Some Studies on Histopathological and Cranio-Facial Structures in Mice on Co-Gestational Exposure of Bifenthrin

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ABSTRACT

Bifenthrin was tested for feto-toxicological manifestations on histology and skeletal anatomy in mice at four maternally sub-toxic doses 0.00, 6.00, 12.00 and 24.00 mg/kg body weight. Each group was further divided into single, (gestation day 6), double (gestation day 9 and 12), triple (gestation day 6, 9 and 12) and multiple (gestation day 6 to 12) subgroups (categories). Dams in 0.00 mg/kg body weight group were treated with vehicle (distilled water only). Fetuses were exteriorized on gestation day 18. Various histopathological and craniofacial abnormalities were observed in fetuses exposed to bifenthrin. The histological studies showed effects related to brain, lever, kidney, heart and lungs, like tissue necrosis of liver and lung, intestinal loops, poorly developed choroid plexus, renal dysplasia, hypoplasia in ventricular walls of heart and non-glandular stomach. Dose dependent decrease in skeletal ossification of cranio-facial, caudal and sacro-limbic regions were observed. Key words: Bifenthrin, Cranio-facial, Skeletal ossification, Histopathological studies

INTRODUCTION

For more than 30 years pyrethroids account for approximately one-fourth of the world insecticide market. These have been used in agricultural and home formulations. After dermal contacts that have led to acute pyrethroid poisonings there have been cases of accidental and occupational exposures (Casida & Quistad, 1998).

In agriculture, pyrethroids have been widely considered as ideal insecticides (Hossain et al., 2001). These are used as mosquito repellent in the form of liquid vaporizer, aerosol, mats and coils in our houses. These are also used to control ectoparasites on both farm animals and pets (Sinha et al., 2006).

From the stand point of mammalian toxicity, pyrethroids that were once known to be the safest insecticide group have now been fairly reported to be genotoxic (Patel et al., 2006), mutagenic, neurotoxic (Sayim et al., 2005), toxic for different aspects of endocrinology, behavioral modulators, reproduction along with their effects as aging enhancing substances and carcinogenic (WHO, 1989).

Srivastava et al. (2005) based on occupational users of bifenthrin developed a single, short-term epidemiologic study. Ten healthy males wearing protective gear sprayed 25 mg/m² of bifenthrin on interior walls six hours daily for five consecutive days. Prior to exposure and on days four and seven following exposure, biochemical and clinical tests were conducted. Tests including hepatic function, nerve conduction, electromyogram and lung function but no significant differences were found in pre and post exposure statistically. This study did not address potential impacts of longer term, chronic exposure but was strictly a short term study.

As very limited work has been carried on histopathological and cranio-facial aspects related to bifenthrin, the present research work is conducted on albino mice (Mus musculus) to obtain the embryotoxicological results for developing guidelines which may be considered for the future use of this insecticide to the human development especially in the country Pakistan.

MATERIALS & METHODS

Swiss Webster strain of albino laboratory mice, Mus musculus were kept under the standard protocol of 12-hour day and night (light/dark) cycles, in 12” x 18” steel cages with room temperature 25±2°C. Three females were caged with one male. For the identification of successful coitus, the females were carefully observed daily. Pregnant females were separated from the males with vaginal plug and that was considered day zero of gestation. Under the approved animal treatment conditions, Ethics Committee of University of the Punjab, Lahore, Pakistan, the above cited protocol was used. Bifenthrin under the brand name TALSTAR prepared by FMC United (Pvt.) Pakistan Ltd. was purchased from the local market. Different concentrations of bifenthrin were prepared through dilutions in water in such a way that each 0.1 ml of the solution contained the desired amount of insecticide.

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The pregnant females were divided into four groups and each group contained forty animals. Then each group was further divided into four different categories, such as single, double, triple and multiple. Doses of bifenthrin dissolved in distilled water containing 6.00 12.00 and 24.00 mg/kg were given to experimental animals. While the animals in vehicle treated (control) group received 0.1ml distilled water only.

The treated dams were anaesthetized with anaesthetic ‘Ether’ on the day 18 of gestation. By giving surgical incision to the anaesthetized dams, intact gravid uteri were dissected out carefully. After that uteri were opened along the inner curvature and developing fetuses were recovered. Then these fetuses were fixed in Bouin’s fixative for 48 hours (Carson, 1992) and then these fetuses were preserved in 70% alcohol (Carson 1992). The preserved fetuses were subjected to histological and skeletal observations.

For histological studies serial sections were prepared through routine histological procedure. Then the serial sections of kidney, brain, liver, heart, eyes and lungs were stained with the help of Ehrlich’s Hematoxylin stain (Ehrlich, 1886) and Eosin stain (Conn, 1930). With the help of Canada balsam (AFIP, 1968), these stained slides were made permanent and brain, spinal cord, eyes, liver, kidneys, lungs and heart were carefully examined for histopathological changes. Fetal skeletons were prepared by the process of Kawamura et al. (1990). For this method fetuses from each dose group, one from each litter size were selected randomly and then preserved in 95% ethanol. Through a small abdominal incision, these fetuses were eviscerated and thoraco-abdominal organs (heart, lung, liver, kidneys, stomach and intestines) were removed carefully. To remove the flesh for clear skeleton studies, the fetuses were placed in 1% KOH solution. Before preserving the stained specimens in 50% ethanolic glycerol for microscopic and macro photographic studies, these fetuses were also placed for about 30 minutes in Alizarin’s Red S (i.e. 1% aqueous solution made alkaline by adding 3-4 drops of 1% KOH solution) and then again in 1% KOH solution.

By placing the slides of serial sections (histopathological studies) and skeleton (skeletal studies) of fetuses under binocular microscope and placing a Panasonic Lumix TZ15 camera micro and macro photographs were taken.

The labelling of deformities was done in Corel Draw 9 and these labelled photographs were pasted in Microsoft Word file of describing and supporting results.

RESULTS

Histological studies through head and different regions were carried out to determine the histological defects. Well-developed right and left ventricles, diencephalon and mesencephalon, left lung, right lung, wall of right ventricle and wall of left ventricle were observed from selected section of vehicle control group (Fig. 5a & Fig. 5b). Sections from dose group 6.00mg/kg BW showed necrosis of liver, lung necrosis and degenerated trabecular zone of heart (Fig. 5c & Fig. 5d). The dose group 12.00mg/kg BW showed abnormalities, like poorly developed choroid plexus and herniated lateral ventricles (Fig. 5e), renal dysplasia, intestinal loops, necrosis of liver, lumen of stomach and prostate (Fig. 5f), middle lobe of lung, hypoplasia in ventricular wall of heart, atrophied body musculature, emphysema of lung and fibrosis of dorsal muscles (Fig. 5g). Histological section observed in dose group 24.00mg/kg BW showed defects, like herniated third and fourth ventricles and herniated lateral ventricle (Fig. 5h), hypoplasia of ventricular walls, middle lobe of lung and dorsal muscles (Fig. 5i).

Well ossified appendicular and axial skeletal i.e. nasal bone, mandible, radius, ulna, parietal bone, exoccipital bone, ribs, vertebral bodies (centra) of lumbar vertebrae, coccygeal vertebrae, right femur, tibia and fibula were seen in the control group (Fig. 6a). Reduced ossification in cranio-facial region was found in dose group 6.00mg/kg BW of bifenthrin (Fig. 6b). In 12.00mg/kg BW dose group of bifenthrin reduced ossification in caudal region (Fig. 6c) while in dose group 24.00mg/kg BW of bifenthrin reduced ossification in cranio-facial and sacro-limbic regions was seen (Fig. 6d).

Fig., 5a: Selected section of an 18 days old fetus recovered from the pregnant mother of control group before treatment. T. S. through cranial region. Note: right ventricle (Red Arrow-A), left ventricle (Blue Arrow-B), mesencephalon (Yellow Arrow-C) and diencephalon (Green Arrow-D).
Fig. 5b: Selected section of an 18 days old fetus recovered from the pregnant mother of control group before treatment. T. S. through lung and heart regions.

Note: Left lung (Red Arrow-A), right lung (Green Arrow-B), wall of right ventricle (Blue Arrow-C), and wall of left ventricle (Yellow Arrow-D).

Fig. 5c: Selected section from an 18 days old fetus recovered from the pregnant mother after treatment with 6.00 mg/kg BW (single) Bifenthrin. T.S. through hepatic region.

Note: Necrosis of liver tissue (Red Star-A).

Fig. 5d: Selected section of an 18 days old fetus recovered from the pregnant mother after treatment with 6.00 mg/kg BW (triple) Bifenthrin. T.S. through heart region.

Note: Lung necrosis (Blue Arrow-A), degeneration of trabecular zone of heart (Red Arrow-B), phalanges (Green Arrow-C).

Fig. 5e: Selected section of an 18 days old fetus recovered from the pregnant mother after treatment with 12.00 mg/kg BW (double) of Bifenthrin. T.S. through cranial region.

Note: Herniated lateral ventricle (Red Arrow-A) and poorly developed choroid plexus (Blue Arrow-B).

Fig. 5f: Selected section from an 18 days old fetus recovered from pregnant mother after treatment with 12.00 mg/kg BW (triple) of Bifenthrin. C.S. through kidney and liver regions.

Note: Renal dysplasia (Kd), intestinal loops (In), necrosis of liver (Lv), lumen of stomach (St) and prostate (Pr).

Fig. 5g: Selected section of an 18 days old fetus recovered from the pregnant mother after treatment with 12.00mg/kg BW (multiple) Bifenthrin. T.S. through hepatic and cardiac regions.

Note: Middle lobe of lung (Purple Arrow-A), fibrosis of dorsal muscles (Blue Arrow-B), hypoplasia in ventricular wall of heart (Green Arrow-C), atrophied body musculature (Red Star-D), emphysema of lung (Orange Arrow-E).
Fig. 5h: Selected section of an 18 days old fetus recovered from the pregnant mother after treatment with 24.00 mg/kg BW (triple) of Bifenthrin. T.S. through cranial region.

**Note:** Herniated fourth ventricle (HV4), Herniated third ventricle (HV3) and herniated lateral ventricle (HLV).

Fig. 5i: Selected section of an 18 days old fetus recovered from the pregnant mother after treatment with 24.00 mg/kg BW (multiple) of Bifenthrin. T.S. through heart region.

**Note:** Hypoplasia of ventricular walls (Green Arrow-A), middle lobe of lung (Yellow Arrow-B) and dorsal muscles (Purple Arrow-C).

Fig. 6a: A macrophotograph showing normal fetal skeletal ossification in vehicle control group.


Fig. 6b: A macrophotograph of fetal skeletal showing slightly reduced ossification in D-I (6.00 mg/kg BW of Bifenthrin).

**Note:** Reduced ossification in craniofacial region (A: reduced eye, B: reduced parietal bone, C: reduced exoccipital bone).

Fig. 6c: A macrophotograph of fetal skeletal showing slightly reduced ossification in D-II (12.00 mg/kg BW of Bifenthrin).

**Note:** Reduced ossification in caudal region (A: reduced vertebral bodies (centra) of coccygeal vertebrae).
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Fig., 6d: A macrophotograph of fetal skeletal showing slightly reduced ossification in D-III (24.00mg/kg BW of Bifenthrin).

Note: reduced ossification in craniofacial and sacro-limbic regions.

DISCUSSION

In target organisms, synthetic pyrethroids are known as neurotoxicants and that is their mode of action. When a nerve cell is excited in mammals, the mechanism of action of neurotoxicity of pyrethroids results from interference with the sodium gate. For hundreds of years, pyrethrins have been used as insecticides (EPA, 2012).

The present study was aimed to discover the toxicological effects of bifenthrin in relation with pregnancy and to report all sorts of developmental abnormalities at histopathological and skeletal levels but not much anomalies were found. However, necrosis of liver, kidney and lung were seen. In some cases ventricles of heart and poorly developed choroids plexus were also observed.

In an experiment on rats exposed to the concentration of bifenthrin no signs of toxicity were observed on kidneys, heart and liver (Peter & Yeboah – Gyan, 2011). When during a study the fish Oreochromits niloticus was given insecticide to test toxicity at histological level, it damaged the tissues particularly liver (Velisek et al., 2009). Bifenthrin is considered to cause liver necrosis in mice (Gammon et al., 2011).

Available information indicated that during some animal studies, decreased liver weights, congestions, hepatocellular hypertrophy and other microscopic signs of liver changes at intermediate and chronic oral exposure to pyrethrins or pyrethroids (bifenthrin, permethrin and deltamethrin) particularly at dose levels also resulting in histopathological signs of neurotoxicity (IRIS 2003). In a study by Schoenig (1995) decreased kidney weight and tubular degeneration in rats consuming pyrethrins (from pyrethrum extracts) in diet at concentrations resulting in dose levels 320mg/kg/day for 90 days were seen.

In an experiment neuro-developmental and neuro-degenerative diseases with chronic exposure to bifenthrin in rats were seen (Nandi et al., 2006). Herniated ventricles were found during this study. These ventricles were confirmed by Shepard & Lemire (1996) in a rat histological study.

Geiger (1986) investigated the bronchiolar-alveolar adenocarcinoma and adenoma in a chronic toxicity study in mice exposure to bifenthrin. In some experimental study Reed (1991) also showed the histopathology of liver and lung in mice fetus due to bifenthrin.

He et al. (1989) find out ventricular ectopics and conjunctival recesses due to exposure of pyrethroids (bifenthrin, deltamethrin and cypermethrin) in a study on mice.

During the present study the fetuses recovered from bifenthrin exposed showed reduced ossification. EPA (1999) reported the developmental toxicity in rats, in which there was decreased fetal body weight and increased incomplete ossification in selected bones. At 5mg/kg/day of bifenthrin, the developmental toxicity based on delayed ossification of the forelimb and hindlimb of digits was seen.

CONCLUSION

On the basis of these findings, it is concluded that oral exposure of bifenthrin is teratogenic and embryotoxic in pregnant mice especially when given at the time of organogenesis even on dose level as low as 6.00mg/kg/day. Bifenthrin damages the vital organs like kidneys, eyes, liver, lungs and nervous system at histopathological level. Furthermore, it also reduces the skeletal and cranio-facial ossification.

The present observations give a strong caution to use this insecticide under a strict control. Furthermore, as the use of bifenthrin in Pakistan is common as insecticide, the children and pregnant women must kept away from this insecticide. Finally the insecticide marketers should label these cautions prominently on the packaging of the insecticide.

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REFERENCES


