Pakistan J. Zool., vol. 44(6), pp. 1515-1519, 2012.

Exposure to β-Cyfluthrin During Pregnancy Induces Teratogenecity in Murine Foetuses

Naveed Ahmad,* Khadija Naveed and Asmatullah
Department of Zoology, University of the Punjab, Lahore, Pakistan

Abstract.- Teratogenecity of a pyrethroid insecticide, β-cyfluthrin was tested in developing foetuses of mice. For this purpose, different concentrations of insecticide i.e., 1.25, 2.50 and 5.00 µg/g body weight were prepared by dissolving it in sterilized distilled water in such a way that each 0.1 ml of the solution contains desired concentration. The doses were given orally on Day 6 of gestation and foetuses were recovered on Day 18 of gestation. Morphological studies of foetuses showed abnormalities including microcephaly, anophthalmia, micromelia, dysmorfogenesis, dysplasia and short tail. Morphometric studies of body weight, crown rump length, brain size, length and width of eye, length of both fore limbs and hind limbs and length of tail of foetuses showed significant ($P < 0.001$) differences against controls. The study indicates that the insecticide is potentially dangerous to developing murine fetuses, and suggests that it may be potentially harmful to foetal development in humans.

Key words: Teratogenecity, β-cyfluthrin, murine foetus.

INTRODUCTION

Pyrethroids are synthetic versions of naturally occurring pesticide pyrethrin, most are safer than the organochlorines, organophosphates and carbamates. They have been modified to increase their stability in the environment (Copping and Hewitt, 1998). Everyone is exposed to some level of pesticides but exposure to pregnant women generates considerable concerns as these chemicals are intentionally designed to damage the nervous system (Robert, 1987).

Developmental toxicity of pyrethroids cannot be denied because certain pyrethroids have been linked to developmental neurobehavioral problems, altered function of immune cells and possibly childhood leukemia. Pyrethroids promote growth in cancers of the reproductive organs including breast cancer and prostate cancer due to significant estroginicity (Frazier, 2007; Joan and Mary, 1998). The synthetic pyrethroids produce sperm abnormalities; affecting mouse sperm head morphology as well as clastogenic effects on root tip cells of *Allium cepa* (Bhunya and Pati, 1990; Kumar *et al*., 2004; Frazier, 2007).

The epidemiological studies show that there is an association between pyrethroids exposure to parents and the incidence of infertility, miscarriage, spontaneous abortion, low birth weight, small-for-gestational-age (SGA) birth, preterm delivery, a deficit of male children, fetal growth retardation and stillbirth. Parental employment in agriculture could increase the risk of congenital malformations in the offspring's, such as orofacial cleft, birthmarks in the form of hemangioma as well as musculoskeletal and nervous system defects. A slower pace of fetal development corresponding to SGA in the population of women exposed to pyrethroids has been recently reported (Hanke and Jurewicz, 2004; Frazier, 2007).

New born babies and children are often exposed to pyrethroids for long periods by the use of liquid vaporizers. Occupational and experimental studies indicate that pyrethroid inhalation during early prenatal/postnatal/perinatal life can cause clinical, biochemical and neurological changes, also exposure to pyrethroids during organogenesis and early developmental period is especially harmful (Sinha *et al*., 2004). There is an association between fetal death due to congenital abnormalities and pyrethroids, when exposure occurs during the 3rd to 8th weeks of pregnancy (Erin *et al*., 2001).

There are few studies on teratogenic effects of cyfluthrin which is a commonly used pyrethroid for pest control in the agricultural fields. It is used in

* Corresponding author naveedahmadzia@hotmail.com
0030-9923/2012/0006-1515 $ 8.00/0

the form of Baythroid, Baythroid H, Attatox, Contur, Laser, Responsar, Solfac, Tempo and Tempo H. (Meister, 1995).

The present study was aimed at determining the developmental toxicity of cyfluthrin.

MATERIALS AND METHODS

Four groups of pregnant mice (Swiss Webster strain of *Mus musculus*) were maintained in constructed conditions *e.g.,* 12 hours light/dark cycles, temperature of 25±1°C and relative humidity 40-55%. β-cyfluthrin was dissolved in water and 4 groups of pregnant mice (n = 15 per group) were administered orally, once after overnight fasting with 0.00, 1.25, 2.50 and 5.00 µg of β-cyfluthrin /g body weight at Day 6 of gestation. At day 18 of gestation, the mothers were weighed and given a cesarean section. Alive, dead/resorbed foetuses were dissected out of the uterus, fixed in Bouin's fixative for 48 hours after which they were stored in 70% ethanol. Fifteen fetuses were selected from each group for morphometric and morphological studies that involved measurement of wet weight, crown rump length, brain size, length and width of eye, lengths of both fore limbs and hind limbs and length of tail of each foetus.

RESULTS

All the foetuses in the control group were normal and a great uniformity was observed for in the fetuses regarding all the morphometric and morphological measurements (Tables I, II, Fig. 1). The data on morphometric observations showed significant differences (P<0.001) between fetuses whose mothers were exposed to 5.00 µg of β-cyfluthrin/g body weight and control groups. These differences were observed in parameters like body weight, crown rump length, brain size, lengths of eye, hind limbs and tail (Table II). Foetuses of this dose group had morphological abnormal of limbs (micromelia, dysmorfogenesis, dysplasia), brain (microcephaly), eye (anophthalmia) and tail (short

Table I.- Developmental anomalies in 18-day old foetuses recovered from pregnant mice administrated orally with different doses of β-Cyfluthrin (n = 15 per group) on day 6 of gestation.

β-Cyfluthrin (µg/g BW)	No. of malformed foetuses (%)	Brain defects (%)	Eye defects (%)	Limb defects (%)	Tail defects (%)
0.00 (controls)	(00.00)	(00.00)	(00.00)	(00.00)	(00.00)
1.25	(20.00)	(00.00)	(00.00)	Micromelia dysplasia (20.00)	(00.00)
2.50	(33.33)	Microcephaly (13.33)	(00.00)	Micromelia dysplasia (20.00)	(00.00)
5.00	(66.66)	Microcephaly (06.66)	Anophthalmia (06.66)	Micromelia dysplasia (46.66)	Short tail (06.66)

Table II.- Morphometric observations of 18-day old fetuses recovered from pregnant mice administrated orally with different concentrations of β-Cyfluthrin on day 6 of gestation.

Parameters	β-cyfluthrin (µg/g BW)			
	0.00	1.25	2.50	5.00
Body weight (mg±SE)	1385.33±37.55	1135.33±15.40***	1030.00±12.03c***	927.33±15.44d***
CR length (mm±SE)	21.7±0.37	21.6±0.54	19.7±0.31**	19.1±0.17***
Eye length (mm±SE)	2.93±0.05	2.7±0.07*	2.6±0.0.07**	2.45±0.06***
Eye width (mm±SE)	2.20±0.11	2.10±0.05	2.00±0.00b	1.90±0.05*
Brain size (mm±SE)	10.33±0.19	10.13±0.19	9.37±0.10***	9.20±0.11***
Fore limb size (mm±SE)	7.83±0.18	7.40±0.16	7.30±0.35	6.97±0.15*
Hind limb size (mm±SE)	8.50±0.12	8.37±0.17	7.77±0.23*	7.27±0.14***
Tail length (mm±SE)	10.93±0.18	10.50±0.17	10.40±0.17	9.83±0.16***

Asterisks show significant difference against controls; *= p<0.05, **= p<0.01, ***= p<0.001

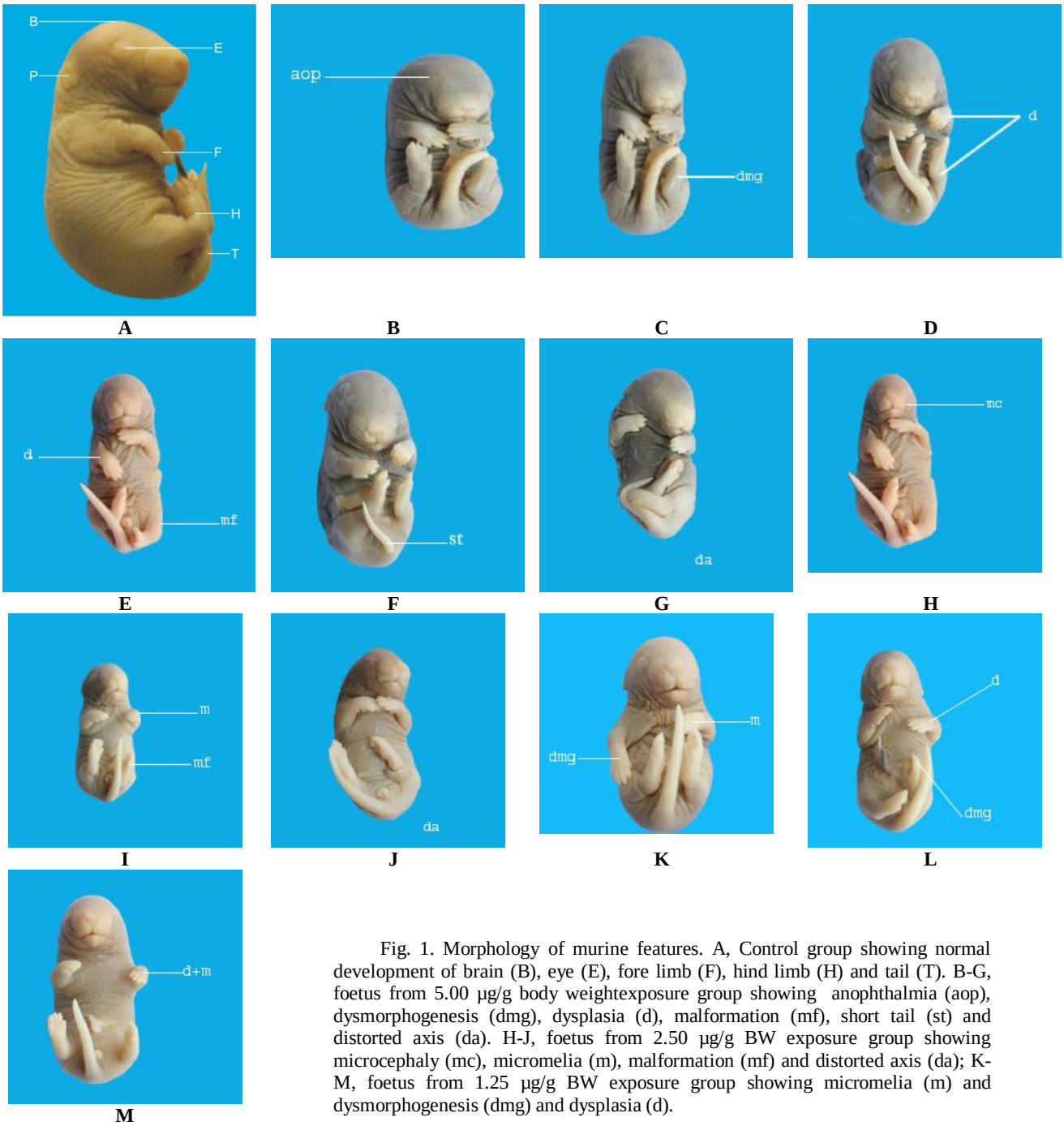

Fig. 1. Morphology of murine features. A, Control group showing normal development of brain (B), eye (E), fore limb (F), hind limb (H) and tail (T). B-G, foetus from 5.00 µg/g body weightexposure group showing anophthalmia (aop), dysmorphogenesis (dmg), dysplasia (d), malformation (mf), short tail (st) and distorted axis (da). H-J, foetus from 2.50 µg/g BW exposure group showing microcephaly (mc), micromelia (m), malformation (mf) and distorted axis (da); K-M, foetus from 1.25 µg/g BW exposure group showing micromelia (m) and dysmorphogenesis (dmg) and dysplasia (d).

tail) (Table I, Figs. 1B-G).

The foetuses from mothers exposed to 2.50 µg of β-cyfluthrin/g BW of mice, had significant different body weights and brain sizes as compared to the fetuses from mice in the control group (Table II). β-cyfluthrin at this dose induced similar type of limb and brain abnormalities as 5.00 µg of β-cyfluthrin/g BW. (Table I, Figs. 1H-J).

Even at the lowest dose of β-cyfluthrin (1.25 µg/g BW) the fetuses showed abnormal body

weights as compared to the controls and did show limb abnormalities. (Tables I, II, Figs. 1K-M).

DISCUSSION

The exposure of pregnant women to environmental toxins is of major concern because of their potential harm on the foetuses. The aim of this study was to determine the effects caused by fetal exposure to β-Cyfluthrin through mother.

The low toxicity to mammals and birds and limited soil persistence has encouraged widespread use of pyrethroids in agriculture as very potent agents against pests (Glickman and Lech, 1982). Infants of mothers performing heavy work inside greenhouse during pregnancy had lower mean birth weight than infants of mothers working out of greenhouse (Joanna *et al*., 2005). In the Hershberger assay, cyfluthrin causes significant decrease in the weight of seminal vesicles, ventral and dorsolateral prostate, LABC, Cowper's glands, though not significant in glans penis and other accessory sex tissues (Jun *et al*., 2008).

Cyfluthrin exerts great toxic effects on the growth, survival, and proper functioning of human astrocytes. By using microarray gene expression profiling, systematic identification and comparison of potential molecular targets of chlorpyrifos and cyfluthrin showed that these affect a similar number of transcripts. These targets include molecular chaperones, signal transducers, transcriptional regulators, transporters, and those involved in behavior and development. Further computational and biochemical analyses showed that cyfluthrin up-regulates certain targets of the interferon-γ and insulin-signaling pathways and increases the protein levels of activated extracellular signal–regulated kinase 1/2, a key component of insulin signaling; interleukin 6, a key inflammatory mediator; and glial fibrillary acidic protein, a marker of inflammatory astrocyte activation. So, inflammatory activation of astrocytes is an important mechanism underlying neurotoxicity of cyfluthrin (Sarah *et al*., 2006).

In another study, it was found that maternal exposure to synthetic pyrethroids in the first or second trimester was associated with a small but statistically significant decrease in birth weight (P=0.02). Mothers who reported involvement in field work had a similar pregnancy duration but delivered infants with a significantly higher birth weight than mothers not reporting such activities in the first or second trimester of pregnancy (P=0.04) (Wojciech *et al*., 2003).

Exposure to transfluthrin, cyfluthrin, and natural pyrethrum has a genotoxic effect on the epithelial cells of human nasal mucosa (Tisch *et al*., 2005). In pregnant rabbits, feeding of cyfluthrin causes both miscarriages and resorption of fetuses. In a three-generation study of rats, feeding of cyfluthrin caused pups to have "decreased viability" and decreased weight (U.S. EPA "Tox one-liners" 1989).

Cyfluthrin may also have more subtle effects on the ability of humans and other animals to reproduce. Synthetic pyrethroids, as well as the naturally occurring pyrethrins, to bind with androgen (a male sex hormone) receptors, and disrupt normal androgen function (Eil and Nisula, 1990). These kinds of effects, broadly called endocrine disruption, have been linked to a variety of health problems, including breast cancer, testicular cancer, and decreased sperm counts. The researchers "advise protection from any form of contact or ingestion of the pyrethroids in order to prevent any undesirable effects on the human reproductive system (Eil and Nisula, 1990).

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(Received 14 December 2011, revised 8 October 2012)