[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

CYPERMETHRIN (CYP) AND LAMBDA-CYHALOTHRIN (λ-CYH) EXPOSURE INDUCED HISTOPATHOLOGICAL DEFECTS OF REPRODUCTIVE ORGANS IN PREGNANT MICE

Tehmeena Ismail,[a,*] Syeda Nadia Ahmad[b,*,†] Khawaja Raees Ahmad,[a,c] Sadia Suleman,[a] Muhammad Ali Kanwal[a] Sitara Shameem,[a] Iram Inayat,[a] Fiza Azhar,[a] Kausar Raees,[d] Asma Younis[a], Anam Nosheen[a]

Sargodha and Chakwal, Pakistan

ABSTRACT: CYP and λ-CYH were tested for their adverse effects on female reproductive organs during pregnancy on co-gestational exposure. The study was conducted on 30 female albino mice randomly divided into three groups (n=10). Vehicle Control group (VcG): This group was given 0.1mL corn oil only to create same psychological stress (if any) which other group have during gavage administration. CYP treated group: this group was given 0.1mL of corn oil containing 5mg/kg (b.w.) of cypermethrin and λ-CYH treated group: This group was given 0.1mL of corn oil containing 5mg/kg (b.w.) of λ-CYH. Corn oil, CYP and λ-CYH were given orally through gavage for 4 days regularly (from 7th to 10th day of pregnancy) to relevant groups. The microanatomical pathologies of λ-CYH includes, increased oocytic degenerations and marginal degenerations in corpus luteum were obvious with many necrotic cells leading to fluid filled spaces and cytoplasmic vacuolization. Great deal of transformation of Large Luteal Cells (LLC's) into Small Luteal Cells (SLC's) was also very obvious. Pathological signs indicate reduction in the thickness of endometrium, wide open uterine glands and damaged circular and longitudinal muscles of muscularis layer. CYP induce marginal degenerations, fibrotic mass accumulations, missing of follicles oocytes, ovarian stroma that shows arrested follicles at different stages of development. The height of the columnar endothelium of the lamina basalis seems to slightly reduce than that of the VcG and the glandular structures of the lamina functionalis appears wider and contained large spaces. Size of luteal cells also decreases as compared to VcG. Significant variations in the morphometric and micrometric parameters such as feto-uterine index significantly decreased in λ-CYH and CYP as compared to VcG group, while the size of oocyte and their nucleus does not show significant variations. The micrometric findings from the uterus show significant decrease in the number and size of the spindle shape cells in the myometrium regions. It may be concluded that the pyrethroid insecticides particularly halogenated type II pyrethroids are highly toxic to the reproductive organs of the females and hence can cause reduced growth rates of embryos

Key words: Cypermethrin (CYP), Lambda-cyhalothrin (λ-CYH), Pregnancy, Ovary, Uterus, Histopathology.

INTRODUCTION

1.1 Cypermethrin ($C_{22}H_{19}Cl_2NO_3$): The major sources of potential environmental hazards are insecticide, herbicides and fungicides.[1,2] Once they enter in the food chain, they cause drastic effects on birds, fish and other animals including the human beings.[3-5] Synthetic pyrethroids are the insecticides which are analogous to naturally occurring insecticidal compound. CYP is synthetic type-II pyrethroid used as insecticide for pest control such as insects of order Lepidoptera, cockroaches and

[a]Department of Zoology, University of Sargodha, Sargodha, Pakistan; [b]Department of Zoology, University of Chakwal, Chakwal, Pakistan; [c]Government Ambala Muslim Graduate College Sargodha, District Sargodha, Pakistan; [d]Chairperson, Board of Intermediate and Secondary Education, Sargodha; *Authors having equal Contribution; †For correspondence: Syeda Nadia Ahmad, Assistant Professor of Zoology, Department of Zoology, University of Chakwal, Chakwal, Pakistan; E-mail: nadia.ahmad@uoc.edu.pk

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

termites.[6-8] Many researches strongly suggested that exposure of CYP results in decreasing body weight; reduce the fertility in both male and females, lessens the number of healthy testicular and epididymal sperms in males.[9] Experiments have revealed that pregnant females exposed to CYP daily from 6th to 17th day of gestation, will adversely affects the females as well as the fetus development.[10]

1.2 Lambda-Cyhalothrin ($C_{23}H_{19}ClF_3NO_3$): Lambda-Cyhalothrin (λ-CYH) is a type-II pyrethroid analogous to the pyrethrin, a naturally occurring compound. It is active ingredient in insect repellent chemicals, farming and household sector.[11] Reproductive potential of animals might reduce when they are exposed to chemicals such as pesticides or insecticides. Pyrethroid administration can result in embryo losses in pregnant rabbits[12] and in rats[13], meanwhile in rat death and fetal resorption.[14] Pyrethroids reduce number of implantation sites, number of viable fetus, pre and post implantation losses and reduction in number of estrous cycles.[15] Corn oil is widely used as a vehicle for water-insoluble agents in drug development and many experiments. In current research work we have used corn oil because Pyrethroid with corn oil has been noted to have much lower LD50 than with water.[16]

The aim of present study was planned to be carried out on a placental mammal (albino mice) so that the result may be easily extrapolatable to human system and to give proposal for the safer utilization and the management of the CYP and λ-CYH, specifically from its teratogenic point of view.

MATERIALS AND METHODS

2.1 Animal Caring & Dose Groups:

All research work was conducted on albino laboratory mice, *Mus musculus* in the animal house in the department of Zoology, University of Sargodha; Sargodha. The females' albino mice were selected having weight between 28 to 30g. Total 30 animals were divided into three groups (10 animals each) randomly as

2.1.1 VcG (Vehicle Control Group): This group was given 0.1mL corn oil only for 4 days regularly (from 7th to 10th day of pregnancy).

2.1.2 CYP (Cypermethrin) group: 0.1mL of corn oil containing 5mg/kg (b.w) of CYP was given orally through gavage for 4 days regularly (from 7th to 10th day of pregnancy).

2.1.3 λ-CYH (Lambda cyhalothrin) group: Through intragastrical gavage of 5mg/kg of λ-CYH was given in 0.1mL of corn oil orally through gavage for 4 days continuously from 7th to 10th day of pregnancy.

On 18th day of pregnancy, after euthanasia the animals from all the groups were dissected.

2.2 Preparation of CYP & λ-CYH solutions:

2.2.1 Dose Preparation:

Stock solutions (20mg/kg) of both insecticides i.e CYP and lambda cyhalothrin was prepared

Calculations for 20mg/kg dose:

Required dose for 1000g mice is = 20mg

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

Required dose for 1g mice is = 20mg/1000g

Required dose for 30g mice is = 20mg/1000g*30 =0.6mg/30g

0.6mg/100μL=0.6mg/0.1mL=600mg/100mL=0.6g/100mL

We can obtain the stock solution by dissolving 0.6g of CYP and λ-CYH in corn oil. By using C1V1=C2V2

2.3. Dissection and Recovery of organ:

On the 18th day of pregnancy, the animals from all groups (VcG, CYP and λ-CYH) were subjected to cervical dislocation for surgical recovery of the all-female reproductive organs including right and left ovaries, fallopian tubes, uterus and placentas. All experimental organs were finally fixed in fixative for further study.

2.4. *Processing of Organs & Histology:*

The organs were further processed by embedding in wax, stained using H & E dyes and mounted in Canada balsam.

2.5. Photography of selected sections using microscope:

Photomicrographs of the selected histological sections from ovaries and uterus of different groups (VcG, CYP and λ-CYH) were obtained using digital camera of 7.2 mega pixel mechanically fitted on Labomid CXR2 trinocular microscope at 40X×, 100× and 400× magnifications.

2.6. Micrometric studies:

Digital micrometery from the soft images of the histological sections of ovary and uterus was carried out by using a computer assisted technique in CorelDraw11.

Calculations for Feto-Uterine Index: The feto-uterine index was calculated by dividing the uterus (after removal of conceptus) (by the weight of gravid uterus (along with the conceptus)). The obtained value was further divided by the number of implantations in each animal.

Feto-uterine index formula: Weight of uterus (without conceptus) / gravid uterus (with conceptus)/ number of implantations per animal

2.7. Analysis of Data:

By using the ANCOVA and ANOVA the obtained data was statistically analyzed which was based on single factor, further on the groups were compared on basis of Tukey's Multiple Range test.

RESULTS

3. Histological Results:

3.1. Histopathology of Follicular Oocyte and Ovarian stroma:

Sections of the VcG ovary show very well-developed corpus lutea and various ovarian follicles at different stages of development. In all developing follicles the oocytes have been found very well placed, surrounded by non-cellular zona pellucida and the cellular layer corona radiata of the cumulus cells embedded in the ovarian stroma. Necrosis of the oocytes in some individual follicles was also seen. Fig 1 (A, A1 and A2)

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at
www.fluorideresearch.online/epub/files/203.pdf]

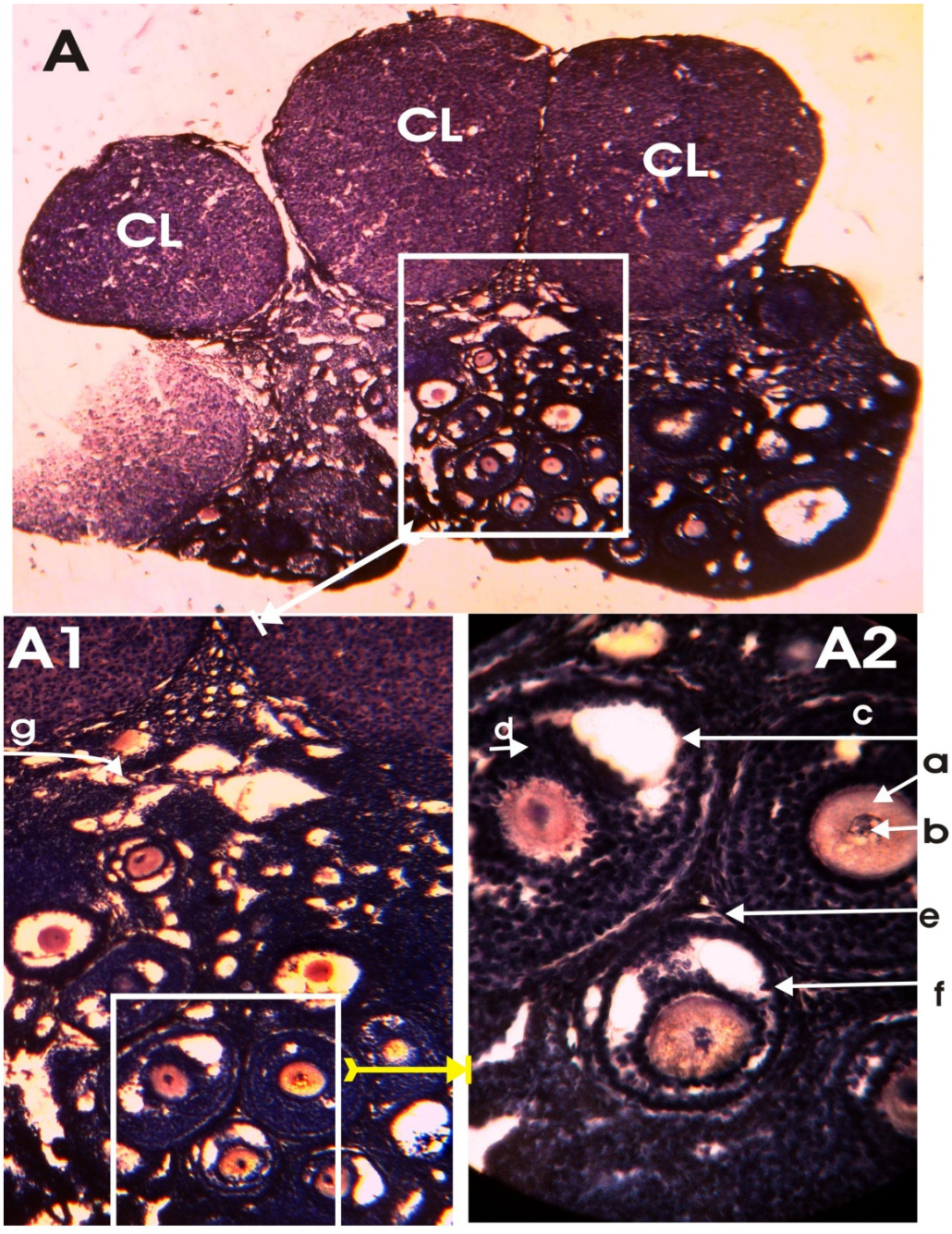

Fig.1: Hematoxylin and Eosin stained histological sections of mice ovary: VcG: A: Histological section of normal mice ovary at 40×; A1: Normal ovary with healthy follicles at different stages of development at 100×; A2: Oocyte at 400×; a: Healthy oocyte cytoplasm; b: oocyte nucleus; c: A clearly defined antral space; d: Cumulus granulosa cell layer; e: Thecal cells; f: Zona Pellucida; g: Ovarian stroma. CL: Healthy Corpus lutea

In CYP treated group ovaries the corpus lutea were well placed. However, the corpus lutea have shown size variations. Some marginal corpus cells degenerations were also obvious living behind fluid accumulation or fibrotic mass. The ovarian

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at
www.fluorideresearch.online/epub/files/203.pdf]

stroma shows follicles arrested at different stages of development whereas in most of the follicles oocytic regression was observed. In most cases the involuting oocytes were surrounded by fluid filled spaces instead of cumulus cells and zona pellucida. In some of the follicles complete absence of oocytes were also observed. Nevertheless a few healthy follicles with proper oocyte were also observed. Fig 2 (B, B1,B2 and B3).

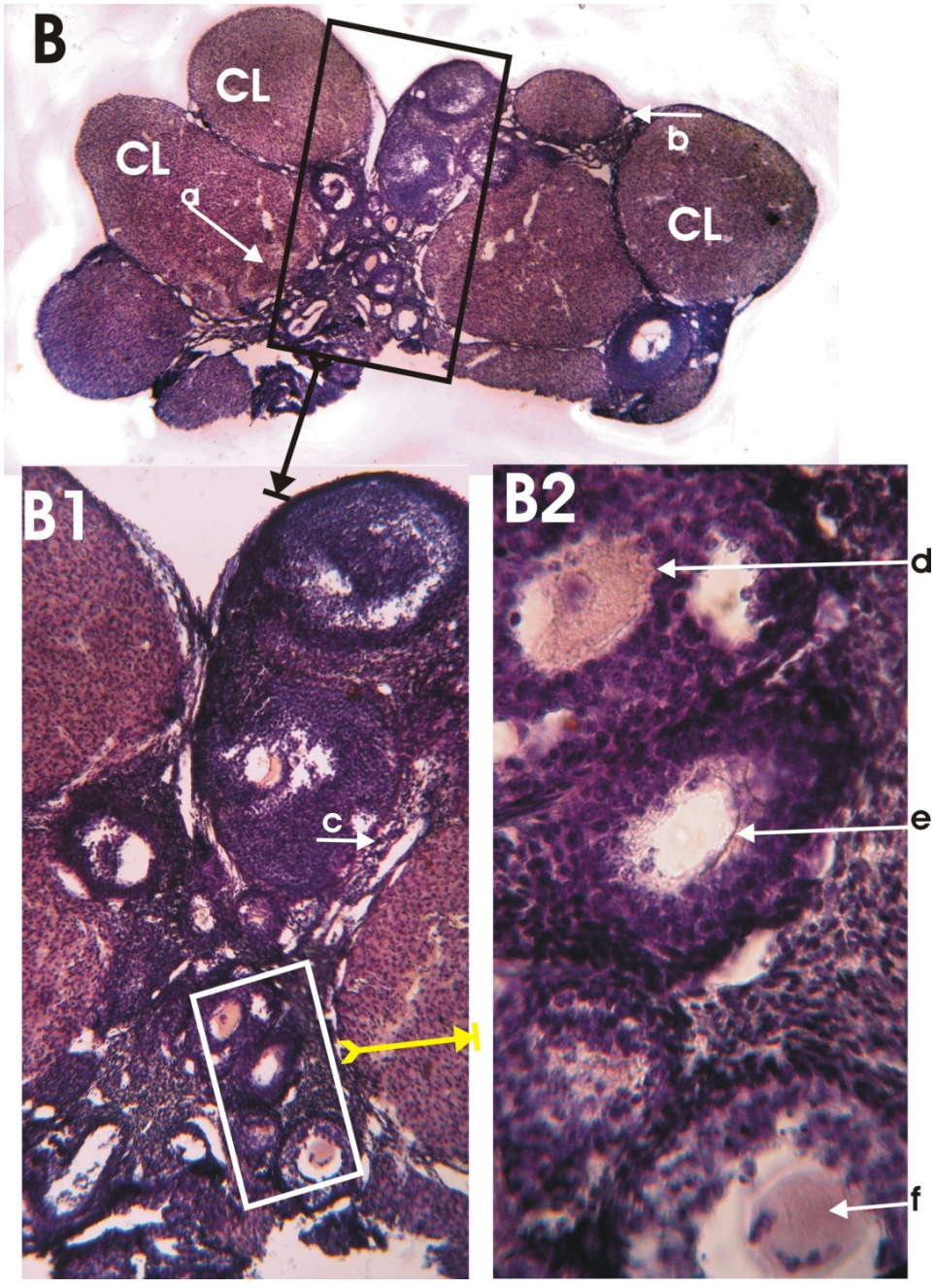

Fig. 2: Hematoxylin and Eosin stained histological sections of mice ovary: CYP: B: Histological section of ovary at 40×; B1: Histological section of ovary at 100×; B2: Histological section of ovary at 400× showing: a: Degeneration and fluid accumulation in Corpus Lutea; b: Fibrotic mass Formation; c: Central Stromal Degenerations; d: Healthy Follicles; e: Fluid filled pace without oocyte; f: Regressed Oocyte without proper cumulus layers.

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at
www.fluorideresearch.online/epub/files/203.pdf]

In λ-CYH group ovaries the corpus lutea were seen more rounded in shape and slightly distinctly placed from each other showing signs of degenerations and cellular necrosis in the cortical region of the corpus lutea. The stroma has shown follicles at various stages. However, in most of the developing follicles the oocytes were at various stages of degenerations and regression. No clear zona pellucida and corona radiata layers were found placed around the involuting oocytes. Instead of these layers, wide fluid filled spaces were observed. Fig. 3 (C, C1 and C2)

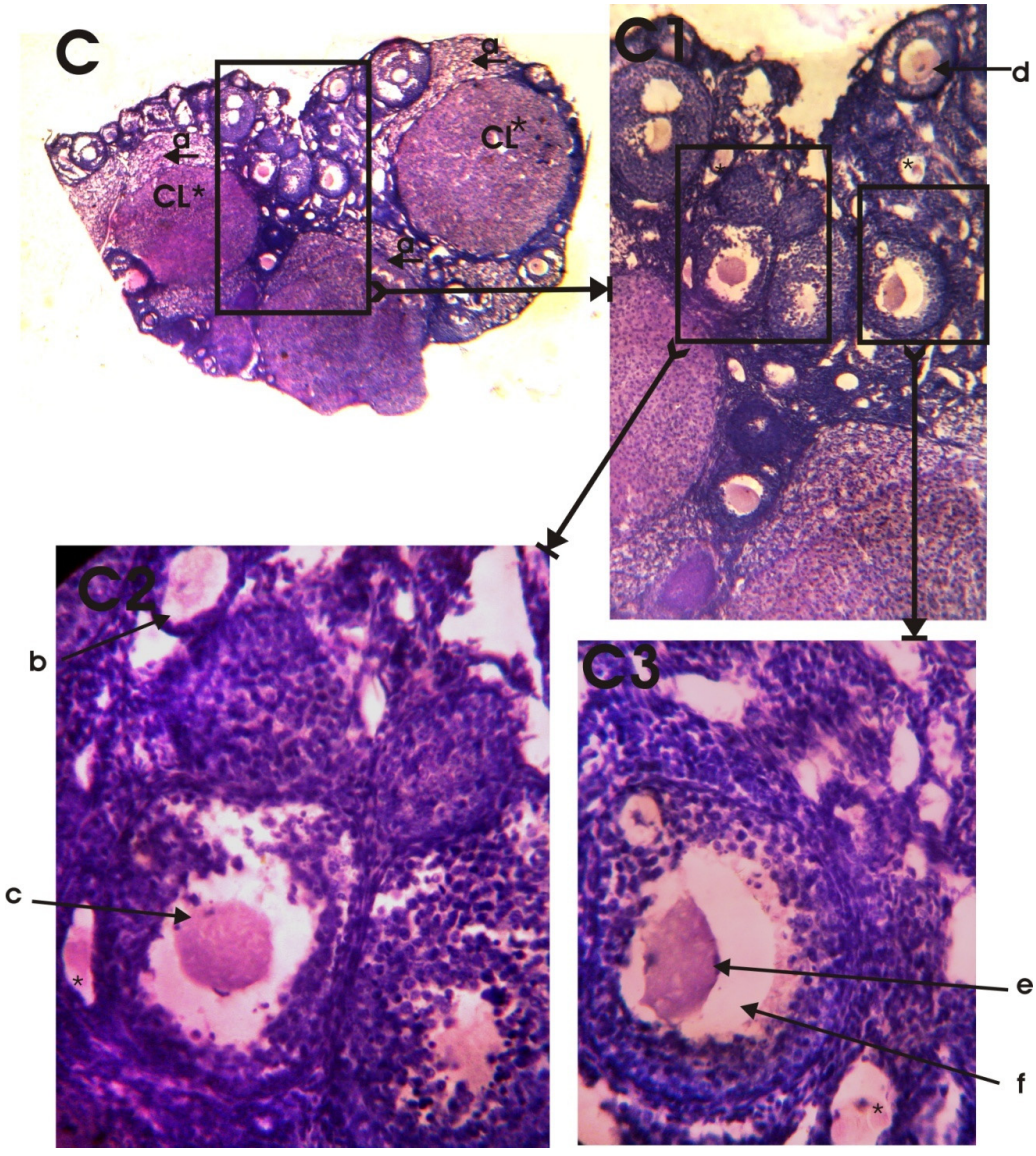

Fig. 3: Hematoxylin and Eosin stained histological sections of mice ovary: λ-CYH: C: Histological section of ovary treated with λ-CYH at 40× showing large number of degenerating follicle pool. C1: Histological section of ovary at 100×; C2 and C3: Histological section of ovary at 400×. CL*: Regressed Corpus Luteum; a: Cortical Region degeneration of Corpus Luteum; b: Atretic Follicle; c: Oocyte without zona pellucida and Corona Radiata layer Empty Follicle; d: Shrinking Follicle; e: Degenerating Nucleus of Oocyte f: Fluid filled space; *: Shows the degenerating Oocytes.

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

3.2. Micrometric results of various parameters of the ovaries:

Micrometric observations such as the mean CSA of oocyte in λ-CYH (2883.37±258μ^2) increased as compared to the CYP (2467.162±280μ^2) and VcG (2395.704±260μ^2) groups. While in CYP group and VcG no significant (P≤0.05) variations were observed. While the mean CSA of oocyte nucleus shows no significant variations among the three groups. Its value almost remains same in treated and VcG. But among the treated groups its shows slight higher values in λ-CYH (217.883±26μ^2) as compared to the CYP (188.768±38μ^2) and VcG (212.7±31μ^2) groups.

3.3. Histopathological and micrometic results of Corpus Luteum: In VcG major steroidogenic and non-steroidogenic types, i.e LLC's, SLC's and fibroblasts respectively, were clearly identifiable. The fibroblasts are dye stained to provide basic structural outlay and networking of corpus luteal tissues, which is thickly populated by the luteal cells. It was observed that LLCs were more concentrated towards the margins whereas the core area of corpus lutea were more thickly populated by SLCs. Corpus lutea were fairly provided with good supply of blood. The distribution of luteal tissues appears t surround the major luteal blood vessels in the center of corpus luteum, from where he arterioles and capillaries distribute the blood to the margins. Fig 4: A and A1.

In CYP treated group as mentioned above the average size of corpus lutea was decreased. Furthermore, it was found that the marginal areas were almost equally populated by the SLCs and LLCs this is in contrast to the VcG group where the marginal areas of the corpus lutea were overwhelmingly populated with LLCs. In the middle region of corpus luteum in CYP treated group fairly big empty spaces were observed, which may be found by premature involutions of LLCs and SLCs which are fairly evenly distributed in this area in VcG. Along with that the core area also shows macrophagic infestations and fibrosis along with various micronuclear formations in surviving luteal cells. Fig 4: B and B2.

The λ-CYH treated group corpus lutea have shown great deal of transformation of the LLCs in to SLCs along with micronuclear formation of luteal cell nuclei at the marginal zones. Some narrow empty spaces appeared due to individual luteal cells necrosis were also observed in the cortical regions of corpus lutea in the group. Whereas the core areas of corpus lutea have shown fairly large fluid filled spaces with great deal of fibrosis indicating corpus luteal cells degenerations, leaving behind the networking of the fibroblasts. The surviving luteal cells in this area have shown big deal of micronuclear formations and cytoplasmic vacuolations, indicative of progression of necrosis. Fig 4: C and C1.

The overall data from the mean number of small luteal cells per unit area significantly (P≤0.05) varied in all three groups. It remains significantly higher (P≤0.05) in the λ-CYH (4.16±0.15b) as compared to CYP (3.45±0.25ab) and VcG (3.08±0.39a). While, the number of LLCs remains high in the VcG (2.50±0.41a) group and does not changed significantly in treated groups. It almost remains same in λ-CYH (3.29±0.37a), CYP (2.45±0.15a) and VcG (2.50±0.41a). But in comparison of the SLCs and LLCs number, the number of SLCs increased significantly in the insecticide treated groups (Fig. 4).

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at
www.fluorideresearch.online/epub/files/203.pdf]

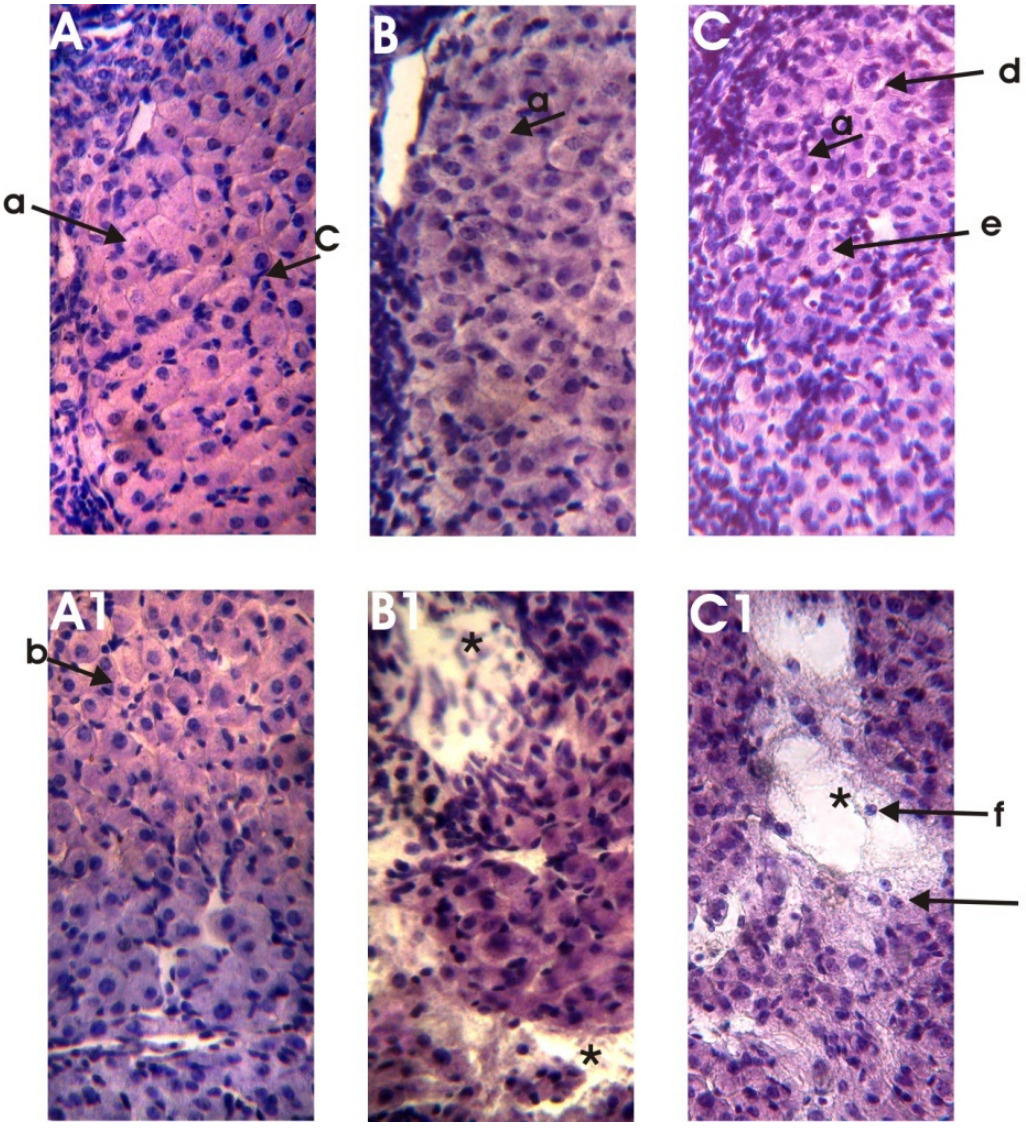

Fig. 4: Hematoxylin and Eosin stained histological sections of mice Corpus luteal Cells at 400×. A: Peripheral area of Corpus lutea of VcG B: Peripheral area of Corpus lutea of CYP C: Peripheral area of Corpus lutea of λ-CYH A1: Central region of Corpus lutea of VcG; B1: Central area of Corpus lutea of CYP; C1: Central area of Corpus lutea of λ-CYH; a: Large Corpus Luteal Cells ; b: Small Luteal; c: Fibroblast Cells; d: Cytoplasmic vacuolation along with micronuclear formations in luteal cells; e: Apoptotic cells; f: Megakaryocytes; g: Fibrotic networking; *:Degeneration in central area forming obvious spaces.

3.4 Gravid Uterine Histopathologies and Micrometric Findings: Walls of pregnant uteri in the VcG have shown typical architectural outlay of uterine structure, containing the longitudinal muscles just beneath the outer mesenteric membranes and the inner circular muscles. While the Inner most or luminal side of the uterine wall contains endometrium distributed in lamina basalis present just inner to the thick circular muscles of the uterine walls and lamina functionalis generally thrown in folds to produce pit and grove like glandular structure of lamina functionalis. The muscular layers were made up of spindle shape cells of the smooth muscles. Fig:5: A

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at
www.fluorideresearch.online/epub/files/203.pdf]

In CYP treated group the muscularis layer was comparatively less developed as compared to VcG, containing various spaces between the longitudinal and circular layers of the muscularis. The height of the columnar endothelium of the lamina basalis seems to slightly reduce than that of the VcG, and the glandular structures of the lamina functionalis appears wider and contained large spaces between. Fig:5: B

The longitudinal muscles show further increased damage in λ-CYH group leaving behind wide fluid filled spaces between the inner circular and outer longitudinal muscles of the muscularis layer. The endothelium was consisting almost slightly columnated cuboidal cells. Thus, the thickness of endothelium was further reduced in this group. The uterine glands were wide open and shallow say consisting of far less branched pit glands as compared to the VcG and CYP treated groups in λ-CYH. Fig:5: C

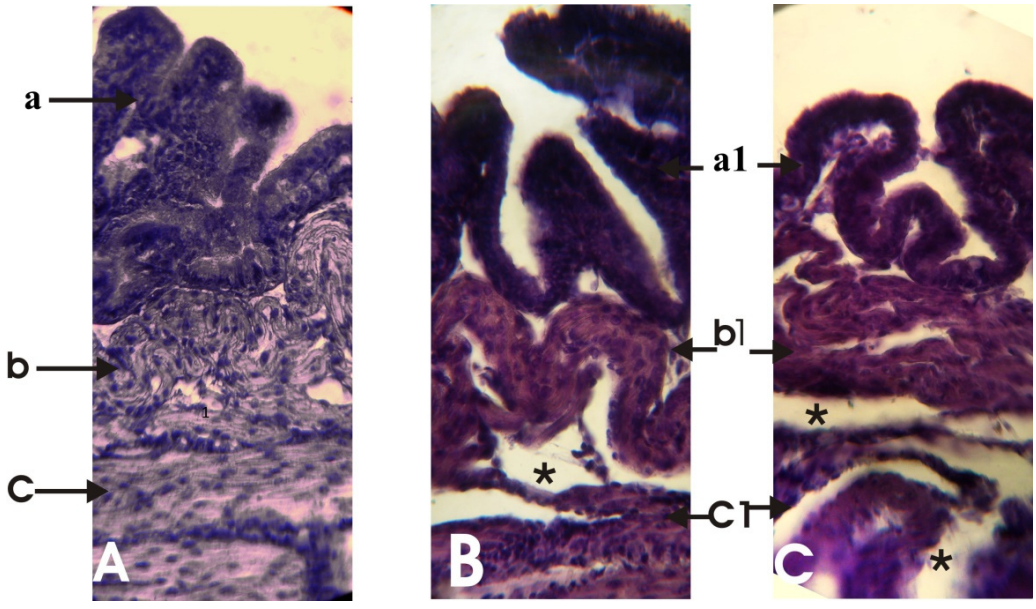

Fig. 5: Hematoxylin and Eosin stained histological sections (400×): of mice uterus. A: VcG B: CYP C: λ-CYH. a: Normal epithelial layer; b: Packed Circular muscles of myometrium; c: Longitudinal muscles of myometrium; b1: Circular muscles with more spaces; c1: Longitudinal Muscles with increased spaces.

Statistical evolution by ANOVA shows a noticeable decline in the feto-uterine index of treated groups. Its value highly significant (P≤0.01) decrease in λ-CYH and CYP, while remains high in the VcG. Micrometric findings for the mean height of epithelial layer significantly (P≤0.05) changes in VcG and treated groups. The height of epithelial layer remains higher in the VcG group (17.02±0.64b) which significantly (P≤0.05) reduced in the treated groups. Among the insecticide groups it is highly reduced in the lambda cyhalothrin (14.51±0.32a) while in CYP (15.19±0.40a) the height of epithelium remains between two groups i.e VcG and λ-CYH.

While the micrometric results for the mean number of the spindle shape cells per unit (5000μm^2) show highly significant variation (P≤0.01) among the three groups. The Post.hoc analysis TMRT (Tukey's multiple range test) have shown that number of spindle shape cells in myometrium per unit area in λ-CYH (10±0.589) were

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

significantly (P≤0.05) lower than that of VcG (15.60±0.67), whereas the CYP (13.550±0.478) shows slight significant variations as compared to λ-CYH group.

Mean size of these spindle shape cells shows slight variations. It does not significantly (P≤0.05) changes in VcG (7.7232±0.15a) and treated groups. Its value remains slightly lower in the λ-CYH group (6.8199±0.4a) as compared to CYP (7.0380±0.5a), showing impaired formation of the myometrium in λ-CYH group.

Table 1: Micrometric Parameters of ovary and uterus among the groups (Values are mean±SEM)

	Micrometric parameters	Mean±SEM		
		VcG	CYP	λ-CYH
Ovary	Mean CSA of oocyte (μm^2)	2395.704±260^a	2883.37±258^a	2467.162±280^a
	Mean CSA of nucleus of oocyte (μm^2)	212.70±31^a	217.883±26^a	188.768±38^a
	Mean CSA of corpus luteal cell (μm^2)	207.230±18^b	142.621±13^a	175.533±7^{ab}
	Number of SLC (2500 $\mu m^{2)}$	3.08±0.39^a	4.16±0.15^b	3.45±0.25^{ab}
	Number of LLC (2500$\mu m^{2)}$	2.50±0.41^a	3.29±0.37^a	2.45±0.15^a
Uterus	Mean height of endothelial layer (μm)	17.02±0.64^b	14.51±0.32^a	14.51±0.32^a
	Number of spindle shape cells in myometrium (5000 μm^2)	15.60±0.67^c	10.00±0.58^a	13.55±0.47^b
	Size of the spindle shapecells in myometrium	7.03±0.5^a	6.81±0.4^a	7.72±0.15^a

*: (p≤0.05), **:(p≤0.001), ***:(p≤0.0001); Any two groups not sharing a common lower-case superscript differ significantly (p≤0.05) with each other.

DISCUSSION

There are quite number of studies indicating the reproductive and developmental toxicities of pyrethroid insecticides. Such as embryo toxicity in terms of co gestational and peri, pre implantation and pan gestational losses due to exposure of pyrethroids. [17] Furthermore the exposure of pyrethroid insecticide, CYP results in many feto-morphic defects including microcephaly, hydrocephaly, free or un-detached pinnae, epinnate ears, twisted neck, meromelia, extradactyly, drooping

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

wrist, round back, hemorragia, forked paws, flipper limbs and decrease in fetal head circumference and crown rump length.[18]

However, combination of some pyrethroid insecticide induced unambiguous alterations in the embryonic growth and development and resulted in axial and appendicular skeleton structure malformations in chick.[19] Teratological effects of fluoridated insecticides (bifenthrin) are unambiguous, the *in ovo* treatment in chick results in many deformations in the 14 day embryo, such as reduced beak length, exocardia and different appendicular malformations such as fore-limb meromelia, twisted and polydactyl hindlimbs, un-clawed digits, and elongated digits corresponding to the index fingers were seen in the embryos. The morphometric data also showed a significant ($p \leq 0.05$) decrease in mean weight of embryo, crown-rump length and occipito-frontal length.[20]

So, the preview of pyrethroid insecticide to be systemically safer than other insecticides groups is rapidly changing. The developmental neurotoxicity of the pyrethroid in terms of effecting the brain development by increased sensitivity towards the sodium channels and enhanced neuronal death on developing embryo has been documented.[21] Similarly, pyrethroid have also been identified as reproductive toxins as CYP and permethrin have been found to cause testicular and spermatogenic toxicities.[22]

However, the toxic effects of pyrethroid on female reproductive organs in general are least understood. In this context many scientists have been reported the ovarian and uterine toxicity. As these pyrethroids are responsible to affect ovulation, cause follicular atresia, reduce the number of follicular cells, oocytes and corpora lutea and induce vesicular atrophy of the endometrial glands. The potential hormonal activity of pyrethroids and their metabolites have shown multiple effects on the endocrine system. They inhibit the production of steroid hormones, such as progesterone and estradiol. They act like inhibitor for estrogen as they mimic estrogen action.[23]

Some metabolites of pyrethroids, specially permethrin and CYP, most probably interact with the cellular estrogen receptors. However, several pyrethroids have low toxicity, but some pyrethroids, such as fenvalerate, CYP, deltamethrin, λ-CYH and bifenthrin have showed substantial toxicity. Similarly, CYP cause adverse effects on the female rats when treated with 50mg/kg (orally) for 4 weeks. As a result, the mean ovarian weight, number of atretic follicles decreased significantly. Further studies show that CYP results in many degenerative activities related to corpus lutea and oocyte. The biochemical analysis shows decrease in the amount of proteins, lipids, phospholipids and cholesterol in ovaries while reduction in the activities of lactate dehydrogenase also observed.[24]

However there exists no comparative study among the pyrethroids in terms of their female reproductive toxicity especially among the halogenated type II pyrethroids. The results of present study are therefore difficult to be compared with the existing knowledge where a fluoridated (λ-CYH) type II pyrethroid insecticide is compared with a non-fluorinated type II pyrethriod (CYP) for their toxic effects on uterine and ovarian structures.

The results of present investigations have shown specific histopathological and microanatomical modifications of female reproductive organs during pregnancy on

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

co gestational exposure of these two insecticides. The microanatomical pathologies of λ-CYH includes, increased oocytic regression and degenerations, no clear formation of non-cellular zona pellucida and cellular corona radiata layer around the involuting oocytes, in corpus luteum marginal degenerations were obvious with many necrotic cells leading to fluid filled spaces, fibrotic networking along with micronuclear formations and cytoplasmic vacuolization. Great deal of transformation of large luteal cells in to small luteal cells was also very obvious.

Many pathological signs of gravid uterus were observed in the lambda cyhalothrin treated group as the thickness of endometrium was reduced significantly and the uterine glands were wide open and shallow consisting of far less branched pit glands. circular and longitudinal muscles of muscularis layer were damaged to an extant showing space formation between them. CYP also shows many toxic effects on the female reproductive system which includes slight marginal degenerations leading to fluid filled spaces and fibrotic mass accumulations while in some follicles oocytes were missing, meanwhile the ovarian stroma shows follicles arrested at different stages of development.

Furthermore, the size of luteal cells also decreases as compared to VcG, while the central area has many empty spaces which may be found by premature involutions of LLCs and SLCS. Which are fairly evenly distributed in this area in VcG the core area also shows macrophagic infestations and fibrosis along with various micronuclear formations in surviving luteal cells. In CYP treated group the muscularis layer was comparatively less developed as compared to VcG, having various spaces between the longitudinal and circular layers of the muscularis. The height of the columnar endothelium of the lamina basalis seems to slightly reduce than that of the VcG, and the glandular structures of the lamina functionalis appears wider and contained large spaces between.

Similarly, significant variations in the morphometric and micrometric parameters such as the feto-uterine index significantly decreased in lambda cyhalothrin and in CYP as compared to control group. While the size of oocyte and their nucleus does not show significant variations while the number of SLCs also significantly increased in both treated groups. The micrometric findings from the uterus show significant decrease in the number and size of the spindle shape cells in the myometrium regions.

The findings indicate that both the insecticide inflicts severe toxicological damage to the pregnant female ovaries and their uteri. However, the results further indicate that among the two λ-CYH have caused more severe damage to these female reproductive organs as compared to the non-fluoridated type II pyrethroid the CYP, indicating that the fluoridation of pyrethroid insecticides can further enhance the toxicological potentials. Whereas, the general inference of present study is that the type II pyrethroid insecticides highly toxic to the pregnant female reproductive organs.

These toxic effects may be mediated through the sustained increase of oxidative stress on the female reproductive organs, particularly the steroidogenic Corpus luteal and follicular cells of ovaries. Additionally, these toxic effects may be inflicted through endocrine regulations of the corpus lutea and the muscularis and

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

endometrium of the gravid uteri. The enhanced toxicity of λ-CYH in comparison to that of CYP may be due to its sustained systemic presence and slower rate of detoxification because of its fluoridated nature. Further in-depth studies involving hormonal estimations and estimations of the oxidative stress on various reproductive tissues and organs are needed to unearth the mechanistic reasons of the presently reported toxic effects.

It may be concluded here that otherwise considered least toxic, the pyrethroid insecticides (particularly halogenated type II pyrethroids) are highly toxic to the reproductive organs of the females and hence can cause reduced growth rates of embryos and to the greater extant decreased reproductive success in the mammals including humans. It is thus suggested that the use of fluoridated insecticides must be curtailed to all possible extent in general and specifically the females should be kept away from exposure to these insecticides at least during pregnancy.

CONCLUSIONS

It may be concluded here that otherwise considered least toxic, the pyrethroid insecticides (particularly halogenated type II pyrethroids) are highly toxic to the reproductive organs of the females and hence can cause reduced growth rates of embryos and to the greater extant decreased reproductive success in the mammals including humans. It is thus suggested that the use of fluoridated insecticides must be curtailed to all possible extent in general and specifically the females should be kept away from exposure to these insecticides at least during pregnancy.

REFERENCES

[1]. . Rajmohan, K. S., Chandrasekaran, R., & Varjani, S. (2020). A Review on Occurrence of Pesticides in Environment and Current Technologies for Their Remediation and Management. Indian journal of microbiology, 60(2), 125–138. https://doi.org/10.1007/s12088-019-00841-x

[2] Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: A systematic review of existing research and methods. PloS one, 14(12), e0225743. https://doi.org/10.1371/journal.pone.0225743

[3] Zaller, J. G., & Brühl, C. A. (2019). Non-target effects of pesticides on organisms inhabiting agroecosystems. Frontiers in Environmental Science, 7, 75. https://doi.org/10.3389/fenvs.2019.00075

[4] Kim, K. H., Kabir, E., & Jahan, S. A. (2017). Exposure to pesticides and the associated human health effects. The Science of the total environment, 575, 525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009

[5] Tesfahunegny, W. (2016). Impact of pesticides on birds from DDT to current fatality: A Literature Review. Journal of Zoology Studies, 3(2), 44-55.

[6] Akhtar ZR, Majid M, Irshad U, Saeed Z, Khalid J, Khan H, Anjum AA, Noreen A and Easha. Resistance development of lepidopteran pests against insecticides in Pakistan: A case study of pink boll worm against different insecticides.EZS 2018; 6(2): 97-101. https://doi.org./10.21162/PAKJAS/20.9765

[7] Nazari, M., Motlagh, B. A., & Nasirian, H. (2016). Toxicity of Cypermethrin and Chlorpyrifos Against German Cockroach [Blattella germanica (Blattaria: Blattellidae)] Strains from Hamadan, Iran. Pakistan journal of biological sciences: PJBS, 19(6), 259–264. https://doi.org/10.3923/pjbs.2016.259.264

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

[8] Mamatha, V., Muthusamy, R., Murugan, J.M. et al. Effect of cypermethrin on worker and soldier termites of subterranean termites Odontotermes brunneus (Hagen) (Termitidae: Isoptera). Proc Zool Soc 73, 40–45 (2020). https://doi.org/10.1007/s12595-018-0284-9

[9] Joshi, S. C., Bansal, B., & Jasuja, N. D. (2011). Evaluation of reproductive and developmental toxicity of cypermethrin in male albino rats. Toxicological & Environmental Chemistry, 93(3), 593-602. https://doi.org./10.4103/0974-1208.138867

[10] Ahmad, L., Khan, A., Khan, M. Z., Hussain, I., Mahmood, F., Sleemi, M. K, Abdullah, I. (2012). Toxico-pathological effects of cypermethrin upon male reproductive system in rabbits. Pesticide biochemistry and physiology, 103(3), 194-201. http://doi.org./10.13040/IJPSR.0975-8232.8(7).2819-31

[11] Djouaka R, Soglo MF, Kusimo MO, Adéoti R, Talom A, Zeukeng F, Paraïso A, Afari-Sefa V, Saethre MG, Manyong V, Tamó M, Waage J, Lines J, Mahuku G. The Rapid Degradation of Lambda-Cyhalothrin Makes Treated Vegetables Relatively Safe for Consumption. Int J Environ Res Public Health. 2018; 15(7):1536. https://doi.org/10.3390/ijerph15071536

[12] Sallam MA., Ahmad M, Ahmad I, Gul ST, Idrees M, Bashir MI, Zubair M. (2015). Toxic effects of cypermethrin on the reproductive functions of female rabbits and their amelioration with vitamin E and selenium.PAKVET J 2015;35(2):193–196.

[13] Obinna, V.C., Agu, G.O. Beta cypermethrin exposure and perinatal reproductive development of female f1 generation of albino rats. JoBAZ 80, 44 (2019). https://doi.org/10.1186/s41936-019-0121-3

[14] Madu EP. Teratogelaminanic and embryotoxic effects of orally administered cypermethrin in pregnant albino rats. J. Toxicol. Environ. Health Sci.2015; 7(7):60-67. https://doi.org.10.4103/0971-6580.75844

[15] Nada MH, Al-Hamdani, Yajurvedi HN. Effect of cypermethrin on the ovarian activity and its impact on fertility and pubertal onset of offspring. Beni-Suef univ. j. basic appl. Sci 2017; 6(4):374-382. https://doi.org/10.1016/j.bjbas.2017.07.003

[16] Babelová J, Šefčíková Z, Ćikoś S, Kovaříková V, Špirková A, Pisko J, Koppel J, Fabian D. In Vitro Exposure to Pyrethroid-Based Products Disrupts Development of Mouse Preimplantation Embryos. Toxicol In Vitro2019; 57:184-193. https://doi.org/10.1016/j.tiv.2019.03.009.

[17] Crofton, K. M., Kehn, L. S., & Gilbert, M. E. (1995). Vehicle and route dependent effects of a pyrethroid insecticide, deltamethrin, on motor function in the rat. Neurotoxicology and teratology, 17(4), 489–495. https://doi.org/10.1016/0892-0362(95)00008-f

[18] Ramon-Yusuf SB, Aliu YO, Salawu OA, Chahoud I, Ambali SF. Maternal and foetal toxicity induced by exposure to mixture of dimethoate and cypermethrin in albino rats. J. Toxicol. Environ. Health Sci 2017; 9(6):59-65. https://doi.org/10.5897/JTEHS2017.0381

[19] Ahmad KR, Shehry K, Raees K, Kanwal MA, Abbas T and Ullah A, 2018. Adverse effects of cypermethrin on the chick (Galusdomesticus) development are reversed by co-treatment with vitamin E and olive oil. Pak Vet J; 38(1): 46-50. http://dx.doi.org/10.29261/pakvetj/2018.009

[20] Khanum Z, Suleman S, Mustanser A, Hassan M WU, Raees K, Kanwal, MA, Ahmad, KR. Comparative teratological outcomes of fluoride ions and a fluoridated insecticide (bifenthrin) in chick embryos. Fluoride 2019; 52(1):59-65.

[21] Laugeray, A., Herzine, A., Perche, O., Richard, O., Montecot-Dubourg, C., Menuet, A., Mazaud-Guittot, S., Lesné, L., Jegou, B., & Mortaud, S. (2017). In utero and lactational exposure to low-doses of the pyrethroid insecticide cypermethrin leads to

[Copyedited version after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/203.pdf]

neurodevelopmental defects in male mice-An ethological and transcriptomic study. PloS one, 12(10), e0184475. https://doi.org/10.1371/journal.pone.0184475

[22] Simon U, David O, Peter R, Joseph R, Ijeoma. Pathological Effects of Cypermethrin on the Testes and Accessory Sexual Glands of Yankasa Rams. Arch Pathol Clin Res. 2018; 2: 006-012. https://doi.org/10.29328/journal.apcr.1001006

[23] Obinna, V., & Kagbo, H. (2017). Endocrine Effect of Beta Cypermethrin on Female Albino Rats. Journal of Advances in Medicine and Medical Research, 23(3), 1-7. https://doi.org/10.9734/JAMMR/2017/33455

[24] Dhar, P., & Singla, N. (2014). Effect of triptolide on reproduction of female lesser bandicoot rat, Bandicota bengalensis. Drug and chemical toxicology, 37(4), 448–458. https://doi.org/10.3109/01480545.2014.884111

Copyright © 2023, ISFR, www.fluorideresearch.online, Dunedin, New Zealand