



Article

Integrated Network Toxicology and Metabolomics Elucidate Mechanisms of Carbosulfan-Induced Respiratory Toxicity in Rats

Xian Ju ^{1,2,3,†}, Di Liang ^{1,2,3,†}, Hongyu Su ⁴, Yachun Zhang ^{1,2,3}, Zhenyu Liang ^{1,2,3}, Yiheng Liu ^{1,2,3}, Wenqi Zhao ^{1,2,3}, Dan Zhang ^{1,2,3}, Zhe Chen ^{1,2,3,5,*} and Keming Yun ^{1,2,3,*}

¹ School of Forensic Medicine, Shanxi Medical University, Jinzhong 030600, China

² Shanxi Key Laboratory of Forensic Medicine, Jinzhong 030600, China

³ Key Laboratory of Forensic Toxicology of Ministry of Public, Jinzhong 030600, China

⁴ West China School of Basic Medical Sciences and Forensic Medicine, Sichuan University, Chengdu 610041, China

⁵ China Institute for Radiation Protection, Taiyuan 030001, China

* Correspondence: chenzhe@sxmu.edu.cn (Z.C.); yunkeming@sxmu.edu.cn (K.Y.)

† These authors contributed equally to this work.

Abstract

Carbosulfan is a widely used carbamate insecticide, yet its mechanisms of respiratory toxicity remain poorly understood. This study integrated network toxicology, untargeted metabolomics, and molecular docking to systematically investigate the potential mechanisms of carbosulfan-induced respiratory toxicity in male Sprague Dawley rats. Rats were administered a single oral dose of carbosulfan (125 or 250 mg/kg) and assessed after 12 h. Exposure resulted in significant pathological lung damage, characterized by disrupted alveolar architecture, inflammatory cell infiltration, and increased serum levels of the pro-inflammatory cytokines IL-6, IL-1 β , and TNF- α . Network toxicology analysis identified 51 potential targets associated with respiratory toxicity, with core targets including SRC, EGFR, PTGS2, CXCL8, CYP3A4, and NR3C1. Enriched pathways were primarily related to neuroactive ligand–receptor interaction, VEGF signaling, and arachidonic acid metabolism. Untargeted metabolomics revealed significant metabolic perturbations in pathways central to antioxidant defense and energy homeostasis, including glutathione metabolism, the tricarboxylic acid cycle, and arginine biosynthesis. Molecular docking confirmed stable *in silico* binding affinities between carbosulfan and the predicted core targets. Integrative analysis suggests that carbosulfan exposure is associated with respiratory damage, potentially through interconnected mechanisms involving oxidative stress, inflammation, and disruption of cell signaling and metabolic enzyme systems. However, given the acute high-dose nature of the model and the interpretative integration of multi-omics data, these findings should be considered hypothesis-generating. This study provides a novel system-level perspective on carbosulfan-induced respiratory toxicity and highlights key pathways and targets for future validation in chronic exposure models.

Keywords: carbosulfan; network toxicology; metabolomics; respiratory toxicity; molecular docking

Received: 30 January 2026

Revised: 18 February 2026

Accepted: 25 February 2026

Published: 25 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

The increasing demand for crop yield and vegetable production has led to a rise in pesticide use in modern agriculture [1,2]. Carbamate insecticides are widely employed for agricultural pest control [3]. Carbosulfan has been reported to be effective against pests resistant to organophosphate insecticides and is also used to control mosquitoes resistant to pyrethroids [4]. Currently, carbosulfan is extensively utilized for pest management in rice fields across many countries [5–7].

Similar to other carbamate pesticides, carbosulfan exerts its toxic effects by inhibiting acetylcholinesterase activity [8,9]. Its overuse may result in residues entering ecosystems via surface runoff, leaching, soil erosion, or volatilization [10,11]. Consequently, although mammals and humans are not its primary targets, carbosulfan may still pose risks to non-target organisms through environmental residues or intentional ingestion. Some studies suggest that carbosulfan may be associated with developmental disorders, reproductive toxicity, and genotoxicity [12–14]. Although acute poisoning cases, including respiratory failure, have been reported [15], the specific molecular and metabolic mechanisms underlying carbosulfan-induced respiratory toxicity remain poorly understood, hindering accurate risk assessment.

Endogenous metabolites participate in various biological processes, and their alterations reflect the internal physiological and pathological states of an organism. Untargeted metabolomics enables the analysis of endogenous metabolites, facilitating the identification of differential metabolites and the elucidation of their metabolic pathways [16]. However, metabolomics primarily captures terminal changes in metabolic processes, often overlooking upstream regulatory mechanisms such as biosynthetic pathways, protein interactions, and molecular targets. Therefore, relying solely on metabolomics may be insufficient for comprehensively revealing the respiratory toxicity mechanisms of carbosulfan. To address this limitation, network toxicology offers a holistic and systematic approach for toxicity studies by constructing interaction networks among genes, proteins, compounds, and toxicological responses [17]. However, network toxicology depends on public databases and often lacks direct experimental validation. Integrating network toxicology with untargeted metabolomics combines the predictive power of the former with the mechanistic insights of the latter, thereby providing a more comprehensive understanding of carbosulfan-induced respiratory toxicity.

This study employed a strategy integrating network toxicology and untargeted metabolomics to elucidate the mechanisms of carbosulfan-induced respiratory toxicity in a rat model. Network toxicology analysis was used to identify key molecular targets and their regulatory networks, while untargeted metabolomics was applied to identify toxicity-associated differential metabolites. This integrated approach leverages both predictive capability and mechanistic insight, offering a systems biology perspective for understanding carbosulfan-induced respiratory toxicity and aiding in uncovering its underlying toxic mechanisms and key functional networks.

2. Results

2.1. Effects on Rat Lung Tissue

Histopathological examination revealed well-defined alveolar structures, absence of alveolar exudate or leukocytes, and only scant interstitial inflammatory infiltration in the control group. In the exposed group, however, lung architecture was disrupted, and extensive inflammatory cell infiltration, thickened alveolar septa, pulmonary congestion, and interstitial edema were observed. Serum levels of IL-1 β , IL-6, and TNF- α were significantly elevated ($p < 0.05$) in exposed rats compared to the control group, indicating activation of systemic inflammatory responses (Figure 1).

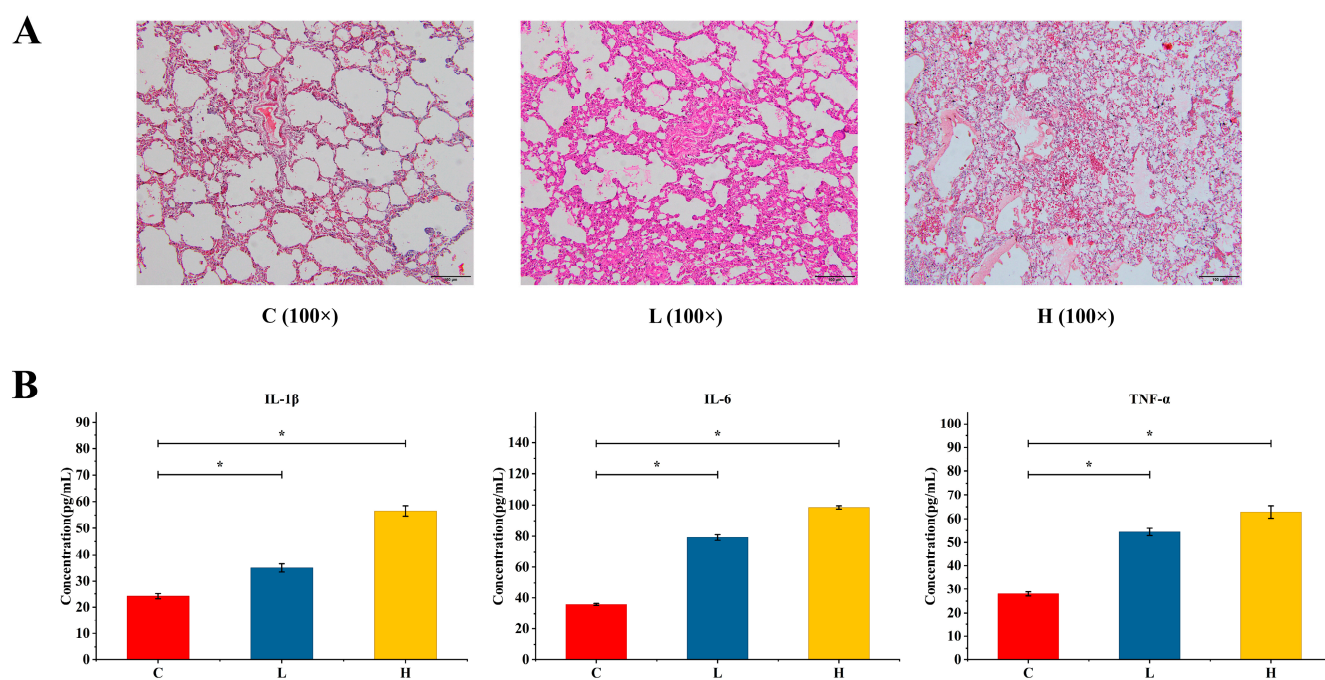


Figure 1. Histopathological changes in lung tissue and serum levels of inflammatory cytokines in rats (C: control group, L: low-dose group, H: high-dose group): (A) HE-stained lung sections (100× magnification). (B) Serum concentrations of IL-1β, IL-6, and TNF-α measured by ELISA. * $p \leq 0.05$ vs. control group.

2.2. Network Toxicology Analysis Results

By integrating the results of carbosulfan toxicity analysis from ProTox and ADMETlab, a basic summary of carbosulfan's respiratory toxicity was obtained. Toxicity prediction results indicated a close association between carbosulfan and respiratory toxicity (Table 1).

Table 1. Toxicity prediction results.

Database	Target	Probability	Comment
ProTox 3.0	Hepatotoxicity	0.690	Inactive
	Neurotoxicity	0.560	Inactive
	Nephrotoxicity	0.550	Inactive
	Respiratory toxicity	0.760	Active
	Cardiotoxicity	0.680	Inactive
ADMETlab 3.0	Human Hepatotoxicity	0.707	Category 1: H-HT-positive; Category 0: H-HT-negative; The output value is the probability of being toxic.
	Drug-induced Neurotoxicity	0.350	Category 0: non-neurotoxic; Category 1: neurotoxic. The output value is the probability of being neurotoxic, within the range of 0 to 1.
	Drug-induced Nephrotoxicity	0.706	Category 0: non-nephrotoxic; Category 1: nephrotoxic. The output value is the probability of being nephrotoxic, within the range of 0 to 1.
	Respiratory	0.996	Category 1: respiratory toxicants; Category 0: non-respiratory toxicants. The output value is the probability of being toxic, within the range of 0 to 1.

Ototoxicity	0.667	Category 0: non-ototoxicity; Category 1: ototoxicity. The output value is the probability of being toxic, within the range of 0 to 1.
Genotoxicity	0.961	Category 0: non-genotoxicity; Category 1: Genotoxicity. The output value is the probability of being genotoxic, within the range of 0 to 1.

Network toxicology was employed to explore the potential mechanisms of carbosulfan-induced respiratory toxicity. A total of 135 carbosulfan-related targets and 2319 respiratory toxicity-related targets were retrieved, with 51 overlapping targets (Figure 2). These 51 common targets were used to construct a protein–protein interaction (PPI) network (Figure 2). Fifteen core targets were identified by selecting nodes with values above the median for multiple topological parameters (betweenness, closeness, degree, eigenvector, network, and LAC). These included SRC, EGFR, PTGS2, CXCL8, CYP3A4, and NR3C1 (Table 2).

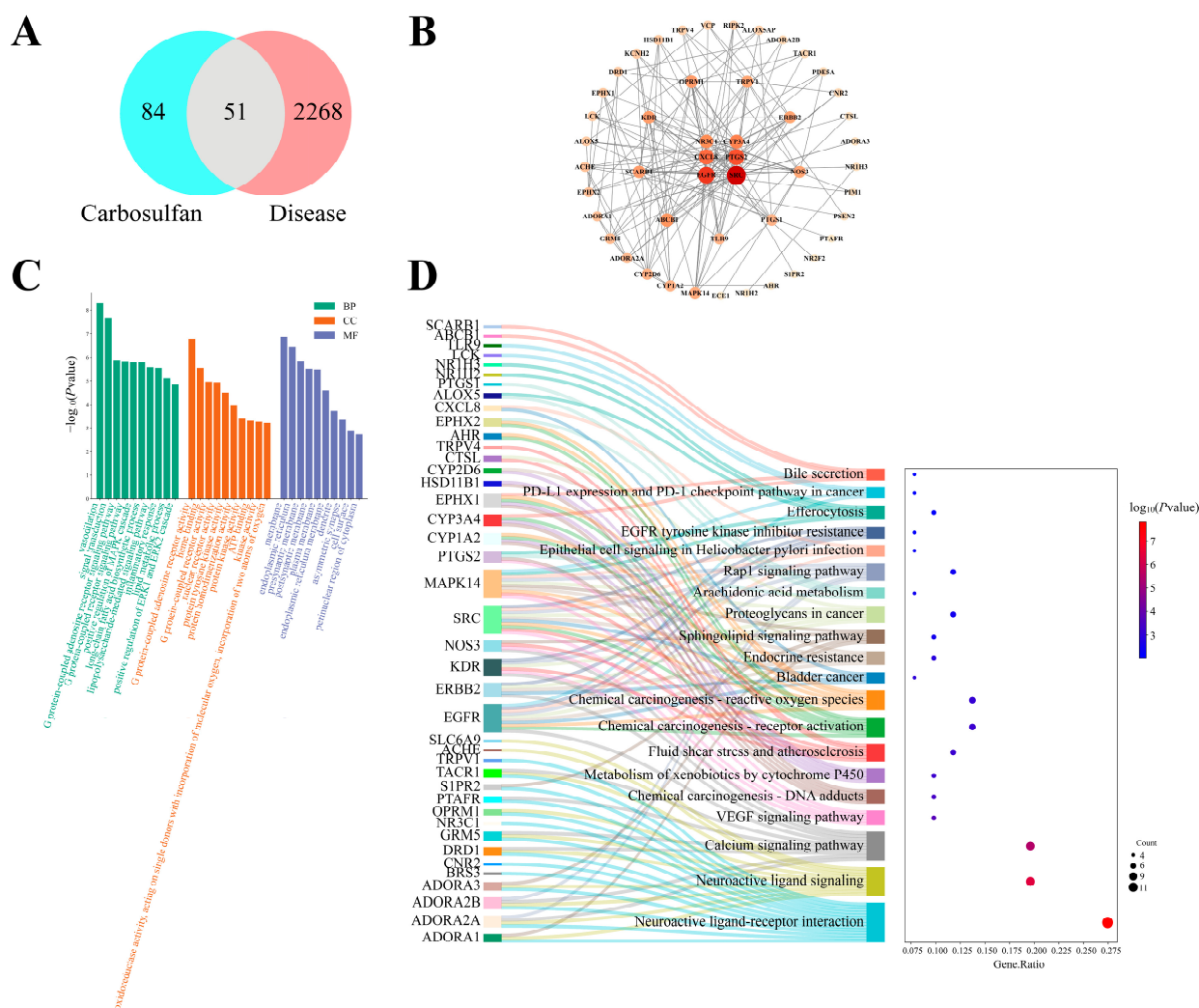


Figure 2. Network toxicology analysis results: (A) Venn diagram of the overlapping targets between the compound and the disease. (B) PPI network of the common targets. The larger and darker the node, the higher the degree it indicates. (C) GO enrichment analysis results, showing the top 10 terms for BP, CC, and MF (from left to right). (D) Bubble chart of the top 20 enriched KEGG pathways.

Table 2. Characteristics of the 15 core targets.

Name	Degree	Betweenness	Closeness	Eigenvector	LAC	Network
SRC	25	604.4856956	0.652777778	0.346923649	5.52	18.91788361
EGFR	20	277.0027803	0.61038961	0.329892367	6	13.37127548
PTGS2	18	234.1150078	0.602564103	0.312265128	6.555555556	12.78026565
CXCL8	16	165.846078	0.55952381	0.279856056	6	11.32936508
CYP3A4	14	141.6259542	0.516483516	0.194849357	4.285714286	8.105544456
NR3C1	13	61.80568757	0.528089888	0.252963573	5.538461538	7.433513709
ABCB1	12	111.4479278	0.528089888	0.217441961	5.333333333	7.582178932
KDR	11	134.0691766	0.546511628	0.212487489	5.090909091	6.736111111
OPRM1	11	68.74671839	0.510869565	0.197384521	4.363636364	6.307936508
SCARB1	11	317.2167795	0.546511628	0.181523725	3.454545455	4.426984127
ERBB2	10	57.46494883	0.522222222	0.205264032	5	6.305555556
TRPV1	10	39.08427128	0.489583333	0.178165957	3.8	6.166666667
NOS3	10	133.1522811	0.510869565	0.167869806	3.4	4.674603175
PTGS1	8	18.9045177	0.447619048	0.154551163	4	5.273809524
TLR9	8	44.19045488	0.494736842	0.167650744	4	5.619047619

Pathway enrichment analysis was performed on the 51 common targets. GO analysis returned 255 items, including 163 biological processes (BP), 35 cellular components (CC), and 57 molecular functions (MF) (Figure 2). Among BP terms, enrichment was observed for vasodilation, signal transduction, the G protein-coupled adenosine receptor signaling pathway, the G protein-coupled receptor signaling pathway, and positive regulation of the MAPK cascade. CC terms mainly included membrane, endoplasmic reticulum, pre-synaptic membrane, postsynaptic membrane, plasma membrane, endoplasmic reticulum membrane, and dendrite. MF terms corresponded to G protein-coupled adenosine receptor activity, heme binding, G protein-coupled receptor activity, nuclear receptor activity, and protein tyrosine kinase activity. KEGG pathway analysis identified 52 signaling pathways, with the most significant including neuroactive ligand–receptor interaction, the calcium signaling pathway, the VEGF signaling pathway, and chemical carcinogenesis–DNA adducts (Figure 2).

Molecular docking was performed for the top 6 core targets ranked by degree score (SRC: 1FMK, EGFR: 1M14, PTGS2: 5F1A, CXCL8: 4XDX, CYP3A4: 5VCC, and NR3C1: 4UDD). Binding affinity was assessed by calculating binding free energy, with more negative values indicating a stronger interaction between the ligand and the receptor. The binding energies of carbosulfan with SRC, EGFR, PTGS2, CXCL8, CYP3A4, and NR3C1 were −6.8, −6.1, −6.2, −5.7, −8.7, and −8.5 kcal/mol, respectively. All six docking runs yielded binding energies lower than −5.0 kcal/mol, indicating stable binding of carbosulfan to the six target proteins and demonstrating high affinity of carbosulfan for each identified target (Figure 3). These *in silico* predictions suggest that carbosulfan has the structural potential to interact with each identified target, providing supportive evidence for the network toxicology predictions.

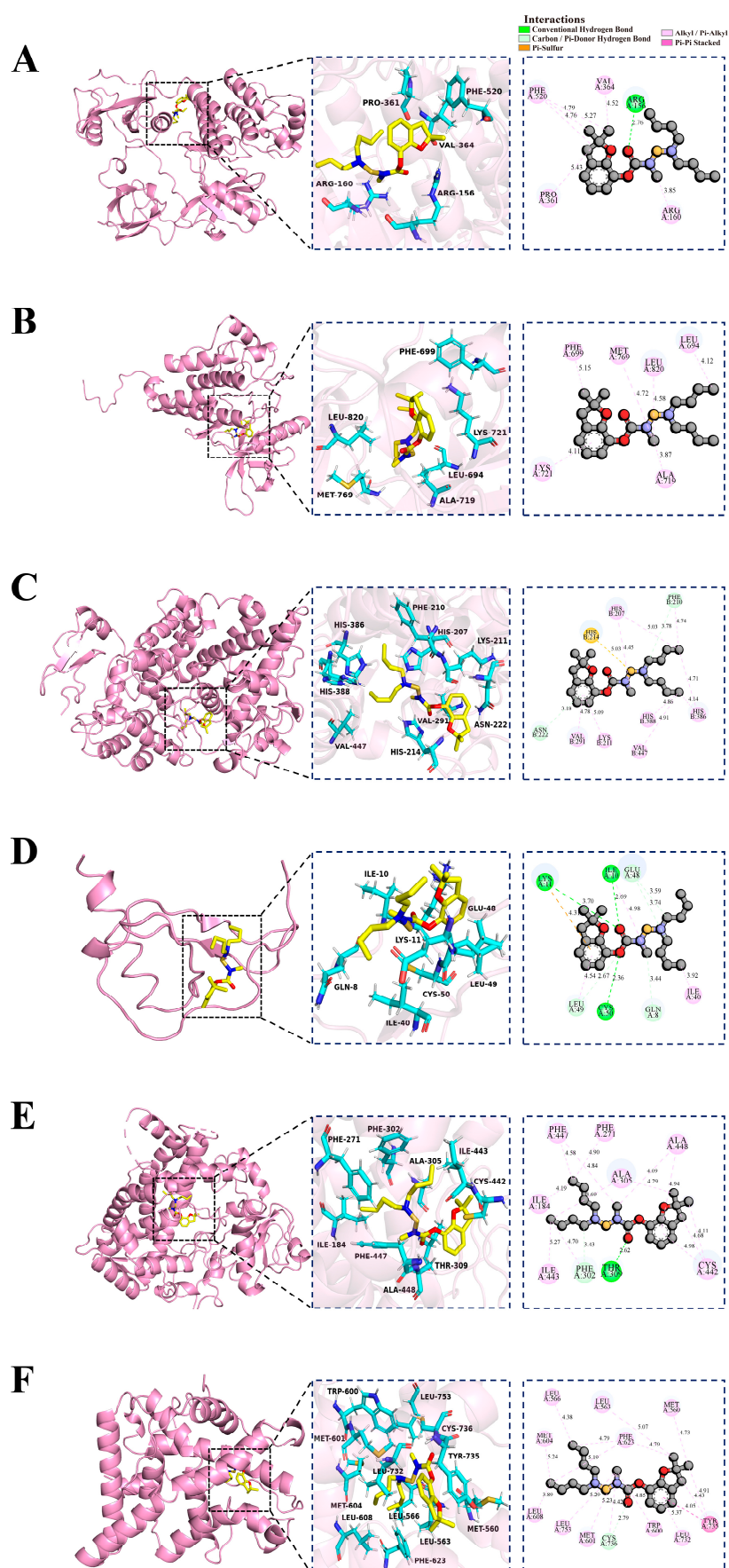


Figure 3. Molecular docking models: (A) carbosulfan with SRC (1FMK, -6.8 Kcal/mol); (B) carbosulfan with EGFR (1M14, -6.1 Kcal/mol); (C) carbosulfan with PTGS2 (5F1A, -6.2 Kcal/mol); (D)

carbosulfan with CXCL8 (4XDX, -5.7 Kcal/mol); (E) carbosulfan with CYP3A4 (5VCC, -8.7 Kcal/mol); (F) carbosulfan with NR3C1 (4UDD, -8.5 Kcal/mol).

2.3. Untargeted Metabolomics Results

2.3.1. Multivariate Statistical Analysis

PCA was performed on the samples to reflect the original data state. Samples within each group showed good clustering, with clear separation trends between groups (Figure 4). The results indicated good sample reproducibility and significant differences between groups. To further resolve inter-group metabolic differences and identify differential metabolites, orthogonal partial least squares-discriminant analysis (OPLS-DA) was employed (Figure 4). In both ESI+ and ESI $^{-}$ modes, clear separation was observed between the high-dose and control groups, as well as between the low-dose and control groups, confirming the significant impact of carbosulfan on rat metabolism. R^2 and Q^2 values indicated that the established models could explain the data matrix information and possessed good predictive capability. The OPLS-DA model was validated by 200 permutation tests (Figure 4). The results demonstrated the significance of the OPLS-DA model, and the VIP values obtained from this model were reliable and suitable for further analysis.

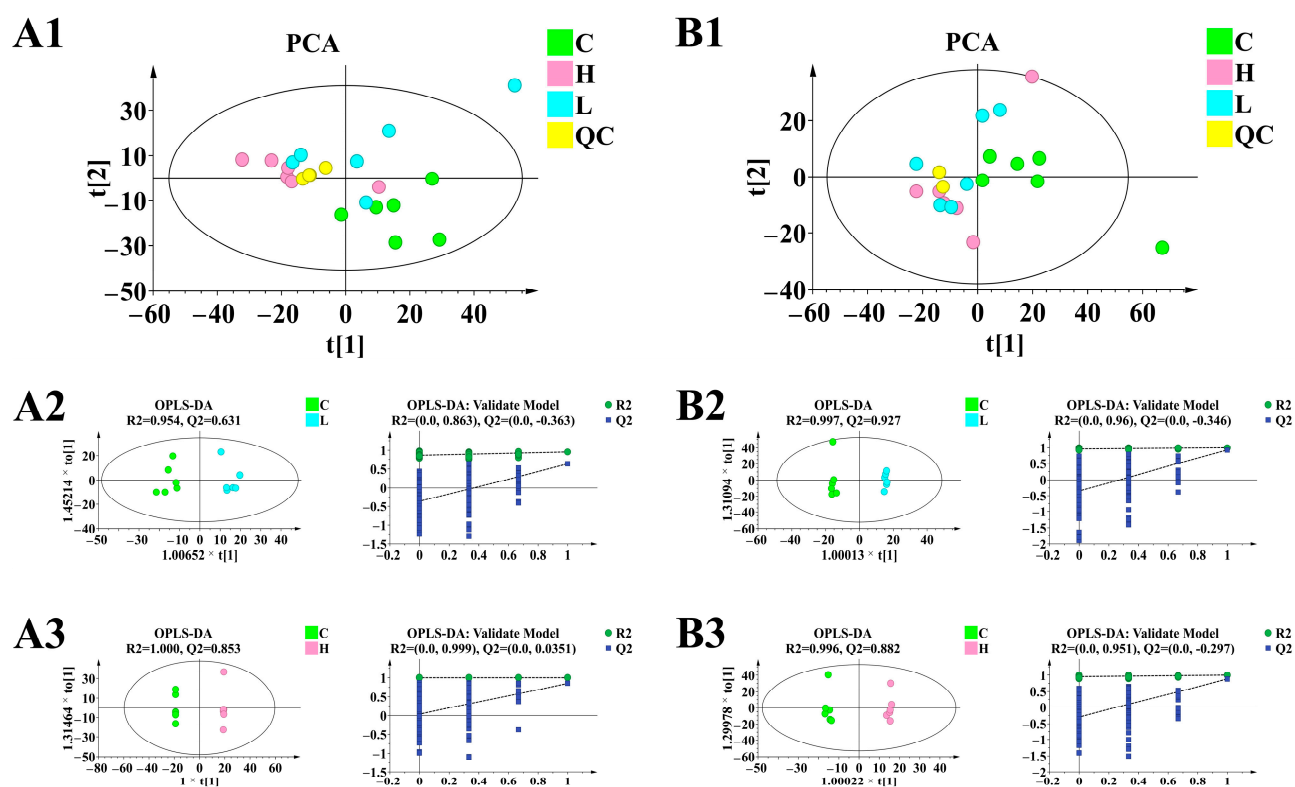


Figure 4. Untargeted metabolomics results. Panels (A1–B3) display the analytical results obtained in the positive and negative ion modes, respectively (C: control group, L: low-dose group, H: high-dose group, QC: quality control group). (A1,B1) PCA score plots of all samples. (A2,B2) Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) score plots for the low-dose group versus the control group and their corresponding permutation tests. (A3,B3) OPLS-DA score plots for the high-dose group versus the control group and their corresponding permutation tests.

2.3.2. Screening and Identification of Differential Metabolites

Differential metabolites were screened by combining VIP values from the OPLS-DA model with descriptive statistics tests, using the criteria $VIP > 1$ and $p < 0.05$. Differential

metabolite identification was performed by matching accurate mass and MS/MS fragmentation patterns with the Human Metabolome Database (HMDB). Compared to the control group (C), the high-dose group (H) identified 15 differential metabolites in positive ion mode and 4 in negative ion mode. The low-dose group (L) identified 6 differential metabolites in positive ion mode and 4 in negative ion mode. Five differential compounds were common to both H and L dose groups: benzamide, glutamine, citrulline, glutamylglutamine, and 2-ketobutyric acid (Table 3).

Table 3. Identification results of potential differential metabolites in rat serum under positive and negative ion mode.

No.	Rt (Min)	m/z	HMDB ID	Metabolites	Ion Mode	Fold-Changes (High Dose)	Fold-Changes (Low Dose)	p-Value
1	0.897	76.07669	HMDB0000925	Trimethylamine N-oxide	positive ion	-	0.486886	0.047611
2	2.801	88.9866	HMDB0002329	Oxalic acid	negative ion	0.512938	-	0.0322651
3	0.966	101.02299	HMDB0000005	2-Ketobutyric acid	negative ion	0.826104	0.742295	0.0031084
4	0.925	112.05144	HMDB0000630	Cytosine	positive ion	0.551351	-	0.0489254
5	0.928	116.07138	HMDB0000162	Proline	positive ion	0.681104	-	0.014223
6	0.898	118.08709	HMDB0000043	Betaine	positive ion	0.458264	-	0.0000709
7	0.817	122.05851	HMDB0004461	Benzamide	positive ion	0.529892	0.829189	0.000344405
8	0.928	126.06696	HMDB0002894	5-Methylcytosine	positive ion	0.452377	-	0.0178167
9	0.87	130.05063	HMDB0000267	Pyroglutamic acid	positive ion	-	0.388074	0.00199171
10	0.889	131.08134	HMDB0000214	Ornithine	negative ion	-	0.567594	0.0129309
11	0.944	133.01294	HMDB0000156	Malic acid	negative ion	7.09436	-	0.00240657
12	0.87	147.07716	HMDB0000641	Glutamine	positive ion	0.42654	0.362517	0.00260668
13	0.852	175.11992	HMDB0000517	L-Arginine	positive ion	0.630218	-	0.0384827
14	0.895	176.10378	HMDB0000904	Citrulline	positive ion	0.646779	0.471208	0.0295454
15	0.971	191.01877	HMDB0000094	Citric acid	negative ion	3.74765	-	0.0478801
16	7.653	192.13908	HMDB0250930	Diethyltoluamide	positive ion	0.564065	-	0.0358488
17	0.925	204.12399	HMDB0000201	L-Acetylcarnitine	positive ion	0.379351	-	0.00658452
18	0.928	276.12003	HMDB0028817	Glutamylglutamine	positive ion	0.415981	0.290292	0.00531233
19	7.355	347.22269	HMDB0001547	Corticosterone	positive ion	2.75211	-	0.0164467
20	7.146	347.22272	HMDB0000015	Cortexolone	positive ion	3.51642	-	0.0298807
21	17.262	359.31674	HMDB0011131	MG(18:0/0:0/0:0)	positive ion	0.490621	-	0.00149531
22	7.436	405.26376	HMDB0000502	3-Oxocholeic acid	negative ion	-	0.278653	0.0179664
23	7.726	407.27945	HMDB0000619	Cholic acid	negative ion	-	0.182922	0.0000516
24	7.952	450.32281	HMDB0000637	Chenodeoxycholic acid glycine conjugate	positive ion	0.188858	-	0.0156555

-: No significant difference was observed compared to the control group.

2.3.3. KEGG Pathway Enrichment Analysis of Differential Metabolites

To elucidate the biological relevance of the differential metabolites, KEGG enrichment analysis was performed using MetaboAnalyst 6.0 (Figure 5). The H group yielded 17 pathways, including arginine biosynthesis, glyoxylate and dicarboxylate metabolism, D-amino acid metabolism, and the citrate cycle (TCA cycle). The L group yielded 14 pathways, including arginine biosynthesis, D-amino acid metabolism, glutathione metabolism, and nitrogen metabolism.

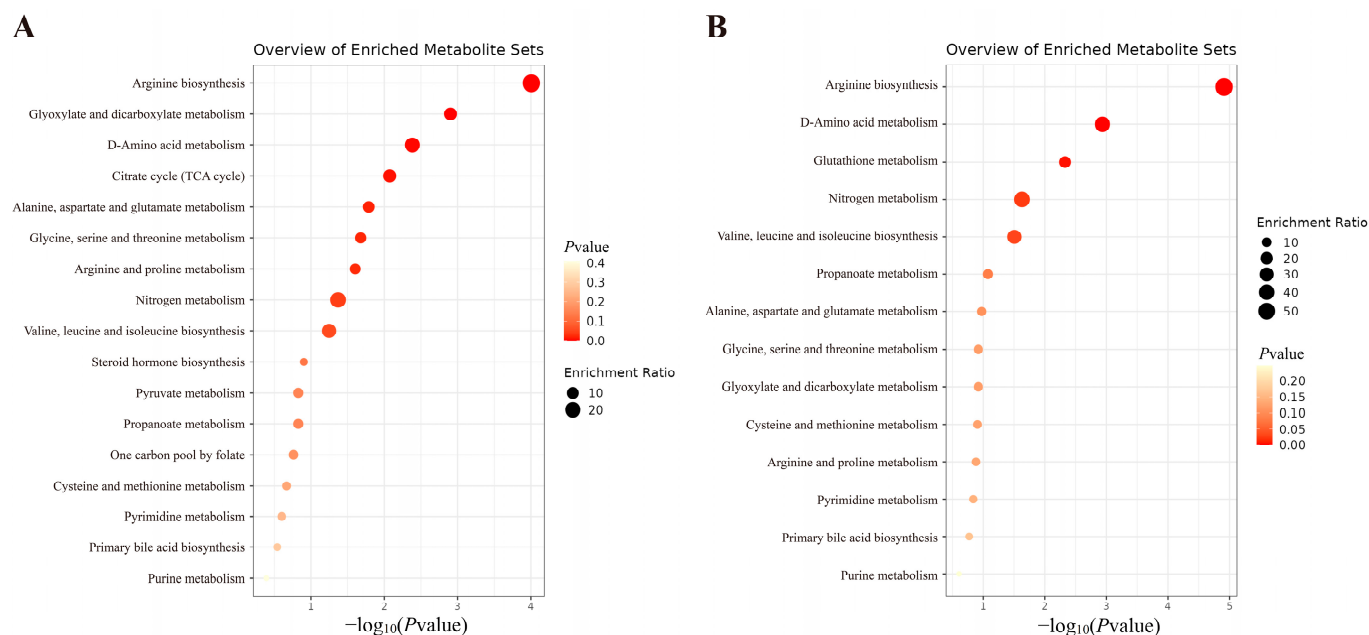


Figure 5. KEGG pathway enrichment analysis of differential metabolites: **(A)** Enriched metabolic pathways for the differential metabolites between the high-dose group and the control group. **(B)** Enriched metabolic pathways for the differential metabolites between the low-dose group and the control group.

3. Discussion

Carbosulfan is a widely used carbamate insecticide. This study comprehensively applied network toxicology, metabolomics, molecular docking, and inflammatory factor detection to systematically investigate the potential mechanisms of its induction of respiratory toxicity in male rats. The integrated analysis suggests that carbosulfan exposure is associated with respiratory system damage in rats, potentially through mechanisms involving oxidative stress, metabolic disturbances, inflammation, immune dysregulation, and disruption of cell signaling and metabolic enzyme systems. However, given the acute high-dose exposure model used in this study, it is important to consider that the observed pulmonary effects may reflect both direct lung injury and secondary consequences of systemic toxicity.

Oxidative stress is defined as a pathological state where the dynamic balance between the generation of reactive oxygen species (ROS) and the scavenging capacity of the antioxidant defense system is disrupted, leading to oxidative damage of biomolecules [18]. Mitochondrial dysfunction and the release of key factors from the oxidative respiratory chain can trigger oxidative stress when ROS accumulate excessively. In the respiratory system, oxidative stress directly damages alveolar epithelial cells and pulmonary vascular endothelial cells, compromising alveolar–capillary barrier integrity, and is a core mechanism in lung injury induced by various toxicants [19]. Multiple lines of evidence in this study support the involvement of oxidative stress in the toxic response to carbosulfan exposure. Nevertheless, it remains unclear whether oxidative damage originates primarily in lung tissue or results from systemic oxidative stress secondary to multi-organ dysfunction. Metabolomics analysis revealed significant perturbations in key antioxidant-related pathways such as glutathione metabolism, arginine and proline metabolism, the citrate cycle (TCA cycle), and alanine, aspartate, and glutamate metabolism. Glutathione (GSH) is one of the most important endogenous intracellular antioxidants, capable of neutralizing free radicals and ROS, thereby reducing oxidative stress and protecting cell membranes from damage [20]. Alterations in GSH metabolism in alveoli and lungs are

widely considered closely associated with the degree of oxidative damage in lung tissue [21]. Significant perturbation of glutathione metabolism may indicate that carbosulfan disrupts the crucial pulmonary antioxidant defense system. Disturbances in the citrate cycle (TCA cycle) and alanine, aspartate, and glutamate metabolism link oxidative stress to a cellular energy crisis. The TCA cycle is central to mitochondrial aerobic respiration and ATP generation and is also a major site of ROS production. Toxicants interfering with TCA cycle enzyme function can lead to the accumulation of metabolic intermediates and dysfunction of the electron transport chain, thereby affecting mitochondrial ROS generation [22]. Simultaneously, glutamate is both a precursor for TCA cycle intermediates and a key amino acid for GSH synthesis [23,24]. Its metabolic disturbance not only exacerbates energy production impairment but also further limits the supply of raw materials for GSH synthesis, creating a vicious cycle. In the respiratory system, alveolar type II epithelial cells are highly dependent on mitochondrial function for synthesizing pulmonary surfactant; their mitochondrial damage directly affects lung mechanical stability and repair capacity [25]. Perturbations in the arginine biosynthesis and arginine and proline metabolism pathways reveal a critical turning point in oxidative stress: nitrosative stress. Arginine metabolism has a dual role. On the one hand, it is a precursor for proline, which has been shown to be an effective compatible solute and antioxidant, stabilizing protein structures and scavenging free radicals [26]. On the other hand, arginine is a substrate for nitric oxide synthase, producing the signaling molecule nitric oxide (NO) [27]. Under physiological conditions, NO is involved in regulating pulmonary vascular tone. However, in the context of oxidative stress, when superoxide anions are overproduced, they react rapidly with NO to form the strong oxidant and nitrating agent peroxynitrite [28]. Peroxynitrite can nitrate tyrosine residues, inhibit protein function, directly damage DNA, and cause further mitochondrial dysfunction [29]. The concurrent disruption of these pathways suggests that carbosulfan may weaken proline-related antioxidant defenses while shifting the arginine–NO pathway toward harmful nitrosative stress. This combined effect may exacerbate damage to the pulmonary capillary endothelium and alveolar epithelium. The “chemical carcinogenesis—reactive oxygen species” pathway identified by network toxicology corroborates the ultimate consequences of the aforementioned metabolic disturbances at the genetic and signaling network level. This pathway encompasses key gene sets involved in ROS-mediated DNA damage, mutation, and cancer initiation. Persistent and severe oxidative stress can lead to the formation of oxidative DNA adducts such as 8-hydroxydeoxyguanosine, potentially activating proto-oncogenes or inactivating tumor suppressor genes, forming the basis for genomic instability in lung cells [30]. The enrichment of this pathway links the acute metabolic oxidative stress induced by carbosulfan with potential risks for respiratory system pathology. Furthermore, molecular docking results showed that carbosulfan has an extremely high binding energy (−8.5 kcal/mol) with NR3C1. Strong binding to NR3C1 may interfere with its normal anti-inflammatory and antioxidant regulatory functions. Studies have confirmed that glucocorticoid receptor signaling is crucial for maintaining pulmonary redox balance, and its dysfunction can exacerbate acute lung injury [31]. In summary, carbosulfan may induce and aggravate oxidative stress damage in lung tissue by disrupting key antioxidant metabolic pathways and affecting endogenous antioxidant regulatory systems.

Inflammation is a defensive response of the body to harmful stimuli, but excessive or uncontrolled inflammation is a key link leading to tissue damage. The ELISA results demonstrated a dose-dependent increase in serum pro-inflammatory cytokines (IL-6, IL-1 β , and TNF- α), indicating a systemic inflammatory response upon carbosulfan exposure. The elevated levels of pro-inflammatory cytokines indicate that carbosulfan exposure triggered a systemic acute inflammatory response, which may contribute to lung injury indi-

rectly through circulating inflammatory mediators. Network toxicology analysis provided mechanistic clues, identifying multiple pathways closely related to inflammation and immunity, such as neuroactive ligand–receptor interaction, the VEGF signaling pathway, and arachidonic acid metabolism. The enrichment of the neuroactive ligand–receptor interaction pathway suggests a possible early mechanism for inflammation initiation. Lung tissue is rich in sensory nerve endings and various neuropeptide receptors. Toxic substances may trigger neurogenic inflammatory responses by stimulating sensory nerves or directly affecting specific receptors. This response can lead to rapid increases in local vascular permeability and promote mast cell degranulation, releasing pre-formed inflammatory mediators like histamine, resulting in immune cell infiltration [32,33]. Another core mechanism is reflected in the activation of the arachidonic acid metabolism pathway. Carbosulfan may induce cell membrane damage or activate phospholipase A2, releasing arachidonic acid. Arachidonic acid is subsequently metabolized via the cyclooxygenase-2 pathway to generate prostaglandin E2 and thromboxane, and via the 5-lipoxygenase pathway to generate leukotriene B4 and cysteinyl leukotrienes [34]. These metabolites are known potent pro-inflammatory factors: prostaglandins cause vasodilation and pain sensitization; leukotrienes are potent neutrophil chemoattractants and smooth muscle contractors, directly leading to bronchoconstriction and increased mucus secretion. This storm of locally produced inflammatory mediators may be the key upstream event driving the surge in serum cytokine levels such as TNF- α , IL-1 β , and IL-6. Research confirms that in various lung injury models, inhibiting the COX-2 or 5-LOX pathways significantly reduces inflammatory cell infiltration and tissue edema [35,36]. Molecular docking results showed that carbosulfan has high binding activity with key inflammation-related targets such as CXCL8 and PTGS2, suggesting it may promote inflammatory cell recruitment in lung tissue and release of inflammatory mediators by regulating the function of these targets, leading to alveolitis and airway inflammation. The detrimental effects of inflammation extend beyond immune cell activation to include structural tissue damage. The enrichment of the VEGF signaling pathway plays a key role at this stage. Vascular endothelial growth factor (VEGF), originally known as vascular permeability factor, is one of the most important substances for increasing microvascular permeability [37]. Pulmonary macrophages and epithelial cells, under strong stimulation by cytokines like TNF- α and IL-6, produce large amounts of VEGF [38]. VEGF binding to its receptors disrupts tight junctions between endothelial cells, leading to a sharp increase in pulmonary capillary permeability. This causes protein-rich fluid to leak extensively from blood vessels into the alveolar interstitium and alveolar spaces, forming non-cardiogenic pulmonary edema [39]. This is the typical pathological basis for acute respiratory distress syndrome (ARDS), directly impairing alveolar gas exchange function and translating biochemical inflammatory signals into lethal physiological dysfunction. Both clinical and animal experiments indicate that during acute lung injury, VEGF levels in lung tissue and plasma are closely related to disease severity and prognosis [40,41]. Notably, network toxicology also identified the PD-L1 expression and PD-1 checkpoint pathway in cancer. This finding provides a new perspective for understanding immune dysregulation in carbosulfan toxicity. The PD-1/PD-L1 pathway is a crucial system for maintaining immune tolerance and preventing excessive immune damage. In acute severe injury or inflammatory states, pulmonary tissue cells may compensatorily upregulate PD-L1 expression to inhibit overactive T-cell immune responses and protect themselves from immune attack [42]. However, this adaptive response may become dysregulated under intense toxic injury. On the one hand, excessive PD-L1 expression may lead to effector T-cell exhaustion or apoptosis, weakening the body's ability to clear damaged cells and pathogens. On the other hand, it may disrupt the functional balance of regulatory T cells, preventing timely and effective termination of the inflammatory response. This abnormality in immune checkpoints may shift acute

inflammation towards a persistent or chronic state, prolonging tissue repair time and potentially setting the stage for subsequent chronic pathologies like fibrosis. Studies have suggested that in acute lung injury induced by sepsis and viral infections, the PD-1/PD-L1 pathway is abnormally activated, and its blockade can improve prognosis to some extent [43].

Pulmonary exposure to toxic substances can lead to damage, abnormal proliferation, or apoptosis of alveolar and bronchial epithelial cells, which is closely related to the development of respiratory dysfunction and chronic lung diseases. In the network toxicology analysis, multiple cancer-related pathways were significantly enriched, including bladder cancer, proteoglycans in cancer, and EGFR tyrosine kinase inhibitor resistance. The commonality of these pathways lies in their regulation of cell survival, proliferation, differentiation, and apoptosis. For example, EGFR plays a key role in the repair and regeneration of alveolar epithelial cells, and its signaling abnormalities are associated with diseases like pulmonary fibrosis. Molecular docking results showed that carbosulfan has strong binding affinity for both EGFR and SRC (binding energies of -6.1 and -6.8 kcal/mol, respectively). SRC is a hub for various cell signaling pathways, integrating growth factor, cytokine, and integrin signals to regulate cell adhesion, migration, and survival [44]. Interference with SRC and EGFR function may disrupt alveolar epithelial barrier integrity, impair repair capacity, and influence inflammatory responses [45,46]. The enrichment of the calcium signaling pathway further emphasizes the possible dysregulation of intracellular second messenger systems; disturbance of calcium ion homeostasis is an important inducer of apoptosis and necrosis [47]. Additionally, efferocytosis is the physiological process of clearing apoptotic cells, and its dysfunction can lead to secondary necrosis and aggravated inflammation. The appearance of this pathway suggests that carbosulfan may affect the ability of pulmonary macrophages to clear apoptotic cells, perpetuating damage. Perturbations in the steroid hormone biosynthesis pathway from metabolomics may also indirectly affect the balance of cell proliferation and apoptosis, as some endogenous hormones regulate lung tissue cells. These pathways suggest that carbosulfan may interfere with cellular processes involved in cell survival and proliferation, which could affect pulmonary epithelial integrity. However, whether these effects are lung-specific or reflect broader systemic disturbances requires further investigation.

CYP450 is a key phase I metabolic enzyme system for xenobiotics in the liver and peripheral tissues, including the lung. The pathways enriched by network toxicology, namely the metabolism of xenobiotics by cytochrome P450 and chemical carcinogenesis—DNA adducts, are directly related to the metabolic activation of carbosulfan and its potential genotoxicity. Molecular docking showed that carbosulfan has extremely strong binding affinity for CYP3A4, suggesting it may be a substrate or modulator of CYP3A4. Carbosulfan may be metabolically activated by CYP enzymes into more reactive intermediates. These intermediates could directly damage pulmonary macromolecules or form DNA adducts, triggering gene mutations or cellular stress, potentially leading to irreversible damage in the long term [48–50]. Furthermore, CYP450 enzyme systems may generate oxygen free radicals during xenobiotic metabolism, and their abnormal activation is a classic pathway for exogenous toxins to induce oxidative stress [51]. Additionally, the enrichment of the bile secretion pathway, while seemingly unrelated to respiratory toxicity, may reflect multi-organ abnormalities common in systemic toxicant exposure. The liver, as the detoxification center, influences systemic inflammation and oxidative stress levels through the systemic circulation. Bile acid metabolism disorders may indirectly exacerbate systemic toxic load, affecting pulmonary repair capacity. Perturbations in glycolipid metabolism-related pathways, such as sphingolipid signaling, may also affect cell membrane stability and signal transduction, participating in toxic responses. Notably, carbosulfan is rapidly metabolized *in vivo* to carbofuran, its primary active metabolite, which

is also a potent acetylcholinesterase inhibitor [48]. Carbofuran has been extensively studied for its toxicity profile, including neurotoxicity, reproductive toxicity, and immunotoxicity [17,52]. The strong binding affinity of carbosulfan to CYP3A4 (-8.7 kcal/mol) observed in our molecular docking studies supports its metabolic activation via this enzyme, which is consistent with previous reports identifying CYP3A4 as a major isoform involved in carbosulfan biotransformation [48].

In summary, our integrated analysis proposes a multifaceted framework for understanding carbosulfan-induced respiratory toxicity, involving interconnected processes such as oxidative stress, inflammation, cell signaling disruption, and metabolic activation. These findings should be interpreted as hypothesis-generating, and further studies are needed to disentangle direct pulmonary effects from systemic contributions. Besides, several limitations of this study should be acknowledged. First, the experimental design employed acute high-dose exposure. While this model is useful for identifying early mechanistic events associated with overt toxicity, it presents an inherent limitation: the inability to definitively discriminate between primary pulmonary toxicity and secondary lung injury resulting from systemic poisoning. The observed pathological changes in lung tissue and metabolic perturbations may, therefore, reflect a combination of direct pulmonary insult and indirect effects of systemic toxicity. Consequently, interpretations of lung-specific mechanisms should be tempered with this consideration. Furthermore, this acute model does not capture the cumulative and adaptive responses that may occur with chronic, low-dose environmental or occupational exposure to carbosulfan. Second, while the integration of network toxicology and metabolomics in this study provides a biologically coherent and mechanistically plausible framework centered on convergent pathways such as oxidative stress and inflammatory signaling, it is important to acknowledge that this integration remains primarily interpretative rather than statistically validated. We did not perform quantitative multi-omics integration, which would be required to establish statistically robust links between predicted molecular targets and observed metabolic changes. Therefore, the proposed mechanistic connections, while biologically logical, should be regarded as hypotheses generated by the integrated analysis rather than as statistically demonstrated causal relationships. Third, although molecular docking provided supportive *in silico* evidence for potential interactions between carbosulfan and core targets, with binding energies below -5.0 kcal/mol suggesting structural compatibility, these predictive data alone cannot establish biological target engagement *in vivo*. Binding affinities calculated *in silico* do not account for factors such as protein conformational dynamics in cellular environments, competition with endogenous ligands, tissue-specific bioavailability, or metabolic transformation of the compound. Thus, these findings should be interpreted as hypothesis-generating indicators of potential interactions that require functional validation through targeted experimental approaches.

4. Materials and Methods

4.1. Reagents

Carbosulfan was purchased from Weiyel Inc. (Xinyang, China). LC-MS grade methanol, acetonitrile, and formic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was provided by Guangzhou Watsons Food & Beverage Co., Ltd. (Guangzhou, China). Rat interleukin-6 (IL-6), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Wuhan Elabscience Biotechnology Co., Ltd. (Wuhan, China).

4.2. Animal Experiments

Eighteen male 8-week-old Sprague Dawley rats, weighing 250 ± 10 g, were purchased from Beijing Yangfang Boyi Biotechnology Co., Ltd. (Beijing, China). Rats were acclimatized for 7 days under controlled conditions with free access to food and water: temperature maintained at 25 ± 2 °C, relative humidity at $50 \pm 10\%$, and a 12:12 h light/dark cycle. Before the experiment, rats were fasted for 12 h with water provided. All experimental procedures were conducted in accordance with the animal experimentation guidelines established by Shanxi Medical University (Taiyuan, China) and were approved by the Institutional Animal Ethics Committee.

Only male rats were used in this study to eliminate potential confounding effects of hormonal fluctuations associated with the estrous cycle in females, which can significantly influence metabolic profiles and inflammatory responses. While this approach enhances internal validity and reduces inter-individual variability, it limits the generalizability of our findings to female populations. Future studies should investigate potential sex differences in carbosulfan-induced respiratory toxicity, as sex-dependent variations in cytochrome P450 enzyme expression and activity have been documented [53,54].

After the 7-day acclimatization period, rats were randomly divided into three groups ($n = 6$ per group): control group, low-dose group, and high-dose group. Control group rats were gavaged with saline, the low-dose group with 125 mg/kg carbosulfan, and the high-dose group with 250 mg/kg carbosulfan. Samples were collected 12 h after gavage.

4.3. Sample Collection

Rats were anesthetized with isoflurane, and blood was collected via the orbital sinus. After standing at room temperature for 1 h, the blood was centrifuged at 3000 rpm for 20 min to obtain serum. One portion of the serum was used for ELISA, and the remainder was stored at -80 °C for metabolomic analysis. Lung tissues were collected and fixed in formaldehyde for hematoxylin and eosin (HE) staining.

4.4. Network Toxicology Analysis

4.4.1. Toxicity Target Organ Prediction

The 2D structure, SMILES string, and InChIKey of carbosulfan were retrieved from PubChem using “carbosulfan” as the keyword. The SMILES string of carbosulfan was imported into ProTox 3.0 and ADMETlab 3.0 for preliminary prediction of respiratory toxicity using integrated network search algorithms. A prediction result of “Active” or an output probability value greater than 0.7 was considered indicative of respiratory toxicity for carbosulfan.

4.4.2. Collection of Compound Potential Targets

Potential human targets were predicted by comparing databases, including TargetNet, Similarity Ensemble Approach (SEA), and SwissTargetPrediction, with the species limited to Homo sapiens and other parameters kept at default. Human databases were used for target prediction because they are more comprehensively annotated than rat-specific databases, and the high sequence homology between human and rat orthologs supports cross-species extrapolation. Targets with a score greater than 0 were considered potential targets of the compound. Targets with a likelihood less than 0 and duplicate targets were removed. All targets were standardized to official gene symbols using the UniProt database. Subsequently, the results were integrated and deduplicated for further analysis.

4.4.3. Acquisition of Toxicity-Related Targets

Targets related to the keywords “lung injury”, “pulmonary toxicity”, “respiratory injury”, and “respiratory toxicity” were retrieved from the OMIM, GeneCards, DisGeNET, and CTD databases. Duplicate targets were removed after merging, forming a disease target list. The compound-related targets and disease-related targets were intersected using WeiShengXin (<https://www.bioinformatics.com.cn/>, accessed on 16 January 2026) to generate common targets, visualized as a Venn diagram.

4.4.4. Protein–Protein Interaction (PPI) Network Construction and Core Target Selection

The common targets of the compound and disease were uploaded to the STRING database, with the species set to *Homo sapiens* and a confidence threshold of 0.4 to generate a PPI network. The resulting data were imported into Cytoscape software (version 3.10.4). Topological attribute analysis was performed using the cytoNCA plugin (version 2.1.6) to obtain scores for betweenness, closeness, degree, eigenvector, network, and the local average connectivity-based method for each target. Core targets were determined by screening based on the median values of these scores. Visualization was performed based on the degree value of the targets, where larger nodes and darker colors indicate higher degree scores.

4.4.5. GO and KEGG Enrichment Analysis

GO and KEGG enrichment analyses for the compound–disease common targets were conducted using DAVID (<https://davidbioinformatics.nih.gov/>, accessed on 16 January 2026), with the species set to *Homo sapiens*. GO results were sorted by $-\log(p)$ value, and the top ten biological processes were plotted as a bar graph. Similarly, KEGG pathways were sorted by $-\log(p)$ value, and the top 20 pathways were visualized as a bubble chart.

4.4.6. Molecular Docking

The molecular structure of carbosulfan was downloaded from the PubChem database and saved in SDF format. The 3D crystal structures of target proteins were downloaded from the PDB database and saved in PDB format. PyMOL software (version 2.6.0) was used to remove water molecules and ligands from the proteins, and the Getbox Plugin (version 20180204) was used to obtain docking pocket parameters. The processed protein and ligand files were imported into AutoDock Tools 1.5.6 and converted to PDBQT format. Molecular docking was performed using AutoDock Vina 1.1.2. Finally, PyMOL 4.6.0 was used to visualize the molecular docking results.

4.5. Metabolomics Analysis

4.5.1. Metabolomics Sample Preparation

A 100 μ L aliquot of each serum sample was transferred to a 1.5 mL centrifuge tube, followed by the addition of 300 μ L of methanol–acetonitrile (2:1, *v/v*) to precipitate proteins. After vortexing and centrifugation, 200 μ L of the supernatant was transferred to an injection vial. An equal volume of supernatant from each sample was pooled to generate quality control (QC) samples, which were analyzed alongside the study samples throughout the batch.

4.5.2. UPLC–Q-Exactive Orbitrap–MS Analysis Conditions

Untargeted metabolomics analysis was performed using a UPLC–Q-Exactive Orbitrap–MS system (Thermo Fisher Scientific, Waltham, MA, USA) with a BEH C18 column (2.1 $\times$ 100 mm, 1.7 μ m, Waters, Milford, MA, USA). Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The

elution gradient was set as follows: 0 min, 2% B; 3 min, 25% B; 5 min, 30%; 6–8 min, 60% B; 15–18 min, 90% B; 18.5–20.5 min, 2% B. The flow rate was 0.3 mL/min, and the injection volume was 5 μ L. Data acquisition was controlled by X-Calibur software (version 4.0.27, Thermo). ESI source conditions were set as follows: sheath gas flow rate, 35 psi; auxiliary gas flow rate, 15 Arb; capillary temperature, 320 $^{\circ}$ C; full scan resolution for primary mass spectrometry, 70,000, with a scan range of 80–1200 m/z ; secondary mass spectrometry scan resolution, 17,500. Both positive and negative ion modes were detected once each, with a spray voltage of 3.2 kV for positive mode and –3.1 kV for negative mode.

4.5.3. Metabolomics Data Analysis

MS-DIAL software (version 4.9.221218) was used for peak detection, peak extraction, retention time correction, peak annotation, and signal drift correction of the serum mass spectrometry data. Parameters in MS-DIAL were set as follows: MS/MS spectrum similarity score threshold of 80%, considering common adduct ions such as $[M+H]^+$ and $[M-H]^-$. After processing the raw data, a dataset containing molecular names, molecular weights, retention times, and relative content (peak area) was obtained. Metabolite identification was based on accurate mass and MS/MS spectral matching, corresponding to confidence level 2 of the Metabolomics Standards Initiative. SIMCA software (version 14.1) was used for principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) of serum samples from each group. Metabolites simultaneously satisfying $VIP > 1$ and $p < 0.05$ were considered differential metabolites. The fold change (FC) relative to the control group was used to assess the regulation of metabolites. $FC > 1.2$ and $FC < 0.8$ were set as thresholds for upregulation and downregulation, respectively. Subsequently, pathway enrichment was performed using MetaboAnalyst 6.0, which incorporates FDR correction for enrichment analyses.

4.6. ELISA Analysis

The levels of IL-6, IL-1 β , and TNF- α in rat serum were measured according to the experimental procedures described in the ELISA kit instructions. The detection sensitivities for each kit were as follows: IL-6 detection sensitivity 7.5 pg/mL; IL-1 β detection sensitivity 9.38 pg/mL; TNF- α detection sensitivity 9.38 pg/mL. The intra-assay and inter-assay coefficients of variation were $<10\%$ for all kits. Specifically, 100 μ L of the sample was added to the corresponding wells and incubated at 37 $^{\circ}$ C for 90 min. After discarding the liquid, 100 μ L of biotinylated antibody working solution was added and incubated at 37 $^{\circ}$ C for 1 h. Following discarding the liquid, 350 μ L of wash buffer was added, incubated for 1 min, and then aspirated; this step was repeated three times. Subsequently, 100 μ L of HRP enzyme conjugate working solution was added to each well and incubated at 37 $^{\circ}$ C for 30 min. After discarding the liquid, the plate was washed five times. Then, 90 μ L of substrate solution was added to each well and incubated at 37 $^{\circ}$ C for 15 min. Finally, 50 μ L of stop solution was added to each well to terminate the reaction. The optical density of each well was immediately measured at 450 nm using a microplate reader.

4.7. HE Staining

Lung tissues were dehydrated using a graded ethanol series. They were then embedded and fixed in liquid paraffin and sectioned to 4 μ m thickness using a microtome. Subsequently, sections were deparaffinized in xylene and hydrated through a graded ethanol series, followed by staining with hematoxylin and eosin. Finally, sections were dehydrated through a graded ethanol series, sealed with neutral resin, and HE-stained lung tissue sections were obtained.

4.8. Statistical Analysis

All analyses were performed using SPSS software (version 22.0). Results are presented as mean $\pm$ standard deviation (SD). Differences between groups were assessed using one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$. Normality of distribution was confirmed by the Shapiro–Wilk test, and homogeneity of variance was verified by Levene’s test.

5. Conclusions

This study employed an integrated approach combining network toxicology and untargeted metabolomics to generate hypotheses regarding the potential mechanisms of carbosulfan-induced respiratory toxicity in rats. The results suggest that the respiratory toxicity of carbosulfan may involve the synergistic perturbation of multiple factors and pathways. The exploratory analysis points to several candidate mechanisms that warrant further investigation, including disruption of oxidative stress and energy metabolism pathways, potentially compromising antioxidant defense systems; activation of inflammation-related pathways, which may contribute to pro-inflammatory cytokine release and pulmonary inflammation; predicted interactions with key signaling proteins that could affect alveolar epithelial cell homeostasis; and *in silico* evidence of strong binding to metabolic enzymes, suggesting possible roles in metabolic activation. However, given the exploratory nature of this study and the aforementioned methodological limitations, including the acute high-dose model, the interpretative rather than statistical integration of multi-omics data, and the predictive nature of molecular docking, these proposed mechanisms should be considered as hypotheses requiring experimental validation rather than as definitively established pathways. Future studies employing chronic exposure models, quantitative multi-omics integration, and functional validation of core targets are essential to confirm the biological relevance of these findings and to elucidate the specific contribution of direct pulmonary toxicity versus systemic effects.

Author Contributions: Conceptualization, D.Z., Z.C., and K.Y.; methodology, D.Z. and Z.C.; software, H.S. and Y.Z.; validation, Z.L., Y.L., and W.Z.; formal analysis, X.J. and D.L.; investigation, H.S. and Y.Z.; resources, D.Z., Z.C., and K.Y.; data curation, X.J. and D.L.; writing—original draft preparation, X.J. and D.L.; writing—review and editing, X.J. and D.L.; visualization, X.J. and D.L.; supervision, D.Z., Z.C., and K.Y.; project administration, D.Z., Z.C., and K.Y.; funding acquisition, D.Z., Z.C., and K.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the National Natural Science Foundation of China (No. 82101980, 82130056, and 82072116), the Shanxi Province Higher Education “Billion Project” Science and Technology Guidance Project (No. SY-BYTZ-2025007), and the Key Research and Development (R&D) Project of Shanxi Province (No. 202302130501007).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Shanxi Medical University (2021GLL051; 3 March 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

IL-6	Interleukin-6
IL-1 β	Interleukin-1 β
TNF- α	Tumor Necrosis Factor- α
TCA	Tricarboxylic Acid Cycle
ELISA	Enzyme-Linked Immunosorbent Assay
MS/MS	Tandem Mass Spectrometry
PCA	Principal Component Analysis
OPLS-DA	Orthogonal Partial Least Squares-Discriminant Analysis
PPI	Protein-Protein Interaction
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
BP	Biological Process
CC	Cellular Component
MF	Molecular Function
HMDB	Human Metabolome Database
ESI	Electrospray Ionization
QC	Quality Control
FC	Fold Change
SD	Standard Deviation
HE	Hematoxylin and Eosin
ANOVA	Analysis of Variance
ROS	Reactive Oxygen Species
ATP	Adenosine Triphosphate
NO	Nitric Oxide
ARDS	Acute Respiratory Distress Syndrome
PD-L1	Programmed Death-Ligand 1
PD-1	Programmed Cell Death Protein 1

References

1. Chukwuka, A.V.; Saha, S.; Mukherjee, D.; Banerjee, P.; Dhara, K.; Saha, N.C. Deltamethrin-Induced Respiratory and Behavioral Effects and Adverse Outcome Pathways (AOP) in Short-Term Exposed Mozambique Tilapia, *Oreochromis mossambicus*. *Toxics* **2022**, *10*, 701.
2. Mukherjee, D.; Ghosh, S.; Mandal, A.H.; Saha, N.C.; Faggio, C.; Pastorino, P.; Saha, S. Silent threats beneath the surface: Unraveling the impact of organophosphate toxicity on fish. *Sci. Total Environ.* **2025**, *985*, 179725.
3. Kumar, S.; Baggi, T.R.; Zughaibi, T. Forensic toxicological and analytical aspects of carbamate poisoning—A review. *J. Forensic Leg. Med.* **2022**, *92*, 102450.
4. Nwani, C.D.; Lakra, W.S.; Nagpure, N.S.; Kumar, R.; Kushwaha, B.; Srivastava, S.K. Mutagenic and genotoxic effects of carbo-sulfan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Food Chem. Toxicol.* **2010**, *48*, 202–208.
5. Pretty, J.; Bharucha, Z.P. Integrated Pest Management for Sustainable Intensification of Agriculture in Asia and Africa. *Insects* **2015**, *6*, 152–182.
6. Cui, J.; Wang, F.; Gao, J.; Zhai, W.; Zhou, Z.; Liu, D.; Wang, P. Bioaccumulation and Metabolism of Carbo-sulfan in Zebrafish (*Danio rerio*) and the Toxic Effects of Its Metabolites. *J. Agric. Food Chem.* **2019**, *67*, 12348–12356.
7. Giri, S.; Giri, A.; Sharma, G.D.; Prasad, S.B. Mutagenic effects of carbo-sulfan, a carbamate pesticide. *Mutat. Res.* **2002**, *519*, 75–82.
8. Umar, A.M.; Aisami, A. Acetylcholinesterase enzyme (AChE) as a biosensor and biomarker for pesticides: A mini review. *Bull. Environ. Sci. Sustain. Manag.* **2020**, *4*, 7–12.
9. Taufikurrahmana, T.; Aryantha, I.N.P.; Purwasena, I.A.; Zahra, M.; Stefania, M.; Fitriyani, A.N. The effect of paraquat dichloride and carbo-sulfan on soil conditions and population dynamic of soil microbes in a cornfield: A case study in Sumedang, West Java. *Curr. Res. Biosci. Biotechnol.* **2024**, *5*, 12–16.

10. Mandal, A.H.; Sadhu, A.; Ghosh, S.; Saha, N.C.; Saha, S. Carbonsulfan-induced physiological, histopathological, and ultrastructural alterations in *Tubifex tubifex* (müller, 1774). *Sci. Rep.* **2025**, *15*, 26321.
11. Trevisan, M.J.; Baptista, G.C.; Trevizan, L.R.P.; Papa, G. Residues of carbosulfan and its carbofuran metabolites and 3-hydroxy-carbofuran in oranges. *Rev. Bras. Frutic.* **2004**, *26*, 230–233.
12. Banji, D.; Banji, O.J.; Ragini, M.; Annamalai, A.R. Carbosulfan exposure during embryonic period can cause developmental disability in rats. *Environ. Toxicol. Pharmacol.* **2014**, *38*, 230–238.
13. Ksheerasagar, R.L.; Kaliwal, B.B. Effects of carbosulfan administration schedules on estrous cycle and follicular dynamics in albino mice. *Ind. Health* **2008**, *46*, 210–216.
14. Topaktaş, M.; Rencüzoğullar, E. Chromosomal aberrations in cultured human lymphocytes treated with Marshal and its effective ingredient Carbosulfan. *Mutat. Res.* **1993**, *319*, 103–111.
15. Xia, L.; Ma, H.; Chen, S. Respiratory failure induced by carbosulfan poisoning: A case report. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue* **2014**, *26*, 444. (In Chinese).
16. Zhai, J.; Yan, H.; Liu, M.; Jiang, C.; Jin, M.; Xie, B.; Ma, C.; Cong, B.; Wen, D. Decoding gelsenicine-induced neurotoxicity in mice via metabolomics and network toxicology. *Phytomedicine* **2025**, *142*, 156753.
17. Liang, D.; Su, H.; Ju, X. Integrated Network Toxicology and Metabolomics Reveal the Ovarian Toxicity Mechanisms of Chronic Carbofuran Exposure in Female Mice. *Int. J. Mol. Sci.* **2025**, *27*, 90.
18. Radulescu, D.; Mihai, F.D.; Trasca, M.E.; Caluianu, E.I.; Calafeteanu, C.D.M.; Radulescu, P.M.; Mercut, R.; Ciupeanu-Calugaru, E.D.; Marinescu, G.A.; Siloși, C.A.; et al. Oxidative Stress in Military Missions-Impact and Management Strategies: A Narrative Analysis. *Life* **2024**, *14*, 567.
19. Belhaj, A.; Dewachter, L.; Rorive, S.; Rummelink, M.; Weynand, B.; Melot, C.; Hupkens, E.; Dewachter, C.; Creteur, J.; Mc Entee, K.; et al. Mechanical versus humoral determinants of brain death-induced lung injury. *PLoS ONE* **2017**, *12*, e0181899.
20. Patanè, G.T.; Putaggio, S.; Tellone, E.; Barreca, D.; Ficarra, S.; Maffei, C.; Calderaro, A.; Laganà, G. Ferroptosis: Emerging Role in Diseases and Potential Implication of Bioactive Compounds. *Int. J. Mol. Sci.* **2023**, *24*, 17279.
21. Rahman, I.; MacNee, W. Oxidative stress and regulation of glutathione in lung inflammation. *Eur. Respir. J.* **2000**, *16*, 534–554.
22. Zorov, D.B.; Juhaszova, M.; Sollott, S.J. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* **2014**, *94*, 909–950.
23. Pei, Y.; Robertson, E.S. The Crosstalk of Epigenetics and Metabolism in Herpesvirus Infection. *Viruses* **2020**, *12*, 1377.
24. Kalinina, E. Glutathione-Dependent Pathways in Cancer Cells. *Int. J. Mol. Sci.* **2024**, *25*, 8423.
25. Hu, X.; Chandler, J.D.; Park, S.; Liu, K.; Fernandes, J.; Orr, M.; Smith, M.R.; Ma, C.; Kang, S.M.; Uppal, K.; et al. Low-dose cadmium disrupts mitochondrial citric acid cycle and lipid metabolism in mouse lung. *Free Radic. Biol. Med.* **2019**, *131*, 209–217.
26. Liang, X.; Zhang, L.; Natarajan, S.K.; Becker, D.F. Proline mechanisms of stress survival. *Antioxid. Redox Signal.* **2013**, *19*, 998–1011.
27. Linz, T.H.; Lunte, S.M. Heat-assisted extraction for the determination of methylarginines in serum by CE. *Electrophoresis* **2013**, *34*, 1693–1700.
28. Pacher, P.; Beckman, J.S.; Liaudet, L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* **2007**, *87*, 315–424.
29. Davis, C.W.; Hawkins, B.J.; Ramasamy, S.; Irrinki, K.M.; Cameron, B.A.; Islam, K.; Daswani, V.P.; Doonan, P.J.; Manevich, Y.; Madesh, M. Nitration of the mitochondrial complex I subunit NDUF8 elicits RIP1- and RIP3-mediated necrosis. *Free Radic. Biol. Med.* **2010**, *48*, 306–317.
30. Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health C* **2009**, *27*, 120–139.
31. Dong, L.L.; Liu, Z.Y.; Chen, K.J.; Li, Z.Y.; Zhou, J.S.; Shen, H.H.; Chen, Z.H. The persistent inflammation in COPD: Is autoimmunity the core mechanism? *Eur. Respir. Rev.* **2024**, *33*, 230137.
32. Zhai, R.; Shu, W.; Hu, Y.; Zhang, F.; Sun, L. Serum histamine in non-small cell lung cancer: An observational study in the context of allerge-oncology. *J. Int. Med. Res.* **2025**, *53*, 3000605251381195.
33. Méndez-García, L.A.; Escobedo, G.; Minguier-Urbe, A.G.; Viurcos-Sanabria, R.; Aguayo-Guerrero, J.A.; Carrillo-Ruiz, J.D.; Solleiro-Villavicencio, H. Role of the renin-angiotensin system in the development of COVID-19-associated neurological manifestations. *Front. Cell. Neurosci.* **2022**, *16*, 977039.
34. Xie, C.; Yang, J.; Gul, A.; Li, Y.; Zhang, R.; Yalikun, M.; Lv, X.; Lin, Y.; Luo, Q.; Gao, H. Immunologic aspects of asthma: From molecular mechanisms to disease pathophysiology and clinical translation. *Front. Immunol.* **2024**, *15*, 1478624.
35. Liu, R.; Luo, J.; Li, J.; Ma, Q.; Sun, J.; Li, Y.; Wang, D. Protective mechanisms of sevoflurane against one-lung ventilation-induced acute lung injury: Role of cyclooxygenase-2 and 5-lipoxygenase pathways. *Nan Fang Yi Ke Da Xue Xue Bao* **2013**, *33*, 625–630.

36. Sharma, S.; Lee, J.; Zhou, J.; Steele, V.E. Chemopreventive efficacy and mechanism of licofelone in a mouse lung tumor model via aspiration. *Cancer Prev. Res.* **2011**, *4*, 1233–1242.
37. Yang, F.; Qin, Z.; Shao, C.; Liu, W.; Ma, L.; Shu, Y.; Shen, H. Association between VEGF Gene Polymorphisms and the Susceptibility to Lung Cancer: An Updated Meta-Analysis. *Biomed Res. Int.* **2018**, *2018*, 9271215.
38. Talotta, R. Impaired VEGF-A-Mediated Neurovascular Crosstalk Induced by SARS-CoV-2 Spike Protein: A Potential Hypothesis Explaining Long COVID-19 Symptoms and COVID-19 Vaccine Side Effects? *Microorganisms* **2022**, *10*, 2452.
39. Luo, Q.; Shen, N.; Liang, J.; Qin, X.; Feng, S.; Chen, Y.; Xu, L. Relationship between VEGF to PEDF ratio and in-hospital mortality in acute respiratory distress syndrome patients. *Sci. Rep.* **2025**, *15*, 1420.
40. Yabo, W.; Dongxu, L.; Xiao, L.; Sandeep, B.; Qi, A. Genetic predisposition to acute lung injury in cardiac surgery ‘The VEGF Factor’: Review article and bibliometric analysis. *Curr. Probl. Cardiol.* **2025**, *50*, 102927.
41. Lin, C.K.; Lin, Y.H.; Huang, T.C.; Shi, C.S.; Yang, C.T.; Yang, Y.L. VEGF mediates fat embolism-induced acute lung injury via VEGF receptor 2 and the MAPK cascade. *Sci. Rep.* **2019**, *9*, 11713.
42. Wang, X.; Lin, W.; Li, H.; Long, S.; Yan, J.; Shi, Y.; Zhang, H.; Yang, X.; Yi, L.; Wang, G. Soluble programmed cell death ligand-1 as a predictive biomarker for severity and poor prognosis in pulmonary tuberculosis. *Ann. Med.* **2025**, *57*, 2527364.
43. Tang, L.; Bai, J.; Chung, C.S.; Lomas-Neira, J.; Chen, Y.; Huang, X.; Ayala, A. Programmed cell death receptor ligand 1 modulates the regulatory T cells’ capacity to repress shock/sepsis-induced indirect acute lung injury by recruiting phosphatase SRC homology region 2 domain-containing phosphatase 1. *Shock* **2015**, *43*, 47–54.
44. Purnell, P.R.; Mack, P.C.; Tepper, C.G.; Evans, C.P.; Green, T.P.; Gumerlock, P.H.; Lara, P.N.; Gandara, D.R.; Kung, H.J.; Gautschi, O. The Src inhibitor AZD0530 blocks invasion and may act as a radiosensitizer in lung cancer cells. *J. Thorac. Oncol.* **2009**, *4*, 448–454.
45. Calligaris, M.; Cuffaro, D.; Bonelli, S.; Spanò, D.P.; Rossello, A.; Nuti, E.; Scilabra, S.D. Strategies to Target ADAM17 in Disease: From its Discovery to the iRhom Revolution. *Molecules* **2021**, *26*, 944.
46. Lin, Z.; Wu, L.; Yu, Y.; Jiang, J.; Yang, Y.; Xiao, G. Case Report: Osimertinib-induced acute interstitial lung disease. *Front. Pharmacol.* **2025**, *16*, 1608733.
47. Zhu, C.; Maharajan, K.; Liu, K.; Zhang, Y. Role of atmospheric particulate matter exposure in COVID-19 and other health risks in human: A review. *Environ. Res.* **2021**, *198*, 111281.
48. Abass, K.; Reponen, P.; Mattila, S.; Pelkonen, O. Metabolism of carbosulfan II. Human interindividual variability in its in vitro hepatic biotransformation and the identification of the cytochrome P450 isoforms involved. *Chem. Biol. Interact.* **2010**, *185*, 163–173.
49. Kangkhetkron, T.; Juntarawijit, C. Pesticide exposure and lung cancer risk: A case-control study in Nakhon Sawan, Thailand. *F1000Res* **2024**, *9*, 492.
50. Zeljezić, D.; Vrdoljak, A.L.; Radić, B.; Fuchs, N.; Berend, S.; Orescanin, V.; Kopjar, N. Comparative evaluation of acetylcholinesterase status and genome damage in blood cells of industrial workers exposed to carbofuran. *Food Chem. Toxicol.* **2007**, *45*, 2488–2498.
51. Li, J.; Li, A.; Luo, K.; Yang, H.; Jiang, S.; Huang, P. Insights into CdTe quantum dots induced hepatotoxicity: Regulation of cytochromes P450 isoenzymes function in liver microsomes from in vivo and in vitro studies. *Arch. Biochem. Biophys.* **2025**, *768*, 110369.
52. Kempuraj, D.; Zhang, E.; Gupta, S.; Gupta, R.C.; Sinha, N.R.; Mohan, R.R. Carbofuran pesticide toxicity to the eye. *Exp. Eye Res.* **2023**, *227*, 109355.
53. Sun, H.; Sun, M.L.; Barr, D.B. Exposure to organophosphorus insecticides and increased risks of health and cancer in US women. *Environ. Toxicol. Pharmacol.* **2020**, *80*, 103474.
54. Ambhore, N.S.; Kalidhindi, R.S.R.; Sathish, V. Sex-Steroid Signaling in Lung Diseases and Inflammation. *Adv. Exp. Med. Biol.* **2021**, *1303*, 243–273.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.