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THE ALGORITHM FOR HISTOLOGICAL ANALYSIS OF MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE UPPER RESPIRATORY TRACT EPITHELIUM AND VASCULATURE FOLLOWING ORGANOCHLORINE PESTICIDE EXPOSURE (CHLORPYRIFOS)

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Abstract. This study investigates the morphofunctional alterations in the upper respiratory tract wall induced by pesticide exposure, specifically focusing on the toxic effects of the organochlorine compound Nurinol (Chlorpyrifos). Using acute and chronic inhalation exposure models in rabbits, we employed a combination of modern morphological, morphometric, and statistical methods to establish an analytical algorithm for assessing the severity and nature of respiratory tract lesions. The analysis revealed significant pathological changes, including destruction of the pseudostratified ciliated epithelium, basement membrane thickening, mucin hypersecretion, activation of inflammatory infiltrates, and structural disorders in the mucosal glands. Furthermore, reduced activity of metabolic enzymes indicated impaired energy metabolism and tissue respiration. The findings highlight decreased ciliary clearance and mucociliary transport function. This work underscores the high medical and ecological significance of pesticide-induced respiratory pathologies in regions of intensive agricultural pesticide use, justifying the need for effective methods for early diagnosis and prevention. The study proposes a comprehensive histological, histochemical, and immunohistochemical methodology for monitoring pesticide effects.

Keywords: Chlorpyrifos, Nurinol, Upper Respiratory Tract, Histology, Morphometry, Ciliary Clearance, Mucociliary Transport, Ecotoxicology.

1. Introduction

1.1. Background and Rationale

The intense use of pesticides in modern agriculture poses a significant environmental and public health concern. Exposure to these compounds, particularly via inhalation in occupational settings or through environmental drift, directly impacts the respiratory system, leading to various pathologies. Organochlorine compounds, such as Chlorpyrifos (marketed here as Nurinol), are neurotoxic agents, yet their sub-lethal effects on non-target organs, particularly the respiratory epithelium, are equally critical for long-term health assessment.

The wall of the upper respiratory tract (URT) serves as the primary barrier against inhaled toxicants. Structural integrity and functional competence, maintained primarily by the pseudostratified ciliated epithelium and the underlying vasculature and glands, are crucial for defense mechanisms like **mucociliary transport (MCT)** and detoxification. Disruption of these elements can initiate or exacerbate chronic respiratory diseases.

1.2. Study Objectives

This investigation aims to:

1. Model the morphological and functional changes in the URT wall following acute and chronic inhalation exposure to Nurinol (Chlorpyrifos) in a validated animal model.

2. Develop and describe a precise, multi-parametric **Algorithm for Histological Analysis** incorporating morphological, morphometric, histochemical, and immunohistochemical techniques to objectively quantify the extent and nature of pesticide-induced damage.

3. Evaluate the systemic implications of the observed structural lesions, particularly concerning energy metabolism and mucociliary function.

The high medico-ecological relevance of this study is rooted in providing a scientific basis for monitoring the impact of pesticide exposure and establishing a scientific foundation for effective prophylactic and therapeutic interventions in environmental medicine.

2. Materials and Methods

2.1. Ethical Statement and Animal Housing

The experimental study was conducted at the Department of Histology and Biology, Fergana Medical Institute of Public Health, adhering strictly to international bioethical standards, specifically the **Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes**.

Subjects: Eighty (80) clinically healthy adult male rabbits (Oryctolagus cuniculus), weighing $2.4 \text{ to } 2.7 \text{ kg}$, were used. Animals were housed individually under natural light cycles, provided ad libitum access to water, and fed a balanced standard diet. All animals underwent a $7-10 \text{ day}$ acclimatization period prior to the experiment commencement.

2.2. Experimental Design and Exposure Model

Toxic Agent: Nurinol (Chlorpyrifos) was used as the toxic agent, administered as an aqueous solution aerosol.

Dose and Duration: The exposure dose was equivalent to $3/4$ of the LD_{50} (216.8 mg/kg). Exposure was conducted twice daily (morning and evening) in a sealed inhalation chamber (45 L volume) equipped with an aerosol delivery, ventilation, and environmental monitoring system. Each exposure session lasted **10 minutes**.

Environmental Parameters: Air temperature was maintained at $+20 \pm 2^{\circ}\text{C}$, relative humidity at $60-70\%$, and atmospheric pressure within the normal range. The **total duration of exposure was 15 days**.

2.3. Sample Processing and Preparation

Upon completion of the experiment, animals were euthanized humanely in accordance with the international bioethical standards (Directive 2010/63/EU). Tissues from the conducting parts of the respiratory tract (upper respiratory tract) were immediately harvested.

Fixation and Sectioning: Tissues were fixed in 10% formalin solution, followed by standard histological processing: dehydration in graded alcohols, infiltration in paraffin wax, and subsequent embedding. Sections of $3-5 \text{ } \mu m$ thickness were prepared for staining.

2.4. The Histological Analysis Algorithm

The methodology employed a multi-faceted approach to comprehensively assess the morphological and functional status of the URT wall.

2.4.1. Morphological and Morphometric Analysis

- **Routine Staining: Hematoxylin and Eosin (H&E)** for general structure, assessment of epithelial integrity, inflammatory infiltration, and glandular structure.
- **Microscopy:** All slides were examined using a digital microscope at magnifications of $\times 200$ and $\times 400$.
- **Morphometric Criteria:** Quantitative evaluation focused on:
  - **Epithelial Integrity:** Measurement of epithelial height and assessment of ciliary layer damage.
  - **Basement Membrane (BM):** Quantitative assessment of BM thickness.
  - **Inflammation:** Counting the density of inflammatory cell infiltrates (lymphocytes, plasma cells, macrophages) per High-Power Field (HPF).

2.4.2. Histochemical Analysis

Histochemical methods were critical for assessing matrix components and functional changes:

- **Van Gieson's Picrofuchsin:** Used for differential staining of collagen fibers (red) to assess potential sub-epithelial fibrosis and vascular wall integrity.
- **Periodic Acid-Schiff (PAS) Reaction:** Used to identify neutral mucins (glycogen, glycoproteins) and basement membrane components, essential for quantifying **mucin hypersecretion** and evaluating potential impairments in carbohydrate metabolism.
- **Alcian Blue Staining (pH 2.5):** Used to stain acidic mucopolysaccharides and mucins, specifically to assess the functional status of goblet cells and mucosal glands related to mucus production.

2.4.3. Immunohistochemical (IHC) Analysis

IHC methods were applied to evaluate the inflammatory and immune response status:

- **Markers:** Assessment of key pro-inflammatory cytokines: **Tumor Necrosis Factor-alpha ($\text{TNF-}\alpha$) and Interleukin-6 ($\text{IL-}6$)$** as markers of activated local and systemic inflammation in the URT wall.

2.5. Statistical Analysis

Data processing was performed using MS Excel and STATISTICA for Windows. The Student's t-test was applied to evaluate the significance of differences between the control and experimental groups. Differences were considered statistically significant at $p < 0.05$. Pearson's and Spearman's coefficients were used for correlation analysis based on data distribution characteristics. The methodology was further directed at the complex assessment of mitochondrial and carbohydrate metabolism using validated biochemical markers, reproducible under standard laboratory conditions.

3. Results of the Study and Discussion

The investigation into the morphofunctional changes in the URT wall following Nurinol (Chlorpyrifos) exposure demonstrated significant structural and functional tissue alterations compared to the control animals. These results unequivocally confirm the toxic impact of the pesticide on the respiratory system.

3.1. Pathological Changes in Epithelium and Glands

The most pronounced changes were observed in the epithelial layer and the underlying mucosal components.

- **Epithelial Destruction and BM Thickening:** H&E staining revealed extensive **destruction of the pseudostratified ciliated epithelium**. This included loss of cellular polarity, vacuolation, and focal areas of epithelial detachment. Crucially, morphometric analysis confirmed a statistically significant **thickening of the basement membrane** (BM) in the experimental group, which is a common indicator of chronic inflammation and sub-epithelial restructuring in response to prolonged toxic insult.
- **Mucin Hypersecretion and Glandular Changes:** PAS and Alcian Blue staining provided clear evidence of **mucin hypersecretion**. This phenomenon was directly linked to the hyperplasia and hyperfunction of goblet cells and profound **structural disorders within the mucosal glands**. This excessive mucus production is a compensatory reaction to protect the damaged epithelium, yet it often impairs proper clearance.

3.2. Impairment of Functional Activity

The morphological changes directly translated into significant functional impairment, particularly concerning the essential defense mechanism of the URT.

- **Decreased Mucociliary Transport (MCT):** The destruction of the ciliated epithelium observed in H&E sections confirms a **reduction in ciliary clearance and overall mucociliary**

transport. MCT is vital for removing inhaled particles and toxins; its failure leads to toxicant accumulation and prolonged tissue contact.

- **Metabolic Disruption:** The observed **reduction in the activity of metabolic enzymes** suggests a severe disruption of **energy metabolism and tissue respiration**. This may be attributed to Chlorpyrifos's known effects on the nervous system and its broader impact on mitochondrial function and oxidative stress, leading to a compromised capacity for tissue repair and maintenance (as evaluated by validated biochemical markers).

3.3. Inflammatory and Immune Response

The IHC analysis confirmed the activation of local inflammatory cascades:

- **Inflammatory Infiltrates and Cytokine Overexpression:** Analysis revealed **activated inflammatory infiltrates** predominantly in the lamina propria and periglandular areas. Furthermore, the immunohistochemical detection of **$\text{TNF-}\alpha$ and $\text{IL-}6$** demonstrated local overexpression of these pro-inflammatory cytokines. This finding suggests that Nurinol exposure not only causes direct tissue damage but also triggers an active, sustained inflammatory response, which contributes to tissue remodeling and chronic pathology.

3.4. Overall Significance

The identified morphological changes (epithelial destruction, BM thickening, hypersecretion, and inflammation) provide the pathological foundation for understanding the **pathogenesis of respiratory diseases** induced by toxic chemical substances. The use of the proposed algorithm—integrating routine, histochemical (mucins, collagen), and IHC ($\text{TNF-}\alpha$, $\text{IL-}6$) methods—ensures a comprehensive and reproducible assessment of pesticide-induced respiratory toxicity.

4. Conclusion and Recommendations

The application of the developed analytical algorithm confirmed that inhalation exposure to Nurinol (Chlorpyrifos) induces significant and multi-layered morphofunctional pathology in the upper respiratory tract of rabbits, characterized by primary epithelial damage, impairment of mucociliary function, inflammatory activation, and metabolic disruption.

These findings are crucial for ecotoxicology and medicine, offering specific morphological biomarkers (BM thickness, inflammation density, mucin scores) that can be utilized for:

1. **Early Diagnosis:** Identifying structural changes indicative of pesticide-induced pathology before the onset of severe clinical symptoms.
2. **Risk Assessment:** Enhancing the scientific basis for regulatory limits on pesticide exposure.
3. **Therapeutic Development:** Guiding the development of prophylactic and therapeutic measures aimed at restoring epithelial integrity and mucociliary clearance in affected populations.

The methodological recommendations provided, incorporating histological, histochemical, and immunohistochemical techniques, form a robust scientific platform for monitoring the effects of pesticide exposure on the organism.

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