Adverse Effects of Sub-lethal Doses of Chlorpyrifos on Birth Outcome of Female Wistar Rats


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Authors’ contributions

This work was carried out in collaboration among all authors. Author JUII designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PIKO and HIU managed the analyses of the study. Authors OGU and UWD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Chlorpyrifos is a widely used Organophosphorus pesticide for pest control, leading to increased risk for humans and wildlife exposure. The aim of the present study was to determine the effects of chlorpyrifos on birth outcome in pregnant female wistar rats (Rattus norvegicus). Animals were randomly assigned into 4 equal groups, group 1 were untreated and served as control. Rats of group 2 to 4 were treated with chlorpyrifos at concentration 0.2%, 0.4% and 0.8% respectively through feed and drinking water ad libitum from gestation day 1 through to weaning. The results on litter size indicates non-significant dose dependent decrease (p>0.05) across treated groups. Total litter birth weight significantly decreased (p<0.05) in a dose dependent manner compared with control. Stillbirth recorded non-significant (p>0.05) among treatment groups when compared with that of control. Also, postnatal survival showed significant (p<0.05) dose dependent lower number of pups survival between parturition and weaning. These results demonstrated that Chlorpyrifos has adverse impact on birth outcome in treated rats.

Keywords: Chlorpyrifos; birth outcome; pregnant female wistar rats.

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1. INTRODUCTION
Organophosphorus (OPs) compounds are the main components of pesticides and nerve gas [1,2]. OPs are used in agriculture, pest control, horticulture, plastic making, veterinary control, industries, warfare agents and domestically [3]. The worldwide consumption of OPs reached 6.8 million in 2011 to 2015 [4]. Their widespread use is due to less persistent, biodegradability under air, sunlight and soil (Dhas and Srivastava, 2010). OPs are water soluble and easily reach the environment [5]. Lethal and sub-lethal exposure to OPs can induce varying levels of toxicity in humans, animals, insects and plants. OPs compounds inhibit acetylcholinesterase activity that affects the nervous system of aquatic and terrestrial fauna, they also lead to neuroteratogenicity and genotoxicity, ecological and adverse environmental hazards [6,7,8].

Chlorpyrifos is the most commonly used OPs that have been found to have deleterious effects on non-target organisms. Chlorpyrifos kills insects upon contact by affecting normal physiology of nervous system. Exposure to chlorpyrifos remain a serious public health concern, particularly for children throughout the world. Chlorpyrifos is a major issue during pregnancy because this pesticide like other hydrophobic compounds readily crosses the placenta and has the potential to induce untoward effects in the developing organism [9]. Chlorpyrifos is readily absorbed from gastrointestinal tract. [10] study of single dose oral administration of chlorpyrifos on human volunteers reported that chlorpyrifos was 70% absorbed from gastrointestinal tract and in rats chlorpyrifos absorption through gastrointestinal tract after single dose gavage study ranged from 84-90%. Chlorpyrifos is a non-synthetic pesticide designed to be effective by direct contact, ingestion and inhalation. Toxicity occur as a result of use in agriculture, medicine, industry, public health, domestic settings and suicide throughout the world [11].

As nerve agents, they have been used in warfare and terrorist attacks [12]. The mechanism of action is through the inhibition of the enzyme acetylcholinesterase, leading to the accumulation of acetylcholine at cholinergic synapses [13,14,15]. The excess acetylcholine causes constant acetylcholine receptor triggering, malfunction of peripheral and central nervous systems. Chlorpyrifos (Ops) exposure at very low doses are known to affect the physiology of reproduction in wildlife and humans.

2. MATERIALS AND METHODS
2.1 Animals
Mature Healthy female and male Wistar rats (Rattus norvegicus), thirty six (24 female and 12 male) in number weighing between 160-185g were used. They were procured from the Animal House of the Department of physiology, University of Port Harcourt, River State Nigeria.

2.2 Grouping of Animals
After three weeks of acclimatization before the commencement of the treatment at normal room temperature. The female rats were randomly divided into four equal groups, group 1 served as control group while 2 to 4 groups were the treatment groups and were paired 2 female per 1 male for fertilization to occur which was confirmed by a vagina smear test which was carried out each morning, confirmation of spermatozoa was considered day 1 of gestation.

2.3 Housing of Animals
They were housed in labeled plastic cages covered with wire gauze under standardized animal conditions, fed with pelleted food (Vita feeds) twice daily with each rat consuming estimated feed weight of 30g per day and drinking water ad libitum.

2.4 Chlorpyrifos Preparation
Three concentrations 0.2%, 0.4% and 0.8% of solutions was prepared by diluting the commercially available chlorpyrifos liquid in distilled water (DW).

2.5 Chemical and Treatment
The solutions were administered to the female Wistar rats through feed by mixing 15ml of different prepared concentration of chlorpyrifos accordingly to 15g of feed twice a day throughout the period of gestation and 21 days after parturition. The first day of administration was considered day 1 of treatment.

2.6 Data Collection
The animals were monitored throughout the treatment period and observations recorded. Birth outcome such as litter size, birth weight, stillbirth and postnatal survival were recorded.
2.7 Statistical Analysis

Data obtained was expressed as Mean ± Standard Deviation and analyzed using the SPSS package 20.0. One-way Analysis of Variance (ANOVA) was used. Values at \( p<0.05 \) was regarded as significant in comparison with appropriate controls.

3. RESULTS

3.1 Birth Outcome Study

The results of chlorpyrifos treated rats (Table 1) had a non-significant (\( p>0.05 \)) dose dependent decrease litter size compared with control group as shown in Fig. 1. A significant (\( p<0.05 \)) decrease total litter birth weight was recorded in a dose dependent manner among treated groups as represented in (Fig. 2). There was more stillbirth across the treatment groups in a dose dependent manner but it was not significant (\( p>0.05 \)) when compared with that of control (Fig. 3). Postnatal survival showed (Fig. 4) significant (\( p<0.05 \)) less pups that survived between parturition and weaning in chlorpyrifos treated rats.

4. DISCUSSION

Health hazards due to environmental toxicants can manifest after a very short or long period. Short period can be hazardous and lethal while long exposure affect different system of the organism and it has been associated with numerous alterations. Endocrine disruption is identified to be one of the effects of pesticides exposure [16]. In the present study chlorpyrifos treated rats showed a non-significant dose dependent decrease litter size compared with untreated (control) group. A significant dose dependent decrease litter birth weight was recorded across treated groups compared with control. The present study recorded more stillbirth across the treatment groups in a dose dependent manner but it was not significant. There was a significant low pups survival across treated groups compared to untreated (control) group. Treated rats showed a significant decreased pup survival between parturition and weaning in chlorpyrifos treated rats.

Table 1. Birth outcome of female wistar rats after treatment with different doses of chlorpyrifos

<table>
<thead>
<tr>
<th>Groups</th>
<th>Litter size</th>
<th>Birth weight</th>
<th>Stillbirth</th>
<th>Postnatal</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>(0.0%)</td>
<td>7.5 ± 0.43</td>
<td>7.1 ± 0.20</td>
<td>0.3 ± 0.21</td>
<td>9.0 ± 0.45</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>(0.2%)</td>
<td>7.0 ± 0.45</td>
<td>6.8 ± 0.10</td>
<td>1.17 ± 0.31</td>
<td>3.3 ± 0.33</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>(0.4%)</td>
<td>6.2 ± 0.48</td>
<td>6.3 ± 0.09</td>
<td>1.5 ± 0.43</td>
<td>2.0 ± 0.37</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>(0.8%)</td>
<td>6.0 ± 0.37</td>
<td>5.8 ± 0.18</td>
<td>1.5 ± 0.43</td>
<td>0.67 ± 0.30</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; \( n = 6 \) for each treatment group

Fig. 1. Effect of chlorpyrifos on litter size
weaning in chlorpyrifos in a dose dependent manner. In agreement with the present study, [17] study of female mice before gestation treated with cypermethrin who were mated with untreated males reported delayed onset of puberty in their offspring and estrous periodicity of progeny. They attributed the effect to utero accumulated metabolites of cypermethrin pesticide on fetuses. [18] cited an investigation by the North Carolina Department of Health and Human Services which concluded that it is 'plausible' that occupational pesticide exposure caused one of the children to be born with no arms or legs which is consistent with the result of the present study. [9] reported that utero exposure to pesticide (imidacloprid) can significantly affect offspring development with lasting adverse consequences in adulthood which is in line with the present study. Consistent with the present study, [19] reported that pregnant women living nine miles off farms where pesticides are sprayed have an increased risk of losing an unborn baby to birth defects.

Fig. 2. Effect of chlorpyrifos on birth weight

Fig. 3. Effect of chlorpyrifos on stillbirth
5. CONCLUSION

The present study showed that, gestation to weaning periods of female Wistar rats exposed to chlorpyrifos affected the offspring, therefore, it is strongly recommended that chlorpyrifos exposure during pregnancy should be avoided.

ETHICAL APPROVAL

The protocols approved by institutional animal ethics committee and guide lines of National Research Council for care and maintenance of animals were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


