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SCIENCE DIRECT
Reproductive Toxicology 20 (2005) 267–271
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Teratogenicity and developmental toxicity of chlorpyrifos Maternal exposure during organogenesis in mice

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Received 11 October 2004; received in revised form 22 December 2004; accepted 31 January 2005
Available online 11 March 2005

Abstract

Chlorpyrifos, an organophosphate pesticide, was evaluated for potential teratogenicity and developmental toxicity in mice. Pregnant females were given a single intraperitoneal injection (40 or 80 mg/kg) on day 10 of gestation and fetuses were evaluated on gestation day 17. At 80 mg/kg, chlorpyrifos treatment resulted in a significant reduction in numbers of live fetuses, and increase in resorptions, versus control litters. There was no indication of maternal toxicity. External and skeletal malformations were observed at 80 mg/kg, but not 40 mg/kg. Rates of fetuses with cleft palate increased significantly ($p < 0.05$) following 80 mg/kg chlorpyrifos (5.97%) versus control litters (0.97%). Similarly, the absence of thoracic vertebrae was increased and the number of caudal vertebrae was significantly decreased. It is suggested that chlorpyrifos is teratogenic and embryotoxic in mice at doses below those that cause significant maternal toxicity.

Keywords: Chlorpyrifos; Malformation; Developmental toxicity; Cleft palate; Mice

1. Introduction

Chlorpyrifos [*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] is a broad-spectrum organophosphate pesticide used extensively in agricultural and domestic pest control. It was one of the most heavily used organophosphorus insecticides in the U.S. [1]. Chlorpyrifos is poorly soluble in water (2 ppm) and readily partitions to the organic phase in the environment; the potential hazard for human exposure is high [2]. It is a neurotoxicant due to inhibition of acetylcholinesterase activity that can cause symptoms such as nausea, dizziness and confusion, and at high exposures respiratory paralysis and death [1]. The U.S. Environmental Protection Agency restricted the use of chlorpyrifos in June 2000 due to the exposure risk especially to children [3]; however, chlorpyrifos is still used extensively in China as an agricultural pesticide [4].

Indoor spraying of chlorpyrifos may pose a considerable risk to human health especially to children [5,6]. In 1996, it was reported that extensive and multiple birth defects in four children were associated with prenatal exposure to chlorpyrifos used in their homes [7]. These malformations included brain abnormalities, microcephaly, palatal defects, developmental delays and mental retardation. That study was criticized because of the absence of data regarding malformations or embryo/fetal mortality in the control group [8]. Therefore, the relationship between maternal chlorpyrifos exposure and birth defects has been controversial despite the importance to public health and concern over prenatal exposure.

Basic studies have recognized cytogenetic toxicity following chlorpyrifos exposure in vitro and in vivo [9–11]. Recently, we demonstrated significant increases in the rate of micronucleus formation, and a dose-dependent reduction in cell number, in the pre-implantation mouse embryo fol-

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doi:10.1016/j.reprotox.2005.01.012

lowing maternal exposure to chlorpyrifos by intraperitoneal injection on day 0 of gestation [12]. These results in mice imply a possible cytogenetic hazard to chlorpyrifos exposure in human development. Furthermore, chlorpyrifos had fetotoxic and teratogenic effects in rats following maternal dosing with 25 mg/kg per day by gavage on gestation days 6–15 [2]. Although maternal oral administration of 25 mg/kg chlorpyrifos to pregnant mice on gestation days 6–15 was not overtly teratogenic it showed minor developmental toxicity (minor skeletal variations, reduced fetal weight and length) [13]. Therefore, the present study was undertaken to evaluate the teratogenicity and developmental toxicity of chlorpyrifos at higher dose levels (40–80 mg/kg) in mice.

2. Materials and methods

2.1. Mating and treatment

The study used virgin female mice of the random-bred Crj:CD-1 (ICR) strain (CLEA JAPAN Co.) at 10–14 weeks of age and at least 30 g in weight. Mice were acclimated to ambient conditions (room temperature 23 ± 2 °C, relative humidity 50 ± 10%, 12-h light–dark cycle) for at least 2 weeks prior to mating. They were fed a standard breeding granulated diet and tap water ad libitum. Females were paired with a male in a 2:1 ratio overnight and examined for a vaginal plug the following morning. The day on which a vaginal plug was observed was designated day 0 of gestation (GD0).

Pregnant females were divided into three groups. Two groups of dams were injected intraperitoneally with a single dose of 40 or 80 mg/kg chlorpyrifos (CAS No. 2921-88-2, Wako Pure Chemical Industries Ltd., Japan), dissolved in olive oil, at 17:00 h on day 10 of gestation ($n = 15$ and 19 litters, respectively). The administered volume of each dose was 10 ml/kg body weight. Control dams ($n = 16$) received an injection of olive oil only. The maximum chlorpyrifos dose was based on the current OECD guidelines for in vivo cytogenetic assays, which suggested that the maximum dose in rodents should be the highest dose that does not induce obvious signs of toxicity to the animals [14].

2.2. Maternal observations

Dams were examined daily throughout the experimental period for signs of toxicity. Body weighs of each dam were recorded 10 and 17 days. The maternal body weight gains were calculated by subtracting the body weight on day 10 of gestation from that on day 17 of gestation.

2.3. Fetal observation

Dams were euthanized by cervical dislocation on day 17 of gestation. The uterine horns were exteriorized through a midline abdominal incision, opened and examined for number of implantation sites, live and dead fetuses, resorption sites. The number of corpora lutea was also counted in each ovary. Each live fetus was weighed and examined for gender and external malformations (exencephaly, cleft palate, abdominal hernia, polydactly, open eyelid, etc.) under a dissecting microscope (LEICA-GZ6, Germany). Alizarin red S staining was used to detect skeletal anomalies [15]. Briefly, fetuses were stained in Alizarin red solution (0.004% in 2.0% KOH) for 3 days, rinsed in E.G.B. solution (70% ethanol:glycerin:benzylalcohol, 2:2:1) for 3 days, and finally stored in 100% glycerin until evaluation.

2.4. Statistical analysis

The litter was used as the experimental unit for statistical analysis [16]. Data were compared by the Mann–Whitney *U*-test, and statistical analyses were carried out using StatView Version 5.0 (Abacus Concepts, Berkley, CA, USA).

Table 1
Effects of chlorpyrifos on reproductive and fetal parameters at day 17 of gestation (mean ± standard error)

Group	Vehicle	Chlorpyrifos	
		40 mg/kg	80 mg/kg
No. of litters (fetuses) examined	16 (216)	15 (225)	19 (276)
No. of corpora lutea/litter	15.7 ± 0.8	17.3 ± 0.7	16.9 ± 0.7
No. of implantation sites/litter	13.5 ± 0.5	15.0 ± 0.6	14.8 ± 0.5
Body weight at day 10 of gestation	39.3 ± 1.0	38.1 ± 1.4	40.5 ± 1.1
Body weight at day 17 of gestation	58.4 ± 1.4	58.2 ± 1.7	56.9 ± 1.1
Maternal weight gain	19.2 ± 3.8	20.1 ± 0.9	16.4 ± 1.0
% of live fetuses	95.9 ± 2.1	93.3 ± 1.7	86.1 ± 2.9**
% of dead fetuses	0.8 ± 0.6	0.8 ± 0.8	2.3 ± 0.8
% of resorbed fetuses	3.3 ± 2.1	5.9 ± 1.7	11.6 ± 2.9*
Fetal body weight (g)/litter			
Males	1.17 ± 0.03	1.13 ± 0.03	1.09 ± 0.03
Females	1.13 ± 0.03	1.06 ± 0.02	1.05 ± 0.03
Ratio of males/total	0.41	0.49	0.49

* $p < 0.05$ compared with controls by Mann–Whitney *U*–test.
** $p < 0.01$ compared with controls by Mann–Whitney *U*–test.