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Time-Dependent Lung Remodeling After Inhalation Exposure to the Combined Insecticide Nurinol in Rabbits and Partial Mitigation by Alpha- Lipoic Acid A Digital Histomorphometry Study

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Research Article

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Abstract

Background

Inhalation of combined pesticide formulations can occur during preparation and application and may contribute to respiratory toxicity. Nurinol contains chlorpyrifos (organophosphate) and cypermethrin (type II pyrethroid), agents with distinct but potentially convergent mechanisms of lung injury. Quantitative data on time-dependent airway and parenchymal remodeling after repeated inhalation of such mixtures are limited.

Aim

To characterize acute and repeated inhalation toxicity of Nurinol in rabbits and to quantify lung remodeling with and without antioxidant intervention using digital morphometry.

Methods

Adult male rabbits were assigned to five groups: control; 30-day exposure; 30-day plus alpha-lipoic acid (ALA); 120-day exposure; 120-day plus ALA. Acute lethality across six dose levels was analyzed by probit regression to derive LD_{16}/LD_{50}/LD_{90}. Repeated exposures were conducted twice daily for 10 minutes at 0.75×LD_{50} in a sealed aerosol chamber. ALA (9 mg/kg/day) was administered orally in intervention groups. Lungs were fixed, paraffin-embedded, H&E-stained, whole-slide scanned, and analyzed using QuPath and ImageJ. Primary endpoints were terminal bronchiole lumen area, bronchiole wall thickness, mean alveolar diameter, alveolar septal thickness, and capillary diameter.

Results

Probit analysis yielded LD_{50} ≈289 mg/kg. Repeated inhalation produced progressive remodeling. At 30 vs 120 days (vs control), terminal bronchiole lumen area decreased by ~ 19% and ~ 35%; bronchiole wall thickness increased by ~ 29% and ~ 57%; alveolar diameter decreased by ~ 24% and ~ 37%; septal thickness increased by ~ 103% and ~ 214%; and capillary diameter increased by ~ 82% and ~ 45%. ALA partially normalized measures, with greater effect at 30 days.

Conclusions

Nurinol inhalation induces dose-dependent lethality and marked, time-dependent bronchiolar–alveolar remodeling consistent with transition from inflammatory to fibrosis-like changes. ALA mitigates early injury but incompletely reverses established structural remodeling.

Introduction

Pesticides remain widely used in agriculture, domestic pest control, and vector management, and multiple reviews conclude that occupational exposure is associated with increased respiratory symptoms and physician-diagnosed airway disease[1]. Importantly, exposure can occur at many steps in the pesticide lifecycle—preparation of concentrated formulations, application, and equipment cleaning—where inhalation of aerosols and contaminated dust contributes to internal dose[2–4]. Nurinol is a combined insecticide containing chlorpyrifos (organophosphate) and cypermethrin (type II pyrethroid) [5]. Organophosphates exert toxicity primarily by inhibiting acetylcholinesterase through the action of bioactivated oxon metabolites that phosphorylate the active-site serine, producing cholinergic overstimulation[6]. Pyrethroids, including cypermethrin, act mainly by altering the gating kinetics of voltage-gated sodium channels, prolonging channel opening and perturbing neuronal excitability; type II compounds additionally affect chloride currents and may provoke more severe systemic toxicity at high exposures[7, 8]. In commercial practice, pyrethroids may be co-formulated with organophosphates, and such combinations can complicate toxicokinetics and toxicodynamics, including via metabolic interactions and non-cholinergic injury pathways[9].

Respiratory toxicity from these classes is biologically plausible and has been documented across experimental and clinical contexts. Animal inhalation studies of chlorpyrifos formulations at very high concentrations have reported lung pathology (e.g., hemorrhagic/hepatized areas) and lethality, while lower intermediate-duration chlorpyrifos exposures in standardized inhalation protocols have sometimes shown normal lung histology—highlighting dose, formulation, and exposure-characterization as key determinants[10]. For pyrethroids, inhalation exposure has been associated with inflammatory and fibrotic lung changes in experimental models, including edema, alveolitis, thickened alveolar walls, and collagen deposition [11].

Clinically, case series and reviews describe severe pulmonary toxicity and interstitial lung disease-like presentations following inhalation of pyrethroid-containing domestic insecticides, though causality is often difficult to establish and susceptibility may be variable [12, 13].

A prominent mechanistic theme across pesticide toxicology is oxidative stress, which can amplify inflammation, endothelial injury, and tissue remodeling. In lung fibrosis biology more broadly, redox imbalance and reactive oxygen species (ROS) are implicated in the progression of injurious-to-fibrotic pathways, including transforming growth factor-β (TGF-β)–linked signaling and fibroblast activation, supporting antioxidant strategies as plausible adjuncts even if monotherapy may be insufficient to reverse established fibrosis[10, 14].

In this context, alpha-lipoic acid (ALA) is a naturally occurring organosulfur compound with antioxidant and anti-inflammatory properties and accumulating evidence of benefit across experimental respiratory disease models [15].

ALA has shown protective effects in a classical lung injury/fibrosis model (bleomycin), reducing oxidative stress markers and fibrotic indices, supporting its evaluation against toxicant-driven lung remodeling [16].

Aim: To characterize acute and repeated inhalation toxicity of Nurinol in rabbits and to quantify time-dependent lung remodeling with and without antioxidant intervention using digital morphometry.

Specific objectives:

To model repeated inhalation exposure over 30 and 120 days at 0.75×LD_{50} and quantify bronchiolar and alveolar remodeling.

To evaluate whether oral alpha-lipoic acid partially mitigates morphometric indicators of airway narrowing, septal thickening, and microvascular changes, consistent with oxidative stress–linked injury pathways.

Materials and Methods

Study setting and ethics: The experiment was conducted in the vivarium at 12 with support from institutional histology/biology units. Ethical approval was granted by the 12 (Ethics Committee protocol No. 7, 26 June 2025). Procedures were performed in accordance with humane standards consistent with 11 Directive 2010/63/EU requirements for minimizing pain, suffering, and distress, including Annex IV provisions on humane killing methods and sedation requirements for rabbits [17].

Animals and husbandry: Adult male rabbits (Oryctolagus cuniculus), 2.4–2.8 kg, were housed individually in metal cages (40×70 cm). Environmental conditions were controlled (18–22°C; 12 h light/dark). Standard diet (hay, vegetables, pellets) and water ad libitum were provided. Individual identification was by ear tags.

Rationale for rabbit model: Rabbits are used in inhalation toxicology and lung disease research due to airway anatomy and inflammatory responsiveness that can approximate relevant facets of human disease biology, while also enabling detailed airway morphometry [18].

Study design and group allocation: The planned design comprised five parallel groups (initially n = 16 each; total planned n = 80), including control, 30-day exposure, 30-day exposure + ALA, 120-day exposure, and 120-day exposure + ALA. Sample-size planning referenced the 3Rs framework (Replacement, Reduction, Refinement) and used G*Power for a one-way ANOVA with five groups (α = 0.05, power = 0.80, effect size f = 0.40), yielding a total sample size of 80. [19]

Pesticide formulation: Nurinol (55% formulation) is a combined insecticide containing chlorpyrifos (500 g/L) and cypermethrin (50 g/L), representing an organophosphate–pyrethroid mixture with distinct but potentially convergent toxicity pathways.

Acute inhalation toxicity and probit regression: Acute toxicity was assessed in six dose groups (n = 6 each) spanning 100–600 mg/kg (study-defined inhalation dose metric). Mortality was recorded for each group. Probit values were assigned to mortality proportions, with 0% and 100% adjusted to 2.28% and 97.72% (probit 3 and 7) to permit regression, consistent with classical probit methodology [20].

A probit–log10(dose) linear regression was fitted, and LD_{16}, LD_{50} and LD_{90} were computed with 95% confidence intervals (CIs) using standard regression-based inference.

Repeated inhalation exposure model: For repeated exposures, the target dose was set at $0.75 \times LD_{50}$ (216.8 mg/kg), based on LD_{50} derived from probit regression. Exposures were conducted in a sealed whole-body chamber (50×30×30 cm; 45 L) equipped with an aerosol generation system (nebulizer; nominal 0.5–0.7 mL/min), twice daily, 10 min per session, for 30 or 120 consecutive days. Animals were monitored for clinical signs and mortality.

In this study, dosing was operationalized via a mass-per-body-weight paradigm and chamber mass balance, rather than continuous real-time aerosol concentration and particle-size distribution measurement (addressed as a limitation).

Alpha-lipoic acid intervention: ALA was administered orally by gastric gavage daily during the exposure period (30 or 120 days) at 9 mg/kg/day. [21]

At study endpoints, animals were euthanized using approved humane methods[17].

Lungs were extracted and fixed in 10% neutral buffered formalin for 24–48 h, processed to paraffin, sectioned at 5–7 μm, stained with hematoxylin and eosin, and mounted.

Digital pathology and morphometry: Whole-slide scanning was performed using a NanoZoomer platform (17). Regions of the respiratory parenchyma were analyzed in ≥ 10 fields per specimen at ×200 equivalent magnification. Digital morphometry employed QuPath (v0.5.0) for segmentation/quantification and ImageJ for supplementary measurement and reconstruction workflows. [22, 23]

Primary morphometric endpoints included terminal bronchiole lumen area, small bronchiole wall thickness, mean alveolar diameter, alveolar septal thickness, and alveolar capillary diameter.

Statistics: Group differences were assessed by comparing each exposed group to control (by p-value annotations in the dataset). For manuscript submission, the recommended approach is one-way ANOVA with multiplicity control versus a single control group (e.g., Dunnett's), after verification of distributional assumptions; non-parametric alternatives should be applied if assumptions are violated.

Results

Quantitative morphometry demonstrated a coherent, time-dependent remodeling pattern (Table 1): bronchiolar narrowing (reduced terminal bronchiole lumen area), airway wall thickening, reduced alveolar airspace size, marked septal thickening, and increased capillary diameter. The magnitude of change was generally larger at 120 days than at 30 days, consistent with progressive structural remodeling.

Table 1
Lung morphometric parameters (mean ± dispersion as recorded) and significance vs control

Parameter	Control	Nurinol 30 days	Nurinol 30 days + ALA	Nurinol 120 days	Nurinol 120 days + ALA
Terminal bronchiole lumen area (μm²)	3571.04 ± 272	2891.71 ± 206	3116.14 ± 3.12	2322.13 ± 296	2614.89 ± 238
Small bronchiole wall thickness (μm)	21.31 ± 1.01	27.44 ± 4.75**	23.40 ± 1.94	33.48 ± 4.2*	29.06 ± 5.93*
Mean alveolar diameter (μm)	81.4 ± 2.76	61.7 ± 5.27	73.32 ± 6.13**	51.20 ± 5.61*	61.32 ± 15.21*
Alveolar septal thickness (μm)	13.44 ± 2.59	27.30 ± 5.54*	20.69 ± 4.36*	42.15 ± 6.88*	30.13 ± 5.44*
Alveolar capillary diameter (μm)	5.11 ± 1.79	9.32 ± 0.16*	7.29 ± 0.04**	7.41 ± 0.06*	6.15 ± 0.05**

Significance coding as provided: *p < 0.05; **p < 0.01; ***p < 0.001 vs control.

To support interpretation and facilitate cross-parameter comparison, percent changes relative to control were computed (Table 5). The most prominent progressive change was alveolar septal thickening (≈ + 103% at 30 days and ≈ + 214% at 120 days), while terminal bronchiole lumen area showed substantial progressive reduction (≈ − 19% to − 35%).

Table 5
Summary of morphometric percent change relative to control (calculated from Table 4 means)

Parameter	Nurinol 30 days	Nurinol 30 days + ALA	Nurinol 120 days	Nurinol 120 days + ALA
Terminal bronchiole lumen area	−19.0%	−12.7%	−35.0%	−26.8%
Small bronchiole wall thickness	+ 28.8%	+ 9.8%	+ 57.1%	+ 36.4%
Mean alveolar diameter	−24.2%	−9.9%	−37.1%	−24.7%
Alveolar septal thickness	+ 103.1%	+ 53.9%	+ 213.6%	+ 124.2%
Alveolar capillary diameter	+ 82.4%	+ 42.7%	+ 45.0%	+ 20.4%

Apparent effect of alpha-lipoic acid on structural endpoints

ALA co-treatment partially shifted morphometric measures toward control values at both timepoints, with generally larger normalization at 30 days than at 120 days (Table 5). In relative terms versus untreated exposure at the same timepoint, ALA increased terminal bronchiole lumen area by + 7.8% (30 days) and + 12.6% (120 days), reduced septal thickness by − 24.2% and − 28.5%, and reduced capillary diameter by − 21.8% and − 17.0%, respectively (computed from Table 4 means).

Discussion

The present morphometric findings allow a quantitative interpretation of how chronic inhalational exposure to Nurinol (chlorpyrifos + cypermethrin) restructures the distal respiratory unit. In contrast to many toxicological studies that rely on descriptive histology, the current data provide micrometric evidence of progressive changes in bronchiolar patency, airway wall structure, alveolar geometry, septal organization, and microvascular caliber. These parameters permit direct comparison with published morphometric and histopathological studies of organophosphate and pyrethroid inhalation toxicity.

A reduction of terminal bronchiole lumen area by 19% at 30 days and 35% at 120 days demonstrates progressive loss of distal airway patency. Similar structural narrowing has been described after cypermethrin inhalation in rats by Sheikh et al., who reported bronchiolar epithelial hyperplasia and peribronchiolar inflammatory infiltration leading to reduced airway lumen[24]. Likewise, Javier et al. (2023) demonstrated bronchiolar constriction and epithelial thickening in a cypermethrin aerosol model[25].

However, these studies described narrowing qualitatively. The current work quantifies the geometric reduction, demonstrating that under chronic exposure this narrowing evolves into structurally fixed remodeling. This aligns with the observations of Thanomsit et al. in mixed chlorpyrifos–cypermethrin exposure, where peribronchiolar fibrosis was described, suggesting that prolonged pesticide inhalation induces airway remodeling rather than transient inflammation [26, 27].

Partial recovery of bronchiolar lumen area with ALA at 30 days, but limited correction at 120 days, parallels findings of Hiller et al. in oxidative lung injury models, where antioxidant therapy was effective during inflammatory stages [28].

Bronchiolar wall thickness increased by 28% at 30 days and 57% at 120 days. Comparable findings were reported by [29] et al. in chlorpyrifos inhalation studies, where septal and peribronchiolar thickening resulted from inflammatory infiltration. Chougule et al. also noted airway wall thickening after CPF exposure[30].

In allergic and toxic airway remodeling models, such as those described by Morsi et al., similar magnitudes of wall thickening correspond to fibro-proliferative processes rather than edema. The

present morphometry confirms that by 120 days, wall thickening exceeds inflammatory parameters and reflects matrix deposition and fibroblast activation.

Mean alveolar diameter decreased by 24% at 30 days and 37% at 120 days. Studies of cypermethrin inhalation by Sheikh et al. described “collapsed” and “distorted” alveoli. Chlorpyrifos studies by Hassani et al. noted alveolar hemorrhage and distortion of alveolar spaces[31].

In fibrosis models, such as bleomycin injury studied by Zhou et al., similar morphometric contraction of alveolar spaces directly corresponds to loss of gas exchange surface[32]. The present data demonstrate that Nurinol inhalation produces quantitatively comparable airspace contraction.

ALA restored alveolar diameter substantially at 30 days, consistent with findings by El-Sayed et al., who showed that ALA prevents oxidative alveolar damage but cannot reverse established interstitial remodeling[15].

Septal thickness increased by 103% at 30 days and 214% at 120 days. This magnitude resembles septal expansion described in pulmonary fibrosis rather than simple inflammatory lung injury. Mediha Demet Okudan Altındaş et al. and Frances C Shaffo et al.[33, 34] reported septal thickening after CPF exposure but did not quantify it. In experimental fibrosis, Zhou et al. demonstrated comparable septal expansion due to collagen deposition.

The present data indicate that chronic Nurinol inhalation reproduces fibrosis-like interstitial expansion. ALA reduced septal thickness during early stages but had limited influence at 120 days, consistent with fibrosis models where antioxidants cannot reverse matrix accumulation.

Alveolar capillary diameter increased by 82% at 30 days and 45% at 120 days. Early capillary dilation corresponds to endothelial dysfunction and congestion described by Thanomsit et al. in mixed pesticide exposure and by Hassani et al. in CPF studies.

The reduction of capillary dilation at 120 days while septal thickness continues to increase indicates a shift from vascular-inflammatory pathology to interstitial-fibrotic remodeling. ALA showed the greatest corrective effect on this parameter, consistent with endothelial protection from oxidative stress as described by El-Sayed et al.

Conclusion

Nurinol inhalation exposure in rabbits produced a clear acute dose–response lethality profile (LD_{50} ≈289 mg/kg by probit regression) and induced progressive, time-dependent lung remodeling over 30 and 120 days characterized by bronchiolar narrowing, airway wall thickening, reduced alveolar airspace size, marked septal thickening, and microvascular dilation. These structural changes were partially mitigated by oral alpha-lipoic acid, with greater apparent benefit during the shorter (30-day) exposure interval, but incomplete reversal after prolonged exposure—consistent with oxidative stress–linked injury contributing to remodeling while established fibrosis remains difficult to fully normalize.

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Declarations

Author Contribution

Conceptualization Ideas; formulation or evolution of overarching research goals and aims.Methodology Development or design of methodology; creation of models.Software Programming, software development; designing computer programs; implementation of code.Validation Verification/replication of results, experiments, or overall research output.Formal Analysis Application of statistical, mathematical, or computational techniques to analyze data.Investigation Conducting the research and investigation process (performing experiments, data collection).Resources Provision of study materials, reagents, patients, laboratory samples, computing resources, etc.

References

1. Tarmure S, Alexescu TG, Orasan O, Negrean V, Sitar-Taut AV, Coste SC, et al. Influence of pesticides on respiratory pathology – a literature review n.d. https://doi.org/10.26444/aaem/121899.
2. Mamane A, Baldi I, Tessier J-F, Raherison C, Bouvier G. Occupational exposure to pesticides and respiratory health. Eur Respir Rev 2015;24:306–19. https://doi.org/10.1183/16000617.00006014.
3. Callahan CL, Al-Batanony M, Ismail AA, Abdel-Rasoul G, Hendy O, Olson JR, et al. Chlorpyrifos Exposure and Respiratory Health among Adolescent Agricultural Workers. International Journal of Environmental Research and Public Health 2014;11:13117–29. https://doi.org/10.3390/ijerph111213117.
4. Barthel E. [Cancer risk in pesticide exposed agricultural workers (author's transl)]. Arch Geschwulstforsch 1981;51:579–85.
5. Darwiche W, Gay-Quéheillard J, Delanaud S, Sabbouri HEKE, 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:e0191237. https://doi.org/10.1371/journal.pone.0191237.
6. Colombia B. Human Pesticide Exposure butyrylcholinesterase and acetylcholinesterase by the organophosphate chlorpyrifos oxon. Toxicology and Applied Pharmacology 2013;241:135–42.
7. Garcia MLB, Santos UP, Perini A, Acencio MMP, Lopes FDTQS, Bueno HMS, et al. Eosinophilic pneumonitis induced by aerosol-administered diesel oil and pyrethrum to mice. Rev Panam Salud Publica 2009;25. https://doi.org/10.1590/S1020-49892009000600008.
8. Field LM, Emyr Davies TG, O'Reilly AO, Williamson MS, Wallace BA. Voltage-gated sodium channels as targets for pyrethroid insecticides. Eur Biophys J 2017;46:675–9. https://doi.org/10.1007/s00249-016-1195-1.
9. Park C-M, Kim K-T, Rhyu D-Y. Exposure to a low concentration of mixed organochlorine pesticides impairs glucose metabolism and mitochondrial function in L6 myotubes and zebrafish. Journal of

Hazardous Materials 2021;414:125437. https://doi.org/10.1016/j.jhazmat.2021.125437.

10. Aly N, EL-Gendy K, Mahmoud F, El-Sebae AK. Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. Pesticide Biochemistry and Physiology 2010;97:7–12. https://doi.org/10.1016/j.pestbp.2009.11.007.
11. Kingsley IA, Sunday IP, Blessing AA. Histological assessment of the Effects of Pyrethroidsinsecticide Morteinon the Lungs of Adult WistarRats n.d.
12. Amizadeh M, Safari-Kamalabadi M, Askari-Saryazdi G, Amizadeh M, Reihani-Kermani H. Pesticide exposure and head and neck cancers: a case-control study in an agricultural region. Iranian Journal of Otorhinolaryngology 2017;29:275.
13. De Stefani E, Boffetta P, Oreggia F, Ronco A, Kogevinas M, Mendilaharsu M. Occupation and the risk of laryngeal cancer in Uruguay. Am J Ind Med 1998;33:537–42. https://doi.org/10.1002/(SICI)1097-0274(199806)33:6%253C537::AID-AJIM3%253E3.0.CO;2-N.
14. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. Med Sci Monit 2004;10:141–7.
15. Ak E, Muhan A, Çalışkan Ş, Oktay Ş, Ak E, Muhan A, et al. Comparison of the Protective Effect of Alpha Lipoic Acid and Quercetin in Methotrexate- Induced Lung Damage. Bagcilar Medical Bulletin 2023. https://doi.org/10.4274/BMB.galenos.2023.2023-01-011.
16. Liu R, Ahmed KM, Nantajit D, Rosenthal FS, Hai C-X, Li JJ. Therapeutic effects of alpha-lipoic acid on bleomycin-induced pulmonary fibrosis in rats. Int J Mol Med 2007;19:865–73.
17. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. vol. 276. 2010.
18. Kamaruzaman NA, Kardia E, Kamaldin N 'Atikah, Latahir AZ, Yahaya BH. The Rabbit as a Model for Studying Lung Disease and Stem Cell Therapy. BioMed Research International 2013;2013:691830. https://doi.org/10.1155/2013/691830.
19. The 3Rs | NC3Rs n.d. https://nc3rs.org.uk/who-we-are/3rs (accessed February 3, 2026).
20. OECD. Test No. 403: Acute Inhalation Toxicity. OECD Guidelines for the Testing of Chemicals, Section 4 2024. https://doi.org/10.1787/9789264070608-en.
21. Guo X, Wu S, Shang J, Dong W, Li Y, Peng Q, et al. The effects of lipoic acid on respiratory diseases. International Immunopharmacology 2023;116:109713. https://doi.org/10.1016/j.intimp.2023.109713.
22. Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: Open source software for digital pathology image analysis. Sci Rep 2017;7:16878. https://doi.org/10.1038/s41598-017-17204-5.
23. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671–5. https://doi.org/10.1038/nmeth.2089.
24. SHEIKH N, JAVED S, Asmatullah A, AHMAD KR, ABBAS T, IQBAL J. Histological changes in the lung and liver tissues in mice exposed to pyrethroid inhalation. Walailak Journal of Science and

Technology (WJST) 2014;11:843–9.
25. Javier M-OMH, Carolina VM-C, Ma M-S. CYPERMETHRIN CAUSE ALLERGIC INFLAMMATORY RESPONSE IN RAT LUNG. World 2023;2.
26. Karimzadeh F, Babazadeh Z, Daraei A, Zabihi-Neyshaburi E, Feizi F, Ranaee M, et al. Repurposing Disulfiram: A Promising Antifibrotic Strategy Against Paraquat-Induced Pulmonary Fibrosis 2025. https://doi.org/10.21203/rs.3.rs-7218465/v1.
27. Tigges J, Worek F, Thiermann H, Wille T. Organophosphorus pesticides exhibit compound specific effects in rat precision-cut lung slices (PCLS): mechanisms involved in airway response, cytotoxicity, inflammatory activation and antioxidative defense. Arch Toxicol 2022;96:321–34. https://doi.org/10.1007/s00204-021-03186-x.
28. Hiller S, DeKroon R, Hamlett ED, Xu L, Osorio C, Robinette J, et al. Alpha-lipoic acid supplementation protects enzymes from damage by nitrosative and oxidative stress. Biochimica et Biophysica Acta (BBA) - General Subjects 2016;1860:36–45. https://doi.org/10.1016/j.bbagen.2015.09.001.
29. Hassani S, Sepand M, Jafari A, Jaafari J, Rezaee R, Zeinali M, et al. Protective effects of curcumin and vitamin E against chlorpyrifos-induced lung oxidative damage. Hum Exp Toxicol 2015;34:668–76. https://doi.org/10.1177/0960327114550888.
30. Chougule AA, Brar RS, Banga HS, Singh ND, Goyal A, Gadhave PD. Concomitant effect of Chlorpyrifos and intranasal endotoxin administration on apoptosis related protein expression in lung of mice. J Environ Anal Toxicol 2013;3:2161–0525.
31. Hulse EJ, Smith SH, Wallace WA, Dorward DA, Simpson AJ, Drummond G, et al. Development of a histopathology scoring system for the pulmonary complications of organophosphorus insecticide poisoning in a pig model. PLoS One 2020;15:e0240563. https://doi.org/10.1371/journal.pone.0240563.
32. Ruscitti F, Ravanetti F, Essers J, Ridwan Y, Belenkov S, Vos W, et al. Longitudinal assessment of bleomycin-induced lung fibrosis by Micro-CT correlates with histological evaluation in mice. Multidiscip Respir Med 2017;12:8. https://doi.org/10.1186/s40248-017-0089-0.
33. Okudan Altındaş MD, Güner A. Astaxanthin Attenuates Chlorpyrifos-Induced Pulmonary Cytotoxicity by Modulating Mitochondrial Redox and Inflammatory Pathways. Current Issues in Molecular Biology 2025;47:663. https://doi.org/10.3390/cimb47080663.
34. Shaffo FC, Grodzki AC, Schelegle ES, Lein PJ. The Organophosphorus Pesticide Chlorpyrifos Induces Sex-Specific Airway Hyperreactivity in Adult Rats. Toxicol Sci 2018;165:244–53. https://doi.org/10.1093/toxsci/kfy158.

Figures

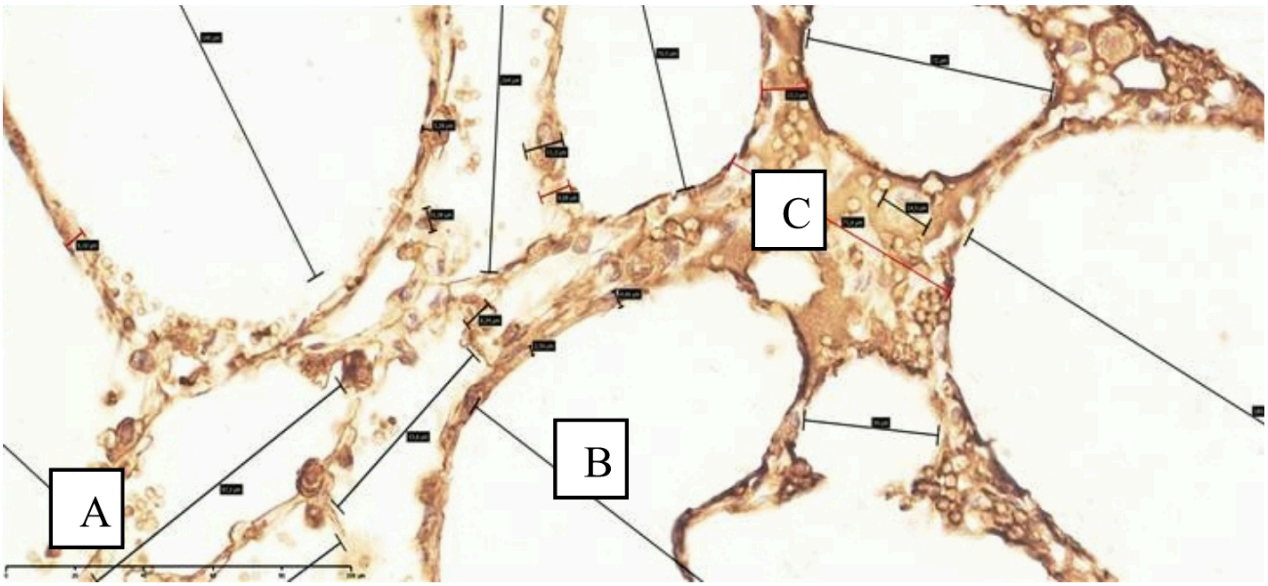


Figure 1

Representative digital morphometry annotation (description for manuscript figure preparation).

(A) Whole-slide region of interest with QuPath segmentation overlay indicating terminal bronchiole lumen boundary and wall thickness measurement vectors.

(B) Alveolar compartment showing alveolar diameter sampling and septal thickness measurements.

(C) Microvascular compartment with capillary diameter measurements.

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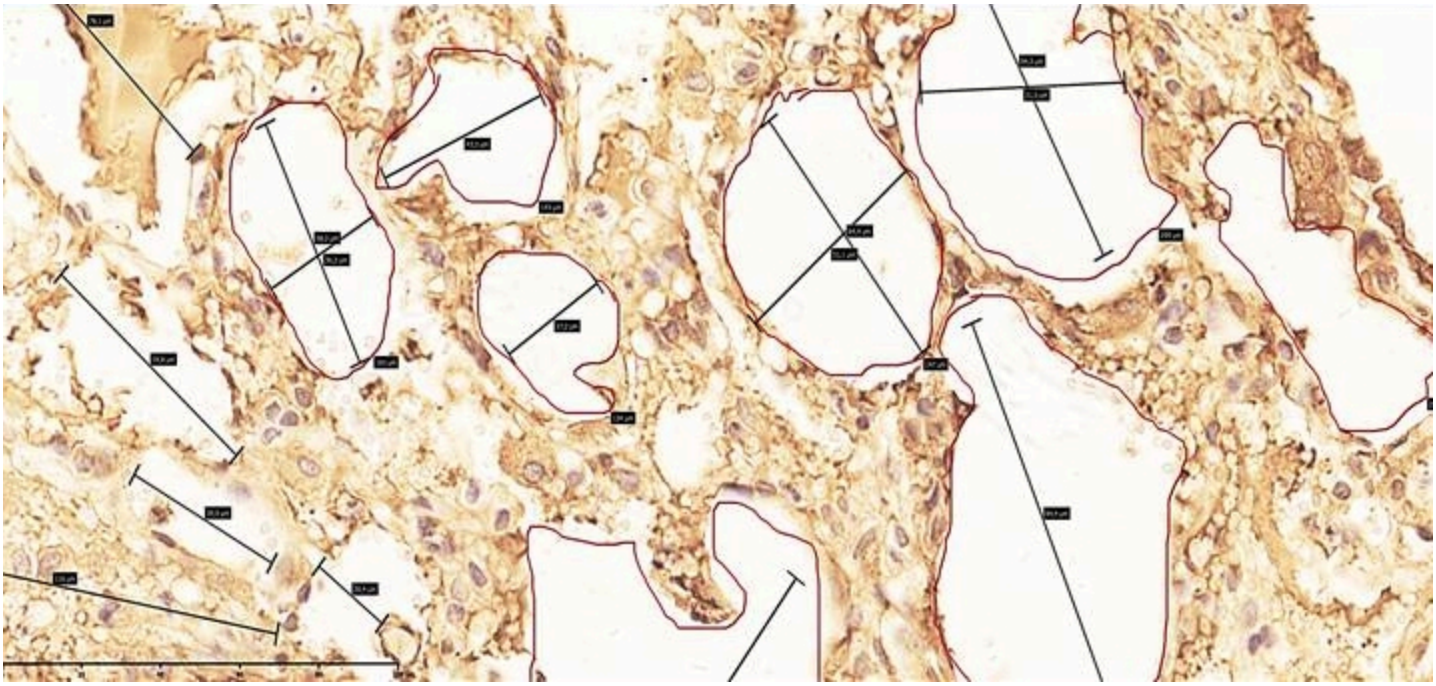


Figure 2

Morphometric analysis of lung tissue.Alveolar lumens are outlined along their contours, and measurements of their areas and diameters were performed. Marked thickening of the alveolar septa is observed, and the alveolar spaces are heterogeneous in shape and size.