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Scanning electron microscopic study of the effect of chlorpyrifos on the developing neural tube in comparison with Arsenic in mouse embryo

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KEYWORDS

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Abstract *Background:* Arsenic is an important environmental toxicant which is usually found in drinking water in inorganic form. Arsenic exposure in pregnant mice causes neural tube defects (NTDs). Chlorpyrifos, an organophosphorus insecticide, recommended universally and in Egypt to control various pests, was evaluated for its potential developmental toxicity. Studies have shown increasing evidence to suggest an association between environmental exposure to this agricultural pesticides and adverse reproductive outcomes. The hypothesis tested in this investigation is chlorpyrifos causes significant defects on the developing central nervous system compared to the proven Arsenic.

Objectives: The aim of this work was to assess congenital malformations induced by the organophosphorus insecticide chlorpyrifos on the neural tube and brain development in comparison with the positive control Arsenic.

Methods: Virgin female ICR (CD-1) mice, approximately 10 weeks old were mated with adult males. The day the vaginal plug was found was considered day 0 of gestation. It consisted of 320 mice. They were subdivided into four groups of 80 bred mice each. Each group was divided into 4 subgroups, and 20 mice per each were treated by gavage as follows: 30 mg/kg/day chlorpyrifos (tested group), 40 mg/kg/day sodium Arsenite (positive control group), and corn oil and distilled water (negative control groups) on days 6–15 of gestation. Maternal observations throughout gestation were reported. In each subgroup the mice proved to be pregnant were sacrificed on gestational days; GD 10, 11, 12 and 16. The day of scarification was determined according to the neural tube developmental stages. The conceptus extraction was done and their number reported to be subjected to the SEM study.

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After mice scarification, the uteri were opened and a total of 30 embryos and fetuses, randomly selected from each subgroup were processed for scanning electron microscopy investigating the neural tube developmental defects.

Results: CPF ingested by gravid mice at dose of 30 mg/kg/day started from 6th day of gestation proved to produce NTDs as compared to Arsenite.

Conclusion: Neural tube defects are due to chlorpyrifos that may directly influence brain cell replication and differentiation.

1. Introduction

Despite restriction on production for home use, chlorpyrifos (CPF), [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphoro thioate], remains one of the most widely used insecticides, and there is concern over the potential consequences of fetal and childhood exposure.[1–5] Uses of CPF, as indoor pests, were restricted in June 2000.[5,6] Despite this restriction CPF is still widely used as indoor pesticide and pet collars in Egypt. Immature organisms are more susceptible to CPF induced toxicity than adults.[7,8] The presence of CPF residue in the umbilical cord and its association with decreases in fetal growth among women during pregnancy raises question about the sensitivity of residue parameter of CPF.[8] Furthermore, it has been emphasized that perinatal exposure of women to CPF would lead to shortening of the gestational period.[9] In addition, several studies have reported that repeated exposure to CPF during gestation can cause fetotoxicity and marked neurochemical changes in the developing brain.[10–13] Chlorpyrifos exposure during the perinatal period is known to evoke deficits in neuritic outgrowth, specifically including the target of cholinergic projections.[14,15] Exposure of pregnant female mice to 30 mg/kg/day CPF through 6–15 days of gestation resulted in increase of gestation length and post implantation loss, decreased litter size and weight, and survivability.[16,17] It is questionable whether malformations produced by a dose of 30 mg/kg/day, particularly neural tube defects, could be increased. Therefore, the present study was conducted to assess the ability of CPF to cause brain and neural tube defects at a dose of 30 mg/kg/day CPF compared to Sodium Arsenite which was used as a positive control and confirmed to produce developmental toxicity and neural tube defects.[18–20]

2. Material and methods

320 female ICR (CD-1) mice, approximately 10 weeks old, were mated. The gravid mice were subdivided into four groups of 80 bred mice each. Each group was divided into 4 subgroups, and 20 mice per each were treated by gavage as follows: 30 mg/kg/ day chlorpyrifos (tested group), 40 mg/kg/day sodium arsenite (positive control group), and corn oil and distilled water (negative control groups) on days 6–15 of gestation. On GD 10, 11, 12 and 16, after mice scarification, the uteri were opened and a total of 30 embryos and fetuses, randomly selected from each subgroup were processed for scanning electron microscopy investigation.[21]

3. Results of the scanning electron microscopic (SEM) study

Study of the neural fold fusion with SEM was done in the chlorpyrifos and sodium arsenite treated groups in mice embryos on gestational days GD10, 11, 12 and in mice fetuses on day 16 of development and compared to the controls (Table 1, Figs. 1–4).

On day 10 of development, examination of embryo specimens showed that the neural folds in the control groups approached each other and the neural groove became concave for the completion of closure of the neural tube. At the same

Table 1 The number of mice embryos and fetuses with neural fold fusion disruption as evident by SEM examination on days 10, 11, 12 and 16 of development randomly selected from each of the 4 groups of the study.

Study groups	Number (%)			
	Negative control (1)[a]	Negative control (2)[b]	Positive control[c]	Chlorpyrifos 30 mg/kg/d
Number of embryos examined in each subgroup	30	30	30	30
Day 10 of development	0 (0)	0 (0)	6 (16)*	9 (30)**
Day 11 of development	0 (0)	0 (0)	7 (23)*	10 (33)**
Day 12 of development	0 (0)	0 (0)	8 (26)*	11 (37)**
Day 16 of development	0 (0)	0 (0)	9 (30)**	12 (40)**

[a] Corn oil as a vehicle of chlorpyrifos.
[b] Distilled water as a vehicle of Arsenic.
[c] Arsenic as Sodium Arsenite (40 mg/kg/day).
* Significantly different from control at $P \leqslant 0.05$.
** Significantly different from control at $P \leqslant 0.01$.