[Copyedited version after the initial publication as an Epub ahead of print November 19, 2023 at www.fluorideresearch.online/epub/files/208.pdf]

TOXIC POTENTIAL OF PYRETHROID INSECTICIDE EXPOSURE *IN UTERO* ON CARDIOVASCULAR DEVELOPMENT IN MICE EMBRYOS

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

Sargodha and Chakwal, Pakistan

ABSTRACT: Cardio-genic disruptions of *in utero* exposure of a non-fluoridated Cypermethrin (CP) and a fluoridated λ-Cyhalothrin (LC) type 2 pyrethroid insecticide were explored in albino mice. Pregnant dams were divided into three groups (n=10); 1) Vehicle Control Group (VCG) (intra-gastric treatment of 0.1mL corn oil); 2) Cypermethrin Group (CPG) and; 3) λ-Cyhalothrin Group (LPG) (intra-gastric treatment of 5mg/kg Cypermethrin (CP) and λ-Cyhalothrin respectively in corn oil). These treatments were applied on 7–10th days of pregnancy. Embryos were removed from the gravid uteri of the euthanized dams on 18th day of pregnancy and fixed in formyl ethanol for 24hrs to finally remove their hearts for histological and histometric analyses. Both insecticides were found to cause micro-anatomical derangements like decreased ventricular wall thickness, rudimentary appearance or complete absence of inter-ventricular (AV) septa, abnormalities of the cardiac valves, and decreased thickness of the muscular layer of the aortic trunk. However, these disruptions were much more pronounced in LCG. Morphometric results revealed a significant decrease (P>0.05) in the thickness of left ventricular wall in CPG (295.90±12.3μ) and LCG (104.27±5.6μ) as compared to VCG (346.68±9.5μ). The mean thickness of interventricular septum was significantly decreased in CPG (440.03±20.4μ) and LCG (214.3±10.2μ) groups than the VCG (2641.75±186.2μ). The thickness of the aortic intima also showed a significant (P>0.05) decrease in CPG (12.70±0.25μ) and LCG (11.03±0.3μ) than the VCG (18.44±0.37μ). Similarly, the thickness of aortic adventitia remained significantly (P>0.05) lower in CPG (26.96±1.1μ) and LCG (23.97±1.5μ) than the VCG (41.09±2.1μ). The relative abundance of myocardiocytes and cardio-myoblasts (per 5000μ^2) also showed significant decrease in both CPG (8.98 ±0.45, 2.50±0.24 respectively) and LCG (4.7 ±0.48, 6.26±0.79) to the VCG (18.19±0.56, 1.02±0.13). The results show that type-2 pyrethroid insecticides inflict a highly toxic impact on the developing heart in mammals and the fluoridated LC is more toxic than the CP in this context.

Keywords: Cypermethrin, Lambda-Cyhalothrin, Fetal heart development

INTRODUCTION

Background:

Pyrethroids have long been considered the safest among the insecticides for mammals. However, recent research reports indicate that pyrethroids may cause serious toxicological implications even in mammals.[1,2] Unfortunately, there are indications for the pyrethroid to cross the placental barrier, and leads to various teratogenic outcomes in developing embryos.[3,4] For example, CP has been reported

[a]University of Sargodha; [b]University of Chakwal; [c]Higher Education Department Government of the Punjab, Pakistan; [d]Chairperson, Board of Intermediate and Secondary Education, Sargodha; [e]Ex-Professor, Department of Zoology, University of Sargodha, Pakistan.

†This article has been extracted from a M.Phil thesis by Sitara Shameem and was presented as an invited oral presentation at the 35th ISFR Conference of the International Society for Fluoride Research, Harbin, People's Republic of China, July 29-30, 2022.

*For correspondence: Professor Dr. Khawaja Raees Ahmad, Ex-Professor, Department of Zoology, University of Sargodha, Sargodha, Pakistan; Email: k.r.ahmad@gmail.com

to increase the level of ROS which results in cellular oxidative stress that may lead to several changes in fetal metabolism.[5] It has been reported that the Japanese rice fish (*Oryzias latipes*) embryos exposed to CP have resulted in visceral and pericardial oedema.[6] In a similar study, the chick embryos exposure to a combination of chlorpyrifos (CL) and CP resulted in defective looping, ventricular non-compaction, and loss of cardiac viability. In domestic chicken, the co-exposure of CL and CP during early development also induced cell death by activating apoptosis.[7] In the same context, LC (type 2 fluoridated pyrethroid) has been found to cause developmental defects like increased pericardial oedema and mortality in zebrafish embryos.[8,9,10] Similarly impaired growth and development were observed in rat pups upon exposure of dams to 8µg/g of LC for 30 days before conception.[11] Earlier on this group has also reported the abnormalities in developing chicks exposed to Bifenthrin (a fluoridated type1 pyrethroid) *in ovo*.[12] The forth going reports suggest that halogenations (especially fluoridations) in pyrethroids (like in Bifenthrin and LC) may increase their persistence as compared to the non-fluoridated pyrethroids (like CP) and thus contributes towards their toxic capacity for the target and non-target organisms especially in the developmental stages.[13,14,15] In light of the above literature it was decided to compare the extent of *in utero* cardiogenic toxicity of a fluoridated type 2 pyrethroid (LC) insecticide with that of a chlorinated type2 pyrethroid (CP) insecticide in mice.

MATERIALS AND METHODS

2.1. Animal: Swiss Webster (*Mus musculus*) estrus dams weighing 30±2g were caged with breeding males. Coitus was determined using an infrared CCTV camera installed in the animal house. Thirty post-coital dams (selected for this study) were randomly distributed into three groups (n=10).

2.2. Dose regime for the experimental groups: Pregnant dams in VCG and the two insecticide groups (CPG and LCG) were provided intra-gastric experimental doses using oral gavages on 7-10days as per detailed below.

2.2.1. Vehicle Control group (VCG): Dams in this group were given 0.1mL corn oil (OD).

2.2.2. Cypermethrin group (CPG): Dams in this group were given 5m/kg CP using corn oil (0.1mL) as a vehicle.

2.2.3. λ-Cyhalothrin group (LCG): Dams in this group were given 5m/kg LC using corn oil (0.1mL) as a vehicle.

2.3. Preparation of stock solution: Desired dose (5m/kg) of technical grade (98%) CP and LC was prepared in corn oil. The single-dose volume for each animal remained 0.1mL with the concentration of CP and LC being adjusted by appropriate dilutions of the standard solutions in accordance with the body weight.

2.4. Recovery of embryos: The gravid uteri were removed intact on day18 from each dam on euthanasia through cervical dislocation. Two well-developed embryos from each dam were placed in alcoholic formalin for 24 hours before further processing.

2.5. *Histological processing:* Entire hearts along with stumps of major blood vessels were removed through micro-dissection of each selected embryo under a

glass magnifier. The organs were gradually dehydrated in 30, 50, 70, 90%, and absolute alcoholic grades for wax embedding. Thin serial sections (3–4µ) of each embryonic heart were obtained on a rotary microtome and affixed to the histological glass slides for H&E staining. The stained serial sections were observed under a trinocular compound microscope (Labomed CXR_2). Snapshots of selected sections were captured at 40× and 100× optic magnifications with a Sony 7.2MP (DSC-W35) Digital Camera.

2.6. Micrometric investigation: Muscular wall thickness of left ventricle and aorta, the thickness of interventricular septum, and relative abundance of myocardiocytes were estimated from the projected embryonic cardiac sections with the help of a pre-calibrated digital scale in CorelDRAW11 software.

2.7. Statistical analysis: The micrometric data were statistically analysed with ANOVA and Tukey's Multiple Range Test (TMRT) using IBM SPSS statistics 23.

RESULTS

3. Micro Anatomy and Histopathology of Developing Cardiac Muscles:

3.1. Anatomy of the Fetal Heart: In VCG, heart sections depict normal development of auricular and ventricular chambers that were completely separated through well-developed intra-auricle and intra-ventricular septa. Moreover, the muscular wall thickness of the auricles and ventricles was appreciable.

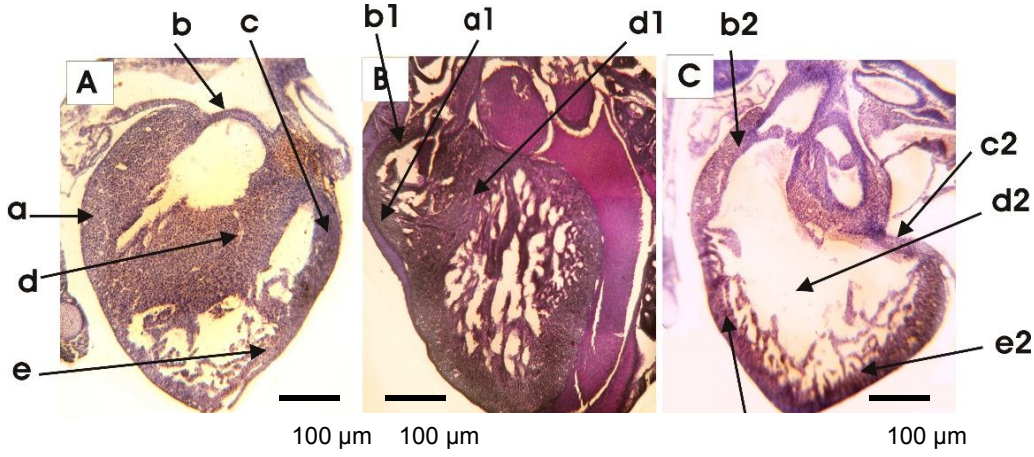

Fig. 1: Digitally calibrated scale 100 µm added on the micrograph. H& E stained transverse sections (100×) of embryonic heart of mice (n=10) A: (VCG);B: (CPG); C: (LCG) a: right ventricle, b: right atrium, c: left atrium, d: interventricular septum, e: left ventricle a1: decreased right ventricular wall thickness b1:thinatrial musculature; d1: perforated inter- ventricular septum; a2 and e2: thin right and left ventricular musculature; b2 and c2:atrial wall defects; d2: missing intra-ventricular septum.

In CPG, an obvious decline in auricular and ventricular wall thickness as compared to VCG was observed. Moreover, the intra-ventricular septa were improperly developed- showing multiple perforations (Fig. 1). In LCG, partial to complete absence of inter-ventricular septum was observed (Fig. 1). Additionally, as compared to VCG, extreme thinning of the ventricular walls with an obvious dilatation of the cardiac chamber sizes was noted in embryonic heart sections in LCG (Fig. 1). Micrometric measurements also revealed a significant decrease in ventricular wall

thickness in CPG and LCG as compared to VCG. Micrometric data also revealed thinnest mean ventricular wall in LCG (Table 1).

Table 1: Alterations in micrometric parameters in developing heart of albino mice fetuses in utero exposed to CP and LC

Micrometric Parameters of fetal heart	Mean+SEM		
	VCG	CPG	LCG
Interventricular septum (IVS) thickness(μ)**	2641.8±186.2^{a}	440.03 ±20.4^{b}	214.3 ±10.2^{b}
Left ventricle wall thickness(μ)**	346.7±9.5^{a}	295.9± 12.3^{b}	104.3 ± 5.6^{c}
Relative abundance of myocardiocytes $(5000\mu)^2$**	18.2 ±0.56^{a}	8.98 ±0.45^{b}	4.77±0.48^{c}
Relative abundance of Cardiomyoblasts$(5000\mu)^2$**	1.02 ±.13^{a}	2.5 ±.24^{ab}	3.26 ±0.8^{b}
Aortic intima thickness(μ)**	18.5±0.37^{a}	12.7±.25^{b}	11.03 ±0.27^{c}
Aortic media thickness(μ)*	22.9 ±0.96^{a}	16±0.33^{b}	14.1±0.38^{b}
Aortic adventitia thickness (μ)**	41.1 ±2.1^{a}	26.96±1.1^{b}	23.97±1.5^{b}

*: (p≤ 0.05), **: (p≤0.01); Any two groups in a single row that do not share a common lower-case superscript differ significantly (p≤0.05) with each other

3.2. Microanatomy of the Aortic Trunk: Although the three basal layers of the aorta (i.e. intima, media, and externa or adventitia) were visible in all three groups however the individual thickness of all the three layers was compromised in CPG and LCG as compare to VCG. Furthermore, intercellular oedema in the medial layer was quite obvious in CPG and LCG (Fig 2: A, B, C).

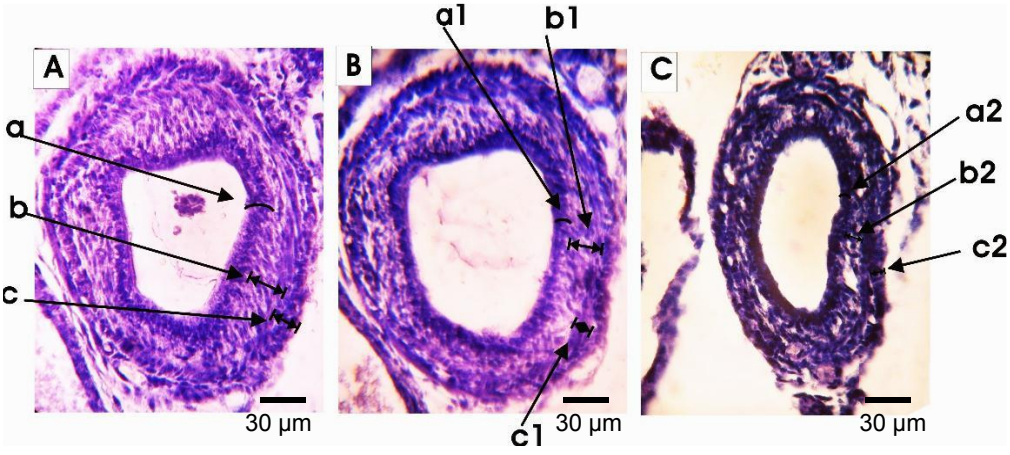

Fig. 2: Digitally calibrated scale 30 μm added on the micrograph. H& E stained transverse sections (400×) of aortic trunk of fetuses of mice (n=10). A: (VCG); B: (CPG); C: (LCG); a: intima thickness; b: media thickness; c: externa thickness; a1 and a2: decreased intimal thickness; b1 and b2: medial layer showing intercellular oedema; c1 and c2: decreased thickness of adventitia.

arising from the left ventricle and the pulmonary valve present at the base of pulmonary trunk- arising from the right ventricle) have shown well-formed cusps and the basal wall thickness of the two major trunks were also appropriate in VCG (Fig 3: A and A1). On the other hand, the cusps of the semilunar valves of aortic and

pulmonary trunk were imperfectly developed and showed rudimentary features in CPG and LCG.

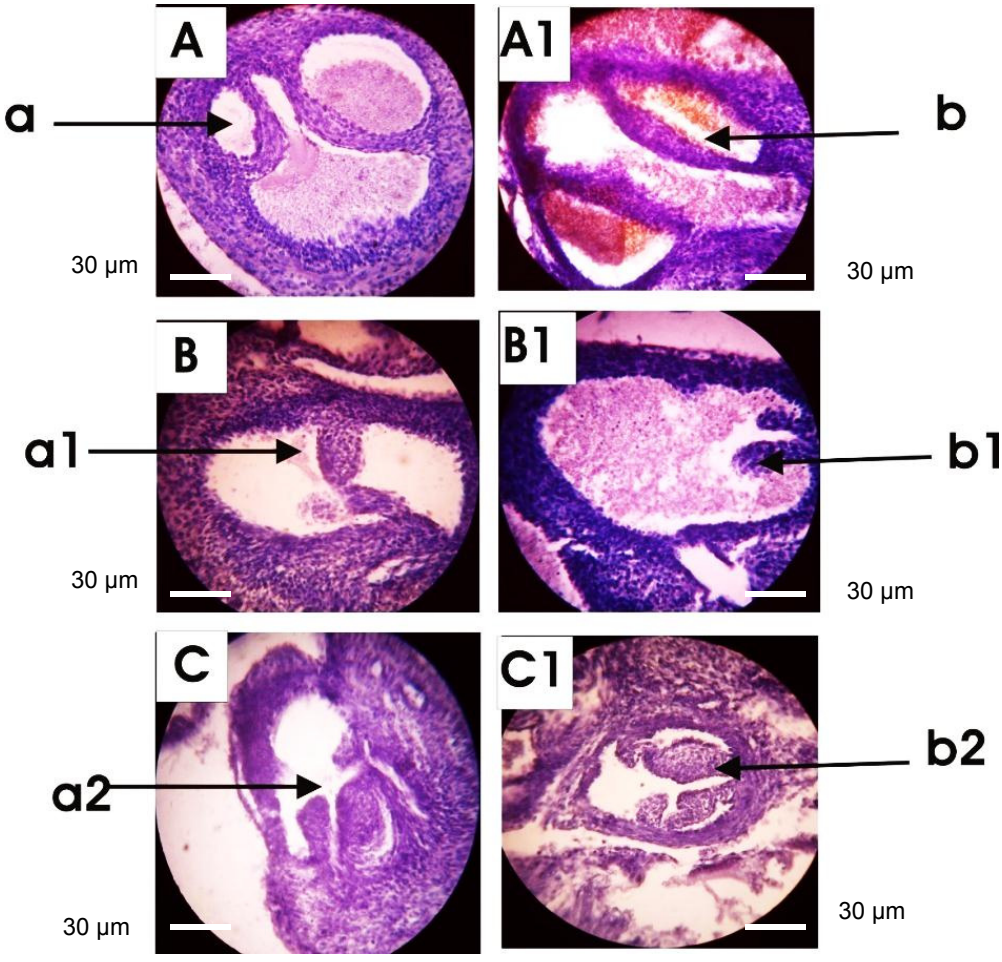

Fig. 3: Digitally calibrated scale 30 µm added on the micrograph. H& E stained sections (400×) of aortic valves (A, B, C) and pulmonary valve (A1, B1, C1) of fetuses of mice (n=10); A and A1: VCG; B and B1: CPG; C and C1: LCG; a and b: showing normal semilunar cusps; a1 and b1: improperly developed semilunar cusps; a2, b2: swollen and poorly developed semilunar cusps.

3.4. Embryonic Cardiac Musculature: The cardiomyoblasts and differentiating myocardiocytes in ventricular walls were clearly visible in VCG. Whereas necrotizing cardiomyoblasts and myocardiocytes along with macrophage infestation and simultaneous fibrosis of the ventricular wall were common features observed in CPG and LCG embryonic hearts. This situation indicates a general delayed differentiation and premature degeneration and necrosis of the differentiating embryonic cardiac musculature in the insecticide-treated groups (Fig 4: B, C). Although a few differentiating and some differentiated myocardiocytes were observed in CPG, the process of differentiation of cardiomyoblasts was severely compromised in LCG - where the developing ventricular walls showed premature myocardiocytes, necrosis of the cardiomyoblasts, and a simultaneous fibrosis and infestation of the macrophages indicating extreme pathological damage of the functional faculties of the ventricular musculature (Fig 4).

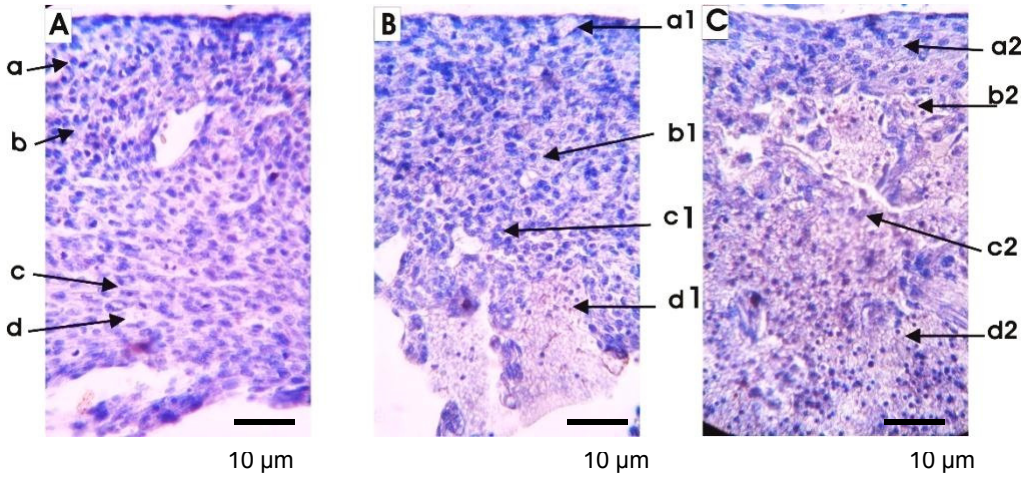

Fig 4: Digitally calibrated scale 10 µm added on the micrograph. H& E stained histological sections (1000×) of left ventricles of fetal hearts of mice (n=10). A: (VCG); B: (CPG) C: (LCG) a: epicardium, b: myocardium mainly consisting of proliferating cardiomyoblasts, c: myocardium containing myocardiocytes, d: endocardium containing loose endothelial tissue, a1: epicardium showing moderate oedema, b1: myocardium containing hypertrophied cardiomyoblasts, c1: myocardium containing aggregates of undifferentiated myocardiocytes, d1:endocardium containing fibrotic endothelial tissue and necrotizing myocardiocytes with infestation of macrophages, a2: epicardium showing intracellular and intercellular oedema, b2: myocardium showing sever necrosis of the cardiomyoblasts, c2: myocardium showing severe destruction of myocardiocytes, d2: endocardium showing debris of the necrotizing myocardiocytes with an extreme infestation of macrophages.

3.5. Micrometric Results: Mean thickness of inter-ventricular septum, left ventricular and aortic walls (intima, media and adventitia) were significantly decreased in CPG and LCG as compared to VCG. However, lowest mean values were recorded in LCG group. The relative abundance of myocardiocytes was highest in VCG and lowest in LCG. However, an opposite trend was observed for the relative abundance of cardiomyoblasts where highest number was recorded in LCG and lowest in VCG (Table: 1).

DISCUSSION

Among the pyrethroids, the type2 pyrethroid insecticides are considered more toxic than the type1 due to the presence of an alpha-cyano moiety in their structure.[10,16] They are more persistent and induce more severe effects on the exposed organisms than the type1 pyrethroid insecticides like tetramethrin, allethrin, d-phenothrin, resmethrin and permethrin.[17-21] Type1 pyrethroids have been reported as potent toxicants for developing organisms.[22] Literature indicates that the *in-ovo* exposure of bifenthrin– a type1 fluoridated pyrethroid –has caused severe abnormalities in chick embryos.[12,23] Likewise, type2 pyrethroid such as cypermethrin– a non-fluoridated pyrethroid insecticide –was also found to induce developmental abnormalities in chick embryos.[24,25] The main focus of the present study was to compare extant of toxicological effects of two type2 pyrethroids i.e. CP (non-fluoridated) and LC (fluoridated) on embryonic heart development in mice.

Unfortunately, the available literature is almost silent upon the embryonic cardiogenic insults of type2 pyrethroids especially the fluoridated LC. Fluoride is a

non-dietary halogen. Its effects on the development of various organs are known to a little extent.[26] Unfortunately, even there exists no comparable study that may address the effects of fluoride on the process of embryonic cardio-genesis. Thus, the results obtained in the present study are not directly comparable with any previous study.

This research work indicates that *in utero* exposure to both CP and LC inflicts toxic effects on embryonic heart development. Whereas, LC appeared to be much more toxic than that of CP in terms of interference in the development of ventricular musculature. The anatomical defects include rudimentary to complete abstinence in the development of interventricular septum, malformations of the aortic and pulmonary valves and decreased wall thickness of the aortic and pulmonary trunks. Differentiation of cardiomyoblasts to myocardiocytes was also severely affected. These histopathological findings were further supported by micrometric results (Table 1).

These findings suggest that LC is highly toxic to myocardial differentiation and development indicating that fluoridation in basic type2 pyrethroid insecticides molecules may further add to their embryo-toxicological impacts. The proposed mode of action may be related to sodium channel openings on the myogenic impulse generating centers of the heart leading to excessive work potential on the developing heart muscles causing pre-mature cellular death of the myocardiocytes as has been seen in LC-treated group embryos (Fig 4). Another reason may be the oxidative stress resulting in premature cellular death and halting the transformation process of cardiomyoblasts to myocardiocytes.[27] Results indicated that type2 pyrethroid insecticides in general and especially the fluoridated pyrethroid insecticides are highly toxic to the development of heart in mammalian embryos.

CONCLUSIONS

The prevailing view that pyrethroids are non-accumulative and thus safer insecticides for human and pet mammals needs a thorough revision. In particular, there is a genuine need for investigation from the standpoint of teratogenic capacities of halogenated (especially the fluoridated) pyrethroids.

ACKNOWLEDGEMENTS

We are thankful to the University of Sargodha for providing the required facilities for this research work. We are also thankful to Auriga Chemical Enterprises, Lahore Pakistan for providing technical-grade insecticides.

REFERENCES

[1] Rehman H, Aziz AT, Saggu SH, Abbas ZK, Mohan A, Ansari AA. Systematic review on pyrethroid toxicity with special reference to deltamethrin. J Entomol Zool Stud 2014;2(6):60-70.

[2] Chrustek A, Hołyńska-Iwan I, Dziembowska I, Bogusiewicz J, Wróblewsk M, Cwynar A, Olszewska-Słonina D. Current research on the safety of pyrethroids used as insecticides. Medicina 2018;54(4):61. https://doi.org/10.3390%2Fmedicina54040061

[3] Dewailly E, Forde M, Robertson L, Kaddar N, Sidi EAL, CÙtÈ S, Ayotte P. Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. Environ Int 2014;63:201-206. https://doi.org/10.1016/j.envint.2013.11.014

[4] Khan MKA. Teratogenic effect of sub lethal doses of deltamethrin in mice. Pak J Zool 2013;45(3):857-864.DOI: 0030-9923/2013/0003-0857

[5] Hocine L, Merzouk H, Merzouk SA, Ghorzi H, Youbi M, Narce M. The effects of alpha-cypermethrin exposure on biochemical and redox parameters in pregnant rats and their newborns. Pestic Biochem Phys 2016;134:49-54. https://doi.org/10.1016/j.pestbp.2016.04.007

[6] Gonzalez-Doncel M, Fernandez-Torija C, Hinton DE, Tarazona JV. Stage-specific toxicity of cypermethrin to medaka (*Oryzias latipes*) eggs and embryos using a refined methodology for an in vitro fertilization bioassay. Arch Environ Contam Toxicol 2005;48:87-98. https://doi.org/10.1007/s00244-003-0223-1

[7] Verma U, Khaire K, Desai I, Sharma S, Balakrishnan S. Early embryonic exposure to chlorpyrifos-cypermethrin combination induces pattern deficits in the heart of domestic hen.Environ Toxicol 2021;36(4):707-721. https://doi.org/10.1002/tox.23074

[8] Demiccoa, Cooperkr, Richardson JR, White LA. Developmental neurotoxicity of pyrethroid insecticides in zebrafish embryos. Toxicol Sci 2010;113(1):177-186. https://doi.org/10.1093/toxsci/kfp258

[9] Zhang Q, Zhang Y, Du J & Zhao M. Environmentally relevant levels of Î-cyhalothrin, fenvalerate, and permethrin cause developmental toxicity and disrupt endocrine system in zebra fish (*Danio rerio*) embryo. Chemosphere 2017;185:1173-1180. https://doi.org/10.1016/j.chemosphere.2017.07.091

[10] Prusty AK, Meena DK, Mohapatra S, Panikkar P, Das P, Gupta SK, Behera BK. Synthetic pyrethroids (Type II) and freshwater fish culture: Perils and mitigations. Inter Aquat Research 2015;7(3):163-191. https://doi.org/10.1007/s40071-015-0106-x

[11] Tu W, Lu B, Niu L, Xu C, Lin C, Liu W. Dynamics of uptake and elimination of pyrethroid insecticides in zebra fish (*Danio rerio*) eleuthero embryos. Ecotoxicol Environ Saf 2014;107:186-191. https://doi.org/10.1016/j.ecoenv.2014.05.013

[12[Khanum Z, Suleman S, Mustanser A, Hassan MWU, 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.

[13] Wakeling EN, Neal AP, Atchison WD. Pyrethroids and their effects on ion channels. Pesticides-advances in Chemical and Botanical Pesticides 2012:39-66. http://dx.doi.org/10.5772/2609

[14] Ubaid Ur Rahman H, Asghar W, Nazir W, Sandhu MA, Ahmed A, Khalid N. A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: Evidence of mechanisms, exposures and mitigation strategies. Sci Total Enviro 2021;755(2):142649. https://doi.org/10.1016/j.scitotenv.2020.142649

[15] Richterova Z, Machova J, Stara A, Tumova J, VelÌsek J, SevcÌkova M, Svobodova Z. Effects of cyhalothrin-based pesticide on early life stages of common carp (*Cyprinus carpio*).BioMed Res Int 2014:107373. https://doi.org/10.1155/2014/107373

[16] Kaviraj A, Gupta A. Biomarkers of type II synthetic pyrethroid pesticides in freshwater fish. BioMed Res Int 2014:928063. https://doi.org/10.1155/2014/928063

[17] Cycon M, Piotrowska-Seget Z. Pyrethroid-Degrading Microorganisms and their Potential for the Bioremediation of Contaminated Soils: A Review. Front Microbiol 2016;7:1463. https://doi.org/10.3389/fmicb.2016.01463

[18] Aelma H, Kilic ET, Salik F, Bicak EA, Yektas A. Pyrethroid intoxication: A rare case report and literature review. Niger J Clin Pract 2019;22(3):442. https://doi.org/10.4103/njcp.njcp_241_18

[19] Henault-Ethier L. Health and environmental impacts of pyrethroid insecticides: what we know, what we don't know and what we should do about It. Equiterre. 2016: 68. http://dx.doi.org/10.13140/RG.2.1.2304.8721

[20] Ramchandra AM, Chacko B, Victor PJ. Pyrethroid poisoning. Indian J Crit Care Med 2019;23(Suppl 4):S267-S271. https://doi.org/10.5005/jp-journals-10071-23304

[21] Khaldoun Oularbi H. Biochemical and histopathological changes in the kidney and adrenal gland of rats Following Repeated Exposure to Lambda-Cyhalothrin. J Xenobiot 2014;4(1):8-13. https://doi.org/10.4081/xeno.2014.2240

[22] Hołyńska-Iwan I, Szewczyk-Golec K. Pyrethroids: how they affect human and animal health? Medicina 2020;56(11):582. https://doi.org/10.3390%2Fmedicina56110582

[23] Dar MA, Raina R, Mir AH, Sultana M, Pankaj NK. Effect of dermal application of bifenthrin on acetyl cholinesterase and oxidative stress indices in rat blood, lung and kidney. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 2015;85(2):431-435. http://dx.doi.org/10.1007/s40011-014-0390-z

[24] Uggini GK, Patel PV, Balakrishnan S. Embryotoxic and teratogenic effects of pesticides in chick embryos: a comparative study using two commercial formulations. Environ Toxicol 2012;27(3):166-174. https://doi.org/10.1002/tox.20627

[25] Andleeb S. Teratogenic potential of pyrethroids: A Review. Punjab Univ J Zool 2016;31(1):107-125. DOI: 57-PUJZ-61024240/16/0107-0125

[26] Zhang Y, Xie L, Li X, Chai L, Chen M, Kong X, Wang H. Effects of fluoride on morphology, growth, development, and thyroid hormone of Chinese toad (*Bufo gargarizans*) embryos. Environ Mol Mutagen 2018;59(2):123-133. https://doi.org/10.1002/em.22147

[27] Li T, Zhang X, Jiang K, Liu J, Liu Z. Dural effects of oxidative stress on cardiomyogenesis via Gata4 transcription and protein ubiquitination. Cell Death Dis 2018;9(2):1-12. https://doi.org/10.1038%2Fs41419-018-0281-y