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RESEARCH ARTICLE

THE TERATOGENIC EFFECTS OF THE INSECTICIDE PYRIPROXYFEN ON THE DEVELOPING CHICK EMBRYOS

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ABSTRACT

Pyriproxyfen (PPF) is a juvenile hormone analogue insecticide that is often used as a larvicide against a wide range of insect pests. The present study was designed to investigate its teratogenic effects on the embryonic development of the chick. First, the median lethal dose (LD_{50}) of the commercial PPF was determined. Accordingly, three sub-lethal doses (15, 30, 45 µg/egg) were selected for injection into the air space of the eggs after 24 hours of incubation. The eggs were opened on the embryonic days (EDs) 7 and 14, and the embryos were examined for morphometric changes and the presence of malformations. The PPF treatment induced growth retardation and reduction in head and eye sizes as reflected by a reduction in wet body weights, crown-rump lengths, anterior-posterior head lengths, and eye diameters. These morphometric alternations were mild or moderate on ED7, but more apparent on ED14. Also, significant reductions in the lengths of the forelimb and hindlimb parts were recorded with high-dose treatment on ED14. Obviously, there was high percentage of malformations among ED7 individuals in the form of hematoma, ventral body wall defect, limb deformities, microphthalmia, and microcephaly. However, on ED14 the embryos exhibited significant hematoma, microcephaly, delay feather, delay beak, microphthalmia in all treated groups, and limb deformities in the group that received the high dose. Other abnormalities included edema, anophthalmia, short neck, short tail, caudal regression, microtia, and microblepharon were also recorded. These findings revealed that PPF has potentially teratogenic effects on the development of the chick embryos.

INTRODUCTION

The widespread utilization of pesticides is a prevalent practice noticed globally. Pesticides are currently causing significant concerns regarding the potential health effects associated with exposure to farmers in treated fields, as well as the general population's exposure to residues in food and drinking water. Despite the manufacturing and distribution of specific hazardous pesticides have been prohibited, other pesticides are still widely employed in many countries, without a complete understanding of their potential adverse consequences on ecosystems and people's health[1,2].

Pyriproxyfen (PPF) is one of third-generation insecticides, the insect growth regulators family. It belongs to juvenile hormone analogues category. It is a pyridine based insecticide with chemical name 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether and chemical formula $C_{20}H_{19}NO_3$. It was first synthesized and manufactured by Sumitomo Chemical Co, Ltd. (Tokyo, Japan) in the 1990's. It can be found in more than 300 registered pesticide products, which are used indoors and outdoors[3,4]. It is frequently employed as a larvicide against a variety of insect pests in household, agricultural, horticulture, public health, and veterinary care. It is also one of the insecticides suggested by the World Health Organization to treat drinking water sources against disease-carrying insects like mosquitoes, with a recommended dosage of 0.01 g/L[3,5,6]. Due to their selective mechanism of action on insect physiology, insect growth regulators like PPF were thought to be harmless to other organisms[7]. However, several previous studies have documented the potential toxicity of PPF to non-target invertebrates, including *Daphnia* and *Artemia*[8-11], as well as crab and shrimp species[12]. Additionally, PPF was found to exhibit toxic effects on various vertebrates, such as fish[13-16], amphibians[17], and mammals[18-20]. In the past decade, PPF has gotten a lot of attention because of strong claims that the widespread use of it may be the cause or contributing factor in the increased frequency of newborn microcephaly observed in Brazil. This is due to its use in drinking water supplies since 2014 to combat the Zika virus vector *Aedes aegypti*[21,22]. Hence, this prompted researchers to investigate the embryotoxicity and developmental toxicity of PPF in vertebrates.

To date, few studies regarding the developmental toxicity of PPF on vertebrates have been evaluated including zebra fish[23-26], amphibians[27], mammals[28], and also a study concerning its neurotoxic effects on developing chick embryos[29]. Therefore, the present study was proposed to investigate the possible embryotoxicity and teratogenic effects of PPF on morphological and morphometric parameters of developing chick embryos on the embryonic days (EDs) 7 and 14 using available PPF commercial formulations that employed in agricultural practices within Egypt.

MATERIAL AND METHODS

Insecticide

The PPF insecticide used in the present study was a commercially available formulation under the trade name Proximo 10% EC, which was marketed by Agrimatco Egypt (Giza) and manufactured by Afraza Company (Valencia, Spain). It is composed of 10% weight/volume pyriproxyfen as an active ingredient.

Egg incubation

Fresh fertilized brown chicken eggs (average weight of about 66 g) were used in the present study under the ethical approval of Suez University committee number 281122. The eggs were weighed, cleaned with a cotton pad moistened with a 70% ethanol solution to eliminate any external contamination, and marked according to their groups. The eggs were then incubated with their broad end in an automatic incubator at 37°C and 55%-60% relative humidity until the desired stages of development (24 hours, EDs 7 and 14).

PPF administration

Pyriproxyfen was applied into egg air space in a volume of 40 µL/egg after 24 hours of incubation through a hole using an insulin syringe. Immediately after the PPF injection, the hole was sealed with melted paraffin wax, and the eggs were re-incubated.

Determination of the PPF LD_{50}

A total of 96 fertilized eggs (after incubation for 24 hours) were used to evaluate the mortality and LD_{50} of PPF. Different dilutions of PPF insecticide were made in corn oil (10, 20, 30, 40, 50, and 60 µg PPF/egg). Eggs were divided into 6 groups

(16 eggs each). The eggs were re-incubated for 24 hours following PPF administration. The eggs were then opened, and the mortality rate of the embryos was recorded. The PPF LD_{50} was calculated according to the Reed and Muench method[30]. The doses selected for the present study were 1/4, 1/2, 3/4 of LD_{50}.

Experimental design

To study the developmental toxicity and teratogenicity of PPF, 320 fertilized eggs were marked, numbered, and divided into ten groups (5 groups for ED7 and 5 groups for ED14, each group had 32 eggs) as follows: fertilized eggs without any treatment (the negative control group); fertilized eggs received corn oil (the vehicle control group); and fertilized eggs received low (15 µg/egg, 1/4 LD_{50}), mid (30 µg/egg, 1/2 LD_{50}), or high (45 µg/egg, 3/4 LD_{50}) does of PPF (the G1, G2 and G3 PPF-treated groups, respectively). The eggs of all groups were re-incubated till EDs 7 or 14.

Morphometric evaluation of body parts

On EDs 7 and 14, the eggs were taken out of the incubator and opened, and the embryos were removed and dissected free of the extraembryonic membranes. All surviving embryos from both the control and treatment groups were weighed and measured to determine their wet body (WB) weights and morphometric data. The eye diameter and crown-rump (C-R) length, as well as the anterior-posterior (A-P) length of the head, were all measured on ED 7. On ED 14, the C-R length, A-P dorsal head length, eye diameter, beak length, humerus, radius, ulna, metacarpus, femur, tibia, fibula, and metatarsus were measured. The venire caliper was used for all measurements.

Examination of external malformations

The number of live, normal and abnormal embryos was recorded. The embryos of the control and treated groups were examined by stereomicroscope, and external malformations were observed carefully and recorded for all embryos. The morphological abnormalities that were recorded included: **(a)** general hematoma (patches of blood under skin) occurred in different body parts, **(b)** edema (abnormal accumulation of fluids beneath skin), **(c)** head region malformations: microcephaly (MIC, small size of head and brain), exencephaly (exposure of brain outside the skull), microphthalmia (MIO, reduced size of eye), anophthalmia (absence of eye), microblepharon (shortening of eye lids), microtia (poorly developed and reduced external auditory meatus), and beak defects (delay of beak, absence of beak), (**d)** short neck region, **(e)** ventral body wall defects (VBWDs) included: thoracic ectopia cordis (malformations in which heart is located partially or completely outside the thoracic cavity through sternal defect), abdominal ectopia cordis (the heart is displaced into abdomen through diaphragmmatic defect), omphalocele (an abdominal wall defect characterized by the absence of abdominal muscles and skin, and abdominal wall covering is replaced by membrane), and gastroschisis (an abdominal wall defect characterized by the absence of abdominal muscles and skin in which parts of internal organs extend outside of the abdomen through the abdominal wall), **(f)** limbs deformities involved: meromelia (absence of a part of limbs), micromyelia (short limbs), brachydactyly (short digits), hypodactyly (losing of one or more digits of feet), clinodactyly (curled digits), simple syndactyly (jointed digit by soft tissue), and bowleg syndrome (a condition where one or both legs curve outward), and **(g)** tail defects included: shortness of tail and caudal regression syndrome (CRS, absence of tail).

Statistical analysis

Data was statistically analyzed using Prism 7 software (Graph pad, San Diego, CA, USA). The data of morphometric parameters were expressed as mean ± standard error and statistically significant difference between groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Fisher's exact test was used for

statistical analysis of malformation rate and types of malformation between groups.

RESULTS

The present study demonstrated that there was no statistically important difference between the negative and vehicle control groups. Consequently, the statistical analysis was performed by comparing the data from the treated groups to those of the negative control group.

PPF LD_{50} and the effect of PPF doses on the mortality (%) of chick embryos

The calculated LD_{50} that induced 50% mortality rate was 60 µg PPF/egg. Moreover, the embryos that were administered doses of 10, 20, or 30 µg PPF/egg exhibited mortality rates of 3.85%, 26.92%, and 34.62% within 24 hours after PPF injection, respectively. The death rates generated by doses of 40 and 50 µg PPF/egg were found to be 38.46% and 46.15%, respectively (Figure 1). The 1/4, 1/2, and 3/4 fractions of the PPF-LD_{50} corresponded to doses of 15, 30, and 45 µg, respectively.

Effect of PPF doses on the morphometric analysis of chick embryos

The 7-day-old chick embryos administered the mid dose (G2) and the high dose (G3) of PPF exhibited a significant decrease (P<0.05) in C-R length compared with the negative control group; whereas, no other significant changes were found in the morphometric analysis of PPF-treated 7-day-old chick embryos (Table 1). The 14-day-old chick embryos administered the low dose of PPF (G1) resulted in a significant reduction (P<0.05-0.0001) in WB weight, C-R length, A-P head length, beak length, humerus length, and metatarsus length compared with the negative control group (Table 2). However, the 14-day-old chick embryos administered the mid dose of PPF (G2) recorded a highly significant reduction (P<0.0001) in A-P head length only compared with the negative control group (Table 2). Whereas, the 14-day-old chick embryos administered the high dose of PPF (G3) showed a significant reduction (P<0.01-0.0001) in WB weight, C-R length, A-P head length, right eye diameter, left eye diameter, humerus length, radius and ulna length, femur length, fibula and tibia length, and metatarsus length compared with the negative control group (Table 2).

Effect of PPF doses on the malformation of chick embryos

On ED7, the highest malformation rate (100%) was seen in the mid-dose group

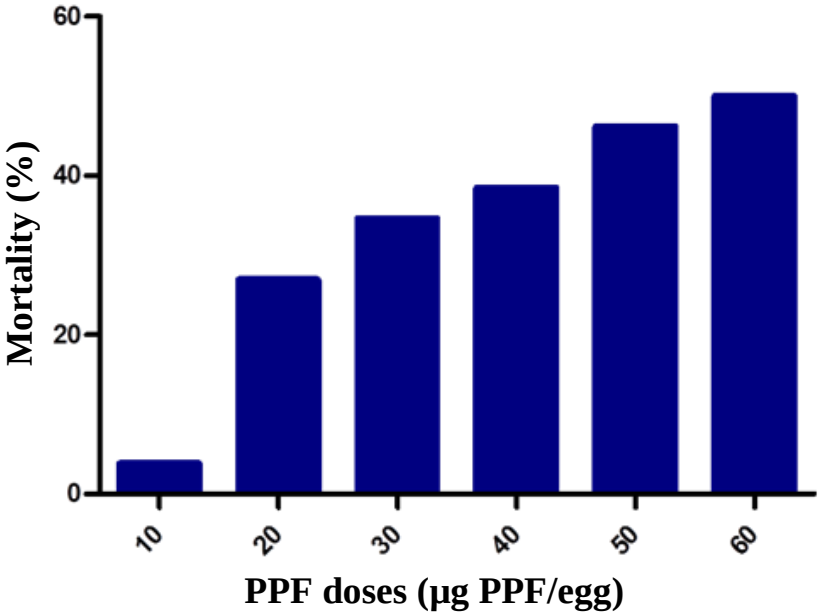

Figure 1: Effect of pyriproxyfen (PPF) doses on the mortality (%) of chick embryos.

Table 1: Body weight and morphometric measurements of the control and pyriproxyfen (PPF)-treated 7-day-old chick embryos.

	Egg groups				
	Negative Control	Vehicle Control	G1	G2	G3
Wet body weight	0.99±0.01	0.94±0.02	0.87±0.04	0.89±0.02	0.91±0.03
C-R length	23.45±0.26	23.42±0.40	22.71±0.60	21.45±0.45*	21.48±0.37*
A-P head length	10.93±0.19	10.96±0.35	9.99±0.61	9.26±0.55	10.29±0.21
Right eye diameter	6.14±0.07	6.10±0.07	5.48±0.36	5.69±0.08	5.82±0.05
Left eye diameter	6.13±0.06	6.10±0.06	5.59±0.35	5.72±0.09	5.35±0.29

The data are means ± standard error. $^{*}P<0.05$ (one-way ANOVA) compared to the negative control group.

Table 2: Body weight and morphometric measurements of the control and pyriproxyfen (PPF)-treated 14-day-old chick embryos.

	Egg groups				
	Negative Control	Vehicle Control	G1	G2	G3
Wet body weight	14.61±0.28	14.25±0.23	12.53±0.68**	13.26±0.31	11.38±0.65****
C-R length	62.82±0.51	62.24±0.57	57.61±1.59*	59.82±0.50	55.03±1.97***
Beak length	9.19±0.14	9.16±0.12	8.00±0.40**	8.64±0.17	8.39±0.21
A-P head length	23.49±0.17	23.17±0.27	20.99±0.44****	21.35±0.21****	19.83±0.29****
Right eye diameter	10.47±0.14	10.41±0.11	9.78±0.18	10.11±0.14	8.55±0.79***
Left eye diameter	10.49±0.14	10.39±0.11	9.64±0.22	10.14±0.14	8.59±0.81***
Humerus length	10.16±0.19	10.10±0.16	8.89±0.24***	9.44±0.23	8.70±0.29***
Radius and ulna length	10.60±0.16	10.47±0.13	10.10±0.32	10.66±0.14	9.57±0.25**
Metacarpus length	8.74±0.18	8.50±0.15	8.07±0.30	8.55±0.19	8.00±0.36
Femur length	11.45±0.31	10.92±0.21	10.56±0.36	10.73±0.17	9.45±0.48***
Fibula and tibia length	14.83±0.32	14.20±0.34	13.46±0.34	14.40±0.19	12.51±0.57***
Metatarsus length	11.95±0.30	11.49±0.25	10.07±0.43**	10.81±0.20	9.51±0.53****

The data are means ± standard error. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ (one-way ANOVA) compared to the negative control group.

of PPF (G2), followed by the high-dose group of PPF (G3, 94.12%), and the low-dose group of PPF (G1, 90.48%). Increased malformations were observed across all three groups (P<0.0001, *versus* the negative control group; Table 3). On ED14, all three PPF-treated groups (G1-G3) had a similarly highly significant malformation rate (100%; P<0.0001, *versus* the negative control group; Table 3).

Table 3: Malformation rates in the control and pyriproxyfen (PPF)-treated groups at embryonic days (EDs) 7 and 14.

	ED7		ED14	
	Number of examined embryos	Malformed embryos (%)	Number of examined embryos	Malformed embryos (%)
Negative Control	31	3.125	30	3.33
Vehicle Control	30	6.25	30	6.67
G1	21	90.48****	15	100****
G2	18	100****	18	100****
G3	17	94.12****	12	100****

****P<0.0001 (Fisher's exact test) compared to the negative control group.

On ED7, the most frequent and significant (P<0.05-0.0001) malformations in the PPF low dose-treated group (G1) were in the following order: hematomas formation > VBWDs (comprised ectopia cordis and completely opened ventral body wall) > limb deformities (consisted of disorientations of limbs, and bowleg syndrome on both hindlimbs), > MIO = short tail > MIC > absence of beak compared with the negative control group (Table 4 and Figure 2B,C). However, the most frequent and significant (P<0.05-0.0001) malformations in the PPF mid dose-treated group (G2) were in the following order: hematoma formations > limb deformities (constituted of limb disorientation, meromelia, and bowleg syndrome in left hindlimb) = VBWDs (comprised ectopia cordis and completely opened ventral body wall) > MIO > MIC > caudal regression compared with the negative control group (Table 4 and Figure 2D,E). Whereas, the most frequent and significant (P<0.05-0.0001) malformations in the PPF high dose-treated group (G3) were in the following order: hematoma formations > limb deformities (represented by limb disorientation, micromyelia, and bowleg syndrome in left hindlimb, and bowleg syndrome in both hindlimbs) > MIO > VBWDs (comprised ectopia cordis, completely opened ventral body wall, gastroschisis, and thin abdominal wall) > MIC > short tail > caudal regression compared with the negative control group (Table 4 and Figure 2F,G).

On ED14, the most frequent and significant (P<0.05-0.0001) malformations in the PPF low dose-treated group (G1) were in the following order: hematomas formation > MIC = delay of feather development > delay in beak development = MIO > limb deformities (represented by meromelia, micromyelia, clinodactyly, brachydactyly, hypodactyly, and simple syndactyly in limb parts and digits) > microtia = microblepharon = edema > VBWDs (included abdominal ectopia cordis accompanied with gastroschisis and abdominal ectopia cordis accompanied with omphalocele) compared with the negative control group (Table 5 and Figures 3B,C and 4B). However, the most frequent and significant (P<0.05-0.0001) malformations in the PPF mid dose-treated group (G2) were in the following order: hematoma formation > MIC > delay feather

Table 4: Frequency of malformations in living embryos of the control and pyriproxyfen (PPF)-treated groups at embryonic day 7.

	Egg groups				
	Negative Control	Vehicle Control	G1	G2	G3
Hematomas formation	3.22	6.67	84.21****	94.44****	100****
Limb deformities	0.00	0.00	57.89****	77.78****	81.25****
Ventral body wall defect	0.00	0.00	63.16****	77.78****	50***
Microphthalmia	0.00	0.00	36.84***	66.66****	68.75****
Microcephaly	0.00	0.00	26.32**	44.44****	43.75***
Short tail	0.00	0.00	36.84***	5.56	31.25**
Caudal regression	0.00	0.00	10.53	16. 67*	18.75*
Delay beak	0.00	0.00	10.53	11.11	12.5
Short neck	0.00	0.00	10.53	5.56	12.5
Exencephaly	0.00	0.00	10.53	0.00	6.25
Anophthalmia	0.00	0.00	10.53	0.00	6.25
Absence of beak	0.00	0.00	15.79*	0.00	0.00
Edema	0.00	0.00	0.00	5.56	6.25

$^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, $^{****}P$<0.0001 (Fisher's exact test) compared to the negative control group.

> delay beak, MIO = limb deformities (included micromyelia and brachydactyly) > microtia, = microblepharon compared with the negative control group (Table 5 and Figures 3D and 4C). Whereas, the most frequent and significant (P<0.05-0.0001) malformations in the PPF high dose-treated group (G3) were in the following order: hematoma formation = MIC = delay of feather development > MIO > limb deformities (represented by micromyelia, brachydactyly, and clinodactyly) > delay in beak development > microtia > caudal regression compared with the negative control group (Table 5 and Figures 3E,F and 4D).

DISCUSSION

The early stages of embryonic development are very sensitive to environmental toxins, and exposure to pesticides during this critical early life period may increase the chance of developmental abnormalities[26,31]. The current study investigated the possible embryotoxicity and teratogenicity of PPF insecticide in chick embryos by measuring the body morphometrics and external malformations, as well as determining the PPF LD_{50}. The present study demonstrated that administration of PPF resulted in growth retardation in both EDs 7 and 14, with moderate effects observed on ED7 as evidenced by a significant reduction in C-R length in G2 and G3 groups. On the other hand, growth retardation was more pronounced on ED14, as reflected by a significant reduction in WB weight and C-R length in G1 and G3 groups. Other scientists reported a significant decrease in weight and size of PPF-treated mice pups with a significant decrease in body weight gain of the PPF-injected pregnant female mice groups[28]. Also, Luckmann, *et al.*[29] authenticated a significant reduction in the

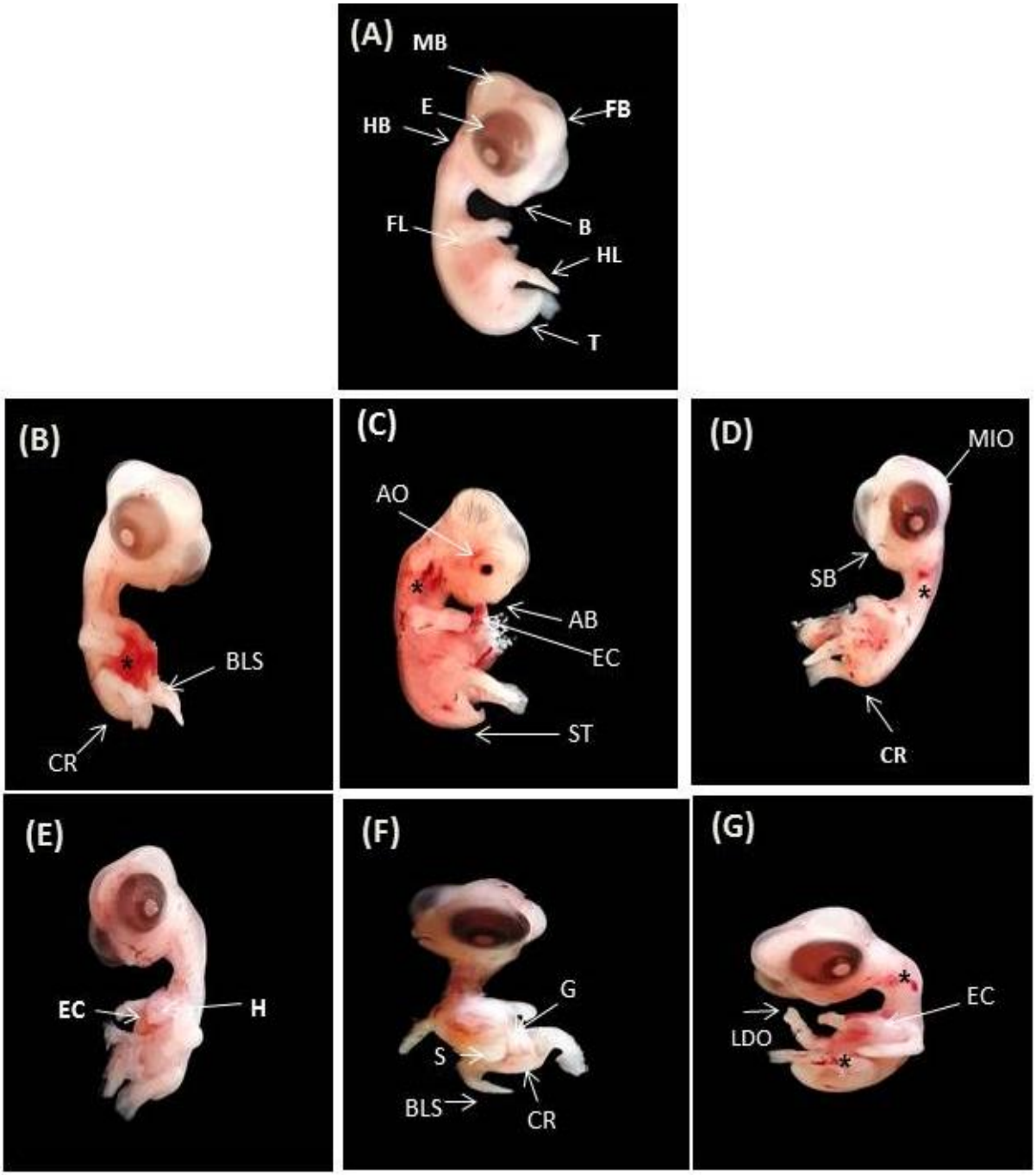

Figure 2: Photographs of 7-day old chick embryos of the control and pyriproxyfen (PPF)-treated groups. **(A)** A control embryo. **(B)** PPF low dose-treated embryo showing hematoma, caudal regression, and bowleg syndrome. **(C)** PPF low dose-treated embryo showing growth retardation, hematoma, microcephaly, anophthalmia, ectopia cordis, short tail, and absence of beak. **(D)** PPF mid dose-treated embryo showing hematoma, growth retardation, microcephaly, microphthalmia, short beak, and caudal regression. **(E)** PPF mid dose-treated embryo showing ectopia cordis. **(F)** PPF high dose-treated embryo showing growth retardation, hematoma, gastroschisis, bowleg syndrome, and caudal regression. **(G)** PPF high dose-treated embryo showing ectopia cordis and limb disorientation. MB: mid-brain; FB: forebrain; HB: hindbrain; E: eye; B: beak; FL: forelimb; HL: hindlimb; T: tail; H: heart; S: stomach; (*****) hematoma; CR: caudal regression; BLS: bowleg syndrome; AO: anophthalmia; AB: absence of beak; EC: ectopia cordis; ST: short tail; MO: microphthalmia; SB: short beak; G: gastroschisis; LDO: limb disorientation.

Table 5: Frequency of malformations in living embryos of the control and pyriproxyfen (PPF)-treated groups at embryonic day 14.

	Egg groups				
	Negative Control	Vehicle Control	G1	G2	G3
Hematomas formation	3.33	3.33	86.67****	100****	100****
Microcephaly	0.00	3.33	73.33****	88.89****	100****
Delay feather	0.00	0.00	73.33****	77.78****	100****
Delay beak	0.00	0.00	53.33****	55.56****	58.33****
Microphthalmia	0.00	0.00	53.33****	22.22*	75****
Limb deformities	0.00	0.00	33.33**	22.22*	66.67****
Microtia	0.00	0.00	26.67**	16.67*	41.67***
Microblepharon	0.00	0.00	26.67**	16.67*	16.67
Edema	0.00	0.00	26.67**	5.56	8.33
Ventral body wall defect	0.00	0.00	20.00*	5.56	16.67
Short neck	0.00	0.00	13.33	0.00	8.33
Caudal regression	0.00	0.00	6.67	0.00	25.00*
Anophthalmia	0.00	0.00	0.00	0.00	8.33

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ (Fisher's exact test) compared to the negative control group.

body mass of chick embryos exposed to PPF at a concentration of 10 mg/L (5 µg/egg) without differences in the body length of treated embryos. Moreover, da Silva *et al.*[14] showed that the exposure of Nile tilapia fish (*Oreochromis niloticus*) to high concentration of PPF-commercial formulation impaired growth rate and reduced body weight gain. Likewise, the exposure of male mice to PPF resulted in a significant decrease in their weights[20]. Therefore, as indicated above, it appears that the decrease in body weight is a common impact of PPF. PPF has been shown to disrupt thyroid hormone activity in zebra-fish and amphibians during their early stages of development[27,32]. The thyroid hormones have been documented to be one of the factors controlling muscle mass, and their alterations may potentially contribute to the development of muscular atrophy[33]. These effects may explain why PPF causes a loss of body weight, or they may be secondary effects of the toxicity due to reduction in feeding capacity[34].

Furthermore, the current morphometric data indicated that PPF also induced a reduction in head size, as shown by the significant decrease in the A-P head length of chick embryos by all PPF doses on ED14. Luckmann *et al.*[29] also reported a significant reduction in external measurements of the head and brain in PPF-treated chick embryos. Additionally, there was a significant dimension in the eye diameter of embryos in the G3 group only on ED14. On the other hand, Dzieciolowska *et al.*[24] did not observe any changes in the eye size of zebrafish embryos when subjected to various concentrations of PPF. This finding could perhaps be attributed to dissimilarities in the sensitivity and response of the embryos from the two species when exposed to PPF. The limb

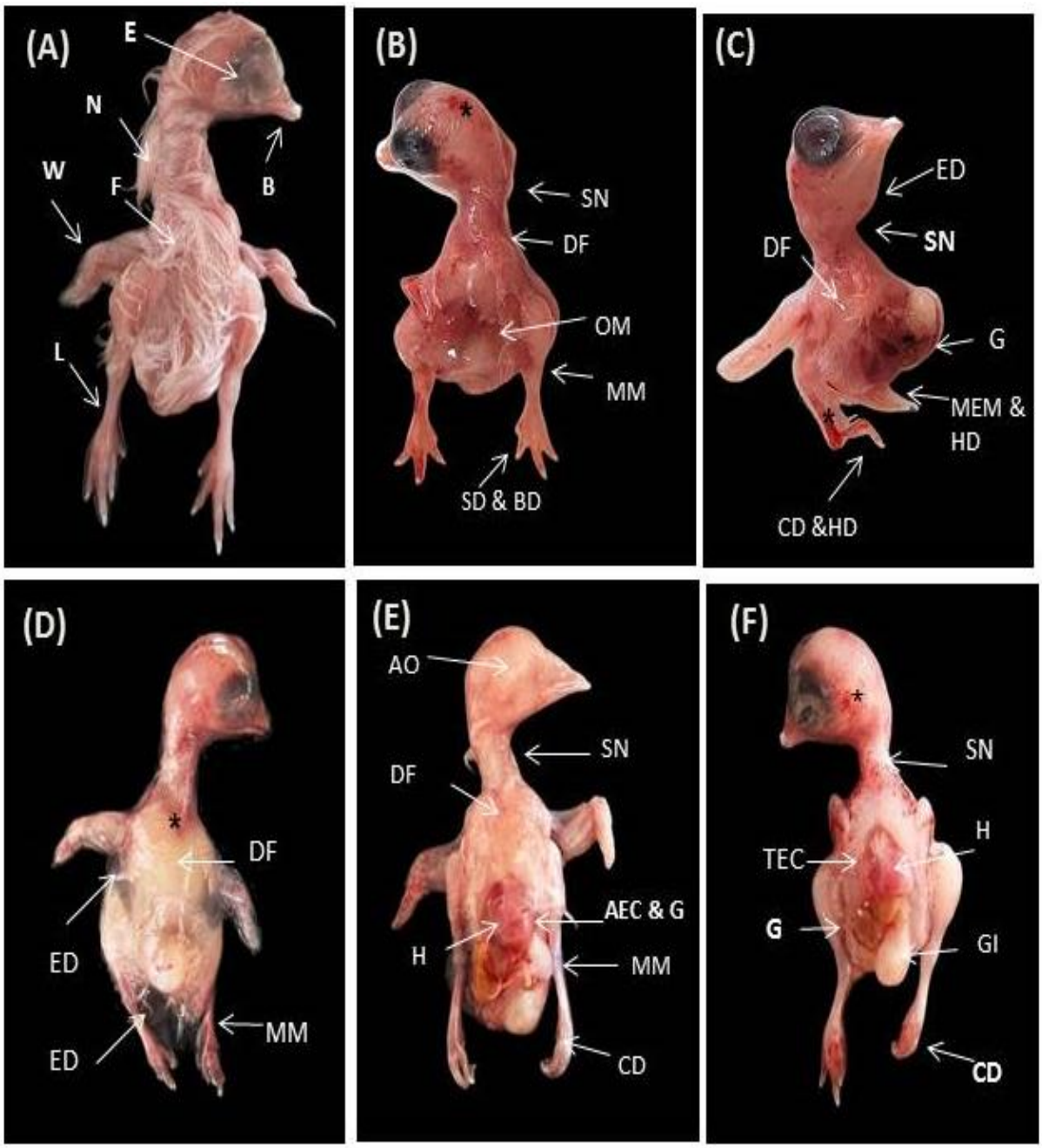

Figure 3: Photographs of 14-day old chick embryos of the control and pyriproxyfen (PPF)-treated groups. **(A)** A control embryo. **(B)** PPF low dose-treated embryo showing hematoma, growth retardation, short neck, delay feather, omphalocele, micromyelia, syndactyly, and brachydactyly. **(C)** PPF low dose-treated embryo showing growth retardation, hematoma, edema, short neck, gastroschisis, meromelia, hypodactyly, and clinodactyly. **(D)** PPF mid dose-treated embryo showing hematoma, growth retardation, edema, delay in feather development, and micromyelia. **(E)** PPF high dose-treated embryo showing growth retardation, anophthalmia, short neck, delay feather, abdominal ectopia cordis and gastroschisis, micromyelia, and clinodactyly. **(F)** PPF high dose-treated embryo showing growth retardation, hematoma, thoracic ectopia cordis and gastroschisis, and clinodactyly. E: eye; B: beak; N: neck; F: feather; W: wing,; L: leg; (*****) hematoma; SN: short neck; ED: edema; DF: delay feather; AO: anophthalmia; OM: omphalocele G: gastroschisis; TCH: thoracic ectopia cordis; AEC: abdominal ectopia cordis; H: heart; GI: gizzard; MEM: meromelia; CD: clinodactyly; HD: hypodactyly; SD: syndactyly; BD: brachydactyly.

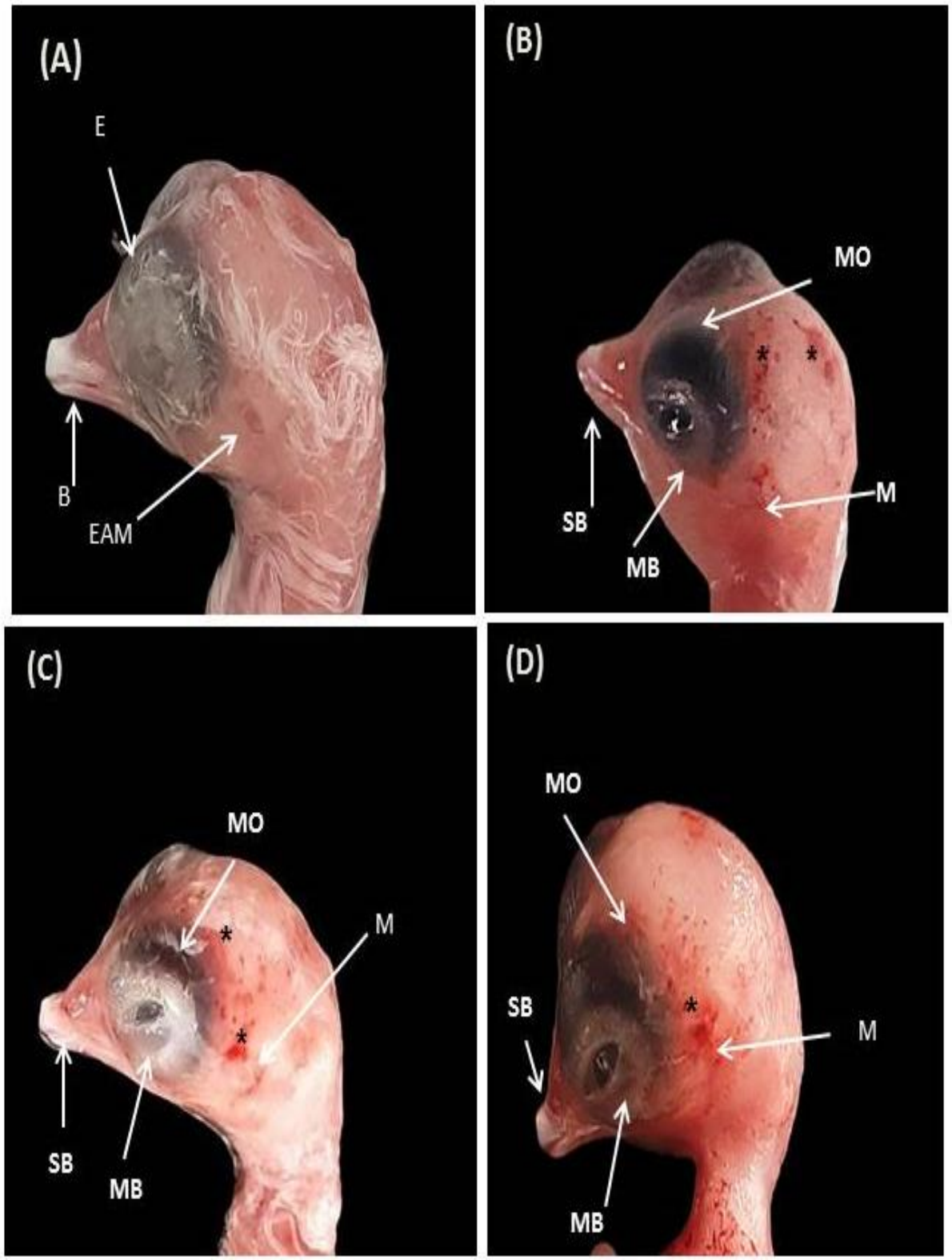

Figure 4: Photographs of the head and neck region of 14-day chick embryo of the control and pyriproxyfen (PPF)-treated groups. **(A)** A head of the control embryo showing a normally developed eye and external auditory meatus. **(B-D)** Embryos treated with low, mid, and high dose of PPF, respectively, showing microcephaly, hematoma, microphthalmia, microblepharon, short beak, and microta (M). E: eye, B: beak; EAM: external auditory meatus; (*****) hematoma; MO: microphthalmia; MB: microblepharon; SB: short beak; M: microtia.

measurements illustrated that the effect was more obvious at high dose on ED14; there was a significant difference in the length of forelimb parts (humerus, and radius and ulna), and in the length of all hindlimb parts (femur, fibula and tibia, metatarsus), in comparison to the negative control group. This was followed by limb length of PPF low dose on ED14, which displayed a significant difference in humerus length of forelimbs and metatarsus of hindlimbs. According to Horie *et al*.[32], this could be attributable to pyriproxyfen's inhibitory action on growth hormone. As previously stated, growth hormone deficiency severely restricts both bone growth and remodeling processes[35].

According to the results of the current investigation, there was a statistically significant increase in the number of chick embryos with malformations on ED7 and ED14 across all three test doses. These findings imply a potential teratogenicity of PPF in developing chick embryos. The predominant types of malformations observed by all PPF doses on ED7 were hematoma, limb deformities, VBWD, MIO, and MIC. On ED14, the highest percentage of malformations across all groups was attributed to hematoma, MIC, delay feather, delay beak, MIO, limb deformities, microtia, and microblepharon. Other studies demonstrated that the exposure of zebrafish embryos to PPF induced teratogenic effects including edema formation, body curvature, craniofacial defects, and abnormal snout and jaw[23,25,26]. Also, in a study conducted by Shahid and Saher[28], it was observed that prenatal mouse pups exposed to PPF had physical defects, including the absence of fur and limb malformations. Moreover, Parens *et al*.[22] documented Sumitomo toxicological results of PPF that revealed low brain mass and one case of microcephaly.

The potential teratogenic effects of PPF on the developing chick embryo may be associated with its capacity to bind to retinoic acid receptors, thereby interfering with the retinoic acid pathway[36]. This pathway is crucial for the proliferation, development, and differentiation of cells. Consequently, such interference can result in the occurrence of malformations or abnormal development in various organs, including the eye, brain, jaw, heart, and limbs[22,37]. Also, prior studies have shown that PPF inhibited the enzyme acetylcholinesterase, and this suppression has the potential to have serious consequences for a number of biological functions including blood pressure, heart rate, respiration, and feeding. Defects in muscle fiber production and innervation were also observed in zebrafish embryos exposed to acetylcholinesterase suppression during embryonic development[26,38]. In addition, previous PPF-toxicity investigations have shown that PPF acts as an oxidative stress inducer, as indicated by an increase in reactive oxygen species and lipid peroxidation, as well as a decrease in glutathione level[26,27]. Researchers have found that oxidative stress is the primary mechanism by which certain pesticides manifest their effects. Delay in embryonic development, retardation of organogenesis, and disruptions of normal early developmental processes that drive the establishment of developmental defects may result from the induction of oxidative stress[39,40].

In conclusion, the present study revealed that pyriproxyfen could be a teratogenic compound as shown here on the developing chick embryos. It induced a reduction in body morphometric measurements accompanied by growth retardation and microcephaly. Also, it caused an increase in malformed embryos at all dosage levels and induced the presence of various malformations on both ED7 and ED14.

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AUTHORS' CONTRIBUTIONS

YMM planned the study and designed the experiments. The experiments, data collection, and statistical analysis were performed by RAS. HSH and RAS drafted the manuscript. The manuscript was revised by YMM, NES, and MEM.

REFERENCES

[1] Damalas, C. A. and Eleftherohorinos, I. G. (2011). Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health, 8(5): 1402-1419.

[2] Garcie, F. B.; Cortés Ascencio, S. Y.; Gaytan Oyarzun, J. C. *et al.* (2012). Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. J Res Environ Sci Toxicol, 1(11): 279-293.

[3] Sullivan, J. J. and Goh, K. S. (2008). Environmental fate and properties of pyriproxyfen. J Pestic Sci, 33(4): 339-350.

[4] Parthasarathy, R. and Palli, R. S. (2021). Stage-specific action of juvenile hormone analogs. J Pestic Sci, 46(1): 16–22.

[5] Jacobs, E. D.; Hutchinson, J. M.; Krieger, J. K. *et al*. (1996). A novel approach to flea control on cats, using pyriproxyfen. Vet Rec, 139(23): 559-561.

[6] WHO (2008): Pyriproxyfen in Drinking-Water: Use for Vector Control in Drinking-water Sources and Containers—Background Document for Development of WHO Guidelines for Drinking-water Quality (WHO/ HSE/AMR/08.03/9). World Health Organization, Geneva, Switzerland.

[7] Mulla, S. M. (1995). The future of insect growth regulators in vector control. J Am Mosq Control Assoc, 11(2 Pt 2): 269-273.

[8] Trayler, K. M. and Davis, J. A. (1996). Sensitivity of *Daphnia carinatasenus lato* to the insect growth regulator, pyriproxyfen. Ecotoxicol Environ Saf, 33(2): 154-165.

[9] Olmstead, A. W. and LeBlanc, G. A. (2003). Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean *Daphnia magna*. Environ Health Perspect, 111(7): 919-924.

[10] Ginjupalli, G. K. and Baldwi, W. S. (2013). The time- and age-dependent effects of juvenile hormone analog pesticide, piriproxifeno on *Daphnia magna* reproduction. Chemosphere, 92(9): 1260-1266.

[11] Vieira Santos, S. V.; Caixeta, E. S.; de Campos Júnior, E. O. *et al*. (2017). Ecotoxicological effects of larvicide used in the control of *Aedes aegyti* on nontarget organisms: redefining the use of pyriproxyfen. J Toxicol Envrn Health A, 80(3): 155-160.

[12] McKenney, C. L. (Jr) (2005). The influence of insect juvenile hormone agonists on metamorphosis and reproduction in estuarine Crustaceans. Integr Comp Biol, 45: 97-105.

[13] Gusso, D.; Reolon, K. G.; Gonzalez, J. B. *et al*. (2020). Pyriproxyfen exposure impairs cognitive parameters and alters cortisol levels in zebrafish. Front Behav Neurosci, 14: 103 (doi: 10.3389/fnbeh.2020.00103).

[14] da Silva, F. F.; da Silva, J. M.; da Silva, T. D. *et al*. (2020). Evaluation of Nile tilapia (*Oreochromis niloticus*) fingerlings exposed to the pesticide pyriproxyfen. Lat Am J Aquat Res, 48(5): 826-835.

[15] Azevedo, R. D. S.; Falcão, K. V. G.; Assis, C. R. D. *et al*. (2021). Effects of pyriproxyfen on zebrafish brain mitochondria and acetylcholinesterase. Chemosphere, 263: 128029 (DOI: 10.1016/j.chemosphere.2020.128029).

[16] de Oliveira, V. S.; Castro, A. J. G.; Marins, K. *et al*. (2021). Pyriproxyfen induces intracellular calcium overload and alters antioxidant defenses in *Danio rerio* testis that may influence ongoing spermatogenesis. Environ Pollut, 270: 116055 (DOI: 10.1016/j. envpol.2020.116055).

[17] Ose, K.; Miyamoto, M.; Fujisawa, T. *et al*. (2017). Bioconcentration and metabolism of pyriproxyfen in tadpoles of African clawed frogs, *Xenopus* lae*vis*. J Agric Food Chem, 65(46): 9980-9986.

[18] Koyama, Y.; Kimura, J.; Yoshioka, K. *et al*. (1989). A six-month chronic dietary toxicity study of pyriproxyfen in rats. J Toxicol Sci, 14: 43-64.

[19] Mehrnoush, G.; Mehrdad, S. and Saeid, K. (2013). Effect of pyriproxyfen on function and tissue of testis in adult rat. Int J Curr Res Rev, 5: 66-74.

[20] Shahid, A.; Zaidi, S. D.; Akbar, H. *et al*. (2019). An investigation on some toxic effects of pyriproxyfen in adult male mice. Iran J Basic Med Sci, 22: 997-1003.

[21] Production Team REDUAS (2016). Report from Physicians in the Crop-Sprayed Villages Regarding Dengue-Zika, Microcephaly, and Mass-Spraying with Chemical Poisons (https://reduas.com.ar/report-from-physicians-in-the-crop-sprayed-town-regarding-dengue-zika-microcephaly-and-massive-spraying-with-chemical-poisons).

[22] Parens, R.; Nijhout, H. F.; Morales, A. *et al*. (2017). A possible link between pyriproxyfen and microcephaly. PLoS Curr, 9: ecurrents. outbreaks.5afb0bfb8cf31d9a4baba7b19b4edbac (DOI: 10. 1371/currents. outbreaks.5afb0bfb8cf31d9a4baba7b19b4edbac).

[23] Truong, L.; Gonnerman, G.; Simonich, M. T. *et al*. (2016). Assessment of the developmental and neurotoxicity of the mosquito control larvicide, pyriproxyfen, using embryonic zebrafish. Environ Pollut, 218: 1089-1093.

[24] Dzieciolowska, S.; Larroque, A.-L.; Kranjec, E.-A. *et al*. (2017). The larvicide pyriproxyfen blamed during the Zika virus outbreak does not cause microcephaly in zebrafish embryos. Sci Rep, 7: 40067 (DOI: 10.1038/srep 40067).

[25] Horie, Y.; Yamagishi, T.; Takahashi, H. *et al*. (2017). Assessment of the lethal and sublethal effects of 20 environmental chemicals in zebrafish embryos and larvae by using OECD TG 212. J Appl Toxicol, 37(10): 1245-1253.

[26] Maharajan, K.; Muthulakshmi, S.; Nataraj, B. *et al*. (2018). Toxicity assessment of pyriproxyfen in vertebrate model zebrafish embryos (*Danio rerio*): a multi biomarker study. Aquat Toxicol, 196: 132-145.

[27] Lajmanovich, R. C.; Peltzer, P. M.; Martinuzzi, C. S. *et al*. (2019). Insecticide pyriproxyfen (Dragón®) damage biotransformation, thyroid hormones, heart rate, and swimming performance of *Odontophrynus americanus* tadpoles. Chemosphere, 220: 714-722.

[28] Shahid, A. and Saher, M. (2020). Repeated exposure of pyriproxyfen to pregnant female mice causes developmental abnormalities in prenatal pups. Environ Sci Pollut Res Int, 27(21): 26998-27009.

[29] Luckmann, M. R., de Melo, M. S.; Spricigo, M. C. *et al*. (2021). Pyriproxyfen exposure induces DNA damage, cell proliferation impairments and apoptosis in the brain vesicles layers of chicken embryos. Toxicology, 464: 152998 (DOI: 10.1016/j.tox.2021. 152998).

[30] Reed, L. J. and Muench, H.(1938). A simple method of estimating fifty percent endpoints. Am J Hyg, 27(3): 493-497.

[31] Hanke, W. and Jurewicz, J. (2004). The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence. Int J Occup Med Environ Health, 17(2): 223-243.

[32] Horie, Y.; Mitsunaga, K. and Yap, C. K. (2023). Pyriproxyfen influences growth as well as thyroid hormone–related and gh/igf-1 gene expression

during the early life stage of zebrafish (*Danio rerio*). Comp Biochem Physiol, C. Toxicol Pharmacol, 269: 109632 (DOI: 10.1016/j.cbpc.2023.109632).

[33] De Stefano, M. A.; Ambrosio, R.; Porcelli, T. *et al*. (2021). Thyroid hormone action in muscle atrophy. Metabolities, 11(11): 730 (DOI: 10.3390/metabo11110730).

[34] Caixeta, E. S.; Silva, C. F.; Santos, V. S. V. *et al*. (2016) Ecotoxicological assessment of pyriproxyfen under environmentally realistic exposure conditions of integrated vector management for *Aedes aegypti* control in Brazil. J Toxicol Environ Health A, 79(18): 799-803.

[35] Olney, R. C. (2003). Regulation of bone mass by growth hormone. Med Pediatr Oncol, 41(3): 228-234.

[36] Inoue, D.; Sei, K. and Ike, M. (2010). Disruption of retinoic acid receptor signaling by environmental pollutants. J Health Sci, 56(3): 221-230.

[37] Mobarak, Y. M. and Al-Asmari, M. A. (2011). Endosulfan impacts on the developing chick embryos: morphological, morphometric, and skeletal changes. Int J Zool Res, 7(2): 107-127.

[38] Behra, M.; Cousin, X.; Bertrand, C. *et al*. (2002). Acetylcholinesterase is required for neuronal and muscular development in the zebrafish embryo. Nat Neurosci, 5(2): 111-118.

[39] Abdollah, M.; Ranjbar, A.; Shadnia, S. *et al*. (2004). Pesticides and oxidative stress: a review. Med Sci Monit, 10(6): RA141-RA147.

[40] Pašková, V.; Hilscherová, K. and Bláha, L. (2011). Teratogenicity and embryotoxicity in aquatic organisms after pesticide exposure and the role of oxidative stress. Rev Environ Contam Toxicol, 211: 25-61.

التأثيرات المُشوِهة لأجنة الدجاج النامية بواسطة مبيد البيربروكسفين

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يُعَد البيربروكسفين من المبيدات الحشرية الهرمونية المماثلة لهرمونات الحداثة في الحشرات، وهو يستخدم عادة كمبيد يرقي ضد العديد من الأفات الحشرية؛ وهدفت هذه الدراسة إلى معرفة تأثيراته المُشوِهة للنمو الجنيني للدجاج. تم تقدير الجرعة المميتة للنصف لهذا المبيد أولا، ثم تم اختيار ثلاث جرعات تحت مميتة (15، 30، 45 ميكروجرام/بيضة) للحقن في الحيز الهوائي للبيض المخصب بعد يوم من التحضين. تم فتح البيض في اليومين "7 و 14" من التكوين الجنيني وفحص الأجنة لتسجيل التغيرات المورفومترية والتشوهات الظاهرية. تسببت المعاملة بالبيربروكسفين في تأخر نمو الأجنة وصغر حجم الرأس والعين الذي تم الاستدلال عليه بالنقص في أوزان الأجنة، وأطوال الأجنه من قمة الرأس إلي بداية الذيل، وأطوال الرأس، وأقطار العين. وقد ظهرت هذه التغيرات المورفومترية بدرجه بسيطة أو متوسطة في أجنة اليوم "7"، بينما كانت أكثر وضوحاً في أجنة اليوم "14". كان هناك أيضا انخفاضات ذات دلالة إحصائية في أطوال الأطراف الأمامية والخلفية لأجنة اليوم "14" المعاملة بالجرعة العالية للمبيد. وهناك نسبة عالية من التشوهات بين أجنة اليوم "7" في صورة تجمعات دموية، وتشوهات الجدار البطني للجسم، وتشوهات الأطراف، وصغر حجم العين وحجم الرأس. ومع ذلك، سجلت أجنة اليوم "14" تشوهات ظاهرية ذات دلالة إحصائية مثل التجمعات الدموية، وصغر حجم الرأس، وتأخر نمو الريش والمنقار، وصغر حجم العين في كل المجموعات التي تم معاملتها بالمبيد، وتشوهات الأطراف في المجموعة التي تلقت الجرعة العالية. أيضا سجلت الدراسة ظهور بعض التشوهات الأخرى مثل الوذمة، وغياب مقلة العين، وقصر العنق والذيل، وغياب الذيل، وصغر حجم الثقب السمعي الخارجي، وقصر الجفون. وقد كشفت هذه النتائج أن مبيد البيربروكسفين له تأثيرات محتملة مُشوِهة لنمو أجنة الدجاج.