

CYPERMETHRIN CAUSE ALLERGIC INFLAMMATORY RESPONSE IN RAT LUNG

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ABSTRACT

Cypermethrin is a type 2 photosensitive liposoluble pyrethroid for agricultural and domestic use, has a half-life in the ambient air for days, its metabolism in human liver generate a cyan group (CN), an alcohol fraction (dimethylcyclopropanecarboxylic acid) (DCCA), and 3-acid phenoxybenzoic acid (3-PBA) with reactive oxygen species (ROS) properties, there are scarce studies when is inhaled. The objective of this work was to evaluate the structural changes and activation of allergic inflammatory response in lower airway components and alveoli induced by inhaled exposure to low doses of cypermethrin. Rats were exposed during 7, 14 and 21 days by inhalation, then were analyzed the serum levels of IgE by ELISA, performed H&E stain on lung tissue for analyze the structural changes, through immunofluorescence were detected positive cells to IL-4, IL-5 and IL-13, finally by qPCR was quantified mRNA for IL-4, our results shows that cypermethrin induce a local and systemic allergic inflammatory reaction in rats, specifically in lungs, induce the production and secretion of products of Th2 cells (IL-4, IL-5, IL-13), eosinophils and, high serum levels of IgE, demonstrating like this, the cytotoxic effect of cypermethrin at 7 and 14 days exposition.

KEYWORDS: Cypermethrin, inhaled toxicity, inflammatory reaction, IL-4, IL-5, IL-13, IgE.

INTRODUCTION

Cypermethrin is a type 2 photosensitive liposoluble pyrethroid for agricultural and domestic use, has a half-life in the ambient air for days (Côté et al. 2014; Amweg, Weston, and Ureda 2005), is widely used in Mexico for control of domestic and field pests (González-Arias et al. 2010). Due to its fat-solubility, it is easily absorbed dermally, gastrointestinal and inhaled. Respiratory exposure requires that the particle size of the respirable fraction be 0.5 to 5 µm, to reach the lower airway and alveoli (Fernández Tena and Casan Clarà 2012). After systemic absorption, cypermethrin is hydrolyzed in the liver by human carboxylesterase 1 (hCE-1), generating a cyan group (CN), an alcohol fraction

(dimethylcyclopropanecarboxylic acid) (DCCA), and 3-acid phenoxybenzoic acid (3-PBA); the cyan and acid fractions form conjugates with several compounds for their elimination, if the cyan group does not conjugate, then it forms the thiocyanate anion (-SCN) that increases oxidative stress, a part of the alcohol fraction is conjugated and removed by urine and feces, while the other part, is oxidized in carbon 4 of the phenoxybenzoic ring to form the compound 4-hydroxyphenoxy benzoic acid (4OH3PBA), the latter compound is oxidized by several isoforms of cytochrome P450 (2E1) in human and in rat generating reactive oxygen species (ROS) (Kaneko 2011). In humans, its half-life is 10 hours (Morgan et al. 2018; Kaneko 2011). Toxicity to exposure to this compound has been reported, manifested as an allergic inflammatory response in lung tissue (Choi et al. 2006). Allergy-like inflammation in mucous membranes, is induced by the disruption of epithelial barriers by allergens and contaminants through two mechanisms (Gandhi and Vliagoftis 2015), a) by the secretion of cytokines derived from the epithelium that activate innate lymphoid cells from group 2 (ILC2) (IL-5, IL-9 and IL-13) and to the dendritic cells (DC) of the lamina propria; b) DC endocytoses the allergens in the lung, they are activated and migrated to the lymph nodes of the bronchial-associated lymphoid tissue (BALT) where they present the antigen to TH2 lymphocyte and activate it, the TH2 cell activation process results in the production and secretion of cytokines IL-4, IL-5, IL-9 and IL-13 (Licona-Limón et al. 2013; Pelletier and Savignac 2018). IL-4, in turn, promotes the production of IgE responsible for activating mast cells, a cell that releases inflammatory mediators (histamine, leukotriene C4 (LTC4) and IL-8) (Cruse et al. 2005), and induce smooth muscle contraction, increase in vascular permeability and recruitment of inflammatory cells. IL-5 is related to the proliferation and differentiation of eosinophils, IL-9 facilitates proliferation of TCD4 + cells and mast cell activation, IL-13 modulates the response of B lymphocytes and promotes the production of IgE (Licona-Limón et al. 2013) (Vitenberga and Pilmane 2017), responsible for airway hyperreactivity (AHR) as occurs in asthma and allergic rhinitis (Manka and Wechsler 2018), although the neurotoxicity of cypermethrin is well established (Côté et al. 2014), damage to other organs has been less studied; For volatile environmental allergens and pollutants, the respiratory system is the main route of exposure, producing adverse health effects (Hénault-Ethier 2016). The objective of this work was to evaluate the structural changes and activation of allergic inflammatory response in components of the lower airway and alveoli induced by inhaled exposure to low doses of cypermethrin.

1. METHODOLOGY

1.1 Inhaled Model

A Wistar rat model of inhaled full body exposure was used, described by Wong (2016). An acrylic camera of 54 L of volume was designed and built where the rats group housed during the course of the experiment. The camera has a window of 2.5 cm to ventilate. Cypermethrin-charged particles were generated with an ultrasonic Citizen® Cun 60 nebulizer with a flow of 0.266 ml / minute, and a produced particle size of 5 µm MAMD (mean aerodynamic mass diameter) in average. The animals were exposed for 30 minutes, once a day during the treatment, at an output flow rate of 0.05 m.h⁻¹.

1.2 Experimental Doses

Technical grade cypermethrin (94.3%, Química Lucava®, Guanajuato, Mexico) was used, preparing a solution with an estimated concentration of 156.64 µg per m³ of air, and using 0.1% deionized water + 0.1% Tween 80 as a vehicle. The dose of cypermethrin was calculated based on the amount sprayed in 30 seconds recommended in the instructions of the housing commercial product Raid® Total Action (cypermethrin 1g / kg, SC Johnson and Son®, State of Mexico, Mexico).

1.3 Experimental groups

32 adult male rats Wistar strain, 250 g average weight, without exposure to pesticides from the gestation period, 8 animals per experimental time: 0, 7, 14 and 21 days were used. They were kept at room temperature (18 ° C to 26 ° C), under a 12-hour light / dark cycle, and Nutricubos® (Purina®, Mexico) ad libitum rodent and water supply. Its use and care were according to the technical specifications of NOM-062-ZOO-1999-2001. At the end of the experimental times, blood and lung samples were obtained, which were preserved according to the study technique until processing. The animals were sacrificed by intraperitoneal sodium pentobarbital overdose (1.5 mg / kg, Pisabental PiSA® Agropecuaria, Hidalgo, Mexico).

1.4 Clinical Hypersensitivity Evaluation

To determine the severity of clinical signs induced by exposure to cypermethrin, the adapted Li et al. (2000). Scale was used, from 0 to 5 where 0 represents no sign; 1 scratched around the nose and / or head; 2 swelling around the mucous membranes of the eyes and mouth, diarrhea, piloerection, lethargy and / or lethargy with increased respiratory rate; 3 wheezing, respiratory distress and cyanosis in mouth and tail; 4 tremor or seizures followed by physical inactivity; and 5 death (Li et al. 2000).

1.5 Immunodetection of IL-4, IL-5, IL-13 positive cells

The intravascular perfusion technique (Prophet and Armed Forces Institute of Pathology (U.S.) 1992) was performed in 3 animals of each experimental group, consisted in applied two washes with 100 ml of a solution 0.9% saline + heparin (5000U / I) and procaine (0.2%) intracardiac via, then perfusion with 40 ml of paraformaldehyde 4%. Subsequently, the right lung was surgically removed and preserved in paraformaldehyde (4%). The proximal third portion of the lung was processed with the histological paraffin inclusion technique (Prophet and Armed Forces Institute of Pathology (U.S.) 1992), to obtain consecutive tissue cuts of 2 µm thickness that were placed on slides treated with 3-aminopropyl-triethoxy-silane (Sigma A3648). Direct double immunofluorescence for IL-5 and IL-13 was performed according to the manufacturer's standard protocol, and indirect immunofluorescence for modified IL-4 by (Sánchez-Alemán et al. 2015). In the double direct immunofluorescence, anti-IL-13 Alexa Fluor® 488 polyclonal antibodies (eBiosciences eBio13A) were used at a dilution of 1: 200, incubation time 60 minutes at 37 ° C and Anti-IL5 Alexa Fluor® 594 (R&D Systems IC605T100UG) at a dilution of 1: 200, incubation time overnight at 4 ° C. In indirect immunofluorescence the Rabbit Anti-IL4 polyclonal primary antibody (Abcam® ab9811) was used at 1:50 dilution and overnight incubation time at room temperature and the secondary GOAT anti rabbit Alexa Fluor® 488 (Invitrogen a11008) dilution secondary antibody 1: 200, incubation 60 minutes at 37 ° C and 60 minutes at room temperature.

From each animal, three histological sections were obtained for observation under a microscope. In each section three random fields were reviewed, using an LSM 700 Zeiss® confocal microscope. Images of each field were captured with the Blue (Zeiss®) software. To quantify the emitted fluorescence, 8-bit color depth images per channel were converted with the Fiji Open Source image processing software package software (<http://fiji.sc>).

1.6 Histopathological evaluation

In histological sections, 2 marking techniques were performed: Hematoxylin & Eosin (Prophet and Armed Forces Institute of Pathology (U.S.) 1992) and Erythrosin B (Erythrosin B 200964-5G, Sigma Aldrich®) (Benítez-Bribiesca, Pérez-Astudillo, and Freyre-Horta 1987). A morphometric study was performed by analyzing 5 random fields (100,000 µm²) at X400 per lamella in 3 cuts of each animal. Image capture was performed on the Axioskop® 40 optical microscope

(Carl Zeiss®) with the CoolSnap Color camera and Image-Pro Plus software (Kodak® 1D Image Analysis Software). The variables studied were: Thickness of the bronchiolar epithelium (μm), number of interruptions of bronchiolar epithelium per field ($20,000 \mu\text{m}^2$) and thickness of the epithelium of the alveolar septum (μm). The number of eosinophils in 6 random fields ($120,000 \mu\text{m}^2$) was quantified at X400 of 3 histological sections of each animal.

1.7 Quantification of IgE by ELISA

Blood was drawn by intracardiac puncture. Serum was obtained by centrifugation at 2500 g for 10 minutes (Eppendorf®-5410 Centrifuge) and divided into two portions, one for quantification of IgE, and another for quantification of cypermethrin. Samples were kept frozen at -80°C until the time of their study. At room temperature, a serum pool was prepared per treatment time with 5 μL of each sample that was mixed in an Eppendorf tube using a Vortex - Genie 2 (Scientific industries®) for 1 minute at 10 revolutions. The IgE measurement was performed in each of the pools with the Abcam® Rat IgE ELISA Kit commercial kit (ab157736) in triplicate, according to the manufacturer's instructions.

1.8 Isolation of total RNA and RT-qPCR

Total RNA was isolated from 100 mg of lung of the control and experimental animals with the SVTotal RNA Isolation System kit (Promega, Madison, WI, USA) following the manufacturer's protocol. Total RNA was quantified with a Biodrop (Isogen life Science, Barcelona, España) and stored at -80°C until use. Reverse transcription was performed with 500 ng of total RNA using the GoScript Reverse Transcription System (Promega). Following, the quantitative PCR was achieved with the Maxima SYBR Green/ROX qPCR Master Mix (2X) (K0221, Thermo Scientific, Waltham, MA, USA) in a StepOne machine (Applied Biosystems) with the next programming: 50°C for 2 min, 95°C for 3 min, 40 cycles of 95°C for 45 sec and 58°C for 45 sec. Oligonucleotides were designed to target on IL-4 and β -actin as reference control (Table 1). Relative expression levels were normalized against β -actin as an internal housekeeping gene and differences were determined using $\Delta\Delta\text{Ct}$ relative method.

1.9 Determination of cypermethrin concentration in tissue by GC/MS

Sample preparation

Serum samples were obtained. Dissected and surgical resection of right lung of 3 animals from each experimental group (0, 7, 14 and 21 days) were performed, tissues were obtained 1-2 h post-treatment and were preserved in glass tubes (KX-45048-15125 test tube 15X125 mm), and placed in a deep-freezer at -80°C until its processing. Tubes were previously washed with extran, distilled water and citric acid until neutralization with distilled water and dried in an oven by evaporation. Analysis of cypermethrin concentration in 1 g of lung tissue and serum was weighted in a 50 mL of centrifuge tube performed by dispersive solid phase extractive technique using QuEChERS® ((© Agilent Technologies, Inc. 5982-5650CH) having a first extraction using 10 mL of HPLC grade acetonitrile (JTBaker) and fractionation with salts such as magnesium sulfate, sodium chloride and citrate salts. The tubes were shaken manually for 1 – 2 min. And they were then centrifuged at 3500 rpm for 2 min at 19°C . 6 mL of supernatant sample extraction was transferred to the QuEChERS clean-up tube a cleaning stage of the dispersive solid phase extract, in both cases using homogenizers, according to the methodology of AOAC 2007.01 (J.AOAC 2007). To determine the concentration of cypermethrin in the tissues, a matrix-matched curve of cypermethrin (45835, Merck) was performed with concentrations from 1 to 40 mg / L.

Cypermethrin quantification by Gas chromatograph (GC / MS)

Readings were made in a GC/MS Perkin Elmer Gas Chromatograph (Perkin Elmer Gas Chromatograph Clarus ® 680), the reference library was NIST and heptacosane as reference gas, at the State Laboratory of Public Health of the State of Aguascalientes, Department of Studies Environmental. An Elite-SMS capillary column 30 m long and internal diameter 0.32 µm was used, under the carrier's parameters: initial setpoint: 1 mL / minute and split flow: 20 mL / minute, using helium as the carrier gas. The oven program included a ramp under the conditions of initial temperature of 220 °C; initial hold: 1.00 minutes, ramp 1: 15.0 ° / min to 300 °, hold for 2.00 min, total run time: 8.33 minutes, maximum temperature: 350 °C, equilibration time: 0.5 minutes.

1.10 Statistical analysis

The data obtained were performed with the single-factor ANOVA statistical tests with a posthoc study with the Tukey test when the data distribution was normal; and Kruskal-Wallis with posthoc study with Dunn's test when the distribution of the data did not meet normality, performed in Graphpad Prism software version 5.01 Graphpad software, Inc.

2. RESULTS

2.1 Clinical Hypersensitivity Evaluation

The animals had nasal irritation and gagging arches from the first day of exposure to cypermethrin. The signs were exacerbated on the seventh day of treatment and epistaxis, piloerection, severe scratching of ears and nose, hyaline rhinorrhea, sialorrhea, diarrhea, respiratory distress, lethargy with anorexia and adipsia were added. These signs correspond to level 3 of the Li scale for the clinical evaluation of hypersensitivity (Li et al. 2000). Between the seventh and tenth day there was the death of 9.4% of the animals, described in level 5 of the scale (Li et al. 2000). From day 11 to 15, the signs of hypersensitivity decreased to level 3; and finally, from day 15 to 21 the signs of hypersensitivity decreased to level 2 (Table 1).

2.2 HE and eosinophils

2.2.1 Histopathological observations H/E

The pulmonary histological structure showed gradual damage in relation to the exposure time, then the normal structure of the pulmonary parenchyma is represented by cubic epithelium, and scarce underlying connective tissue, surrounded by a layer of smooth muscle and integral alveolar septa in the control group (Fig. 1Aa, b), at 7 days, epithelium suffer an oedema observed in bronchiole (arrow in Fig. 1Ba), and in the socket begins the acute phase of damage or exudative, with neutrophil and macrophage infiltrate, erythrocyte congestion and edema at the expense of exudate (arrow in Fig. 1Bb). At 14 days, in bronchiole there are damage manifested by exfoliation of epithelial cells that tend to form clusters previously described as Creola bodies (Yamada, Yoshihara, and Arisaka 2004) (arrow in Fig. 1Ca); and in the alveoli the proliferative phase of damage due to the thickening of the alveolar walls, glandular appearance, recruitment of neutrophils and macrophages is observed (arrow in Fig. 1Cb). Finally, at 21 days, there is a transition of the proliferative phase, even with a large inflammatory infiltrate (Fig. 1Da), but with a tendency to return to the normal structure (Fig. 1Db).

2.2.2 Eosinophils

The quantification of eosinophils in the pulmonary stroma (Fig. 3), showed a significant increase in the tissue, with a statistically significant difference with $p < 0.0001$ between the control group with 7, 14 and 21; and of the group treated 7 days with the group treated 21 days (Fig. 2d).

2.2.3 Morphometry

In addition to the histological description, *epithelium thickness bronchiolar* morphometry (Red arrow in Fig. 1Aa) was also performed, which increased towards 7 days of exposure (Red arrow in Fig. 1Ba), and then decreased at 14 and 21 days of treatment (Red arrow in Figs. 1Cb, Db) (Fig. 2a) ($p < 0.0001$). *Alveolar septum thickness* (yellow line in Figs. 1Ab, Bb, Cb and Db) behaved similarly to that of the epithelium bronchiolar, increasing towards 7 and 14 days with a tendency to recovery at 21 days and presented statistically significant difference between the control group with the exposed group 7, 14 and 21 days, respectively ($p < 0.0001$) (Fig. 2b). *Bronchiolar Epithelial disruption* did not occur in the control group, and they were presented in relation to the time of exposure, being its highest peak at 21 days ($p < 0.001$) (fig. 2c).

2.3 Detection of IL-4, IL-5 e IL-13 by Immunofluorescence

2.3.1 IL-4

The control group showed positivity in the cytoplasm of the IL-4 lung parenchyma cells. Change in the distribution of IL-4 was observed based on the time of exposure to cypermethrin.

The cytoplasmic distribution of positivity changed in relation to the exposure time, displacement towards the extracellular matrix was observed in the alveolar septum and increased fluorescence intensity (Fig. 4), with a significant difference between the control group and the treated groups at 7, 14 and 21 days respectively, and the group 14 days with the group 21 days ($p < 0.0001$) (Fig. 4).

2.3.2 IL-5 and IL-13

They are reported in parallel by the double marking carried out. In the control group, localized positivity was observed in bronchiolar epithelial cells whose intensity increased in relation to the exposure time and persists in epithelial location, the participation of IL-5 and IL-13 in the damage phase is confirmed, based on fluorescence intensity for IL-5, we detected a difference among the control group respect to the groups exposed at 7, 14 and 21 days respectively, and 7 with 14 days and 14 with 21 days ($p < 0.0001$) (Fig. 5). A significant difference in the fluorescence intensity in IL-13 among control group with 7, 14 and 21 days ($p < 0.0001$) was observed (Fig. 5).

2.4 IL-4 mRNA detection by qPCR

Cypermethrin induced decreased expression of the IL-4 mRNA. Analysis of the lung tissue exposed to cypermethrin decreased the expression of the IL-4 mRNA at 14 (* $P < 0.05$) and 21 days (** $P < 0.01$) during exposure in relation to the control group, while at 7 days there is no modification regarding control (Fig. 6).

2.5 IgE

An increase in concentration was observed in relation to the exposure time and a difference was observed between the control group vs 7 days ($P < 0.01$), and vs 14 and 21 days ($p < 0.001$) (Fig. 7).

2.6 Cypermethrin detection by GC/MS

Cypermethrin was not detected in serum samples at any of the study times. In the lung samples, cypermethrin was not detected at time 0, however, at 7, 14 and 21 days of exposure, a concentration of 9.125, 7.861 and 7.875 mg of cypermethrin / Kg of lung was observed, respectively, observing a significant difference with respect to time zero ($p < 0.0001$ ****) (Figure 8).

Table 1: Oligonucleotides used for qPCR determination of IL-4 expression levels.

Gene	Accession Number	Oligonucleotides
IL-4	NM_201270.1	Fw: TCCTTACGGCAACAAGGAAC
		Rv: GTGAGTTCAGACCGCTGACA
β-actin	NM_031144.3	Fw: GTCGTACCACTGGCATTGTG
		Rv: GCTGTGGTGGTGAAGCTGTA

Table 2: Clinical Signs severity of hypersensitivity.

Treatment days	Severity
0	0
1	1
2	1
3	1
4	2
5	2
6	2
7	3
8	5
9	5
10	5
11	3
12	3
13	3
14	3
15	3
16	2
17	2
18	2
19	2
20	2
21	2

0 = No sign; 1 = Scratched around the nose and / or head; 2 = Swelling around the mucous membranes in the head (eyes and mouth), diarrhea, piloerection, lethargy and / or lethargy with increased respiratory rate; 3 = Wheezing, respiratory distress and cyanosis in mouth and tail; 4 = Tremor or seizures followed by physical inactivity; 5 = high severity of clinical signs and Death.

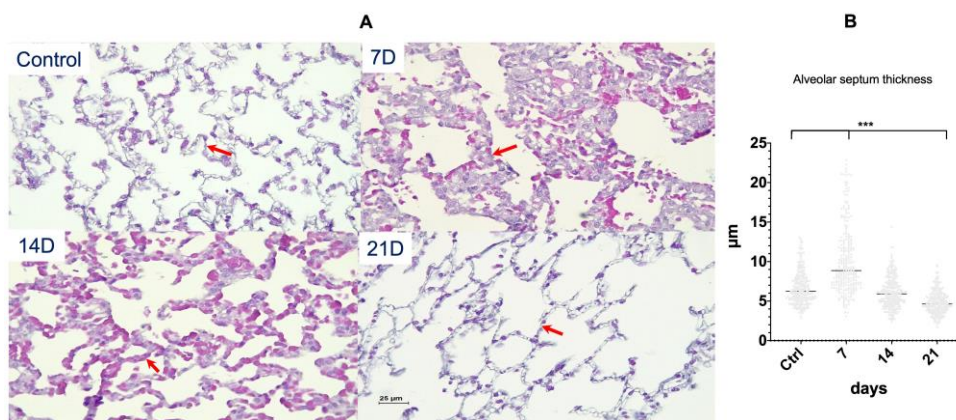


Figure 1: Morphology and Morphometry. A) Description of rat lung damaged by cypermethrin (H/E). (Control) normal lung tissue, 7D) 7 days lung exposed to cypermethrin, acute phase with oedema and infiltrate inflammatory cells. 15D) Alveoli wall with oedema and scarce inflammatory infiltrate cells. 21D) lung tissue shows a histological recovery to normal tissue. **B)** Morphometry analysis for Alveolar septum thickness (red arrows) in Figs. 1B (control), 7D, 14D and 21D) increasing towards 7 days ($p < 0.001$ ***) and 14 days, with a tendency to recovery at 21 days ($p < 0.001$ ***) (Fig. 2b).

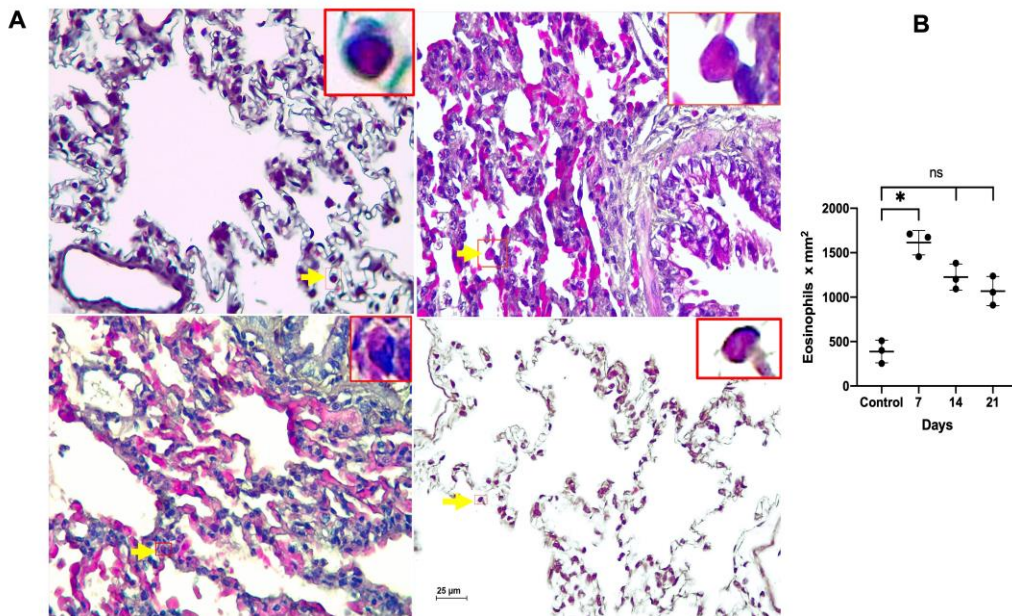


Figure 2: Eosinophils detected by Erythrosine B (blue arrow). a) normal lung (control), b) 7 days post-exposed, were different in relation to control ($p < 0.01$ **); c) 14 days post-exposed, and d) 21 days post-exposed to cypermethrin not difference statistically were found vs control.

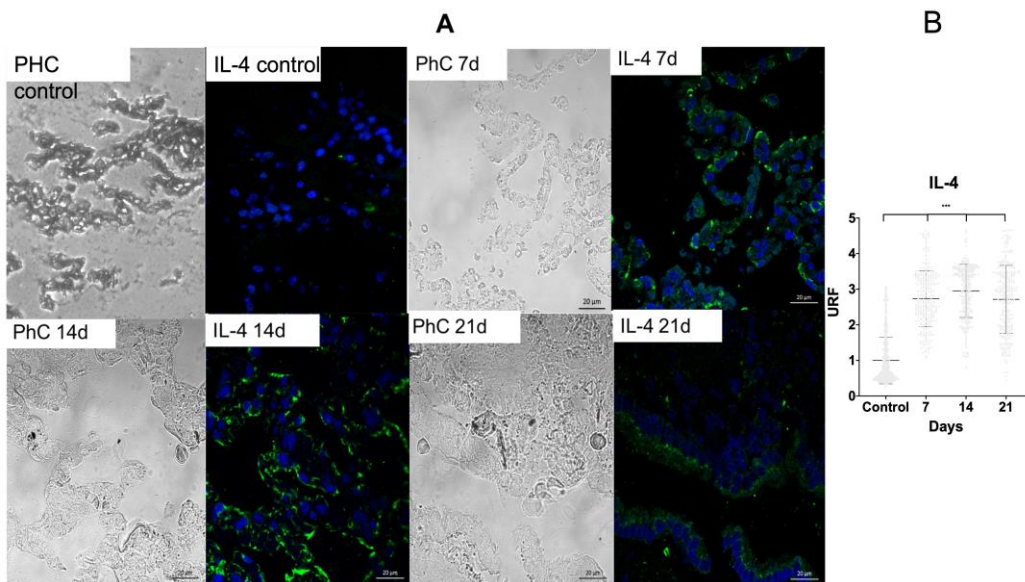


Figure 3: Immunofluorescence for IL-4, confocal microscopy images. A) PhC control= Phase Contrast control, IL-4 control= Detection of IL-4 in normal lung tissue (control), PhC 7d= Phase Contrast 7 days post-exposition to cypermethrin, IL-4 7d= Detection of IL-4 positive cells in lung tissue 7 days post-exposition, PhC 14d = Phase Contrast 14 days post-exposition, IL-4 14d = Detection of IL-4 in positive cells in lung tissue 14 days post-exposition, PhC 21d = Phase contrast 21 days post-exposition, IL-4 21d = Detection of IL-4 positive cells in lung tissue 21 days post-exposition. B) Graphics of fluorescence intensity.

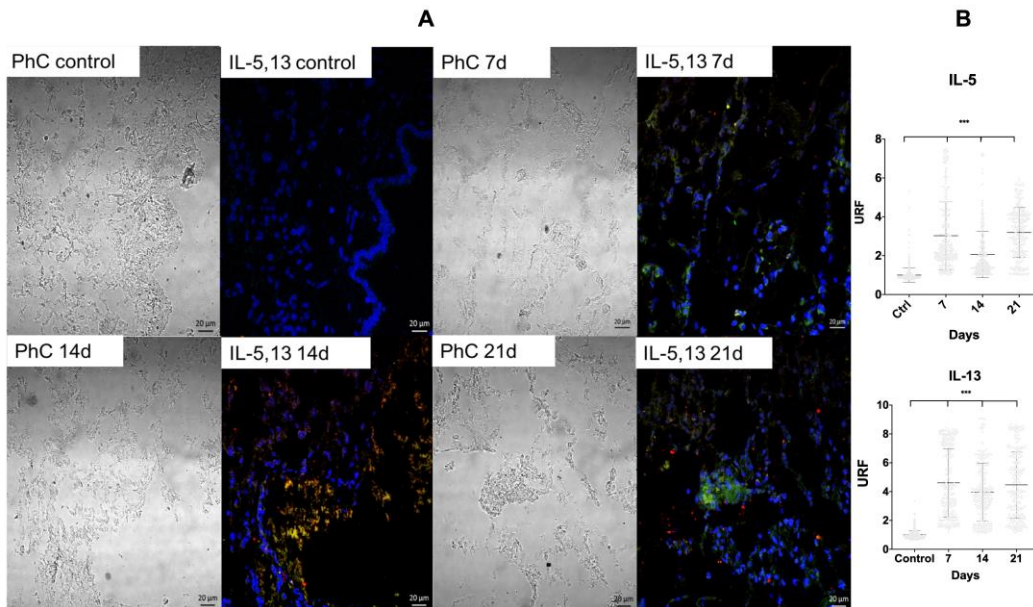


Figure 4: Immunofluorescence for IL-5 and IL-13.

Double immunodetection for IL-5 and IL-13 on rat lung tissue. PhC control = phase contrast of normal rat lung tissue; IL-5,13 control = double Immunofluorescence for both cytokines on normal rat lung tissue; PhC 7d = Phase contrast of lung tissue during 7 days exposition to cypermethrin; IL-5,13 7d= Double immunodetection for both cytokines, scarce positive cells for IL-5, high positive cells for IL-13 (right graphics); PhC 14d = Phase contrast of lung tissue during 14 days exposition to cypermethrin; IL-5,13 14d = Double immunodetection for IL-5,13, at 14 days exposition, middle expression of IL-5 and scarce cells positive to IL-13 (right graphics); PhC 21d = Phase contrast of lung tissue during 21 days exposition to cypermethrin; IL-5,13 14d = Double immunodetection for IL-5,13, at 21 days exposition, high expression of IL-5 and IL-13 positive cells (right graphics).

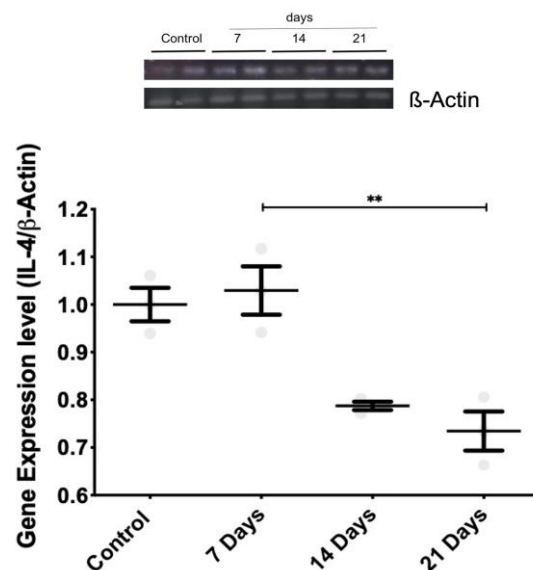


Figure 5: Cypermethrin IL-4 expression in a toxicity chronic Wistar rat model.

Lung analysis shows that IL-4 had a lesser expression after exposition at 14 and 21 days to cypermethrin in relation to control, meantime, at 7 days exposition was observed some increase without different to control. Data represent a mean ± standard deviation. **P<0.01.

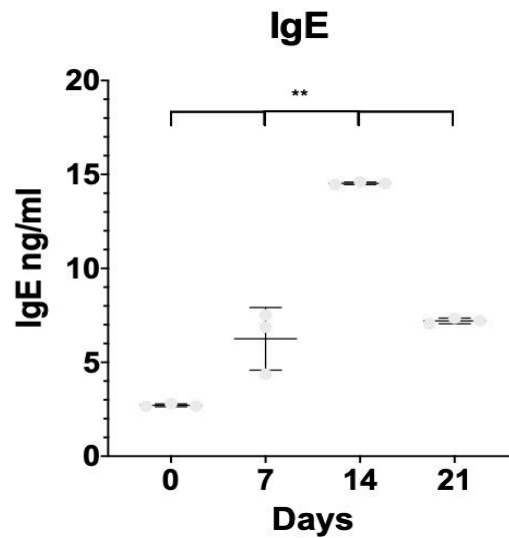


Figure 6: IgE detection by ELISA. The level of IgE antibody is raised at 7, 14 and 21 days exposition to cypermethrin, note that a higher raise was at 14 days.

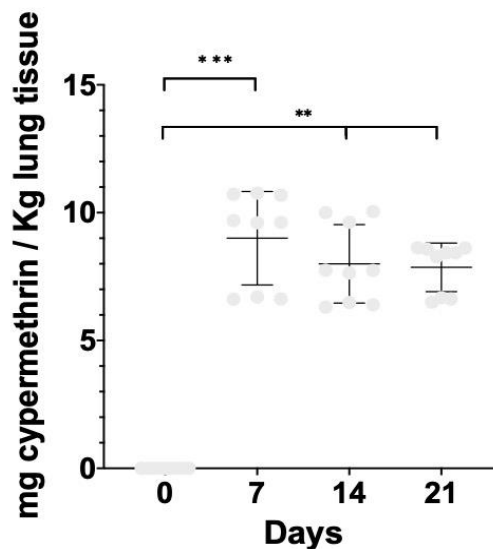


Figure 7: Cypermethrin concentration in lung tissue. at 7, 14 and 21 days exposition to cypermethrin were detected raising of concentration in lung tissue ($p < 0.001$ **).

DISCUSSION

Although there are several presentations of cypermethrin as an inhalable insecticide for domestic use, they are part of the aeroallergens and main triggers of respiratory allergies, there are few studies that review the adverse effects on the respiratory tract, so, hence the importance of studying cypermethrin inhaled to reproduce its effects before exposure and study the mechanisms related to the pulmonary immune system. In this work it is observed that cypermethrin induces an allergy response in bronchioles and alveoli between 7 and 14 days of exposure, represented by classic clinical signs of respiratory allergy syndrome (Li et al. 2000), damage in epithelium and bronchial stroma, local inflammatory reaction, timely increased expression of cytokines IL-5 and IL-13; but decrease in IL-4 with exception at 14 days, as well as sustained increase in serum IgE levels. Of the few studies of cypermethrin in rat Wistar, Tulinska et al., (1995), applied cypermethrin orally for 28 days to analyze its effect on splenocytes for phagocytosis, ability to respond to mitogens and activity of NK cells, found increased proliferative capacity in response to phytohemagglutinin and concanavalin A,

improved NK activity, but there was no effect on phagocytic capacity, did not study the effects on the respiratory system. The near study of the effects of cypermethrin to our study, resides in what was reported by Lessenger (1992), he described five cases of people accidentally intoxicated with cypermethrin, reported that people manifested immediately, shortness of breath, cough, congestion, burning eyes and itchy skin at the time of exposure, in chronicity it is described that one of the patients developed respiratory distress syndrome, as a result of this accident, it was established that cypermethrin has a toxic effect. In the rat, what can be compared with the previous signs is the arch, simulating the cough sign, increased mucus secretions by nose, scratched eyes and nose, which allows us to say that the rat has a burning sensation that and causes that behavior.

The respiratory epithelium is a structural barrier that constitutes the first line of defense against inhaled particles containing aeroallergens that triggers a pro-inflammatory innate immune response, at the level of bronchioles we can find Clara cells with microvilli, without cilia, in her cytoplasm there are secretion granules, which reflects its function as a steam secretory cell, its number increases with decreasing hair cells and constitutes 22% of the cell mass in the bronchioles(Reynolds and Malkinson 2010; Gandhi and Vliagoftis 2015).

Normally, the lungs when exposed to irritating, allergic or infectious stimuli, respond with a significant increase in mucin-producing cells in both mice and humans, which is dependent on IL-13 signaling. This increased mucosal response generates a differentiated cell that can no longer enter the cell cycle, which provides critical protection to the respiratory tract but leads to loss of repair potential (Reynolds and Malkinson 2010).

Gandhi and Vliagoftis (2015) has described the effect of allergens on the epithelium by irritating them with the consequent mucous secretion, increased permeability of the epithelia, which facilitates the entry of allergens and their capture by dendritic cells that leads to Dendritic cell interactions towards the activation of Th2 cells, that are part of type 1 hypersensitivity, is characterized by the secretion of cytokines that signal eosinophils, basophils, mast cells and lymphocytes, which produce the effector cytokines that characterize the allergic response such as IL4, IL-5 and IL-13 and production of IgE (Nakayama et al. 2017). In humans, the relationship of IgE production from birth and its relationship with IL-4, IL-13, IL-4 is essential for the differentiation of lymphocytes to CD4+ producers of IL-13 that leads to airway hyperreactivity, mucus production and subepithelial fibrosis (Nakayama et al. 2017). IL-4 and IL-13 share a receptor complex: (IL4R α / IL-13R α 1) and work to promote the isotype change to IgE of B lymphocytes. When IgE binds to its receptor in mast cells and basophils, cellular activation and degranulation occurs with the recruitment of other inflammatory cells in the airways (Nakayama et al. 2017). Another type 2 cytokine is IL-5, which is critical for the proliferation, maturation and chemotaxis of eosinophils, that release the cationic protein content in their granules, the neurotoxin derived from eosinophils, and the main basic protein that causes local inflammation in airway tissue (Crestani et al. 2007).

The mechanisms described above coincide with our results, that is, we detect a pattern of cells that produce and secrete IL-4, IL-5, IL-13 that regulate the production of systemic IgE that is characteristic of the allergic type inflammatory response, as well as inflammatory modifications in the lung tissue, we also corroborate it with the detection of the expression of the IL-4 mRNA in tissues, so we can say that cypermethrin induces both local and systemic allergic inflammation when administered by inhalation.

In this study, cypermethrin was detected in lung tissue at 7, 14 and 21 days, with a tendency to decrease during the time

without statistical difference. Detected cypermethrin is consistent with inflammatory damage described in lungs, and with biomarkers of allergy-type inflammatory response described in this study which have not been previously described for inhaled exposure (Chrustek et al. 2018).

As for the human, cypermethrin effects have been shown in long-term exposure, most studied in workers exposition. Because of the toxicokinetic characteristics of cypermethrin, no reproductive toxicity has been reported as a manifestation of systemic effects or bioaccumulation of this pyrethroid; however, other effects reported such as skin lesions, neurologic and immune changes, and oxidative stress have been described and also the association with no metabolites of this pesticide in the urine, showing similar patterns in the rat and the human metabolism and toxic behavior of the cypermethrin (Ramchandra et al. 2019).

3. CONCLUSIONS

In Wistar rat exposed to cypermethrin in a model of chronic poisoning via inhalation:

1. There were clinical signs of hypersensitivity with greater severity between 7 and 14 days of treatment.
2. There was an inflammatory response in the lung characterized by the expression of IL-4, IL-5 and IL-13 and an increase in eosinophils recruited according to the exposure time.
3. A 5-fold increase in serum IgE concentration was detected after 14 days of treatment with respect to the control.
4. Cypermethrin was not detected in serum at any experimental time.
5. Cypermethrin was detected in lung tissue at seventh day of treatment, with a tendency to decrease timeline.

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Coauthor declare not conflict of interest.

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