ORIGINAL ARTICLE

Teratological Effects of Chlorpyrifos on Crown Rump Length and Weight of Developing Mice

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ABSTRACT

An organophosphorus insecticide, chlorpyrifos was tested for the embryotoxicity and teratogenecity in mice. Different sublethal doses of the insecticide i.e. 20, 40, 60 and 80ug/gm body weight were prepared by dissolving it in corn oil in such a way that each 0.1ml of the solution contains desired concentration. The doses were given orally on day 6 of gestation to different experimental groups. The embryos were recovered on day 15 of gestation. A significant decrease in body weight and crown rump length of embryos was noted. Comparatively higher doses proved more toxic and also caused many developmental defects. it is concluded that chlorpyrifos is toxic to developing embryos, especially in the quantities used in this study..

Keywords: Organophosphates, Chlorpyrifos, Embryotoxity.

INTRODUCTION

Ever since the time human being came to occupy the earth they were ought to compete with other animals for existence. This competition was for food as well as space. With the increase in their agricultural skills, they started growing and cultivating many new crops to supplement their food. But to their dismay, they found that many animals like their crops and take away a lot of their labour. Among these animals insects were the main culprits. Many poisons were used to kill these insects and were named insecticides.[1]

In Pakistan the use of pesticides started in 1954 with an import of 254 metric ton of formulated product, but in 1976-77, the import of pesticides increased to 16,226 metric ton and is increasing since now.[2] A craze has been developed in Pakistan for using mosquito repellent coils and mats, use of DDT as antilice measures. All the chemicals used as pesticides are poisonous leading to environmental pollution.[3]

Chlorpyrifos is moderately toxic following acute oral, dermal and inhalation exposures [toxicity category II].[4] The acute toxicity of organophosphates reflects their ability to inhibit acetyl cholinesterases[5,6], thus eliciting cholinergic hyperstimulation.[7] For chlorpyrifos, this effect is caused by the active metabolite, chlorpyrifos oxon.[8]

In another study, Malathion was tested for its embryotoxic and teratogenic properties in mice embryos. Following relative high doses (125, 250 and 500mg/g) of the insecticide. It was observed that along with a decrease in body weight and crown rump length, the embryos showed a significant lag in the development of main body parts such as brain, snout, external pinnae and limbs while significant increase in uncovered area of eyeball [9]

Methamidophos was administered in higher doses 15-20ug/g body weight to female mice, mothers died immediately after treatment. At different concentration of dose applied some. fetuses recovered from survived mothers were studied. There was tendency towards dwarfism, body weight and CR length was reduced.[10,]

The most common toxic effects of fenitrothion observed in female rats were significant decreased in body weight, food consumption, decreased absolute weight of liver and kidney [11], Recent epidemiology studies reported associations between umbilical cord plasma chlorpyrifos levels and fetal birth weight decreases among minority women living in New York City during pregnancy.[12] Due to lack of research to evaluate the efficacy and toxicity of particular pesticide, Pakistan suffered a mishap in 1976 when "Malathion" sprayed for malaria eradication programme, resulted in 2800 cases of acute intoxication.[13]

In another study conducted at Civil Hospital, Quetta, there were 46 females admitted through emergency with diagnosis of medicolegal cases of poisoning, attempted deliberate self harm due to organophosphate, the clinical signs like excessive salivation, miosis, bradycardia, wheezing, excessive bronchial secretions, typical breath odor of organophosphate and response to atropine were noticed.[14]

All these studies have indicated that organophosphate insecticides are potentially dangerous to animal and human health. It is quite

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apparent that much more research will have to be carried out before we know more specifically, about the effect of these chemicals on the unborn and thus plan and designs, ways and means for the use of these insecticides in the most appropriate ways.

Objective: To record the teratological effects of Chlorpyrifos on crown rump length and weight of developing mice.

MATERIALS AND METHODS

This experimental study was conducted at Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-Azam Campus, University of the Punjab between 2003-2005. In this experimental study 100 pregnant mice, of Swiss Weister Variety of Mus musculus, were used. All animals were kept separately in animal house of Zoology Department, University of the Punjab, Lahore and were acclimatized for 15 days. The food and water was provided ad libitum. Chlorpyrifos, (liquid form) in a dose concentration of 20.0, 40.0, 60.0 and 80.0ug/g body weight was given to experimental groups A, B, C and D respectively. These concentrations were prepared by dissolving the insecticide in corn oil in such a way that each 0.1 ml of solution contained the desired concentration. The doses were given at day 6 of gestation. The treated female pregnant mice who survive after 24 hours of dose application in each experimental group i.e. A, B, C and D were kept singly in different cages till day 15 of gestation. The control and vehicle control groups were also maintained.. On the 15th day of gestation, all the animals were weighed and anaesthetized with anaesthetic ether and then sacrificed. The two horns of gravid uterus was dissected out and weighed. The fetuses were carefully dissected out of uterus. The alive and dead/resorbed embryos were counted and fixed in Bouin's fixative for 48 hours. The emrbyos were then washed with 70% alcohol for 15-30 minutes to remove extra fixative. Both control as well as treated fetuses were then prepared for further morphological studies. Such fetuses were washed in 70% alcohol for overnight and then preserved in 80% alcohol.

RESULTS

The crown rump length of control embryos was 19.735±0.51 mm and their average weight turned out to be 0.89±0.06 mg. The crown rump length of vehicle control embryos was 16.34±0.88mm and their average weight turned out to be 0.78±0.13mg. The crown rump length of experimental group A embryos was 14.22±1.158 mm and their average weight turned out to be 0.68±0.279mg. The crown rump length of experimental group B embryos was 11.76±2.22mm and their average weight turned out to be 0.51±0.19mg. The crown rump length of experimental group C embryos was 9.19±1.63mm and their average weight turned out to be 0.45±0.12mg. The crown rump length of group D was 7.75±0.768mm and their average weight turned out to be 0.38±0.03mg (Figs. 1-2).

DISCUSSION

Developing countries bear the brunt of damage to health from environmental hazards.[15] Due to some known hazardous effects, the use of organophosphate insecticides have been reduced in many countries but they are still being used heavily in developing countries including Pakistan.[16]

So in this context, the present study was designed to see the teratological effects of chlorpyrifos on the crown rump length ad weight of the developing mice. Chlorpyrifos, an acetyl cholinesterase (AChE) inhibitor is a widely used organophosphate pesticide.[17] The oral LD50 of chlorpyrifos in mice is 60mg/kg body weight.[18] Four sublethal doses 20.0, 40.0, 60.0 and 80.0µg/g body weight were selected for experimental group A, B, C and D respectively. Asmatullah et al described the morphometric studies of the fetuses recovered following different concentrations of Malathion showed reduction in body weight and crown rump length which was basically dose dependent..[19]

As far as the effects of chlorpyrifos on body weight and crown rump length are concerned, there was significant morphometrical decrease in all treated embryos but statistically non significant data was extracted as compared to control groups. It is quite apparent from the results that chlorpyrifos is embryotoxic. These results are not surprising because many of the previous investigations have also shown that organophosphorus insecticides are highly toxic to mammalian embryonic system, even at lower concentrations.[20-21]

CONCLUSION

The present study indicates that this insecticide "chlorpyrifos" is potentially dangerous to pregnant mice and developing fetuses. A significant decrease in body weight and crown rump length of embryos were noted. Pregnant population and growing children in particular, should be protected from the hazards of these compounds It is quite apparent that this insecticide must be used with utmost care and according to a well thought out plan.

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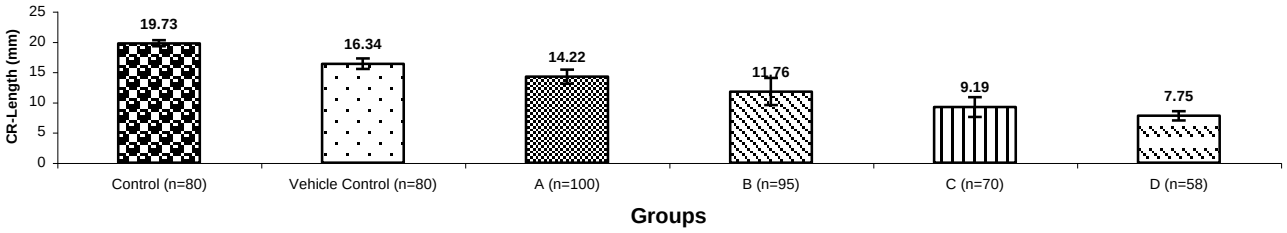

Fig.1: Effects of Chlorpyrifos on crown-rump length (mean±SD) among control and experimental groups

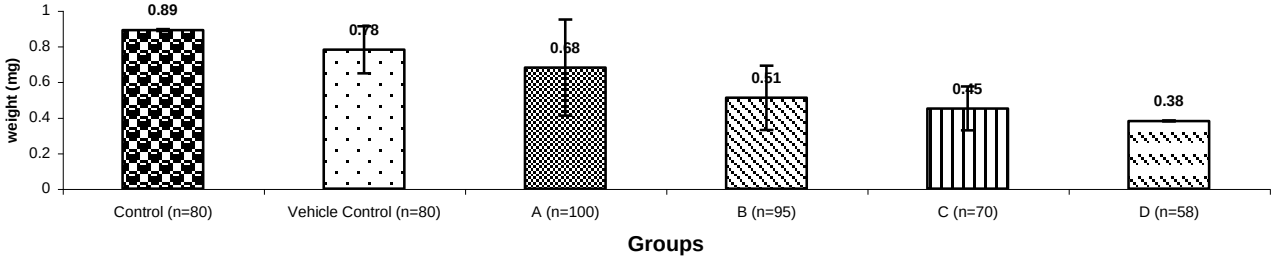

Fig.2: Effects of Chlorpyrifos on weight (mean±SD) among control and experimental groups

REFERENCES

1. WHO. Public health impact of pesticides used in agriculture. WHO 1990.
2. Inayatullah C, Haseeb M. Poisoning by pesticides. Pak J Med Res 1996; 35:57-8.
3. Eskenazi B, Harley K, Bradman A, Weltzein E, Jewell NP, Barr DB et al. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 2004; 112:1116-24.
4. PAN Pan international website. September 1998. https://secure.virtuality.net/ panukcom/subs.htm
5. Zhang H, Liu J, Pope CN. Age related effects of chlorpyrifos on muscarinic receptor mediated signaling in rat cortex. Arch Toxicol 2002; 75:676-84.
6. Castorina R, Bradman A, McKone TE, Barr B, Harnly ME, Eskenazi B. Cumulative organophosphate pesticide exposure risk assessment among pregnant women living in agricultural community: A case study from the CHAMACOS cohort. Environ Health Perspectives 2003; 111:1640-8.
7. Betancourt AM, Carr RL. Neurotoxicology: The effect of chlorpyrifos and cholinesterase, muscarinic receptor binding, and neurotrophin levels in rats following early postnatal exposure. Toxicol Sci 2004; 77:63-71.
8. Timchalk C, Nolan RJ, Mendrala AL, Dittenber DA, Brzak KA, Mattsson JL. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci 2002; 66:34-53.
9. Asmatullah, Mufti SA, Cheema AM, Iqbal J. Embryotoxocity and teratogenicity of malathion in mice. Punjab Univ J Zool 1993; 8:53-61.
10. Asmatullah, Aslam T. Toxicity of methamidophos in pregnant mice and developing fetuses. Punjab University J zool 1999; 14:141-51.
11. Berlinska B, Sitarek K. Disturbances of prenatal development in rats exposed to fenitrothion. Rocz Banstw Zakl Hig 1997; 48:217-28.
12. Zhao Q, Gadagbui B, Dourson M.Lower birth weight as a critical effect of chlorpyrifos:A Comparison of human and animal data:Regulatory toxicology and pharmacology*;2005;42:55-63*
13. WHO. Principles for evaluating health risk to reproduction associatd with exposure to chemicals. WHO 2001
14. Khan MN, Hanif S. Deliberate self harm due to organophosphates. J Pak Inst Med Sci 2003; 14:784.
15. Smith KR. Environmental health for the rich or for all? Bulletin WHO 2000; 78:1156-7.

16. Yanez L, Ortiz D, Calderon J, Batres L, Carrizales L, Mejia J et al. Overview of human health and chemical mixtures: problems facing developing countries. Environ Health Prospect 2002; 110:901-9.
17. Hood E. New chlorpyrifos link? Environ Health Prospect 2005; 113:1-10.
18. 18,. Extoxnet. Extension toxicology network pesticide information profiles. Revised June 1996. file://I:\EXTOXNET%20-%20CHLORPYRIFOS.htm
19. Asmatullah, Mufti SA, Cheema AM, Iqbal J. Embryotoxocity and teratogenicity of malathion in mice. Punjab Univ J Zool 1993; 8:53-61.
20. Whyatt RM, Barr DB, Camann DE, Kinney PL, Barr JR, Andrews HF, et al. Contemporary use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspectives 2003; 111:749-55.
21. Cavieres MF, Jaeger J, Porter W. Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size. Environ Health Perspect 2002; 110:1081-5.