
this paper was performed on the known. Therefore, the
aim of this paper was performed on the cytogenetic and
biochemical effects of Nuvacron as an OP pesticide. The
study was focused on chromosomes of bone marrow,
liver, embryo and germ cells in addition to some
biochemical parameters: DNA, RNA, proteins and enzyme
activities.

MATERIALS AND METHODS

Chemicals: Pesticide used; Nuvacron (MCP, Azidin or
Monocrotophos) is an organophosphorus insecticide
with a chemical name, Phosphoric acid dimethyl
{1-methyl-3(methyl amine)-3-oxo-n-propenyl} ester.
Colchicine, giemsa stain and sodium bisulphate were
purchased from BDH chemicals AT. All other chemicals
used were from Sigma, while kits were purchased from
Quimica Clinica Aplicada (QCA).

Animals: Forty adult fertile male and adult virgin female
Swiss albino mice, 8-12 weeks old weighted 25-30 g were
used in the study and obtained from the animals house
Colony, National Research Centre, Dokki, Giza, Egypt.
The animals were kept under normal laboratory and
nutritional conditions throughout the experimental period.

Experimental design and methods: Twenty virgin female
and twenty fertile male mice were used in this study.
Female mice were placed in the cage of adult fertile males
at a ratio of 2:1. The next day, females exhibiting a vaginal
plug of coagulated ejaculate were considered pregnant
and designated as zero day. The pregnant females were
divided into four groups five animals each and at the 11th
day which is the critical time of bone structure, the
animals were given the pesticide for four days (single
dose/day/animal as follows: the first group was
administered with 0.25 ml distilled water instead of
pesticide and served as control. Each animal of the
second group was administered with 0.25 ml solution
containing 0.65 mg/kg of Nuvacron (1/4 LD$_{so}$) and
repeated for another 3 days$^{[20]}$. The same trend was
applied for group three and four but the pesticide doses
used were 0.3mg/kg (1/8 of LD50) and 0.25 mg/kg (1/10 of
LD50) respectively.

The male animal groups were also divided into four
groups (5 animals each) and the same trend of pesticide
administration was used as in the pregnant female
animals.

Cytogenetic analysis:

1- For cytogenetic effects, fifty metaphase spreads$^{[21]}$
were prepared from each maternal bone marrow cells.
The types and frequency of aberrations were
recorded and photographed. Mitotic activity of the
cells was calculated as the number of dividing cells
including prophase and metaphases per 1000 cells.

2- Embryos-liver analysis: after killing the mothers
which were injected with 0.5 ml colchicines (0.05%) three hours before decapitation, twenty five embryos
were randomly selected from each group to study the
abnormalities in the metaphase chromosomes. Metaphase spreads were prepared according to$^{[22]}$.

3- Germ cells technique of$^{[23]}$ was used for meiotic
preparation from mammalian testes chromosomes.

Biochemical analysis:

1- Determination of nucleic acids: liver tissues were
homogenized$^{[24]}$, then DNA content was
determined$^{[20]}$, and RNA was also measured$^{[25]}$.

2- Total protein in plasma was determined using
proteinase-kit$^{[26]}$.

3- Cholinesterase activity was measured in plasma
using QCA-kit$^{[27]}$.

4- Gamma glutamyl transferase activity in plasma was
determined using QCA-kit$^{[28]}$.

5- Statistical analysis for scoring the metaphases in
both somatic and germ cells was carried out using the
method of T test$^{[29]}$.

RESULTS AND DISCUSSIONS

The cytogenetic analysis: In this study, various
chromosomal aberrations were recorded in bone marrow
cells of male, pregnant mice and their fetuses. The
numerical aberration was only polyploidy. Total structural
chromosomal aberrations were gaps, breaks, deletions,
end to end association, centromeric attenuation and
centric fusion. Mitotic activity was recorded in all treated
groups.

Table (1) showed highly significant differences in
total aberrant cells and mitotic activity between control
and treated groups in maternal bone marrow cells and
embryo cells treated with 0.65 mg/kg b.w.

In male somatic cells, there were significant increases
in both structural and numerical chromosomal aberrations,
when compared with control after using the mentioned
three pesticide doses. The increase in structural
chromosomal aberration was dose dependent (Table 1).
The highest change value (37.6± 1.148) was obtained by
the high dose (0.65mg/kg b.w). The medium change value (26.8± 0.935) was obtained by the second dose used (0.3 mg/kg b.w). The low dose (0.25mg/kg body weight) also showed a significant effect (20.0± 0.77) as compared with control (4.4± 0.197).

The frequency of chromosomal aberration in the germ cells of male mice was significantly increased in the three treated groups when compared with control (Table 2).

Total chromosomal aberrations were dose dependent and the highest number of aberrations (37.2± 1.33) was obtained by the highest pesticide dose, 0.65 mg/kg body weight.

The present results are similar to some studies who mentioned that MCP caused chromosomal aberrations in bone marrow cells of mice. Many types of these aberrations such as gaps, breaks, deletions, fragments and interchanges were also detected who confirmed the mutagenic action of the pesticide. In addition, centromeric attenuation, endomitosis, polyploidy and segmented chromosomes were also detected. The increase in chromosomal aberration and SCE as well as the decrease in mitotic index was dose dependent which agreed with that of.

The frequency of chromosomal aberrations in germ cells by MCP was also detected. Their results suggested that the MCP may have mutagenic potential in mice leading to a decrease in the reproductive performance. The autosomal and X-Y univalents, gaps, fragments, breaks and translocations in germ cells of treated mice were also observed which may lead to a decrease in the number of spermatocytes per seminiferous tubules in section of testes of adult male mice.

The abnormalities found in chromosomes of bone marrow cells of pregnant female mice and liver cells of their embryos by MCP were also detected. The effect was due to the toxicity of the pesticide as a potent teratogen.

**The biochemical changes:**

**Protein and nucleic acids contents:** Data in Table (2) showed that the administration of Nuvaron into male mice reduced plasma protein contents and the reduction was dose dependent since the high dose gave 23% reduction. The same trend was found in pregnant female mice but the highest reduction was 28.09% by the high dose.

The obtained results agreed with other studies who found that organophosphorous pesticides decreased total serum proteins in treated animals. They added that albumin content was decreased while the globulins were increased and therefore A/G was decreased (from 1.5 to 1.12). These changes were related to the physiological state and the health of animals hence the insecticide may affect the gastrointestinal tract and induce the decrease in the absorption and assimilation of protein.

The content of DNA in liver of male mice was significantly reduced by pesticide treatment and the reduction was 27.6%, 49% and 60% for the low, medium and high dose respectively (Table 2). The same reduction was nearly obtained for liver-RNA content in both male and pregnant female mice by Nuvaron treatment (Table 2). The reduction in DNA was also observed by several authors who attributed this effect to the inhibition of DNA synthesis or DNA damage by the organophosphorous compounds. The insecticide was
The results showed that the pesticide administration in a conclusion, the present study showed that the
Enzyme activities: enzyme activities of Cholinesterase and gamma glutamyl transferase (GGT) in blood plasma and liver of pregnant female mice and embryos.

Table 2: Effects of organophosphorous pesticide monocrotophos on total values and percentages of different parameters (protein, DNA, RNA, Cholinesterase & GGT) in blood plasma and liver of adult male mice.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Control</th>
<th>Low dose</th>
<th>Med. Dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>%</td>
<td>Value</td>
<td>%</td>
<td>Value</td>
</tr>
<tr>
<td>Protein content in blood plasma (g/l)</td>
<td>81.65±0.153</td>
<td>78.67±0.104</td>
<td>70.02±0.177</td>
<td>62.84±0.326</td>
</tr>
<tr>
<td>DNA content in liver Tissue (mg/g)</td>
<td>1.328±0.009</td>
<td>0.962±0.006</td>
<td>0.68±0.057</td>
<td>0.532±0.006</td>
</tr>
<tr>
<td>RNA content in liver Tissue (mg/g)</td>
<td>0.762±0.008</td>
<td>0.531±0.008</td>
<td>0.357±0.004</td>
<td>0.296±0.0037</td>
</tr>
<tr>
<td>Cholinesterase enzyme activity (U/L)</td>
<td>6.85±0.071</td>
<td>5.189±0.646</td>
<td>3.774±0.714</td>
<td>2.818±0.0506</td>
</tr>
<tr>
<td>GGT enzyme activity (U/L)</td>
<td>4.483±0.082</td>
<td>6.518±0.105</td>
<td>7.406±0.105</td>
<td>8.502±0.159</td>
</tr>
</tbody>
</table>

Table 2 (continued): Effects of organophosphorous pesticide monocrotophos on total values and percentages of different parameters (protein, DNA, RNA, Cholinesterase & GGT) in blood plasma and liver of pregnant female mice and embryos.

<table>
<thead>
<tr>
<th>Case</th>
<th>Pregnant female</th>
<th>Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Control</td>
<td>Low dose</td>
</tr>
<tr>
<td>Value</td>
<td>%</td>
<td>Value</td>
</tr>
<tr>
<td>Protein content in blood plasma (g/l)</td>
<td>80.723±0.191</td>
<td>76.72±0.153</td>
</tr>
<tr>
<td>DNA content in liver Tissue (mg/g)</td>
<td>1.205±0.009</td>
<td>0.862±0.005</td>
</tr>
<tr>
<td>RNA content in liver Tissue (mg/g)</td>
<td>0.664±0.0099</td>
<td>0.465±0.005</td>
</tr>
<tr>
<td>Cholinesterase enzyme activity (U/L)</td>
<td>6.053±0.0332</td>
<td>4.347±0.0384</td>
</tr>
<tr>
<td>GGT-G,T enzyme activity (U/L)</td>
<td>2.666±0.105</td>
<td>3.897±0.128</td>
</tr>
</tbody>
</table>

also found to be genotoxic and inducing severe DNA lesions[48]. The reduction in RNA contents was dependent on the decrease of total nucleic acids and total protein in liver and brain of animals using insecticides[49], inhibition of its synthesis[50] or to the general inhibition of DNA dependent RNA polymerase[50].

Enzyme activities: The effect of three Nuvacron doses on choline esterase and gamma glutamyl transferase (GGT) activity in plasma of treated mice was studied (Table 2). The results showed that the pesticide administration reduced choline esterase activity and the effect was dose dependent, hence the high dose gave 59% reduction in the plasma of male mice. The reduction in the enzyme activity was relatively higher in pregnant female mice by the same treatment.

Similar results were obtained[48,51] in animals exposed to organophosphorous pesticides. Female rats showed less considerable decrease in the blood enzyme activity than did in male animals because the cholinergic status is significantly higher in female rats than in males[52]. The change in the AchE activity was used as biosensor for organophosphates[53] and therefore, used as important marker in individuals who are exposed to organophosphorous compounds. It was found that many of the organophosphorous insecticides used in Gulf War have neurotoxic effects[54].

Concerning the activity of gamma glutamyl transferase the study showed that the Nuvacron administration greatly increased enzyme activity in plasma of treated animals. The increase was raised by dose and the effect was more pronounced in pregnant female mice.

In a conclusion, the present study showed that the administration of Monocrotophos (MCP) into mice gave high incidence of structural and numerical chromosomal aberrations in both somatic and germ cells of males and liver and embryos of pregnant females. The aberrations were associated with a lower mitotic index as well as low incidence of protein, DNA, RNA contents and choline esterase (CHE) activity together with a high incidence in gamma glutamyl transferase (GGT) activity. These effects may result in either genetic ill health or cancer and therefore, the study recommends the prevention of this pesticide and/or its analogues or at least used cautiously.
REFERENCES

25. Dische, Z., 1930. Some new characteristic colour tests for thymonucleic and microchemical method for determining the same in animal organs by means of these tests. Mikrochemie, 8:4-32.


