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Evaluation of the pulmonary epithelium in rats treated with malathion

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Avaliação do epitélio pulmonar em ratos submetidos ao malation

RESUMO

Objetivo - Analisar as alterações do epitélio pulmonar em ratos submetidos por via oral ao inseticida, Malation, através da análise histopatológica.

Metodologia - Foram utilizados 30 ratos fêmeas da linhagem Wistar, com idade inicial de 21 dias, provenientes do Biotério Central da Universidade Estadual de Londrina – UEL, Protocolo CEUA- 7109. Os animais foram distribuídos casualmente em três grupos experimentais (n = 10). Dois grupos de animais foram tratados com Malation nas doses de 10 mg/kg ou 50 mg/kg de peso corpóreo. Essas doses correspondem a 0,5% e 2,5%, respectivamente da DL50 oral para ratos (DL50 oral = 2000 mg/kg). O outro grupo (grupo controle) recebeu apenas o veículo (óleo de soja) em igual volume. Resultados apresentados em média (desvio padrão), p<0,05, Teste de ANOVA de 2 fatores.

Originalidade/relevância - A originalidade do artigo reside na escassez de estudos histopatológicos sobre os efeitos do Malation no epitélio pulmonar, especialmente com enfoque morfométrico em modelos animais, contribuindo para a compreensão dos impactos tóxicos respiratórios de inseticidas organofosforados.

Resultados - Os resultados foram obtidos através de análise histopatológica. Embora tenha existido um aumento das células caliciformes no grupo M50, não tivemos valores significativos, pois as médias foram próximas. Em relação à área do bronquíolo comparando o grupo controle com o M10 tivemos um aumento da área, considerado valor significativo. Em relação à área da luz quase não observamos diferença nas médias dos grupos, porém com redução no grupo M50, constatamos aumento da espessura da camada muscular no grupo M50 e aumento do score de inflamação.

Contribuições teóricas/ metodológicas - As contribuições teóricas e metodológicas do estudo incluem o aprimoramento da compreensão dos mecanismos de toxicidade pulmonar induzida por organofosforados, além da aplicação de análise histopatológica e morfométrica como abordagem eficaz para avaliar alterações estruturais em tecidos respiratórios expostos a agrotóxicos.

Contribuições sociais e ambientais - Os achados do estudo evidenciam riscos respiratórios associados à exposição ao Malation, reforçando a necessidade de políticas públicas voltadas à regulação do uso de agrotóxicos e à proteção de populações expostas, contribuindo para práticas agrícolas mais seguras e ambientalmente responsáveis.

PALAVRAS-CHAVE: Inseticidas. Sistema Respiratório. Organofosforado.

Evaluation of the pulmonary epithelium in rats treated with malathion

ABSTRACT

Objective – To analyze alterations in the pulmonary epithelium of rats subjected to oral administration of the insecticide Malathion through histopathological analysis.

Methodology – Thirty female Wistar rats, initially aged 21 days, from the Central Animal Facility of the State University of Londrina – UEL, Protocol CEUA-7109, were used. The animals were randomly distributed into three experimental groups (n = 10). Two groups were treated with Malathion at doses of 10 mg/kg or 50 mg/kg of body weight. These doses correspond to 0.5% and 2.5%, respectively, of the oral LD50 for rats (oral LD50 = 2000 mg/kg). The other group (control group) received only the vehicle (soybean oil) in an equal volume. Results are presented as mean (standard deviation), p<0.05, two-factor ANOVA test.

Originality/Relevance – The originality of this article lies in the scarcity of histopathological studies on the effects of Malathion on pulmonary epithelium, especially with a morphometric focus in animal models, contributing to the understanding of respiratory toxic impacts of organophosphate insecticides.

Results – The results were obtained through histopathological analysis. Although there was an increase in goblet cells in the M50 group, the values were not significant due to similar means. Regarding bronchiole area, a significant increase was observed when comparing the control group with M10. For the lumen area, no major differences were observed among group means, although the M50 group showed a reduction. An increase in muscle layer thickness and inflammation score was noted in the M50 group.

Theoretical/Methodological Contributions – The study contributes to improving the understanding of pulmonary toxicity mechanisms induced by organophosphates, and supports the use of histopathological and morphometric analysis as effective approaches to assess structural changes in respiratory tissues exposed to agrochemicals.
Social and Environmental Contributions – The findings highlight respiratory risks associated with Malathion exposure, reinforcing the need for public policies aimed at regulating pesticide use and protecting exposed populations, thus promoting safer and more environmentally responsible agricultural practices.

KEYWORDS: Insecticides. Respiratory system. Organophosphate.

Evaluación del epitelio pulmonar en ratas tratadas con malation

RESUMEN
Objetivo – Analizar las alteraciones en el epitelio pulmonar de ratas tratadas por vía oral con el insecticida Malatión mediante análisis histopatológico.
Metodología – Se utilizaron treinta ratas Wistar hembras, de 21 días de edad inicial, provenientes del Bioterio Central de la Universidad Estatal de Londrina – UEL, Protocolo CEUA-7109. Los animales fueron distribuidos aleatoriamente en tres grupos experimentales (n = 10). Dos grupos fueron tratados con Malatión en dosis de 10 mg/kg o 50 mg/kg de peso corporal. Estas dosis corresponden al 0,5% y 2,5%, respectivamente, de la DL50 oral para ratas (DL50 oral = 2000 mg/kg). El grupo control recibió solo el vehículo (aceite de soya) en volumen equivalente. Los resultados se presentan como media (desviación estándar), p<0,05, prueba ANOVA de dos factores.
Originalidad/Relevancia – La originalidad del artículo radica en la escasez de estudios histopatológicos sobre los efectos del Malatión en el epitelio pulmonar, especialmente con un enfoque morfométrico en modelos animales, lo que contribuye a la comprensión de los impactos tóxicos respiratorios de insecticidas organofosforados.
Resultados – Los resultados se obtuvieron mediante análisis histopatológico. Aunque se observó un aumento de las células caliciformes en el grupo M50, los valores no fueron significativos debido a medias similares. En cuanto al área del bronquiolo, se observó un aumento significativo al comparar el grupo control con el M10. Para el área de la luz, no se observaron grandes diferencias entre las medias de los grupos, aunque hubo una reducción en el grupo M50. Se constató un aumento en el grosor de la capa muscular y en el puntaje de inflamación en el grupo M50.
Contribuciones Teóricas/Metodológicas – El estudio mejora la comprensión de los mecanismos de toxicidad pulmonar inducida por organofosforados, y respalda el uso del análisis histopatológico y morfométrico como enfoques eficaces para evaluar los cambios estructurales en tejidos respiratorios expuestos a agroquímicos.
Contribuciones Sociales y Ambientales – Los hallazgos destacan los riesgos respiratorios asociados a la exposición al Malatión, reforzando la necesidad de políticas públicas orientadas a regular el uso de pesticidas y proteger a las poblaciones expuestas, promoviendo así prácticas agrícolas más seguras y responsables con el medio ambiente.

PALABRAS CLAVE: Insecticidas. Sistema respiratorio. Organofosforado.

ISSN 2966-2931 - v. 21, n. 3, 2025

1 INTRODUCTION

The use of pesticides in Brazil has been increasing steadily, causing alterations in human health and ecosystems. The health impacts can affect both workers and consumers of food contaminated with residues, with the applicators of these products being the most affected. Some problems that can be caused by pesticide exposure include respiratory diseases (such as asthmatic bronchitis) and gastrointestinal effects. Additionally, certain compounds like organophosphates and organochlorines can cause muscle disorders, motor debility, and weakness (Bowles and Webster, 1995, p. 418; Simões et al., 2024; Zhou and Achal, 2024, p. 100410).

The use of pesticides in agriculture began in 1920, but little was known about their side effects. Around 1960, pesticides started to be used more intensively in agriculture; previously, they were used in public health programs, such as combating vectors and controlling parasites (Ribeiro and Mella, 207, p. 125).

It is known that 20% of the global pesticide market consists of developing countries. Thus, Brazil stands out as the largest individual market, representing 35% of the total. Brazil is classified in the market as the largest consumer of pesticides per hectare in the world (Peres et al., 2001, p. 564; Pires et al., 2005, p. 598; Tudi et al., 2021, p. 1112).

Pesticide consumption has increased considerably in Brazil, leading it to rank as the fourth country globally in pesticide consumption. From a historical perspective, the population is more exposed to pesticides due to the increased consumption of agricultural products and direct contact with rural applicators or handlers. It is also worth noting suicide attempts or indirect exposure, as seen in populations that require the use of pesticides for vector control in endemic areas (Faria et al., 2007, p. 25; Zúñiga-Venegas, 2022, p. 096002).

Pesticides are classified according to their chemical nature and function. Regarding their chemical nature, they are divided into organic and inorganic. Organic insecticides, based on their mechanism of action, are divided into cholinesterase inhibitors (organophosphates and carbamates), pyrethrins, pyrethroids, and organochlorines. On the other hand, inorganic insecticides do not contain any carbon atoms in their chemical structure and can be classified as arsenicals, fluorides, and miscellaneous. These are rarely used due to their high environmental risk, high toxicity, poor insect control, and lack of antidotes (Alengebawy et al., 2021, p. 42; Faria et al., 2007, p. 25).

Due to the practicality of organophosphates, they began to be more widely used because they degrade rapidly in the environment, causing less significant environmental impact. Cholinesterase inhibitor insecticides classified as organophosphates are absorbed through the skin, ingestion, or inhalation (Ganie et al., 2022, p. 153181).

1.1 Diethyl dimethylthiophosphoryl ester (Malation)

Malathion (diethyl dimethylthiophosphoryl ester) is one of the most widely used organophosphates in Brazil. It is highly useful in rural and urban areas for a variety of purposes (eradication of insects, ants, and even lice). It is extensively used due to its high effectiveness as an insecticide and its low toxicity in mammals compared to other organophosphates. However, some impurities present in its formulation may increase its toxicity (Azizan et al., 2023, p. 100291).

There is inhibition of the enzyme cholinesterase, especially acetylcholinesterase, causing the accumulation of acetylcholine in nerve synapses, leading to a series of parasympathomimetic effects. Inhibition of cholinesterase by phosphorus compounds causes the accumulation of acetylcholine, and the organism begins to present many manifestations, such as muscarinic, nicotinic, and central effects. The action of cholinesterase is derived from two enzymes: one found in the membrane of erythrocytes (erythrocyte cholinesterase or acetylcholinesterase) and another in serum (plasma cholinesterase or butyrylcholinesterase). The reduction in plasma cholinesterase can last for thirty days and in red blood cells for ninety days after the last contact with organophosphates (Chen et al., 2024, p. 2237-2254; Guevara and Pueyo, 1995; Opas, 1996).

The toxic action of organophosphate compounds is related to the inhibition of numerous enzymes. The inhibition of AChE occurs through the process of phosphorylation of the hydroxyl group of the serine residue of the enzyme. Consequently, the hydrolysis of the neurotransmitter acetylcholine (ACh) is compromised, leading to the accumulation of this neurotransmitter in the synapses of the central and peripheral nervous systems. Thus, there will be hyperstimulation of muscarinic and nicotinic receptors (cholinergic receptors), triggering a variety of signs and symptoms that characterize the "cholinergic syndrome" (Ankarberg et al., 2004, p. 555; Chanda and Pope, 1995, p. 771; Jaiswal, et al., 2024, p. 118888).

1.2 Effects of exposure to pesticides

The greatest use of these substances occurs in agriculture, but they are also used in public health for the elimination and control of vectors transmitting endemic diseases such as Chagas disease, Malaria, and Dengue (Domingues et al., 2004, p. 45).

Brazilian government is not giving adequate attention to the problem of occupational pesticide poisoning, while simultaneously encouraging the increase in agricultural production, as the export of agricultural products accounts for a significant portion of Brazil's trade balance. According to the National Toxic-Pharmacological Information System (SINITOX), in 2014, 4,758 cases of pesticide intoxication were registered in agriculture and household management, with the age group most affected being one to four years old, representing 20.45% of the total. Following closely are individuals between twenty to twenty-nine years old, accounting for 14.2% of cases, with the highest number of cases recorded in rural areas (Moreira et al., 2002, p. 299; SINITOX, 2021).

The success of plantations often depends on weed control actions, such as the use of pesticides. Since 2008, Brazil has been the world leader in pesticide consumption. As a result, the abusive use of pesticides is directly related to numerous health problems, affecting workers in the agribusiness production chain, residents of areas near production centers, and consumers who ingest derivatives of crops assisted by herbicides (Meira et al., 2025, p. 100215).

The pesticides used in agricultural activities produce various effects on human and environmental health. Contamination resulting from pesticide use for humans and animals can be acute or chronic. In the former case, there is direct exposure to a single dose of the product, resulting in rapid or slow death. In the latter case, there is constant exposure to relatively low

ISSN 2966-2931 - v. 21, n. 3, 2025

doses, leading to the appearance of signs or symptoms related to chronic intoxication (Elumalai et al., 2025, p. 100597).

The most widely used pesticides in agriculture are organophosphate compounds (OPs), which derive from phosphoric and thiophosphoric acid and are generally applied as insecticides (Oboh et al., 2024, p. 797).

Exposure to OPs generally occurs in individuals who come into contact with these chemicals as a result of their occupation, eating habits during work, product preparation, or individuals residing near storage locations of these products. It is known that the general population is exposed through the spread of chemicals in the environment by consuming contaminated food, in addition to intentional poisonings (homicide and suicides) (Mahajan et al., 2022, p. 473-490).

We know that the absorption of OPs by the human body occurs through dermal, respiratory, and digestive routes. The oral route is particularly important in cases of digestive intoxication, especially in children in accidental cases, and in adults through the consumption of contaminated food (Ganie et al., 2022, p. 153181).

Commercial formulations of malathion may contain up to twelve impurities formed during manufacturing and storage. The mutagenic capacity and high carcinogenic potential are being studied and discussed. However, further studies are still needed on the association between malathion, cancer, and respiratory effects (Bastos et al., 2020, p. 3273).

Given the impactful data on the use of pesticides, especially organophosphates, in health, both through direct or indirect exposure, there is an increasing need for studies to assess their effects on the human body systems. Since insecticides are used in agriculture and in the control of vector-borne diseases, which are considered public health threats, we need to evaluate the symptoms of intoxication and exposure so that healthcare professionals are able to identify the symptoms and administer appropriate treatment.

Agricultural workers are exposed to the risk of intoxication most of the time due to intense contact with insecticides. These compounds are potentially toxic to humans, capable of causing adverse effects on the central and peripheral nervous systems, having immunosuppressive action, or even being carcinogenic, among other effects. Organophosphates are among the most widely used pesticides and are major causes of occupational intoxication, belonging to the class of cholinesterase enzyme inhibitors. Both carbamates and organophosphates are absorbed orally, respiratorily, and cutaneously, increasing intoxication rates worldwide (Toniasso et al., 2025)

With the increase in population and technological advances, the use of organophosphate insecticides has been increasingly employed in agriculture and for the control of vector-borne diseases. Therefore, it is necessary to analyze the possible damage to the respiratory system through histopathological analysis of the lungs of rats exposed to Malathion. This aims to enhance knowledge about the signs of intoxication and permanent changes in the pulmonary epithelium. The present study aims to analyze the alterations in the pulmonary epithelium of rats subjected to the organophosphate insecticide, Malathion.

2 METHODS

2.1 Ethical Considerations

ISSN 2966-2931 - v. 21, n. 3, 2025

The project was submitted to the Ethics Committee for Animal Use of the University of Western São Paulo (UNOESTE) and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (USA). Protocol CEUA-7109.

This project utilized lungs from the rats used in the study titled "Evaluation of the effects of malathion on the development of the female genital system in rats from the juvenile period to puberty," approved on November 17, 2021.

2.2 Experimental Design

Thirty female Wistar rats, 21 days old, from the Central Animal Facility of the State University of Londrina - UEL, were used. The animals were randomly distributed into three experimental groups (n = 10). Two groups of animals were treated with Malathion at doses of 10 mg/kg or 50 mg/kg of body weight via gavage. These doses correspond to 0.5% and 2.5%, respectively, of the oral LD50 for rats (oral LD50 = 2000 mg/kg) (U S EPA, 2000). The other group (control group) received only the vehicle (soybean oil) in the same volume.

2.3 Sample Collections

At the Londrina animal facility, the female rats were anesthetized with a combination of xylazine and ketamine and euthanized by cardiac puncture during the estrous phase for lung collection, which were then subjected to histological processing. The lungs were fixed in Alfac fixative solution (85% 80% alcohol, 10% formalin, and 5% glacial acetic acid). At the UNOESTE laboratory, the lungs were processed in paraffin and histological sections of 5 µm thickness (three non-serial sections per animal) were prepared and stained with hematoxylin and eosin.

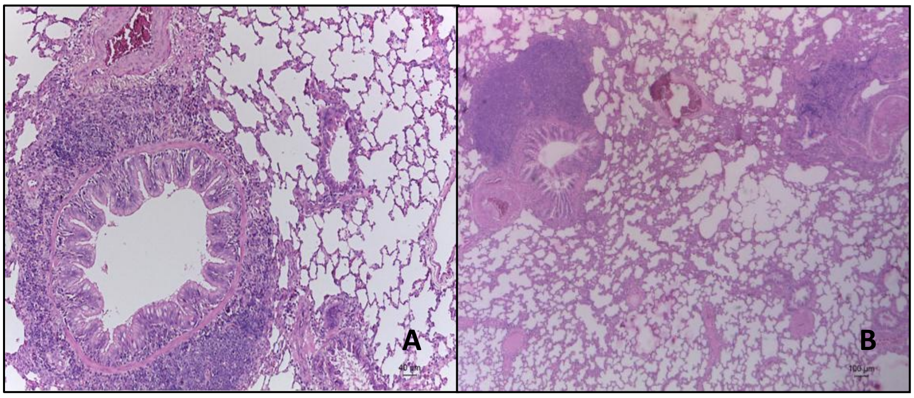
2.4 Data Analysis

The evaluation of the pulmonary epithelium, after the preparation of slides, was performed by capturing images using a microscope with an attached camera (Leica ICC50 HD). Histopathological and morphometric analysis of the lung tissue was conducted at microscope magnifications of 400X and 1000X (Figure 1). A total of 42 slides, with 3 images each, totaling 126 images, were analyzed.

After capturing the images, Image-Pro Plus® software (version 7.0, Media Cybernetics, Silver Spring, MD, USA) was used to quantify goblet cells, bronchiolar area, and light area of the rat lung tissue. Blinded analysis was performed to avoid false results.

ISSN 2966-2931 - v. 21, n. 3, 2025

Figure 1 - Bronchiole and alveoli stained with hematoxylin eosin, at 400x magnification (A). Bronchiole and alveoli stained with hematoxylin and eosin, at 1000x magnification (B).



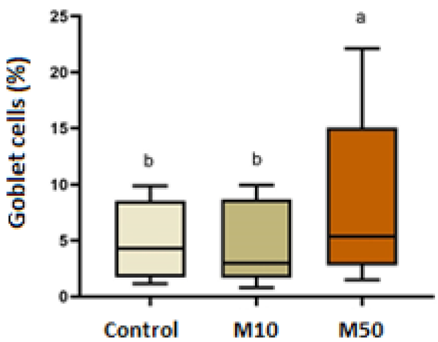
Source: Authors, 2020.

3 RESULTS

The present study analyzed the pulmonary epithelium of 30 female Wistar rats through the administration of Malathion by gavage at doses of 10 mg/kg and 50 mg/kg, with a control group receiving soybean oil. The results were obtained through the following analyses: goblet cells; bronchiolar area, luminal area, muscle layer thickness, and inflammation score. No animals died during the experimental period.

In figure 2, the relationships between the control group and those that received malathion at different dosages are compared with the percentage of goblet cells. We observed an increase in goblet cells in the group that received the higher dosage of malathion (M50).

Figure 2 - Comparison of the means of animals that received malathion and the increase in goblet cells.



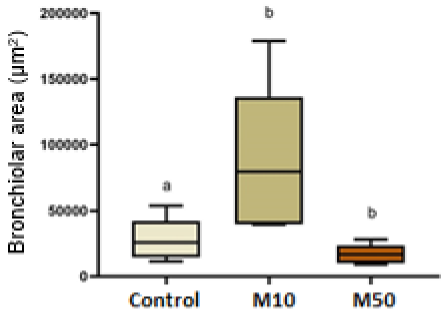
Results presented as mean (Standard Deviation), value obtained by two-factor ANOVA test, with a significance level of $p<0.05$. Legend: different lowercase letters represent statistical differences in the comparison between groups according to malathion dosages. Source: Authors, 2020.

We can observe (Figure 3) the comparison between the control group and the different dosages of malathion with the bronchiole area. We observed a large increase in bronchiole area in the M10 group, both compared to the control and the M50 group. Comparing the control

ISSN 2966-2931 - v. 21, n. 3, 2025

group with the M10, we had a significant increase in the bronchiole area, however, when comparing the control group with the M50, we did not obtain statistically significant values.

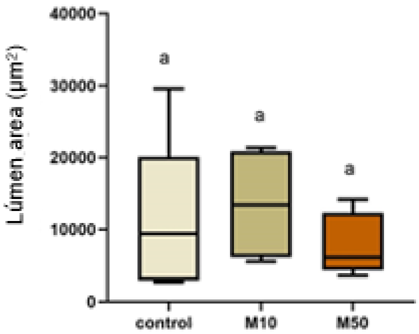
Figure 3 – Comparison between the control group with different dosages of malathion about the bronchiolar área.



Results presented as mean (Standard Deviation), value obtained by two-factor ANOVA test, with a significance level of $p<0.05$. Legend: different lowercase letters represent statistical differences in the comparison between groups according to malathion dosages. Source: Authors, 2020.

We analyzed the changes in the lumen area of the bronchiole in relation to malathion dosages (Figure 4). We observed a reduction in the lumen area of the bronchioles in the group that received the highest dosage (M50) of malathion.

Figure 4 – Comparison between different dosages of malathion about the area of the bronchiole lumen.

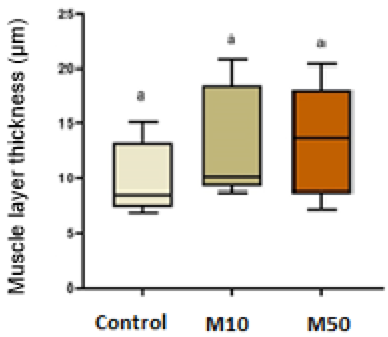


Results presented as mean (Standard Deviation), value obtained by two-factor ANOVA test, with a significance level of $p<0.05$. Legend: different lowercase letters represent statistical differences in the comparison between groups according to malathion dosages. Source: Authors, 2020.

In figure 5, there is a comparison between malathion dosages and changes in muscle layer thickness. We found an increase in muscle layer thickness in both groups that received malathion (M10 and M50). It can also be observed that the mean of the M50 group showed a greater increase in muscle layer thickness compared to the other groups, although not significant.

ISSN 2966-2931 - v. 21, n. 3, 2025

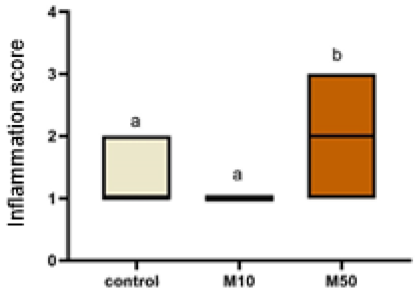
Figure 5 – Comparison of the increase in muscle layer thickness with malathion dosages.



Results presented as mean (Standard Deviation), value obtained by two-factor ANOVA test, with a significance level of $p<0.05$. Legend: different lowercase letters represent statistical differences in the comparison between groups according to malathion dosages. Source: Authors, 2020.

In Figure 6, the inflammation score is compared between the control group and the groups that received malathion. We observed an increase in the inflammation score in the M50 group, which received the highest dosage of malathion, with statistically significant values.

Figure 6 – Comparison of groups about inflammation score.



Results presented as mean (Standard Deviation), value obtained by two-factor ANOVA test, with a significance level of $p<0.05$. Legend: different lowercase letters represent statistical differences in the comparison between groups according to malathion dosages. Source: Authors, 2020.

4 DISCUSSION

The results of the present study demonstrated that the concentrations used caused alterations in the pulmonary histology of rats subjected to malathion at different dosages. This was also verified by Inouye et al. (2014, p. 103), showing an increase in inflammatory infiltrate, alveolar and bronchiolar thickening, and pulmonary congestion. The use of chemical pesticides in developing countries like Brazil and their impacts on human health and food safety is a global concern. Due to this fact, our study analyzed the effects that the insecticide malathion can have on the pulmonary epithelium.

Imamura et al. (1983, p. 73) conducted a study on the oral administration of one of the impurities of malathion (O, O, S-trimethylphosphorothioate-OOS), where they observed morphological alterations in the bronchiolar epithelium of the rat lungs. The lungs of rats treated with OOS had fewer Clara cells (non-ciliated) but were larger than the lungs of control rats that

ISSN 2966-2931 - v. 21, n. 3, 2025

received corn oil or purified malathion. Additionally, lactate dehydrogenase (LDH) activity in the bronchoalveolar lavage fluid was significantly higher in OOS-treated rats than in control rats. These data demonstrate that OOS and/or its metabolites cause lung injury, probably due to inflammatory factors. According to this study, the increased levels of LDH in the bronchoalveolar lavage fluid have been shown to be a useful marker for detecting lung injury resulting from pneumotoxicants.

The present study analyzed the lungs of 30 female Wistar rats subjected to malathion. The animals were randomly divided into three experimental groups. Two groups of animals were treated with malathion at doses of 10 mg/kg (M10) or 50 mg/kg (M50) of body weight via gavage. The other group (control group) received only the vehicle (soybean oil) in an equal volume.

Histopathological analysis of lung tissue was performed, stained with Hematoxylin and Eosin, at a microscopic magnification of 400X and 1000X. To evaluate the organization of the pulmonary epithelium after the use of malathion, we conducted analyses regarding goblet cells, bronchiole area, muscle layer thickness, bronchiole lumen area, and inflammation score.

The majority of the respiratory epithelium is called pseudostratified columnar ciliated epithelium and is composed of goblet cells, which are responsible for the production and secretion of mucus (Luis, 1997, p. 433).

We observed an increase in the percentage of goblet cells in the group that received the highest dosage of malathion compared to the control group and the M10 group. According to the statistical test, with a p-value <0.05, no statistically significant change was observed (Figure 2). Regarding the bronchiole area, we observed a significant increase in the group that received the lower amount of malathion (M10), even when compared to the control group and the group that received the higher dosage. What also draws our attention is the reduction in the bronchiole area in the M50 group, which may indicate bronchiolar remodeling (Figure 3).

OPs act by inhibiting the function of two types of proteins (enzymes of the acetylcholinesterase class - AChEs): a) specific acetylcholinesterase, true or acetylcholinesterase (AChE), found mainly in the synapses of the central nervous system, peripheral parasympathetic system, and neuromuscular junction; b) butyrylcholinesterase, pseudocholinesterase, or nonspecific acetylcholinesterase (BChE), found in the plasma, intestine, and, in lower concentration, in other tissues, and also present in erythrocytes (Carvalho and Ribeiro, 2001, p. 11; Kushik and Chandrabhan, 2003, p. 171).

Through hepatic metabolism, OPs undergo metabolization, producing inhibitors of cholinesterase enzymes. Biochemical and physiological alterations in erythrocytes and lymphocytes can be caused by malathion, in addition to inducing a series of chromosomal aberrations in animals (Amer et al., 2002, p. 1; Banerjee et al., 1999, p. 33; Santos, 2009).

Póvoa et al. (1997, p. 1997) state that studies have shown the ability of malathion to alter the cell cycle and produce distortions at the level of cellular DNA replication, chromosomal aberrations, and associate its action with various pathologies such as cancer, arteriosclerosis, Parkinson's, Alzheimer's, congenital malformations, infertility, and sterility.

Inoyue et al. (2014, p. 103) conducted an experimental study aimed at analyzing the morphological alterations occurring in the liver and lungs of adult Wistar rats subjected to doses of the insecticide malathion for 7, 14, and 21 days. The animals were divided into four groups, of which three were treated with malathion and one control group received saline solution. The

ISSN 2966-2931 - v. 21, n. 3, 2025

investigation included 39 albinos, adult, male and female Wistar rats, weighing between 240 g and 500 g. These were divided into four groups designated as 1, 2, and 3, each composed of eight animals treated with malathion (volume of 10 mL and concentration of 250 mg/kg of body weight) and a control group formed by 15 animals, administered with 0.9% saline solution in variable volume according to weight. In the histopathology of the lungs of these control rats, normal aspects were found in the alveolar and bronchiolar regions. Similar results were also observed in our study.

The groups treated with the drug for different durations showed considerable alterations. In group 1 (received malathion for 7 days), fibrotic areas, marked inflammatory infiltrate, and alveolar thickening were observed, but with preservation of the alveolar lumen. According to our study, the doses of malathion at 10 mg/kg and 50 mg/kg showed little change in the lumen area, although the sample that received the higher dosage showed a reduction in the lumen area (Figure 4). In group 2, evident pulmonary congestion was observed, followed by thickening of the alveolar septa with the presence of capillaries in these septa. In group 3, a mononuclear inflammatory infiltrate consisting of macrophages and lymphocytes, epithelial desquamation in the bronchioles with the presence of red blood cells in the lumen, and thickening of the bronchiolar connective tissue (submucosa) were also observed.

Regarding the thickness of the bronchiole muscle layer, no statistically significant change was observed (Figure 5). According to the study by Inoyue et al.35, with a longer exposure time (21 days) and a higher dosage than used in our study, an increase in the bronchiole muscle layer was observed.

According to the evaluation of the inflammation score, we noticed a significant increase in the M50 group compared to the control and M10 groups. The statistical data are more relevant because the mean of the group that received the highest dosage (M50) consequently showed a higher inflammation score (Figure 6). 12

In the same study by Inoyue et al. (2014, p. 103), in addition to analyzing the pulmonary histopathology of rats exposed to malathion, they also analyzed the presence of hepatic alterations. The presence of pyknotic nuclei, vascular congestion, cytoplasmic vacuolization, and inflammatory infiltrate was observed. Thus, we can conclude that exposure to malathion presents systemic alterations.

Rezg et al. (2007, p. 143), when analyzing the effects of malathion, calculated the hematocrit value, hemoglobin content, and blood glucose concentration in animals subjected to subchronic exposure to the product via intragastric administration daily for 32 days. In fact, the only noticeable change was in the amount of hemoglobin, which increased significantly. This result is justified as an adaptation for better oxygenation, since malathion causes lung damage. This finding reinforces our observation of pulmonary alterations.

Wyatt et al. (1985, p. 42) published a study on the intrabronchial instillation of paraquat in rats and conducted a study on the morphology and retention of the insecticide. Various amounts of paraquat (10(-5) to 10(-12) g) in 0.1 ml of saline solution were instilled directly into the left bronchus of adult male rats. Gravimetric, macroscopic, and microscopic studies of the left lung lobe showed that 10(-5) g of paraquat led to pulmonary edema and macroscopic lesions two and 14 days after application. The lung pathology was similar to that observed after systemic poisoning. When 10(-6) g of paraquat were instilled, some animals

ISSN 2966-2931 - v. 21, n. 3, 2025

developed pulmonary edema and macroscopic lesions. However, microscopic examination showed subtle changes in the lung parenchyma.

Shaikat et al. (2022, p. 23) evaluated the health hazards posed by chemicals such as malathion and nitrobenzene in humans using a rabbit model. Among pesticides, organophosphates are associated with growth and reproductive abnormalities in animals. Malathion is an organophosphate pesticide that exerts its toxic effect by inhibiting acetylcholinesterase. However, this pesticide is now being considered a probable carcinogen by the International Agency for Research on Cancer (IARC), with deleterious effects reported in humans and animals (Badr, 2020, p. 26036; Getenga et al., 2002).

Malathion is widely used today because it is considered to have low toxicity compared to other organophosphates. However, widespread use can lead to excessive exposure from multiple sources. The mechanisms of malathion toxicity include the inhibition of the enzyme acetylcholinesterase, alteration of the oxidant/antioxidant balance, DNA damage, and facilitation of apoptotic cellular damage. Malathion exposure has been related to various toxicities affecting nearly all organs of our bodies, with central nervous system (CNS) toxicity being the most well-documented. The toxic effects of malathion on the liver, kidneys, testicles, ovaries, lungs, pancreas, and blood have also been reported. Furthermore, it has been considered a genotoxic and carcinogenic chemical compound. There is evidence of adverse effects associated with both prenatal and postnatal exposure in animals and humans.

In our literature review, we found few recent studies on the alterations that malathion can cause in the lungs, which made it difficult to compare our results. Therefore, more studies on this subject are necessary.

Another factor that draws attention is related to the residual impact of pesticides, as people are exposed to chemical pesticides through skin contact, ingestion, or inhalation. The type of pesticide, the duration and route of exposure, and individual health status are risk factors that influence health in a given community (Damalas and Koutroubas, 2016, p. 1; Shafy and Mansour, 2016, p. 107).

Epidemiologists in the USA have observed an unusual increase in non-Hodgkin lymphoma in areas of high pesticide use. The numerous health effects associated with pesticides include dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects.

Exposure to organophosphate pesticides may be a risk factor for congenital anomalies and other adverse birth outcomes. In the context of safety levels, concentrations may not exceed the safe levels determined by legislation. However, these "safe limits" may underestimate the practical aspect of the residual impact of pesticides on human health (Emery and Carrell, 2006, p. 131; Recio et al., 2001, p. 1237).

Furthermore, the identification of pesticides in breast milk highlights the importance of public health concerns arising from residual pesticides (Brody and Rudel, 2003, p. 1007).

5 CONCLUSIONS

The results of the present study demonstrated that exposure to malathion causes alterations in pulmonary histology, such as the presence of bronchiolar inflammation. We observed that the rats that received the highest dosage of malathion (M50) showed an increase in goblet cells, a reduction in the bronchiole area, a reduction in the lumen area, preservation of muscle layer thickness, and an increase in the inflammation score, compared to the control group and the group that received a lower dosage of malathion (M10). This indicates the presence of pulmonary histopathological alterations, although at times we did not obtain statistically significant values.

Thus, it is important to conduct further research on the subject to gather more data for the implementation of safer alternatives, both for the environment and the population. Current public health policies and agricultural practices should implement environmentally friendly protocols that pose fewer health risks.

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