Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 1923-1928

Received: 02-07-2020
Accepted: 06-08-2020

Nasrul I Shaikh
Research Scholar, Department of Biotechnology, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

RS Sethi
Professor and Head, Department of Biotechnology, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Corresponding Author:
Nasrul I Shaikh
Research Scholar, Department of Biotechnology, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Exposure to chlorpyrifos and cypermethrin alone or in combination induces developmental abnormalities and lung damage in animal models: A review

Nasrul I Shaikh and RS Sethi

DOI: https://doi.org/10.22271/j.ento.2020.v8.i5aa.7769

Abstract
Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate) is an organophosphate used for indoor and outdoor pest control whereas cypermethrin belongs to Type 2 pyrethroid insecticide commonly used against agricultural and domestic pests. This review focuses on the effect of these pesticides alone and in combination on physio developmental and the lung toxicity in different animal models in the light of histochemistry, immunohistochemistry and molecular level. Information such as experimental protocol focusing on the type of animal used, conditions in which experiment was conducted, dosage of pesticide and its mode of administration, duration of exposure and their main finding were extracted from research papers, which were available on pubmed database and google scholar. After the review, It was found that chlorpyrifos and cypermethrin alone or in combination elicits developmental defects and lung damage in different animal models.

Keywords: Pesticide exposure, chlorpyrifos, cypermethrin, chlorpyrifos and cypermethrin combination, lung damage

1. Introduction
Indian agriculture sector plays a vital role in the of the economic development of a country [1]. Each year this sector loses approximately 45% of its outcomes as a result of pest infestation. A wide range of pesticide is used for pest management to oppose pest and to enhance the agriculture yield [2]. The unrestrained pesticide usage is increasing in developing countries that can be attributed to demand of expanded crop production from the limited agriculture land [3, 4]. About 4.6 million tons of pesticides are used worldwide, and a population of 1.8 billion utilizes pesticides globally to deter or kill pests in agricultural settings [5, 7]. India holds a position among top ten pesticide consuming countries worldwide after China [8]. Indian pesticide industries have become one of the leading pesticides manufacturing countries in Asia with 90,000 tons of annual production [9] that include pesticide such as Mancozeb, Cypermethrin, Sulphur, Acephate and Chlorpyrifos which are exported to USA, Brazil and France [10].
In agricultural settings, the use of two pesticides in mixture/combination form has become common. One such combination is of chlorpyrifos and cypermethrin [11] Chlorpyrifos ((O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate) is an organophosphate used for indoor and outdoor pest-control [12]. Chlorpyrifos degrades and produces metabolite 3,5,6-trichloro-2-pyridinol (TCP). The degradation rate and pH are inter-related in a manner that lower pH leads to longer half-life of metabolite [13]. Studies have reported the presence of traces of metabolites in plants and algae that doubled within a short two-year period [14, 15]. On the other hand, cypermethrin belongs to Type 2 pyrethroid insecticide commonly used against agricultural and domestic pests [16]. Cypermethrin besides causing environmental pollution also causes developmental neurotoxicity, [17, 18] oxidative stress, [19] cellular infiltration, necrotic changes and characterized by thickening of alveolar septa and inflammation of lung tissues [20].
The pesticide applied in an unstrained manner remains on the crop surface, becomes an aerosol, settles down in soil and finally reaches to various water bodies making severe interference in food chain [21, 23]. Humans and others get exposed to these pesticides through the most common route such as dietary intake, respiratory inhalation, and dermal contact [24]. Recent studies have reported the presence of cypermethrin and chlorpyrifos in water body's

nearby agriculture area causing health issues [25, 27].
Through decades, the individual pesticide such as chlorpyrifos and cypermethrin has been studied for their toxicity in humans and animal but recently the trend of pesticide combination has taken a vital place in the agricultural sector. Moreover, the developemental and lung toxicity of these pesticides alone and in combination has not been summarized in any other review. This review thus focuses on the effect of these pesticides alone and in combination on developmental and the lung toxicity in different animal models in the light of histochemistry, immunohistochemistry and molecular basis.

2. Methodology
Data were obtained from an advanced search on pubmed database and google scholar using key words: chlorpyrifos lung damage, cypermethrin lung damage and chlorpyrifos cypermethrin lung damage without any restriction such as language of article, country, type and date of publication. Moreover, further publications were taken from cross references. Only the required information such as experimental protocol focusing on animal used, conditions of experiment, dosage of pesticide and its mode of administration, duration of exposure and their main findings were extracted.

3. Results
3.1 Mechanism of inducing toxicity
Acetylcholinestrase (AchE) is the enzyme that primarily functions to catalyse and facilitate the breakdown of neurotransmitter called acetylcholine. Organophosphates causes termination of synaptic function via dysregulation of AChE in the biological system in order to elicit the toxicity [28]. Exposure of chlorpyrifos to Mediterranean crab (Carcinus maenas) [29], Frog (Lysapsus limellium) [30], Chicken [31], Earthworms [32] and Caracal (Caracal caracal) [33] decreased the AChE and butChE level in hepatopanreas and gills. Cypermethrin exposure to rat [34] and mice [35] also resulted in inhibition of AchE activity. On the other hand, rats fed with 10 mg of individual compound or 5 mg of each per kg body weight daily dissolved in rapeseed oil for 14 and 28 days markedly depressed cholinesterase to a different degree in plasma and brain of animals receiving chlorpyrifos and cypermethrin alone or in combination [36]. Additionally, cypermethrin induces neurotoxicity by increasing the level of Gamma-amino butyric acid (GABA) in rat's cerebellum [37]. The depression in antioxidant inside the body elevates stress on the biological system that results in malfunctioning of vital organ as reported in various studies [38, 43].

3.2 Developmental effects
A study on sea urchins (Paracentrotus lividus) exposed to low and high doses chlorpyrifos showed that low dose of chlorpyrifos altered the pattern of metamorphosis while the high dose did not allow larval growth and differentiation. However, exposures at later stages caused reabsorption of larval structures within a few hours and precocious release of the immature rudiments, followed by death of the juveniles [44]. In another study embryo mortality and developmental anomalies were increased when exposed to chlorpyrifos [45]. Repeated oral exposure of cypermethrin has been shown to produce a harmful effects on various organ developments. Relative weight of liver and heart increased with decrease in weight of brain, kidneys and testes when exposed to cypermethrin [46]. Few studies contradicted in a way that cypermethrin exposure decreases weight of liver, spleen and kidney [67]. Effect of cypermethrin in ovulation has been reported where atresia of follicles was observed with a decreased number of follicular cells, oocytes and corpora lutea followed by induction of vesicular atrophy of the endometrial glands [47]. Cypermethrin has been reported to disturb motor development without modifying sensory and communicative skill which later in life offsprings expresses maladaptive behaviors in response to highly challenging tasks alongside abnormal sociability. Furthermore, transcriptomic analyses performed in the offspring's brain highlighted mitochondrial dysfunction and dysregulate several genes involved in proteostasis maintenance [48]. In case of pisces, Gills were found more sensitive to oxidative damage than the digestive glands. The gill filament exhibited a reduction or loss of cilia, vacuolization of the columnar cells when exposed to pesticides. At high concentrations of cypermethrin caused disruptions in the columnar muscle fibers [49]. The pyrethroid caused decreases in reproductive organ's weight, sperm count, sperm motility meanwhile changing the architecture of testes [39]. Similar observations were seen when chlorpyrifos (6.75 mg/kg body weight) and cypermethrin (12.5 mg/kg body weight) were given in combination to male albino rats where exposure resulted in dysfunctioning of reproductive organs with decreased testicular weight, abnormal development of spermatozoa, sperm count and motality [50]. Combined exposure of these pesticides down regulated hedgehog signaling contribute to pesticide-mediated bone marrow aplasia [51].

3.3 Lung damage
Lung has been found to be the first organ to come in contact with after inhalation/ingestion [52]. Toxicants present in the breathing zone may be absorbed in the nasopharyngeal, tracheobronchial, or pulmonary exchange surfaces of the lung, depending upon the physical and chemical properties of the toxicant [53]. We have earlier reported that oral dietary exposure to various pesticides alters the histomorphology [72] and transcription in lung of mice [73]. Several *in vivo* studies on Mice, Guinea pigs, and rats exposed to different doses of Chlorpyrifos alone dissolved in corn or linseed oil administered in either intranasal, intramuscular subcutaneous injection or orally elicited lung toxicity, pulmonary dysfunction, airway hyperactivity, abnormal lung development (Table 1). Cypermethin exposure to Mice, Guinea pigs and rats at varied doses administered orally, aerosolized or orally caused congestion of lungs, pulmonary hemorrhage, irritant effect on lung tissue and hyper responsiveness (Table 2). The exposure of combination of chlorpyrifos and cypermethrin increased expression of TNF-α resulting synergistic effect on the expression of TNF-α mRNA that triggers a strong inflammatory response with massive neutrophil infiltration during lung injury. Moreover, the study showed that in combination these pesticide results in lung injury characterized by infiltration of mononuclear cells around perivascular and peribronchiolar regions, sloughing of epithelium and thickening of the alveolar septa in mice [54]. The effect of the same has also been seen fresh water crab, Paratelphusa jacquemontii (Rathbun) where the exposure altered the histology of lung [29]. At lower concentration of 0.0187 ppm enlargement of intralamellar spaces and loss of gill structure took place whereas higher concentration of 0.0374 ppm resulted in thickened gill lamellae, hemocytic infiltration, epithelial necrosis and hyperplasia [55].

Table 1: Chlorpyrifos lung toxicity

References	Dose (Body weight)	Route	Animal model	Type of damage	Main findings
Chougule *et al.* 2013 [56]	3mg/kg	Intranasal	Mice	Pulmonary dysfunction	Increased critical apoptosis related proteins such as p53, Bax and Caspase-3 in lung of mice
Fryer *et al.* 2004 [57]	70 mg/kg or 390 mg/kg	Subcutaneous injection	Guinea pigs	Airway hyper-reactivity	Decreased responsiveness of neuronal M2 receptors
Uzun *et al.* 2010 [58]	5.4 mg/kg	Oral gavage	Wistar rats	Histopathological alteration in lung	Increased the levels of malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) Decreased glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities in lung tissues
Darwiche *et al.* 2018 [59]	1 or 5mg/kg b.wt/day	Oral gavage	Wistar rats	Respiratory dysfunction	Accumulation of acetylcholine in the synaptic clefts
Shaffo *et al.* 2018 [60]	30 mg/kg in males & 7 mg/kg	Oral gavage	Female Sprague Dawley rats	Airway Hyper-reactivity	Induced airway resistance and tissue elastance at 7 d post-exposure in males and at 24 h and 7 d post-exposure in females
Karaoz *et al.* 2002 [61]	41 mg CE per kg	Intramuscularly	Rats	Lung toxicity	Increased inflammatory mononuclear cells in peribronchial and perivascular areas
Yazdinezhad *et al.* 2017 [62]	6.75 mg/kg	Oral gavage	Rats	Lung dysfunction	Oxidative stress and induction of cell death signaling leading to lung failure
Hassani *et al.* 2014 [63]	13.5 mg/kg	Oral gavage	Rats	Lung toxicity	Histopathological damages were observed
Shalaby *et al.* 2013 [64]	10 mg /kg	Oral gavage	female Sprague Dawley rats	Abnormal lung development	Hypoplasia of the lungs

Table 2: Cypermethrin lung toxicity

References	Dose (Body weight)	Route	Animal model	Type of damage	main findings
Arafa MH *et al.* (2015) [20]	14.5 mg kg	Oral gavage	Rats	Lung damage	Collapsed alveoli, congested blood capillaries with thickened wall and extravasations of red cells within the alveolar lumen.
Nair *et al.* 2011 [65]	40 mg/kg, 80 mg/kg, and 120mg/kg	Oral gavage	Adult Sprague Dawley male rats	Congestion of lungs, pulmonary hemorrhage	Pulmonary hemorrhage
Al-Shaikh 2012 [66]	2.8 mg/kg	Oral gavage	Male mice	Lung injury	Bleeding, inflammatory cells within alveolar spaces and inside the cavity of bronchioles Decrease in alveolar spaces alongwith inflammatory cell infiltration Capillary congestion
Grewal *et al.* 2009 [67]	14.5 mg/kg	Oral gavage	Rats	Lung toxicity	Congestion, oedema and marked diffuse chronic interstitial pneumonia with diffuse lymphomononuclear (LMN) cell infilteration and fibroplasia
Grewal at al 2010 [68]	5 and 20 mg/kg/day	Oral gavage	Albino rats	Irritantion effect on the pulmonary tissue	Congestion, hemorrhage, and thickening of interalveolar septa
Garcia *et al.* 2009 [69]	1-2% cypermethrin	Aerosolized	Swiss mice	Lung hyperrespon-siveness	Increased polymorphonuclear cells (eosinophils and neutrophils) in blood and lungs
Shaikh *et al.* 2013 [70]	0.5% dilution of cypermethrin	Inhalation	Swiss albino mice	Hyperplasia, clumping of cells and necrosis in the lungs	Pulmonary edema, alveolitis, and pulmonary fibrosis by the deposition of collagen
Manna *et al.* 2004 [71]	145 mg/kg	Oral gavage	Wistar rats	Lung hemorrhages	Lung hemorrhages

4. Conclusion

The exposure of chlorpyrifos and cypermethrin alone or in combination causes developmental defects and pulmonary impairment signified by alveolar congestion, hemorrhage, neutrophil infiltration, emphysematous changes and cellular aggregation in vascular walls or air spaces. Moreover, these pesticides alters the expression of genes involved in molecular pathways.

5. References

1. Limbore NV, Khillare SK. An analytical study of Indian agriculture crop production and export with reference to wheat. Review of Research. 2015; 4(6):1-8.
2. Abhilash PC, Singh N. Pesticide use and application: an Indian scenario. Journal of hazardous materials. 2009; 165(1-3):1-2.
3. Pandit AA, Gandham RK, Mukhopadhyay CS, Verma R, Sethi RS. Transcriptome analysis reveals the role of the PCP pathway in fipronil and endotoxin-induced lung damage. Respiratory research. 2019; 20(1):1-6.
4. Schreinemachers P, Tipraqsa P. Agricultural pesticides and land use intensification in high, middle and low income countries. Food policy. 2012; 37(6):616-26.
5. Fenske RA, Kissel JC, Lu C, Kalman DA, Simcox NJ, Allen EH *et al.* Biologically based pesticide dose estimates for children in an agricultural community. Environmental Health Perspectives. 2000; 108(6):515-20.
6. Zhang W, Jiang F, Ou J. Global pesticide consumption

and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences. 2011; 1(2):125.
7. Alavanja MC. Pesticides use and exposure extensive worldwide. Reviews on environmental health. 2009; 24(4):303.
8. Worldatlas, 2018. https://www.world atlas. com/artic les/toppesti cide-consu ming-count ries-of-the-world. html.
9. Khan MJ, Zia MS, Qasim M. Use of pesticides and their role in environmental pollution. World Acad Sci Eng Technol. 2010; 72:122-8.
10. Subash SP, Chand P, Pavithra S, Balaji SJ, Pal, S. Pesticide use in Indian agriculture: trends, market structure and policy issues. National Institute of Agricultural Economics and Policy Research, 2017, 43**.**
11. Tripathi M, Pandey R, Ambesh SP, Pandey M. A mixture of organophosphate and pyrethroid intoxication requiring intensive care unit admission: a diagnostic dilemma and therapeutic approach. Anesthesia & Analgesia. 2006; 103(2):410-2.
12. Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG *et al.* Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical reviews in toxicology. 2008; 38(sup2):1-25.
13. Watts M. Chlorpyrifos as a possible global POP. Pesticide Action Network North America, Oakland, CA. www.pan-europe.info/News/PR/121009_Chlorpyrifos_as_POP_fin al. pdf, 2012.
14. Prasertsup P, Ariyakanon N. Removal of chlorpyrifos by water lettuce (*Pistia stratiotes* L.) and duckweed (*Lemna minor* L.). International journal of phytoremediation. 2011; 13(4):383-95.
15. Lal S, Lal R, Saxena DM. Bioconcentration and metabolism of DDT, fenitrothion and chlorpyrifos by the blue-green algae Anabaena sp. and *Aulosira fertilissima*. Environmental Pollution. 1987; 46(3):187-96.
16. Carriquiriborde P, Díaz J, Mugni H, Bonetto C, Ronco AE. Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production. Chemosphere. 2007; 68(4):613-21
17. Maurya SK, Rai A, Rai NK, Deshpande S, Jain R, Mudiam MK *et al.* Cypermethrin induces astrocyte apoptosis by the disruption of the autocrine/paracrine mode of epidermal growth factor receptor signaling. Toxicological Sciences. 2012; 125(2):473-87.
18. Flaskos J, Harris W, Sachana M, Munoz D, Tack J, Hargreaves AJ *et al.* The effects of diazinon and cypermethrin on the differentiation of neuronal and glial cell lines. Toxicology and applied pharmacology. 2007; 219(2-3):172-80.
19. Abdelhafidh K, Mhadhbi L, Mezni A, Badreddine S, Beyrem H, Mahmoudi E *et al.* Protective effect of Zizyphus lotus jujube fruits against cypermethrin-induced oxidative stress and neurotoxicity in mice. Biomarkers. 2018; 23(2):167-73.
20. Arafa MH, Mohamed DA, Atteia HH. Ameliorative effect of N-acetyl cysteine on alpha-cypermethrin-induced pulmonary toxicity in male rats. Environmental toxicology. 2015; 30(1):26-43.
21. Sharma A, Kumar V, Bhardwaj R, Thukral AK. Seed pre-soaking with 24-epibrassinolide reduces the imidacloprid pesticide residues in green pods of Brassica juncea L. 2017; 99:95-103.
22. Sharma BM, Bharat GK, Tayal S, Nizzetto L, Čupr P, Larssen T *et al.* Environment and human exposure to persistent organic pollutants (POPs) in India: A systematic review of recent and historical data. Environment international. 2014; 66:48-64
23. Karr JR. River conservation in the United States and Canada. Global perspectives on river conservation: science, policy, and practice. *Wiley.* 2000.
24. Sharma A, Kumar V, Bhardwaj R, Thukral AK. Seed pre-soaking with 24-epibrassinolide reduces the imidacloprid pesticide residues in green pods of Brassica juncea L. Toxicological & Environmental Chemistry. 2017; 99(1):95-103.
25. Li YF, Macdonald RW, Li YF, Macdonald RW. Sources and pathways of selected organochlorine pesticides to the Arctic and the effect of pathway divergence on HCH trends in biota: a review. Science of the total environment. Science of the total environment. 2005; 342:87-106.
26. Hunt L, Bonetto C, Resh VH, Buss DF, Fanelli S, Marrochi N *et al.* Insecticide concentrations in stream sediments of soy production regions of South America. Science of the Total Environment. 2016; 547:114-24.
27. Jergentz S, Mugni H, Bonetto C, Schulz R. Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. Chemosphere. 2005; 61(6):817-26.
28. Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG *et al.* Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical reviews in toxicology. 2008; 38(sup2):1-25.
29. Ghedira J, Jebali J, Bouraoui Z, Banni M, Chouba L, Boussetta H *et al.* Acute effects of chlorpyryphos-ethyl and secondary treated effluents on acetylcholinesterase and butyrylcholinesterase activities in Carcinus maenas. Journal of Environmental Sciences. 2009; 21(10):1467-72.
30. Attademo AM, Peltzer PM, Lajmanovich RC, Cabagna-Zenklusen M, Junges CM, Lorenzatti E *et al.* Biochemical changes in certain enzymes of Lysapsus limellium (Anura: Hylidae) exposed to chlorpyrifos. Ecotoxicology and environmental safety. 2015; 113:287-94.
31. Begum SA, Upadhyaya TN, Rahman T, Pathak DC, Sarma K, Barua CC *et al.* Hematobiochemical and pathological alterations due to chronic chlorpyrifos intoxication in indigenous chicken. Indian journal of Pharmacology. 2015; 47(2):206.
32. Bednarska AJ, Choczyński M, Laskowski R, Walczak M. Combined effects of chlorpyriphos, copper and temperature on acetylcholinesterase activity and toxicokinetics of the chemicals in the earthworm Eisenia fetida. Environmental Pollution. 2017; 220:567-76.
33. Merbl Y, Aroch I, Klainbart S, Aizenberg Z, Kelmer E. Intermediate Syndrome of Chlorpyriphos Toxicity (Polaris®) in a Caracal (Caracal caracal). Journal of Zoo and Wildlife Medicine. 2011; 42(1):144-8.
34. Elsawy H, Al-Omair MA, Sedky A, Al-Otaibi L. Protective effect of α-lipoic acid against α-cypermethrin-induced changes in rat cerebellum. Journal of Chemical Neuroanatomy. 2017; 86:52-8.

35. Abdelhafidh K, Mhadhbi L, Mezni A, Badreddine S, Beyrem H, Mahmoudi E *et al.* Protective effect of Zizyphus lotus jujube fruits against cypermethrin-induced oxidative stress and neurotoxicity in mice. Biomarkers. 2018; 23(2):167-73.
36. Wielgomas B, Krechniak J. Effect of α-Cypermethrin and Chlorpyrifos in a 28-Day Study on Free Radical Parameters and Cholinesterase Activity in Wistar Rats. Polish Journal of Environmental Studies. 2007; 16(1).
37. Gómez-Giménez B, Llansola M, Hernández-Rabaza V, Cabrera-Pastor A, Malaguarnera M, Agusti A *et al.* Sex-dependent effects of developmental exposure to different pesticides on spatial learning. The role of induced neuroinflammation in the hippocampus. Food and Chemical Toxicology. 2017; 99:135-48.
38. Paravani EV, Simoniello MF, Poletta GL, Zolessi FR, Casco VH. Cypermethrin: Oxidative stress and genotoxicity in retinal cells of the adult zebrafish. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2018; 826:25-32.
39. Sharma P, Khan IA, Singh R. Curcumin and quercetin ameliorated cypermethrin and deltamethrin-induced reproductive system impairment in male wistar rats by upregulating the activity of pituitary-gonadal hormones and steroidogenic enzymes. International journal of fertility & sterility. 2018; 12(1):72.
40. Arafa MH, Mohamed DA, Atteia HH. Ameliorative effect of N-acetyl cysteine on alpha-cypermethrin-induced pulmonary toxicity in male rats. Environmental toxicology. 2015; 30(1):26-43.
41. Dahamna S, Belguet A, Bouamra D, Guendouz A, Mergham M, Harzallah D *et al.* Evaluation of the toxicity of cypermethrin pesticide on organs weight loss and some biochemical and histological parameters. Communications in agricultural and applied biological sciences. 2011; 76(4):915-21.
42. Ozkan U, Osun A, Basarslan K, Senol S, Kaplan I, Alp H *et al.* Effects of intralipid and caffeic acid phenethyl ester on neurotoxicity, oxidative stress, and acetylcholinesterase activity in acute chlorpyriphos intoxication. International Journal of Clinical and Experimental Medicine. 2014; 7(4):837.
43. Pankaj M, Krishna K. Acute organophosphorus poisoning complicated by acute coronary syndrome. J Assoc Physicians India. 2014; 62(7):614.
44. Aluigi MG, Falugi C, Mugno MG, Privitera D, Chiantore M. Dose-dependent effects of chlorpyriphos, an organophosphate pesticide, on metamorphosis of the sea urchin, Paracentrotus lividus. Ecotoxicology. 2010; 19(3):520-9.
45. Budai P, Grúz A, Várnagy L, Kormos E, Somlyay IM, Lehel J *et al.* Toxicity of chlorpyriphos containing formulation and heavy elements (Cd, Pb) to chicken embryos. Commun. Agric. Appl. Biol. Sci. 2015; 80(3):393-6.
46. Grewal KK, Sandhu GS, Kaur R, Brar RS, Sandhu HS. Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicology international. 2010; 17(2):94.
47. Marettova E, Maretta M, Legáth J. Effect of pyrethroids on female genital system. Review. Animal Reproduction Science. 2017; 184:132-8.
48. Laugeray A, Herzine A, Perche O, Richard O, Montecot-Dubourg C, Menuet A *et al.* In utero and lactational exposure to low-doses of the pyrethroid insecticide cypermethrin leads to neurodevelopmental defects in male mice-An ethological and transcriptomic study. PloS one. 2017; 12(10):e0184475.
49. Arrighetti F, Ambrosio E, Astiz M, Capítulo AR, Lavarías S. Differential response between histological and biochemical biomarkers in the apple snail Pomacea canaliculata (Gasteropoda: Amullariidae) exposed to cypermethrin. Aquatic Toxicology. 2018; 194:140-51.
50. Alaa-Eldin EA, El-Shafei DA, Abouhashem NS. Individual and combined effect of chlorpyrifos and cypermethrin on reproductive system of adult male albino rats. Environmental Science and Pollution Research. 2017; 24(2):1532-43.
51. Chaklader M, Das P, Pereira JA, Chaudhuri S, Law S. Altered canonical hedgehog-gli signalling axis in pesticide-induced bone marrow aplasia mouse model. Archives of Industrial Hygiene and Toxicology. 2012; 63(3):271-82.
52. Dixon D, Herbert RA, Kissling GE, Brix AE, Miller RA, Maronpot RR *et al.* Summary of chemically induced pulmonary lesions in the National Toxicology Program (NTP) toxicology and carcinogenesis studies. Toxicologic pathology. 2008; 36(3):428-39.
53. Richards IS. Principles and Practices of Toxicology in Public Health. 1st Ed. Jones and Bartlett Publishers, USA, 2008, pp.115-190.
54. Nazki M, Sethi RS. Pulmonary Expression of TNF-Following Exposure to Mixture of Chlorpyriphos and Cypermethrin with or without Endotoxin. Indian Journal of Veterinary Anatomy. 2019; 31(2):148-50.
55. Maharajan A, Narayanasamy Y, Ganapiriya V, Shanmugavel K. Histological alterations of a combination of Chlorpyrifos and Cypermethrin (Nurocombi) insecticide in the fresh water crab, Paratelphusa jacquemontii (Rathbun). The journal of Basic & Applied Zoology. 2015; 72:104-12.
56. Chougule AA, Sethi R, Schneberger D, Brar RS, Gill JP, Singh B *et al.* Chlorpyriphos induces lung inflammation and alters response to E. coli lipopolysaccharide challenge. The FASEB Journal. 2013; 27, 1166.
57. Fryer AD, Lein PJ, Howard AS, Yost BL, Beckles RA, Jett DA *et al.* Mechanisms of organophosphate insecticide-induced airway hyperreactivity. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2004; 286(5):L963-9.
58. Uzun FG, Demir F, Kalender S, Bas H, Kalender Y. Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food and Chemical Toxicology. 2010; 48(6):1714-20.
59. Darwiche W, Gay-Quéheillard J, Delanaud S, El Khayat El Sabbouri H, Khachfe H, Joumaa W *et al.* Impact of chronic exposure to the pesticide chlorpyrifos on respiratory parameters and sleep apnea in juvenile and adult rats. PloS one. 2018; 13(1):e0191237.
60. Shaffo FC, Grodzki AC, Fryer AD, Lein PJ. Mechanisms of organophosphorus pesticide toxicity in the context of airway hyperreactivity and asthma. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2018; 315(4):L485-501.
61. Karaoz E, Gultekin F, Akdogan M, Oncu M, Gokcimen A. Protective role of melatonin and a combination of vitamin C and vitamin E on lung toxicity induced by chlorpyrifos-ethyl in rats. Experimental and Toxicologic

Pathology. 2002; 54(2):97-108.
62. Yazdinezhad A, Abbasian M, Hojjat Hosseini S, Naserzadeh P, Agh-Atabay AH, Hosseini MJ *et al.* Protective effects of *Ziziphora tenuior* extract against chlorpyrifos induced liver and lung toxicity in rat: Mechanistic approaches in subchronic study. Environmental toxicology. 2017; 32(9):2191-202.
63. Hassani S, Sepand MR, Jafari A, Jaafari J, Rezaee R, Zeinali M *et al.* Protective effects of curcumin and vitamin E against chlorpyrifos-induced lung oxidative damage. Human & experimental toxicology. 2015; 34(6):668-76.
64. Shalaby MA, Abo-El-Sooud K, Hamoda AA. Assessment of toxicity of chlorpyrifos insecticide on fetuses and suckling pups of rats. Insight Ecol. 2013; 2:1-7.
65. Nair RR, Abraham MJ, Lalithakunjamma CR, Nair ND, Aravindakshan CM. A pathomorphological study of the sublethal toxicity of cypermethrin in Sprague Dawley rats. International journal of nutrition, pharmacology, neurological diseases. 2011; 1(2):179.
66. Al-Shaikh TM. Protective antioxidant effect of garlic against cypermethrin induced lung toxicity in adult male mice: Biochemical and Histopathological studies. Life Science Journal. 2012; 9(4):4017-25.
67. Grewal G, Verma PK, Dhar VJ, Srivastava AK. Toxicity of subacute oral administration of cypermethrin in rats with special reference to histopathological changes. International Journal of Green Pharmacy (Medknow Publications & Media Pvt. Ltd.). 2009; 3(4).
68. Grewal KK, Sandhu GS, Kaur R, Brar RS, Sandhu HS. Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicology international. 2010; 17(2):94.
69. Garcia ML, Santos UP, Perini A, Acencio MM, Lopes FD, Bueno H, Saldiva PH *et al.* Eosinophilic pneumonitis induced by aerosol-administered diesel oil and pyrethrum to mice. Revista Panamericana de Salud Pública. 2009; 25:518-23.
70. Sheikh N, Javed S, Asmatullah A, Ahmad KR, Abbas T, Iqbal J *et al.* Histological changes in the lung and liver tissues in mice exposed to pyrethroid inhalation. Walailak Journal of Science and Technology (WJST). 2014; 11(10):843-9.
71. Manna S, Bhattacharyya D, Basak DK, Mandal TK. Single oral dose toxicity study of a-cypermethrin in rats. Indian journal of pharmacology. 2004; 36(1):25.
72. Sethi RS, Shaikh NI, Mukhopadhyay CS, Sodhi SS, Ramneek. Chronic dietary exposures of chlorpyriphos and cypermethrin dysregulate apoptosis pathway in mouse lung. Proceedings international e- conference on Immunology in 21st Century for Improvising One Health, 2020.
73. Sethi RS, Shaikh NI, Singh B. Pulmonary expression of genes in response to exposure to chlorpyriphos and cypermethrin. *Antomia histologia*, embryologia Journal of veterinary medicine, 2019, 18.