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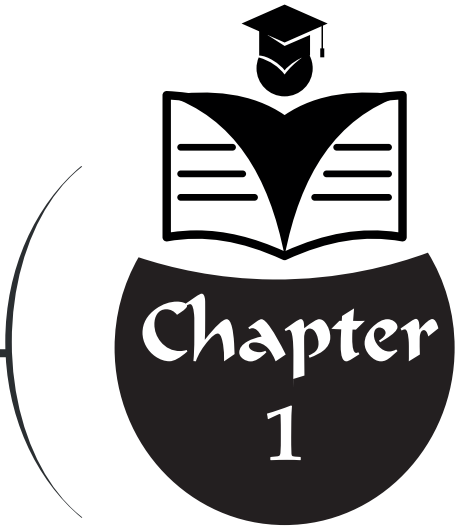

POTENTIAL RISKS OF MODERN HERBICIDES TO HUMAN HEALTH

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1. Introduction

Herbicides are the cornerstone of modern crop protection. They account for the largest share of global pesticide consumption and have become indispensable to the intensive, high-yield agricultural systems that feed a growing world population. By suppressing weeds that compete with crops for light, water, and nutrients, herbicides reduce yield losses, lower the labour intensity of cultivation, and enable conservation-tillage practices that limit soil erosion. The economic logic of chemical weed control is therefore compelling, and the volume of herbicides applied worldwide has expanded enormously over the past five decades (Benbrook, 2016).

Nowhere is this expansion more visible than in the rise of glyphosate. Since the commercialization of genetically engineered, glyphosate-tolerant ("Roundup Ready") crops in 1996, the use of glyphosate-based herbicides has increased almost fifteen-fold, making glyphosate the single most heavily applied agrochemical in recorded history; by 2014 roughly 8.6 billion kilograms had been sprayed globally (Benbrook, 2016). Parallel trends are evident for other active ingredients: the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and the benzoic-acid herbicide dicamba have experienced a resurgence as growers confront the spread of glyphosate-resistant weeds and adopt new herbicide-tolerant crop systems (Lerro et al., 2020). Glufosinate ammonium, the imidazolinones such as imazethapyr, and newer molecules such as the triazolinone ipfencarbazone illustrate the continuing diversification of the herbicide market (Kido, Okita, Okamura, Takeuchi, & Morita, 2016).

This intensification of use has been accompanied by widespread, low-level human exposure. People are exposed to herbicides through several converging routes. Occupational exposure affects farmers, applicators, formulators, and field workers, who may contact concentrated products dermally or by inhalation. Environmental exposure arises from spray drift, contaminated soil, and volatilization affecting residents near treated fields. Dietary exposure occurs because residues persist on and within food crops, particularly where pre-harvest desiccation is practised, and water-borne exposure follows the leaching and run-off of herbicides and their degradation products into surface water and groundwater (Van Bruggen et al., 2018; Sang, Mejuto, Xiao, & Simal-Gandara, 2021). Biomonitoring surveys now detect glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in the urine of the majority of sampled populations, including young children, confirming that exposure is no longer confined to agricultural settings (Gillezeau et al., 2019; Buekers et al., 2022; Ospina et al., 2022).

The purpose of this chapter is to provide a critical, evidence-based synthesis of the potential human-health risks posed by modern herbicides. Rather than cataloguing findings uncritically, the chapter weighs the strength,

consistency, and limitations of the available data, gives particular attention to contradictory results and to the well-documented divergence between regulatory and research-based assessments, and identifies the principal knowledge gaps. The scope encompasses the most widely used modern active ingredients, the recognized routes of human exposure, the mechanistic basis of herbicide toxicity, and the major organ-system and carcinogenic outcomes, followed by an overview of the current regulatory landscape and future research priorities. The aim is a balanced appraisal that neither overstates speculative hazards nor dismisses genuine signals of concern.

2. Modern Herbicides: Definition and Classification

Herbicides are classified in several complementary ways: by chemical family, by mode of action (the biochemical target site in the plant), by spectrum (selective versus non-selective), and by timing of application (pre- or post-emergence). For the purposes of human-health risk assessment, the mode of action is informative because it constrains the herbicidal target but does not necessarily predict mammalian toxicity, which frequently arises from off-target effects such as oxidative stress, mitochondrial impairment, or endocrine interference. The following subsections introduce the most relevant modern active ingredients; Table 1 summarizes their chemical class, herbicidal target, and current carcinogenicity status.

2.1. Glyphosate

Glyphosate [N-(phosphonomethyl)glycine] is a non-selective, broad-spectrum, post-emergent herbicide first marketed in 1974. It acts by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme of the shikimate pathway that synthesizes aromatic amino acids. Because this pathway is absent in mammals, glyphosate was historically presumed to pose minimal risk to humans, and its acute mammalian toxicity is indeed low (Williams, Kroes, & Munro, 2000). However, the shikimate pathway is present in many bacteria, including members of the human gut microbiota, providing a plausible indirect route to mammalian effects (Van Bruggen et al., 2018). Glyphosate is almost always applied as a formulated product containing surfactants and adjuvants; these co-formulants can substantially modify the toxicity of the active ingredient, a point of recurring importance throughout this chapter (Myers et al., 2016).

2.2. Glufosinate

Glufosinate ammonium (phosphinothricin) is a non-selective, broad-spectrum herbicide and the active ingredient of glufosinate-tolerant crop systems. It inhibits glutamine synthetase, causing ammonia accumulation and disruption of nitrogen metabolism in plants. Critically, glutamine synthetase is also essential in the mammalian central nervous system, where it

regulates glutamate and ammonia homeostasis. Glufosinate is a structural analogue of glutamate, and experimental data indicate neurotoxic and developmental effects that are mechanistically distinct from those of glyphosate (U.S. Environmental Protection Agency [EPA], 2012; Watanabe & Iwase, 1996).

2.3. Imazethapyr

Imazethapyr is an imidazolinone herbicide used for broad-spectrum weed control in legumes and other crops. Like other imidazolinones, it inhibits acetohydroxyacid synthase (AHAS, also called acetolactate synthase), the first enzyme in the biosynthesis of branched-chain amino acids. Although mammalian acute toxicity is low, imazethapyr and related imidazolinones have produced positive signals in plant and invertebrate genotoxicity assays and in amphibian models, indicating that low systemic toxicity should not be equated with the absence of genotoxic potential (Fragiorge, Alves, de Rezende, Graf, & Spanó, 2008; Liman, Ciğerci, & Öztürk, 2015; Magdaleno et al., 2015; Pérez-Iglesias et al., 2015).

2.4. Ipfencarbazone

Ipfencarbazone is a triazolinone-class rice herbicide developed in Japan and registered there since 2013. It is a pre- and early post-emergence herbicide that controls annual grasses, sedges, and some broadleaf weeds, acting by inhibiting the biosynthesis of very-long-chain fatty acids (VLCFAs). Manufacturer-reported toxicological studies describe low acute mammalian toxicity, rapid metabolism and excretion in rats, and low residue translocation to edible plant parts (Kido et al., 2016). Ipfencarbazone exemplifies a broader challenge in herbicide risk assessment: for many recently introduced molecules, independent human-relevant toxicology data are scarce, and the available information derives largely from regulatory dossiers rather than peer-reviewed academic study.

2.5. Dicamba

Dicamba is a selective benzoic-acid (synthetic auxin) herbicide used against broadleaf weeds. Its agricultural footprint has grown sharply following the approval of dicamba-tolerant cotton and soybean, raising both off-target drift concerns and renewed interest in its chronic health effects (Lerro et al., 2020).

2.6. 2,4-D

2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic-auxin phenoxy herbicide in continuous use since the 1940s and, like dicamba, has seen renewed deployment in herbicide-tolerant crop systems. It is among the most extensively used herbicides worldwide. Its long history, its historical associa-

tion with phenoxy-herbicide formulations, and a substantial epidemiological literature make it an important comparator for assessing herbicide health risks (Agency for Toxic Substances and Disease Registry [ATSDR], 2020; Schinasi & Leon, 2014).

2.7. Other new-generation herbicides

Beyond the molecules above, the modern herbicide portfolio includes numerous other classes — additional imidazolinones, sulfonylureas, triketones, and protoporphyrinogen-oxidase inhibitors, among others. A recurring theme is that the diversification driven by herbicide resistance increases the number and combination of residues to which humans are simultaneously exposed, complicating both exposure assessment and the interpretation of health outcomes.

Table 1

Principal Modern Herbicides: Chemical Class, Herbicidal Target, and Carcinogenicity Classification

Active ingredient	Chemical class	Herbicidal target site	Carcinogenicity status
Glyphosate	Phosphonoglycine	EPSPS (shikimate pathway)	IARC: Group 2A (probable); EFSA/ECHA/US EPA: not classified as carcinogenic
Glufosinate	Phosphinic acid	Glutamine synthetase	Not classified as a human carcinogen; flagged for neuro- and reproductive toxicity
Imazethapyr	Imidazolinone	AHAS / acetolactate synthase	Not classified; positive in several non-mammalian genotoxicity assays
Ipfencarbazone	Triazolinone	VLCFA biosynthesis	Not classified; limited independent toxicology data
Dicamba	Benzoic acid (synthetic auxin)	Auxin signalling	US EPA Group D (not classifiable); cohort signals for liver and lymphoid cancers
2,4-D	Phenoxy (synthetic auxin)	Auxin signalling	IARC: Group 2B (possible); US EPA: not classifiable

Note. EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase; AHAS = acetohydroxyacid synthase; VLCFA = very-long-chain fatty acid; IARC = International Agency for Research on Cancer; EFSA = European Food Safety Authority; ECHA = European Chemicals Agency. Classifications summarize positions discussed in Sections 7 and 9.

3. Routes of Human Exposure

Accurate characterization of exposure is the foundation of any credible risk assessment, yet it is also one of the weakest links in the herbicide lite-

rature. Many epidemiological studies rely on self-reported use or surrogate measures rather than measured internal dose, introducing exposure misclassification that tends to bias associations toward the null (Connolly, Coggins, & Koch, 2020).

3.1. Occupational exposure

Farmers, commercial applicators, formulators, and field workers experience the highest and most direct exposures, primarily through dermal contact and inhalation during mixing, loading, and spraying. Biomonitoring of agricultural workers consistently shows higher urinary glyphosate concentrations than in the general population, with personal protective equipment and application method acting as important determinants of dose (Connolly et al., 2020). Occupational cohorts therefore provide the most informative — though still imperfect — setting for evaluating chronic effects.

3.2. Environmental exposure

Spray drift, volatilization, and contaminated dust expose populations living near treated land even in the absence of direct handling. Glyphosate and AMPA are relatively persistent in soil and water and are detected across diverse environmental matrices, establishing a diffuse background of exposure for the wider community (Van Bruggen et al., 2018).

3.3. Dietary exposure

For the general population, diet is generally considered the dominant exposure pathway. Residues enter the food supply both from in-crop application and, importantly, from pre-harvest desiccation of cereals and pulses, a practice that can leave comparatively high residues on grains destined for direct human consumption (Sang et al., 2021). The detection of glyphosate in a large majority of urine samples in population surveys is consistent with continuous, low-level dietary intake (Ospina et al., 2022).

3.4. Water-borne exposure

Leaching and run-off transfer herbicides and their degradation products into surface water and groundwater. Where drinking-water sources are affected, this constitutes an additional, often under-quantified, exposure route; it is also central to hypotheses linking agrochemical exposure to regional epidemics of chronic kidney disease, discussed in Section 8 (Jayasumana, Gunatilake, & Senanayake, 2014).

3.5. Children and sensitive subpopulations

Children are of particular concern because of their higher intake of food and water relative to body weight, their developing organ systems, and their hand-to-mouth behaviour. Biomonitoring confirms that glyphosate is detectable in children as young as three years of age, and harmonized European

surveys document measurable exposure across paediatric populations (Buekers et al., 2022; Ospina et al., 2022). Pregnant women and the developing fetus represent an additional window of vulnerability, given evidence that glyphosate and AMPA can be detected in maternal biological samples. Standard adult-based reference doses may not adequately protect these groups, and dedicated paediatric toxicity values are largely lacking.

4. Toxicological Mechanisms of Herbicide Action

The mammalian toxicity of modern herbicides is mediated by a recurring set of interconnected mechanisms that operate downstream of, and independently from, the herbicidal target. These mechanisms — oxidative stress, cellular and DNA damage, mitochondrial dysfunction, apoptosis, and inflammation — are also recognized "key characteristics" of many human carcinogens and toxicants, which is why mechanistic data have carried substantial weight in hazard evaluations.

4.1. Oxidative stress

Induction of oxidative stress through excess generation of reactive oxygen species (ROS) and depletion of antioxidant defences is the most consistently reported mechanism across herbicide classes. For glyphosate, the evidence that it can induce oxidative stress operative in humans was judged strong by international hazard reviewers, and an analogous conclusion was reached for 2,4-D (IARC, 2015; ATSDR, 2020). Importantly, oxidative stress has now been documented in humans rather than only in vitro: in a large occupational cohort, higher glyphosate exposure was associated with elevated urinary biomarkers of oxidative DNA and lipid damage (Chang et al., 2023). Oxidative stress provides a unifying link between the molecular initiating events of herbicide exposure and downstream genotoxic, endocrine, hepatic, renal, and neurological effects.

4.2. Cellular damage and DNA damage

ROS and reactive metabolites can damage lipids, proteins, and nucleic acids. DNA damage — including single- and double-strand breaks, oxidized bases, and chromosomal alterations — is the genotoxic endpoint of greatest concern for carcinogenesis and is examined in detail in Section 5. The mechanistic plausibility of glyphosate genotoxicity rests substantially on its capacity to generate oxidative DNA lesions, even where direct mutagenicity in standard bacterial assays is weak or absent (Benbrook, 2019).

4.3. Mitochondrial dysfunction

Several herbicides and their formulations impair mitochondrial bioenergetics, reducing membrane potential and ATP synthesis and further increasing ROS production. Multi-omic analyses of rodents chronically exposed

to ultra-low doses of a glyphosate-based formulation revealed disturbances in fatty-acid β-oxidation and energy metabolism consistent with mitochondrial and peroxisomal stress, alongside histological steatosis and necrosis (Mesnage, Renney, Séralini, Ward, & Antoniou, 2017; Mesnage et al., 2015).

4.4. Apoptosis

Sustained oxidative and mitochondrial stress can trigger programmed cell death. Glufosinate, for example, induces apoptosis in the neuroepithelium of developing embryos, a finding mechanistically linked to its disruption of glutamate and glutamine homeostasis (Watanabe & Iwase, 1996). Dysregulated apoptosis is relevant both to developmental toxicity and, paradoxically, to carcinogenesis, where evasion of apoptosis is a hallmark.

4.5. Inflammatory responses

Herbicide exposure can activate pro-inflammatory signalling and, for glyphosate, may do so partly by perturbing the gut microbiome via inhibition of the bacterial shikimate pathway. The resulting dysbiosis and low-grade systemic inflammation have been proposed as mechanisms connecting glyphosate exposure to metabolic, hepatic, and neurological outcomes (Van Bruggen et al., 2018; Kulcsarova, Bang, Berg, & Schaeffer, 2023). Maternal exposure has likewise been associated with altered expression of antioxidant- and inflammation-related genes in offspring brain tissue (de Souza et al., 2019).

5. Cytotoxic and Genotoxic Effects

Genotoxicity occupies a central place in the herbicide debate because DNA damage is an early, mechanistically necessary step in chemical carcinogenesis. The literature on glyphosate genotoxicity is, however, markedly heterogeneous, and this heterogeneity is itself a key finding: results depend strongly on the test system, the endpoint, the dose, and especially on whether the pure active ingredient or a complete commercial formulation is tested.

5.1. In vitro studies

Across in vitro systems, glyphosate-based formulations are more potent than pure glyphosate, frequently inducing DNA strand breaks and micronuclei at concentrations far below those required for the active ingredient alone (Benbrook, 2019). This pattern implicates surfactants and other co-formulants as important genotoxic contributors and undermines risk assessments based solely on technical-grade glyphosate. Controlled studies using assays designed to detect irreversible DNA damage (micronucleus and bacterial reverse-mutation tests) have nonetheless often returned negative results for pure glyphosate, reinforcing the view that direct, classical mutagenicity is weak and that oxidative, formulation-dependent mechanisms predominate (Smith-Roe et al., 2023).

5.2. Human lymphocyte studies

In cultured human lymphocytes, pure glyphosate typically fails to induce chromosomal aberrations at low, environmentally relevant concentrations, with positive responses appearing mainly at high concentrations. Formulations and certain adjuvants, by contrast, can produce genotoxic signals at lower concentrations. The disparity again highlights that conclusions drawn from the active ingredient cannot be extrapolated uncritically to the products to which people are actually exposed.

5.3. Micronucleus test findings

The micronucleus assay, a validated biomarker of chromosomal damage, has been widely applied to herbicides. Pooled analyses of predominantly experimental studies indicate that both glyphosate and its formulations can increase micronucleus frequency in exposed organisms, although effects in standardized in vivo mammalian assays are inconsistent (Smith-Roe et al., 2023). For the imidazolinones, imazethapyr formulations have induced micronuclei and chromosomal aberrations in plant and amphibian models, providing convergent evidence of clastogenic and aneugenic potential even where mammalian data are sparse (Magdaleno et al., 2015; Pérez-Iglesias et al., 2015; Liman et al., 2015).

5.4. Comet assay findings

The single-cell gel electrophoresis (comet) assay, which detects DNA strand breakage, is among the most frequently positive endpoints for glyphosate-based herbicides, consistent with an oxidative mechanism of DNA damage. Its sensitivity is also a limitation, because it detects potentially repairable lesions rather than fixed mutations; positive comet results therefore signal hazard but require corroboration by assays of irreversible damage (Smith-Roe et al., 2023).

5.5. Chromosomal abnormalities and biomonitoring

Human biomonitoring of occupationally exposed populations provides the most directly relevant evidence. Studies of agricultural workers exposed to glyphosate-based herbicides have reported increased markers of cytogenetic damage relative to unexposed referents, although confounding by co-exposure to other pesticides and uncertainty in actual dose limit causal inference (Bolognesi, Carrasquilla, Volpi, Solomon, & Marshall, 2009). Taken together, the genotoxicity evidence supports a biologically plausible hazard, concentrated in commercial formulations and oxidative endpoints, while underscoring that exposure characterization and formulation composition are decisive for interpreting individual studies.

6. Endocrine-Disrupting Effects

6.1. Effects on the hormonal system

A growing experimental literature indicates that glyphosate and glyphosate-based herbicides can act as endocrine-disrupting chemicals. In vitro and in vivo studies report inhibition of aromatase (the enzyme converting testosterone to estradiol), interference with steroidogenic regulatory proteins such as StAR, and altered expression of steroidogenic enzymes, with consequent changes in sex-hormone levels (Ingaramo, Alarcón, Muñoz-de-Toro, & Luque, 2020). A systematic review and meta-analysis of experimental studies found that glyphosate exposure was associated with alterations in testosterone and other reproductive hormones, supporting an endocrine mode of action (Mohammadi et al., 2022).

6.2. Reproductive toxicity

Endocrine disruption translates into reproductive effects in animal models, including impaired ovarian and uterine function in females and reduced testosterone, abnormal sperm parameters, and seminiferous-tubule damage in males (Ingaramo et al., 2020). Glufosinate poses distinct reproductive hazards: by inhibiting glutamine synthetase it can compromise embryo implantation during the narrow window in which the conceptus depends on maternal glutamine, an effect demonstrated at high maternal doses (Watanabe & Iwase, 1996; EPA, 2012). The extent to which these experimental findings translate to human reproductive health at environmental exposure levels remains uncertain and is a priority for further research.

6.3. Developmental toxicity

The developing organism is especially sensitive to endocrine and neurochemical perturbation. Animal studies report developmental and dysmorphogenic effects of glufosinate on embryos and developmental neurotoxicity following perinatal exposure (Watanabe & Iwase, 1996). For glyphosate, maternal exposure has been associated with altered antioxidant gene expression and metabolite profiles in offspring, and human studies have begun to examine associations between gestational exposure and birth outcomes (de Souza et al., 2019). These findings collectively argue for precaution regarding exposures during pregnancy and early life.

7. Cancer Risk and Epidemiological Findings

No single issue has generated more controversy than the carcinogenic potential of glyphosate, and the evidence must be appraised with care because authoritative bodies have reached opposing conclusions from largely the same data.

7.1. The IARC evaluation

In 2015 the International Agency for Research on Cancer classified glyphosate as "probably carcinogenic to humans" (Group 2A), citing limited evidence of carcinogenicity in humans (principally for non-Hodgkin lymphoma, NHL), sufficient evidence in experimental animals, and strong mechanistic evidence of genotoxicity and oxidative stress (IARC, 2015; Guyton et al., 2015). In the same volume, 2,4-D was classified as "possibly carcinogenic to humans" (Group 2B), with strong evidence that it induces oxidative stress (ATSDR, 2020).

7.2. Cohort and case-control evidence

The epidemiological evidence is mixed. The Agricultural Health Study (AHS), the largest prospective cohort of pesticide applicators, found no statistically significant association between glyphosate use and NHL or most solid tumours after extended follow-up, although a non-significant signal for acute myeloid leukaemia in the most-exposed group was noted (Andreotti et al., 2018). In contrast, several case-control studies have reported positive associations between glyphosate-based herbicide exposure and NHL. A pooled analysis of agricultural cohorts from France, Norway, and the USA reported heterogeneous results across regions (Leon et al., 2019), while a widely cited meta-analysis emphasizing the highest-exposure groups estimated an approximately 41% increase in relative risk of NHL (Zhang, Rana, Shaffer, Taioli, & Sheppard, 2019). Other meta-analyses applying different inclusion criteria and weighting the null AHS data more heavily concluded that the evidence does not establish a causal association (Chang & Delzell, 2016; Donato, Pira, Ciocan, & Boffetta, 2020). The phenoxy-herbicide and glyphosate associations with B-cell lymphoma identified in earlier systematic reviews add historical context to these debates (Schinasi & Leon, 2014).

For other herbicides, the AHS has provided informative updates. With extended follow-up, increasing dicamba use was associated with elevated risk of liver and intrahepatic bile-duct cancer and chronic lymphocytic leukaemia, whereas earlier signals for lung and colon cancer attenuated (Lerro et al., 2020). 2,4-D has been examined extensively, and although individual studies have linked phenoxy herbicides to lymphoid malignancies, the overall epidemiological evidence has been judged insufficient to establish causation (ATSDR, 2020; Schinasi & Leon, 2014).

7.3. Critical analysis of contradictory findings

The divergence between the IARC classification and the conclusions of the European Food Safety Authority, the European Chemicals Agency, and the US EPA reflects genuine methodological differences rather than simple disagreement (Tarazona et al., 2017; Benbrook, 2019). IARC conducts hazard

identification using publicly available, peer-reviewed literature and gives substantial weight to formulation and mechanistic data; regulatory agencies perform risk assessment, rely heavily on unpublished, guideline-compliant industry studies of the pure active ingredient, and focus on exposures considered realistic (Williams et al., 2016; EFSA, 2023). Each approach has strengths and weaknesses: hazard identification may overstate real-world risk if it does not account for dose, whereas regulatory risk assessment may understate hazard if it excludes formulation effects and underweights independent research. Industry-funded reviews have tended to conclude that glyphosate is neither genotoxic nor carcinogenic (Williams, Kroes, & Munro, 2000; Williams et al., 2016; Mink et al., 2012), while independent researchers have urged reassessment of current safety standards (Myers et al., 2016; Vandenberg et al., 2017). Some influential primary studies, such as long-term rodent carcinogenicity experiments reporting tumours at low doses, remain methodologically contested (Séralini et al., 2014; Mesnage & Antoniou, 2017). A defensible reading is that glyphosate-based herbicides present a plausible carcinogenic hazard — strongest for NHL and most credible for highly exposed individuals and for complete formulations — but that the magnitude of population-level risk at typical exposures remains uncertain.

Table 2

Summary of Selected Epidemiological Findings on Modern Herbicides and Cancer

Herbicide	Study / design	Principal outcome	Direction of finding
Glyphosate	Andreotti et al. (2018), AHS cohort	NHL; solid tumours; AML	No significant association overall; non-significant AML signal at highest exposure
Glyphosate	Zhang et al. (2019), meta-analysis	Non-Hodgkin lymphoma	~41% increased relative risk in highly exposed
Glyphosate	Chang & Delzell (2016); Donato et al. (2020), meta-analyses	Lymphohematopoietic cancers	No clear causal association
Dicamba	Lerro et al. (2020), AHS cohort	Liver/bile-duct cancer; CLL	Elevated risk at highest exposure
2,4-D	Schinasi & Leon (2014), systematic review	Non-Hodgkin lymphoma	Positive associations for phenoxy herbicides; evidence judged insufficient for causation

Note. AHS = Agricultural Health Study; NHL = non-Hodgkin lymphoma; AML = acute myeloid leukaemia; CLL = chronic lymphocytic leukaemia. The table is illustrative and not exhaustive.

8. Neurotoxicity and Other Systemic Effects

8.1. Nervous system

Concern about herbicide neurotoxicity has intensified as exposures have become ubiquitous. A systematic review of experimental studies concluded

that glyphosate can exert toxic effects on the nervous system through oxidative stress, mitochondrial dysfunction, neuroinflammation, and disruption of neurotransmitter systems (Costas-Ferreira, Durán, & Faro, 2022). A prominent emerging hypothesis links glyphosate to Parkinson's disease and other neurodegenerative conditions via the microbiome-gut-brain axis, whereby shikimate-pathway inhibition perturbs the gut microbiota, promoting dysbiosis, intestinal and systemic inflammation, and — potentially — neurodegeneration (Kulcsarova et al., 2023). Glufosinate poses a more direct neurological threat: as a glutamate analogue that inhibits glutamine synthetase, it disrupts glutamate homeostasis and has produced memory impairment, structural brain changes, and developmental neurotoxicity in experimental models (EPA, 2012; Watanabe & Iwase, 1996). Maternal glyphosate exposure has also been associated with altered brain antioxidant gene expression in offspring (de Souza et al., 2019).

8.2. Hepatotoxicity

The liver, as the principal site of xenobiotic metabolism, is a sensitive target. Chronic exposure of rats to ultra-low, environmentally relevant doses of a glyphosate-based formulation produced biochemical, transcriptomic, proteomic, and metabolomic signatures consistent with non-alcoholic fatty liver disease, including steatosis, oxidative stress, and disturbed fatty-acid metabolism (Mesnage et al., 2017; Mesnage et al., 2015). These findings are striking for occurring well below regulatory no-effect levels, though their human relevance and the influence of formulation adjuvants continue to be debated.

8.3. Nephrotoxicity

The kidney is the most sensitive target of 2,4-D in animal studies (ATSDR, 2020). For glyphosate, the most prominent — and contested — hypothesis concerns chronic kidney disease of unknown etiology (CKDu), an epidemic affecting agricultural communities in Sri Lanka, Central America, and elsewhere. It has been proposed that glyphosate may form nephrotoxic complexes with hard-water metals and arsenic, concentrating renal toxicity in affected regions (Jayasumana et al., 2014). This hypothesis remains unproven and is regarded by many investigators as multifactorial, with heat stress, dehydration, and other agrochemicals also implicated; it nonetheless illustrates how water-borne herbicide exposure may interact with local environmental factors to produce regional disease clusters.

8.4. Immune-system effects

Immunomodulation is a less thoroughly characterized but biologically plausible outcome. IARC judged the evidence that 2,4-D causes immunosuppression to be moderate (ATSDR, 2020), and disruption of the gut microbiome by glyphosate provides an additional, indirect route to altered immune function (Van Bruggen et al., 2018). The immunological consequences of ch-

ronic low-level herbicide exposure in humans remain insufficiently studied.

9. Current Regulations and Risk Assessment

Regulatory positions on modern herbicides, particularly glyphosate, diverge across jurisdictions, reflecting both scientific disagreement and differing risk-management philosophies.

9.1. European Union

Following assessments by EFSA and ECHA that did not identify critical areas of concern and concluded that glyphosate does not meet the criteria for classification as carcinogenic, mutagenic, or toxic for reproduction, the European Commission renewed the approval of glyphosate for ten years, until December 2033, subject to new conditions including a prohibition on pre-harvest desiccant use and measures to protect non-target organisms (EFSA, 2023; European Commission, 2023). The renewal was adopted in the absence of a qualified majority among Member States and remains politically contested, with several states maintaining national restrictions (Tarazona et al., 2017).

9.2. Türkiye

In Türkiye, plant protection products are authorized and regulated by the Ministry of Agriculture and Forestry through its Directorate of Plant Protection Products, and glyphosate-based products remain registered for use (Ministry of Agriculture and Forestry, 2024). Maximum residue limits in food are set under the Turkish Food Codex Regulation on Maximum Residue Limits of Pesticides, which is harmonized with the European Union framework (Regulation 396/2005/EC) as part of Türkiye's broader alignment of food-safety legislation (Turkish Food Codex, 2014). Türkiye thus largely follows EU residue standards while authorizing continued domestic use.

9.3. United States

The US EPA has concluded that glyphosate is "not likely to be carcinogenic to humans" and that there are no risks of concern to human health when the product is used according to the label (EPA, 2020). This position, like its European counterpart, has been challenged in litigation and in the scientific literature, and it contrasts sharply with the IARC hazard classification (Benbrook, 2019).

9.4. Current legislation and limits

Across jurisdictions, exposure limits are expressed primarily as acceptable daily intakes (ADIs) and reference doses. A notable and instructive discrepancy is that the US EPA chronic reference dose for glyphosate has historically been set several-fold higher than the European ADI, meaning that the two systems implicitly tolerate very different exposures (Myers et al., 2016). Such differences, together with the reliance on industry-generated data and

the limited incorporation of formulation toxicity and endocrine endpoints, have led independent scientists to call for a reassessment of current safety standards (Vandenberg et al., 2017; Mesnage & Antoniou, 2017).

10. Knowledge Gaps and Future Perspectives

10.1. Research deficiencies

Several deficiencies recur throughout the literature. First, the great majority of regulatory toxicology has tested pure active ingredients rather than the formulated products to which humans are actually exposed, despite consistent evidence that adjuvants increase toxicity (Myers et al., 2016; Benbrook, 2019). Second, exposure assessment in epidemiology has relied heavily on self-report and surrogate measures rather than measured internal dose, biasing many studies toward the null (Connolly et al., 2020). Third, data on chronic, low-dose, and mixture exposures — the realistic human scenario — are sparse, and dedicated studies of vulnerable groups, especially children and pregnant women, are limited. Fourth, for newer molecules such as ipfencarbazone, independent human-relevant toxicology is almost entirely absent (Kido et al., 2016).

10.2. Novel biomarkers

Progress will depend on validated biomarkers of both exposure and effect. Urinary glyphosate and AMPA are established exposure biomarkers, while oxidative-stress markers measured in human cohorts now offer biomarkers of early biological effect (Chang et al., 2023; Ospina et al., 2022). Extending and standardizing such biomarkers across populations would substantially strengthen exposure-response inference.

10.3. Omics technologies

High-throughput omics — transcriptomics, proteomics, metabolomics, and microbiome profiling — have already revealed effects of glyphosate-based formulations at doses below regulatory thresholds and can illuminate mechanisms and adverse outcome pathways that conventional toxicology misses (Mesnage et al., 2017; Kulcsarova et al., 2023). Integrating omics endpoints into regulatory frameworks is a promising avenue for next-generation risk assessment.

10.4. Human biomonitoring

Large-scale, harmonized human biomonitoring programmes, such as those conducted in Europe and the United States, provide the population-level exposure data needed to interpret health outcomes and to evaluate the impact of regulatory measures over time (Buekers et al., 2022; Ospina et al., 2022; Connolly et al., 2020). Sustained, internationally comparable biomonitoring — coupled with longitudinal health follow-up — represents perhaps the single most valuable investment for resolving current uncertainties.

11. Conclusion

Modern herbicides are agriculturally indispensable, yet the evidence reviewed here indicates that they are not without potential human-health risk. A coherent mechanistic picture has emerged in which oxidative stress acts as a central node linking herbicide exposure to genotoxic, endocrine, hepatic, renal, and neurological effects, with mitochondrial dysfunction, apoptosis, inflammation, and gut-microbiome perturbation as interconnected contributors. The genotoxicity and toxicity of glyphosate are consistently greater for commercial formulations than for the pure active ingredient, a finding that exposes a fundamental limitation of risk assessments based on the technical material alone.

The carcinogenicity debate remains unresolved, and the divergence between IARC's hazard classification and the conclusions of regulatory agencies arises chiefly from differences in methodology, data sources, and the weight assigned to formulation and mechanistic evidence. On balance, the data support treating glyphosate-based herbicides as a plausible carcinogenic hazard — most credibly for non-Hodgkin lymphoma among highly exposed individuals — while acknowledging genuine uncertainty about population-level risk at typical exposures. For other modern herbicides, the evidence is more fragmentary: dicamba shows cohort signals for hepatic and lymphoid cancers, 2,4-D carries a possible-carcinogen classification and clear nephrotoxic and oxidative effects, glufosinate presents distinctive neuro- and reproductive toxicity through its glutamate-related mechanism, the imidazolinones display genotoxic potential in non-mammalian systems, and for the newest molecules such as ipfencarbazone independent data are largely lacking.

From a public-health perspective, the prudent course is precautionary risk management informed by continued, rigorous science. Priorities include testing formulated products rather than active ingredients alone, improving exposure assessment through measured internal dose, protecting vulnerable subpopulations with appropriate reference values, harmonizing international biomonitoring, and integrating omics-based biomarkers and adverse outcome pathways into regulatory evaluation. Resolving the current contradictions will require well-designed prospective cohorts with quantitative exposure data, standardized genotoxicity testing of formulations, and transparent, independent re-analysis of both published and proprietary studies. Until such evidence matures, reducing unnecessary human exposure to modern herbicides represents a reasonable and defensible objective.

REFERENCES

Agency for Toxic Substances and Disease Registry. (2020). *Toxicological profile for 2,4-dichlorophenoxyacetic acid (2,4-D)*. U.S. Department of Health and Human Services.

Andreotti, G., Koutros, S., Hofmann, J. N., Sandler, D. P., Lubin, J. H., Lynch, C. F., … Beane Freeman, L. E. (2018). Glyphosate use and cancer incidence in the Agricultural Health Study. *JNCI: Journal of the National Cancer Institute, 110*(5), 509–516. https://doi.org/10.1093/jnci/djx233

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe, 28*(1), 3. https://doi.org/10.1186/s12302-016-0070-0

Benbrook, C. M. (2019). How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides? *Environmental Sciences Europe, 31*(1), 2. https://doi.org/10.1186/s12302-018-0184-7

Bolognesi, C., Carrasquilla, G., Volpi, S., Solomon, K. R., & Marshall, E. J. P. (2009). Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: Association to occupational exposure to glyphosate. *Journal of Toxicology and Environmental Health, Part A, 72*(15–16), 986–997. https://doi.org/10.1080/15287390902929741

Buekers, J., Remy, S., Bessems, J., Govarts, E., Rambaud, L., Riou, M., … Schoeters, G. (2022). Glyphosate and AMPA in human urine of HBM4EU-aligned studies: Part A children. *Toxics, 10*(8), 470. https://doi.org/10.3390/toxics10080470

Chang, E. T., & Delzell, E. (2016). Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. *Journal of Environmental Science and Health, Part B, 51*(6), 402–434. https://doi.org/10.1080/03601234.2016.1142748

Chang, V. C., Andreotti, G., Ospina, M., Parks, C. G., Liu, D., Shearer, J. J., … Beane Freeman, L. E. (2023). Glyphosate exposure and urinary oxidative stress biomarkers in the Agricultural Health Study. *JNCI: Journal of the National Cancer Institute, 115*(4), 394–404. https://doi.org/10.1093/jnci/djac242

Connolly, A., Coggins, M. A., & Koch, H. M. (2020). Human biomonitoring of glyphosate exposures: State-of-the-art and future research challenges. *Toxics, 8*(3), 60. https://doi.org/10.3390/toxics8030060

Costas-Ferreira, C., Durán, R., & Faro, L. R. F. (2022). Toxic effects of glyphosate on the nervous system: A systematic review. *International Journal of Molecular Sciences, 23*(9), 4605. https://doi.org/10.3390/ijms23094605

de Souza, J. S., Laureano-Melo, R., Herai, R. H., da Conceição, R. R., Oliveira, K. C., da Silva, I. D. C. G., … Giannocco, G. (2019). Maternal glyphosate-based herbicide exposure alters antioxidant-related genes in the brain and serum metabolites of male rat offspring. *NeuroToxicology, 74*, 121–131. https://doi.org/10.1016/j.neuro.2019.06.004

Donato, F., Pira, E., Ciocan, C., & Boffetta, P. (2020). Exposure to glyphosate and risk of non-Hodgkin lymphoma and multiple myeloma: An updated meta-analysis. *La Medicina del Lavoro, 111*(1), 63–73. https://doi.org/10.23749/mdl.v111i1.8459

European Commission. (2023). *Commission Implementing Regulation (EU) 2023/2660 of 28 November 2023 renewing the approval of the active substance glyphosate*. Official Journal of the European Union.

European Food Safety Authority. (2023). *Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate*. European Food Safety Authority.

Fragiorge, E. J., Alves, A., de Rezende, A. A. A., Graf, U., & Spanó, M. A. (2008). Comparative genotoxicity evaluation of imidazolinone herbicides in somatic cells of *Drosophila melanogaster*. *Food and Chemical Toxicology, 46*(1), 393–401. https://doi.org/10.1016/j.fct.2007.08.022

Gillezeau, C., van Gerwen, M., Shaffer, R. M., Rana, I., Zhang, L., Sheppard, L., & Taioli, E. (2019). The evidence of human exposure to glyphosate: A review. *Environmental Health, 18*(1), 2. https://doi.org/10.1186/s12940-018-0435-5

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., … Straif, K. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *The Lancet Oncology, 16*(5), 490–491. https://doi.org/10.1016/S1470-2045(15)70134-8

Ingaramo, P., Alarcón, R., Muñoz-de-Toro, M., & Luque, E. H. (2020). Are glyphosate and glyphosate-based herbicides endocrine disruptors that alter female fertility? *Molecular and Cellular Endocrinology, 518*, 110934. https://doi.org/10.1016/j.mce.2020.110934

International Agency for Research on Cancer. (2015). *Some organophosphate insecticides and herbicides (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 112)*. World Health Organization.

Jayasumana, C., Gunatilake, S., & Senanayake, P. (2014). Glyphosate, hard water and nephrotoxic metals: Are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? *International Journal of Environmental Research and Public Health, 11*(2), 2125–2147. https://doi.org/10.3390/ijerph110202125

Kido, T., Okita, H., Okamura, M., Takeuchi, T., & Morita, K. (2016). Development of a rice herbicide, ipfencarbazone. *Journal of Pesticide Science, 41*(3), 113–119. https://doi.org/10.1584/jpestics.J16-06

Kulcsarova, K., Bang, C., Berg, D., & Schaeffer, E. (2023). Pesticides and the microbiome-gut-brain axis: Convergent pathways in the pathogenesis of Parkinson's disease. *Journal of Parkinson's Disease, 13*(7), 1079–1106. https://doi.org/10.3233/JPD-230206

Leon, M. E., Schinasi, L. H., Lebailly, P., Beane Freeman, L. E., Nordby, K. C., Ferro, G., … Schüz, J. (2019). Pesticide use and risk of non-Hodgkin lymphoid ma-

lignancies in agricultural cohorts from France, Norway and the USA: A pooled analysis from the AGRICOH consortium. *International Journal of Epidemiology, 48*(5), 1519–1535. https://doi.org/10.1093/ije/dyz017

Lerro, C. C., Hofmann, J. N., Andreotti, G., Koutros, S., Parks, C. G., Blair, A., … Beane Freeman, L. E. (2020). Dicamba use and cancer incidence in the Agricultural Health Study: An updated analysis. *International Journal of Epidemiology, 49*(4), 1326–1337. https://doi.org/10.1093/ije/dyaa066

Liman, R., Ciğerci, İ. H., & Öztürk, N. S. (2015). Determination of genotoxic effects of imazethapyr herbicide in *Allium cepa* root cells by mitotic activity, chromosome aberration, and comet assay. *Pesticide Biochemistry and Physiology, 118*, 38–42. https://doi.org/10.1016/j.pestbp.2014.11.007

Magdaleno, A., Peralta Gavensky, M., Fassiano, A. V., Ríos de Molina, M. C., Santos, M., March, H., … Juárez, Á. B. (2015). Phytotoxicity and genotoxicity assessment of imazethapyr herbicide using a battery of bioassays. *Environmental Science and Pollution Research, 22*(24), 19194–19202. https://doi.org/10.1007/s11356-015-5103-5

Mesnage, R., & Antoniou, M. N. (2017). Facts and fallacies in the debate on glyphosate toxicity. *Frontiers in Public Health, 5*, 316. https://doi.org/10.3389/fpubh.2017.00316

Mesnage, R., Arno, M., Costanzo, M., Malatesta, M., Séralini, G.-E., & Antoniou, M. N. (2015). Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. *Environmental Health, 14*, 70. https://doi.org/10.1186/s12940-015-0056-1

Mesnage, R., Renney, G., Séralini, G.-E., Ward, M., & Antoniou, M. N. (2017). Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. *Scientific Reports, 7*, 39328. https://doi.org/10.1038/srep39328

Mink, P. J., Mandel, J. S., Sceurman, B. K., & Lundin, J. I. (2012). Epidemiologic studies of glyphosate and cancer: A review. *Regulatory Toxicology and Pharmacology, 63*(3), 440–452. https://doi.org/10.1016/j.yrtph.2012.05.012

Ministry of Agriculture and Forestry. (2024). *Plant protection products database [Bitki Koruma Ürünleri Veri Tabanı]*. Republic of Türkiye, Ministry of Agriculture and Forestry. https://bku.tarimorman.gov.tr

Mohammadi, K., Sani, M. A., Safaei, P., Rahmani, J., Molaee-Aghaee, E., & Jafari, S. M. (2022). A systematic review and meta-analysis of the impacts of glyphosate on the reproductive hormones. *Environmental Science and Pollution Research, 29*, 62030–62041. https://doi.org/10.1007/s11356-021-16145-x

Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., … Benbrook, C. M. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environmental Health, 15*, 19. https://doi.org/10.1186/s12940-016-0117-0

Ospina, M., Schütze, A., Morales-Agudelo, P., Vidal, M., Wong, L.-Y., & Calafat, A. M.

(2022). Exposure to glyphosate in the United States: Data from the 2013–2014 National Health and Nutrition Examination Survey. *Environment International, 170*, 107620. https://doi.org/10.1016/j.envint.2022.107620

Pérez-Iglesias, J. M., Soloneski, S., Nikoloff, N., Natale, G. S., & Larramendy, M. L. (2015). Toxic and genotoxic effects of the imazethapyr-based herbicide formulation Pivot H on montevideo tree frog *Hypsiboas pulchellus* tadpoles (Anura, Hylidae). *Ecotoxicology and Environmental Safety, 119*, 15–24. https://doi.org/10.1016/j.ecoenv.2015.04.045

Sang, Y., Mejuto, J. C., Xiao, J., & Simal-Gandara, J. (2021). Assessment of glyphosate impact on the agrofood ecosystem. *Plants, 10*(2), 405. https://doi.org/10.3390/plants10020405

Schinasi, L., & Leon, M. E. (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis. *International Journal of Environmental Research and Public Health, 11*(4), 4449–4527. https://doi.org/10.3390/ijerph110404449

Séralini, G.-E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., … de Vendômois, J. S. (2014). Republished study: Long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe, 26*, 14. https://doi.org/10.1186/s12302-014-0014-5

Smith-Roe, S. L., Swartz, C. D., Shepard, K. G., Bryce, S. M., Dertinger, S. D., Waidyanatha, S., … Witt, K. L. (2023). Evaluation of the herbicide glyphosate, (aminomethyl)phosphonic acid, and glyphosate-based formulations for genotoxic activity using in vitro assays. *Environmental and Molecular Mutagenesis, 64*(4), 202–233. https://doi.org/10.1002/em.22534

Tarazona, J. V., Court-Marques, D., Tiramani, M., Reich, H., Pfeil, R., Istace, F., & Crivellente, F. (2017). Glyphosate toxicity and carcinogenicity: A review of the scientific basis of the European Union assessment and its differences with IARC. *Archives of Toxicology, 91*(8), 2723–2743. https://doi.org/10.1007/s00204-017-1962-5

Turkish Food Codex. (2014). *Regulation on maximum residue limits of pesticides [Türk Gıda Kodeksi Pestisitlerin Maksimum Kalıntı Limitleri Yönetmeliği]* (Official Gazette No. 29099, 25 August 2014). Republic of Türkiye.

U.S. Environmental Protection Agency. (2012). Glufosinate ammonium; pesticide tolerances. *Federal Register, 77*(187), 59101–59110.

U.S. Environmental Protection Agency. (2020). *Glyphosate: Interim registration review decision (Case No. 0178)*. U.S. Environmental Protection Agency.

Van Bruggen, A. H. C., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., & Morris, J. G. (2018). Environmental and health effects of the herbicide glyphosate. *Science of the Total Environment, 616–617*, 255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Vandenberg, L. N., Blumberg, B., Antoniou, M. N., Benbrook, C. M., Carroll, L., Colborn, T., … Myers, J. P. (2017). Is it time to reassess current safety standards for

glyphosate-based herbicides? *Journal of Epidemiology and Community Health, 71*(6), 613–618. https://doi.org/10.1136/jech-2016-208463

Watanabe, T., & Iwase, T. (1996). Developmental and dysmorphogenic effects of glufosinate ammonium on mouse embryos in culture. *Teratogenesis, Carcinogenesis, and Mutagenesis, 16*(6), 287–299.

Williams, G. M., Aardema, M., Acquavella, J., Berry, S. C., Brusick, D., Burns, M. M., … Greim, H. (2016). A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment. *Critical Reviews in Toxicology, 46*(sup1), 3–20. https://doi.org/10.1080/10408444.2016.1214677

Williams, G. M., Kroes, R., & Munro, I. C. (2000). Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regulatory Toxicology and Pharmacology, 31*(2), 117–165. https://doi.org/10.1006/rtph.1999.1371

Zhang, L., Rana, I., Shaffer, R. M., Taioli, E., & Sheppard, L. (2019). Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence. *Mutation Research/Reviews in Mutation Research, 781*, 186–206. https://doi.org/10.1016/j.mrrev.2019.02.001

Chapter 2

CLIMATE CRISIS AND ITS IMPACTS ON CROP PRODUCTION

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1. INTRODUCTION

The climate crisis is widely recognized as one of the most pressing environmental challenges of the twenty-first century, with profound implications for ecosystems, economies, and human well-being. Unlike the broader concept of climate change, the term climate crisis emphasizes the accelerating pace, increasing severity, and potentially irreversible consequences of ongoing environmental transformations. Scientific evidence demonstrates that anthropogenic greenhouse gas emissions are the primary driver of global warming, disrupting the Earth's energy balance and intensifying environmental risks worldwide koymaktadır ((IPCC), 2023a, 2023b).

The climate system operates through complex interactions among the atmosphere, hydrosphere, lithosphere, and biosphere, where relatively small disturbances may trigger large-scale changes through feedback mechanisms. Consequently, climate change often follows nonlinear pathways characterized by threshold-dependent responses and abrupt transitions. The increasing likelihood of crossing climate tipping points, particularly beyond 1.5°C and 2°C of global warming, raises concerns about irreversible changes such as large-scale ice loss, sea-level rise, and ecosystem degradation ((IPCC), 2023a; Timothy M. Lenton et al., 2019)

Agriculture is among the sectors most vulnerable to the climate crisis because crop production depends directly on environmental factors including temperature, precipitation, solar radiation, atmospheric CO_2 concentration, soil properties, and nutrient availability (Sun et al., 2024; Yang et al., 2024). At the same time, agricultural activities contribute to climate change through greenhouse gas emissions associated with land-use change, fertilizer application, and livestock production, creating a reciprocal relationship between climate and agricultural systems (Yang et al., 2024).

The impacts of the climate crisis on crop production are primarily mediated through rising temperatures, altered precipitation patterns, increasing atmospheric CO_2 concentrations, and the growing occurrence of extreme weather events. Temperature is particularly critical because it regulates plant development, phenology, photosynthesis, and reproductive processes. When temperatures exceed species-specific thresholds, reductions in pollen viability, fertilization success, and grain filling can substantially decrease crop yields (Saleem & Smirnova, 2026; Tran et al., 2025; Yuan et al., 2024). Evidence suggests that yield losses accelerate beyond critical temperature thresholds and are especially severe in warm and semi-arid regions already exposed to heat and water stress (Tran et al., 2025).

Changes in precipitation patterns further exacerbate production risks. Insufficient rainfall limits photosynthesis, biomass accumulation, and nutrient uptake, whereas excessive precipitation may cause flooding, soil erosion,

nutrient leaching, and increased disease incidence (Farah et al., 2025; Saleem & Smirnova, 2026; Xu et al., 2024). Importantly, climatic stresses frequently occur simultaneously, and the combined effects of heat and drought generally result in greater yield reductions than either stress alone (Janni et al., 2024).

Atmospheric CO_2 enrichment may stimulate photosynthesis and biomass production, particularly in C3 species, by reducing photorespiration and enhancing carbon assimilation. However, these potential benefits are often constrained by elevated temperatures, water scarcity, and nutrient limitations. Furthermore, increasing CO_2 concentrations may reduce the nutritional quality of agricultural products through changes in biochemical composition and nutrient content (Adireddy et al., 2024).

In addition to gradual climatic shifts, the increasing frequency and intensity of extreme weather events including heat waves, frost, hailstorms, and severe storms pose major threats to agricultural productivity and stability. These events can directly reduce crop yield and quality while also promoting the spread of pests, pathogens, and invasive species, thereby increasing production risks and vulnerability (Alotaibi, 2023; Nóia Júnior et al., 2025). A recent synthesis of approximately 230 studies demonstrated that even a 1°C increase in temperature is associated with measurable yield reductions in major crops such as maize, wheat, and soybean, highlighting the sensitivity of agricultural systems to climate variability and extremes (Hu et al., 2024).

Beyond biophysical impacts, the climate crisis also has significant socioeconomic consequences. Reduced agricultural productivity can increase food prices, disrupt supply chains, and exacerbate food insecurity, particularly in developing regions (Hultgren et al., 2025). Therefore, understanding the interactions between climate and crop production requires holistic and interdisciplinary approaches that integrate environmental, agronomic, and socioeconomic dimensions. Such approaches are essential for developing resilient agricultural systems capable of maintaining productivity, sustainability, and food security under increasingly uncertain climatic conditions (Abebaw, 2025; Dietrich et al., 2025; Liaqat et al., 2022).

2. TEMPERATURE INCREASE

Crop production is highly sensitive to temperature, which regulates plant growth, enzymatic activity, carbon metabolism, reproductive development, and yield. The effects of global warming on agriculture are driven not only by rising mean temperatures but also by the increasing frequency and severity of heat stress events. Exposure to high temperatures during critical developmental stages can disrupt metabolic processes, induce oxidative damage, and impair growth and reproduction, ultimately reducing crop productivity and yield (A. Kumar et al., 2025; Tran et al., 2025).

2.1 Physiological Responses of Plants to Heat Stress

Heat stress occurs when temperatures exceed the optimal range for plant growth, negatively affecting productivity and performance. Elevated temperatures disrupt membrane stability, damage proteins, promote reactive oxygen species (ROS) accumulation, and accelerate chlorophyll degradation and senescence, ultimately reducing yield potential. Plants respond through mechanisms such as heat shock protein (HSP) synthesis, antioxidant defenses, osmotic adjustment, and hormonal signaling. However, these responses vary among species, genotypes, and developmental stages, leading to differences in heat tolerance across crops (A. Kumar et al., 2025; Samat et al., 2025).

The effects of heat stress extend beyond leaf-level processes to plant development, reproduction, and yield. High temperatures disrupt plant water relations and stomatal function; while transpiration may initially increase, prolonged heat stress often causes stomatal closure, limiting carbon dioxide uptake and photosynthesis. Consequently, plant growth and productivity decline. Evidence indicates that recurrent heat stress is generally more damaging than a single short-term event. In sorghum, repeated heat stress during flowering and post-flowering stages significantly reduced grain number and thousand-grain weight, resulting in progressively greater yield losses (Berger et al., 2025).

2.2 Effects of Rising Temperature on Photosynthesis and Respiration

Elevated temperatures reduce photosynthetic carbon assimilation, the physiological basis of crop productivity. Heat stress increases the sensitivity of photosystem II (PSII), disrupts thylakoid membranes, reduces chlorophyll stability, and decreases carbon fixation through lower Rubisco activity. Stomatal closure under high temperatures further limits intercellular CO_2 availability and photosynthesis. Consequently, heat stress impairs both the structure and function of the photosynthetic apparatus, reducing carbon acquisition and biomass production (A. Kumar et al., 2025; Sher et al., 2024).

The effects of rising temperature on respiration are often more complex than those on photosynthesis. Elevated temperatures generally increase mitochondrial respiration, causing a larger proportion of photosynthetically fixed carbon to be used for maintenance and stress-response processes. Studies indicate that 30–60% of assimilated carbon may be lost through respiration, depending on species, developmental stage, and environmental conditions. As a result, higher respiratory costs reduce the carbon available for growth, biomass accumulation, and grain filling, thereby limiting crop productivity under warming conditions (Amthor, 2025).

Rising temperatures impose a dual constraint on plant carbon balance by reducing photosynthetic carbon gain while increasing respiratory carbon

losses. This imbalance weakens source–sink relationships, limiting dry matter accumulation and assimilate transport to reproductive organs, ultimately reducing growth and yield. These negative effects are often intensified when heat stress occurs together with drought, further restricting carbon assimilation and increasing yield losses (A. Kumar et al., 2025; Sher et al., 2024).

2.3 Effects of Rising Temperature on Flowering and Yield

The reproductive stage is among the most heat-sensitive phases of the plant life cycle. Elevated temperatures during flowering can impair pollen viability, fertilization, and grain set, making reproductive failure a major cause of yield loss. Studies show that heat stress during flowering and grain filling can induce pollen sterility, increase flower abortion, damage ovule development, and reduce grain-setting rates, ultimately lowering crop productivity and yield (A. Kumar et al., 2025; Sher et al., 2024).

Experimental studies have demonstrated the negative effects of heat-induced reproductive disturbances on crop yield. In chickpea, heat stress accelerated flowering by approximately four days and shortened maturity by nearly twenty-four days, resulting in an average yield reduction of 34.7%. Significant declines in biomass production and yield components were also observed, indicating that elevated temperatures accelerate phenological development while reducing yield potential (Jha et al., 2025).

In cotton, heat stress reduced pollen germination by 71%, while seed cotton and fiber yields declined by 19% and 26%, respectively. These reductions were accompanied by decreases in chlorophyll index and other morphophysiological disturbances, indicating that heat stress during flowering impairs both reproductive processes and assimilate production required for yield formation. Thus, the effects of high temperature extend beyond fertilization failure to the physiological processes underlying crop productivity (Bista et al., 2025).

Large-scale analyses confirm the negative effects of warming on crop yield, particularly when critical temperature thresholds are exceeded. Tran et al. (2025) reported that in wheat, each 1°C temperature increase reduced yield by about 6.1% below the threshold, with losses rising to 8.2% beyond it. Similar responses were observed in rice, where productivity declines intensified at higher temperatures. These findings highlight the vulnerability of sensitive stages such as flowering and grain set to heat stress and underscore the risks that continued warming poses to crop productivity and global food security (Tran et al., 2025).

In conclusion, rising temperatures are among the most important climatic pressures affecting crop production. Heat stress causes oxidative damage, disrupts cellular functions, and alters the balance between photosynthesis

and respiration, thereby reducing carbon availability for growth and yield formation. Its effects are particularly severe during reproductive stages, where declines in pollen viability, fertilization success, and grain set can result in substantial yield losses. Although responses vary among species and developmental stages, current evidence indicates that increasing temperatures pose a serious threat to crop productivity, especially when heat stress coincides with critical phenological periods (Amthor, 2025; A. Kumar et al., 2025; Sher et al., 2024; Tran et al., 2025).

3. WATER STRESS AND DROUGHT

Water stress and drought are among the most important environmental pressures affecting crop production under the climate crisis. Water is essential for maintaining cell turgor, nutrient transport, stomatal regulation, photosynthesis, growth, and reproductive development; therefore, reduced water availability directly limits agricultural productivity. Recent studies emphasize that drought is a multidimensional stress influenced not only by precipitation deficits but also by soil water-holding capacity, evapotranspiration, root-zone moisture, irrigation availability, and the resilience of production systems. Consequently, its effects on crop performance are shaped by the interaction of climatic, soil, hydrological, and management factors.

3.1 Water Stress

Water stress occurs when water availability in the root zone is insufficient to meet plant transpiration and metabolic demands. Early responses include declines in cellular water potential and turgor, followed by adaptive mechanisms such as stomatal closure, osmotic adjustment, changes in root architecture, activation of antioxidant defenses, and abscisic acid (ABA)-mediated signaling. Water stress causes not only dehydration but also osmotic stress, oxidative damage, membrane impairment, and disruption of the photosynthetic apparatus. These responses help plants reduce water loss, maintain cellular homeostasis, and sustain metabolic activity under limited water availability (Haghpanah et al., 2024).

Plant responses to drought are commonly classified as drought avoidance, drought tolerance, and drought escape. Avoidance mechanisms help maintain plant water status through traits such as deeper root systems, improved stomatal regulation, and reduced water loss. Tolerance mechanisms involve physiological and biochemical adjustments, including osmotic regulation, accumulation of compatible solutes, activation of reactive oxygen species (ROS) scavenging systems, and synthesis of protective proteins. These responses are closely associated with improved water-use efficiency and the maintenance of metabolic activity under drought conditions. However, their effectiveness varies among species, genotypes, and developmental stages, resulting in differences in drought resilience across crop systems (Franco-Navarro et al., 2025;

Haghpanah et al., 2024).

Plant responses to water stress vary among species and developmental stages. Drought occurring during critical phases such as flowering and grain filling generally causes greater yield losses than stress during vegetative growth. Accelerated leaf senescence, reduced photosynthetically active leaf area, and limited assimilate translocation during reproductive development restrict the resources available for grain formation and yield accumulation. In maize, drought stress during flowering accelerated leaf senescence, whereas effective water management helped maintain photosynthetically active leaf area and dry matter translocation, thereby improving yield under water-limited conditions (Yan et al., 2025).

3.2 Drought

Drought is a multidimensional phenomenon affecting climate, soil, plants, and water resources, and cannot be explained solely by reduced precipitation. It is commonly classified as meteorological, agricultural, and hydrological drought. Meteorological drought results from prolonged precipitation deficits, agricultural drought arises when soil moisture becomes insufficient for crop growth, and hydrological drought is characterized by reduced water availability in surface and groundwater resources. These forms of drought are closely interconnected and often develop sequentially, with meteorological drought progressing into agricultural and eventually hydrological drought, thereby intensifying impacts on agricultural and environmental systems (Disasa et al., 2026).

An emerging focus in drought research is the concept of flash droughts, which develop rapidly when high evaporative demand, heat waves, and sharp declines in soil moisture occur simultaneously. These conditions can quickly intensify plant water deficits, leaving little time for adaptation or management responses. Consequently, drought is increasingly viewed not simply as a precipitation deficit but as a dynamic process driven by interactions among rainfall, atmospheric demand, soil moisture, and the balance between water supply and demand. This perspective provides a more comprehensive understanding of drought impacts on agricultural systems under changing climatic conditions (Disasa et al., 2026).

3.3 Impacts of Water Stress and Drought on Crop Production

The most evident effect of drought on crop production is yield reduction, resulting from multiple physiological constraints rather than a single mechanism. Water deficits restrict leaf expansion and stomatal conductance, reducing photosynthesis, biomass accumulation, and reproductive development. Yield losses generally increase with the severity and duration of drought and depend strongly on the phenological stage affected. A review of Brassica crops

reported that drought can cause yield reductions ranging from moderate to severe levels; in canola, annual losses may exceed 30%, highlighting the vulnerability of oilseed crops to water scarcity (Mohan et al., 2025).

One of the earliest plant responses to drought is stomatal closure, which reduces water loss but also limits carbon dioxide uptake and photosynthesis. As a result, carbon assimilation, biomass production, leaf expansion, and shoot growth decline. Drought further impairs photosynthesis through reductions in chlorophyll content, photosystem II (PSII) efficiency, electron transport, and increased reactive oxygen species (ROS) accumulation. Together, these changes reduce photosynthetic capacity and contribute to lower growth and productivity under water-limited conditions (Dietz et al., 2021; Qiao et al., 2024).

The impact of drought on yield depends not only on its severity but also on the developmental stage at which it occurs (Dietz et al., 2021). During vegetative growth, water deficits mainly reduce leaf expansion and biomass accumulation, whereas drought during reproductive stages impairs flowering, fertilization, grain set, grain filling, and fruit development (Dietz et al., 2021; Qiao et al., 2024). In cereals, these effects often reduce grain number, grain weight, and harvest index, leading to substantial yield losses. The impacts become even greater when drought coincides with high temperatures, as combined stress shortens crop life cycles and causes larger productivity reductions than either stress alone (Cohen et al., 2021).

The effects of drought on yield vary according to the harvested plant organ. In leafy vegetables, water stress reduces leaf expansion and fresh biomass production, whereas in root and tuber crops it limits assimilate accumulation in storage organs. In fruit- and seed-producing crops, drought can impair fertilization, fruit set, grain filling, and product quality. Therefore, drought impacts should be evaluated not only in terms of biomass reduction but also through their effects on marketable yield and quality, which ultimately determine the economic value of crops (Dietz et al., 2021).

Under water-limited conditions, plants develop adaptive responses such as deeper rooting, increased root-to-shoot ratios, reduced leaf area, and osmolyte accumulation to maintain water balance. However, these adaptations often involve trade-offs with growth and productivity. For example, reduced transpiration conserves water but also limits carbon dioxide uptake and carbon assimilation, lowering yield potential (Qiao et al., 2024). Therefore, drought tolerance should be evaluated not only by plant survival but also by the ability to maintain growth and productivity under water stress (Dietz et al., 2021).

In summary, water stress and drought reduce crop productivity through interconnected physiological, morphological, and biochemical mechanisms. Yield losses are primarily associated with stomatal closure, reduced photosynthesis, oxidative stress, impaired nutrient uptake, and disruptions in reproductive processes such as flowering and grain filling (Dietz et al., 2021; Qiao et al., 2024). The severity of these effects depends on crop species, developmental stage, stress duration, and environmental conditions, with reproductive-stage drought generally causing the greatest economic losses (Cohen et al., 2021; Dietz et al., 2021). Therefore, drought-tolerant cultivars, efficient irrigation, soil moisture conservation, and improved water-use efficiency are critical for sustaining crop production under climate change (Dietz et al., 2021; Wang et al., 2024).

4. CHANGES IN PRECIPITATION REGIMES

Climate change affects precipitation regimes not only through changes in total rainfall but also through shifts in the timing, frequency, intensity, duration, and seasonal distribution of precipitation events. Rainfall patterns are becoming increasingly irregular, particularly in monsoon-dependent and rainfed agricultural systems, directly influencing key stages such as sowing, emergence, vegetative growth, and grain filling. In addition, the concentration of rainfall into shorter periods can reduce the effective availability of water for crops even when annual precipitation remains unchanged. Therefore, precipitation regimes should be evaluated not only by total rainfall amounts but also by their timing, intensity, and seasonal distribution, which are critical determinants of agricultural productivity and water availability (Deng et al., 2025).

The agricultural value of precipitation depends not only on its amount but also on its timing and intensity. High-intensity rainfall over short periods can reduce soil water storage and plant water availability, while prolonged dry intervals increase root-zone moisture deficits. Consequently, crop productivity may decline even when annual rainfall remains unchanged if precipitation becomes more irregular and concentrated. This effect is especially important in rainfed systems, where yield depends largely on the coincidence of rainfall with critical phenological stages. Therefore, rainfall timing and distribution are often as important as total precipitation in determining crop productivity (Deng et al., 2025).

One of the major agricultural consequences of increasing precipitation intensity is the greater occurrence of flooding and waterlogging. Flooded soils restrict oxygen diffusion, causing hypoxia or, under prolonged conditions, anoxia in the root zone. These conditions inhibit root respiration, reduce nutrient uptake, and trigger physiological responses such as ethylene accumulation, fermentative metabolism, reactive oxygen species (ROS) production,

reduced photosynthesis, and changes in root architecture. Together, these effects suppress crop growth and yield. Thus, the impacts of flooding arise not only from excess water but also from disruptions in plant–soil–atmosphere interactions (Zhang et al., 2025).

Regional studies indicate that the effects of flooding and waterlogging on crop production vary with crop species, developmental stage, and inundation duration. In the Middle and Lower Yangtze River Basin, flood events caused greater yield losses when they occurred during intermediate and late growth stages, and the relationship between flood intensity and yield reduction was consistently observed across different meteorological indicators (Meng et al., 2024). Similarly, soybean studies showed that temporary waterlogging reduced root volume, biomass accumulation, leaf area, and grain yield, with impacts differing according to the developmental stage at which flooding occurred (Gangana Gowdra et al., 2025).These findings highlight the importance of considering both flood characteristics and crop phenology when assessing flood risk.

Soil moisture is a key mechanism linking changes in precipitation regimes to crop production. The agricultural value of rainfall depends not only on the amount received but also on how much water is retained in the root zone and remains available to plants. Soil moisture is widely recognized as an important indicator for agricultural sustainability, irrigation management, and drought monitoring. However, it is influenced not only by precipitation but also by soil texture, organic matter, topography, vegetation cover, temperature, atmospheric evaporative demand, and antecedent moisture conditions. Consequently, similar rainfall events can produce very different soil moisture responses depending on soil characteristics and seasonal conditions (Teixeira et al., 2025).

The importance of soil moisture extends beyond drought and waterlogging to maintaining suitable conditions throughout the growing season. Excessive rainfall can cause prolonged soil saturation and reduced root-zone aeration, whereas extended dry periods can rapidly create moisture deficits. As precipitation regimes become more irregular, soil moisture variability increases, affecting crop performance differently across developmental stages. Climate projections indicate that changes in the timing and intensity of rainfall may alter the occurrence and seasonal distribution of agricultural stress events. Therefore, soil moisture dynamics should be evaluated not only through long-term averages but also in relation to the timing, duration, and intensity of events that influence water availability during critical growth stages (Deng et al., 2025).

In conclusion, precipitation regime changes affect crop production through shifts in rainfall distribution and intensity, increased flooding and

waterlogging, and altered soil moisture dynamics. Current evidence shows that total rainfall alone is insufficient to assess agricultural risk; the timing, intensity, and antecedent soil moisture conditions of precipitation are equally important determinants of crop performance. Therefore, adaptation strategies should prioritize improved drainage systems, enhanced soil water-holding capacity, soil moisture monitoring, and planting schedules aligned with crop phenology and changing climatic conditions. These measures are essential for reducing climate-related risks and strengthening the resilience of agricultural production systems (Qi et al., 2025; Teixeira et al., 2025; Zhang et al., 2025).

5. EXTREME CLIMATE EVENTS

5.1 Heat Waves

Among extreme climate events, heat waves are among the most important stressors affecting crop production. Unlike gradual warming, they involve rapid exceedance of critical temperature thresholds and are often accompanied by high atmospheric water demand, causing severe physiological stress. Studies indicate that warming increases not only yield losses but also inter annual yield variability, while extreme heat events, particularly when combined with water scarcity, can greatly intensify crop damage. These impacts are especially severe in arid and semi-arid regions, where temperatures frequently exceed crop-specific physiological limits (Proctor et al., 2025; Tran et al., 2025).

The effects of heat waves on crop production depend strongly on their timing within the crop growth cycle. During vegetative growth, extreme temperatures can reduce photosynthesis, disrupt plant water relations, and limit carbon assimilation. However, heat waves occurring during flowering and grain set are generally more damaging because they impair pollen viability, fertilization, and grain filling. Thus, heat stress severity is determined not only by temperature magnitude but also by event duration, recurrence, and coincidence with sensitive phenological stages (Ullah et al., 2024). In addition, yield losses in major crops increase rapidly once critical thermal thresholds are exceeded, highlighting the growing risks posed by extreme heat under future climate scenarios (Tran et al., 2025).

Regional and multi-hazard studies indicate that heat waves are becoming an increasingly important source of agricultural risk. A European assessment showed that temperature-related hazards during the growing season increased substantially in 2011–2020 compared with 1981–1990, reflecting the growing influence of extreme heat on agricultural systems. The study also emphasized that heat waves should be evaluated together with drought and other extreme climate events, as compound stresses often cause greater crop

losses than individual hazards acting alone. Therefore, a multi-risk perspective provides a more realistic assessment of agricultural vulnerability under climate change (Lakatos & Csabai, 2025).

5.2 Frost, Hailstorms, and Severe Storms

Frost events, particularly late spring frosts, remain an important agricultural risk despite rising average temperatures. Frost damage depends not only on frost frequency but also on temperature variability, event duration, timing, and crop developmental stage. A global study identified temperature variability as a key factor explaining frost-related yield losses in crops such as maize and winter wheat, indicating that late frosts may continue to cause significant damage under climate warming (Guo et al., 2024). Similarly, a recent review reported that late spring frosts can severely reduce yields in cool-season cereals, especially when they occur during flowering and early grain-filling stages, with damage largely determined by the timing, intensity, and duration of the event (Richetti et al., 2025).

Hail causes agricultural losses mainly through direct physical damage, including reductions in leaf area, injury to stems and reproductive organs, and loss of photosynthetically active tissue, all of which reduce carbon assimilation (Dąbrowski et al., 2024). A study in Switzerland showed that hail damage in field crops and vineyards can be effectively assessed using radar-based approaches and varies according to crop type and production system characteristics (Portmann et al., 2024). In winter oilseed rape, simulated hail events reduced yield by 53.9% and significantly decreased CO_2 assimilation, stomatal conductance, transpiration, and photosystem II (PSII) efficiency (Dąbrowski et al., 2024). These findings indicate that hail not only causes mechanical injury but also disrupts photosynthetic processes and induces physiological stress that further reduces crop productivity (Dąbrowski et al., 2024; Portmann et al., 2024).

Storm events, particularly tropical cyclones and intense wind systems, affect crop production through stem breakage, lodging, leaf and fruit loss, flooding, and reductions in product quality. A study from the coastal southern United States showed that tropical cyclones can significantly reduce crop performance, especially when they occur late in the growing season, with major hurricanes causing average yield losses of about 6%. The study also demonstrated that agricultural risk depends not only on wind intensity but also on event timing and antecedent soil moisture conditions. Therefore, storms should be considered compound hazards arising from the interaction of strong winds, heavy rainfall, and flooding, which together can substantially reduce crop productivity and sustainability (Bundy et al., 2023).

6. EFFECTS OF CLIMATE CRISIS ON SOIL PROPERTIES AND FUNCTIONS

6.1 Soil Moisture and Soil Organic Matter

The climate crisis alters soil functions by affecting both soil moisture dynamics and organic matter cycling. Rising temperatures increase evapotranspiration, while irregular precipitation and more frequent droughts intensify soil moisture deficits, particularly in surface layers. These changes influence soil carbon cycling and may reduce the long-term stability of soil organic carbon stocks. Studies using Earth System Models indicate that warming can accelerate soil carbon losses, strengthening climate carbon feedbacks (Ren et al., 2024). In addition, declining soil organic carbon reduces water-holding capacity, further increasing moisture loss and drought vulnerability (McDermid et al., 2022). Therefore, soil carbon depletion should be considered both a carbon-cycle issue and a feedback mechanism that amplifies climate change impacts on agricultural and ecosystem functioning.

Long-term warming experiments indicate that soil organic carbon stocks can decline substantially, particularly in surface soils. Mineral-associated organic carbon (MAOC), a relatively stable and important component of long-term carbon sequestration, appears to be especially vulnerable to warming. Chen et al. (2023) showed that long-term warming reduced surface soil organic carbon largely through losses from the MAOC pool. Likewise, Chen et al. (2024) reported significant soil carbon emissions under whole-profile warming treatments in alpine grasslands. These findings suggest that rising temperatures affect not only labile organic matter fractions but also stable soil carbon reservoirs previously considered resistant to environmental change (Chen et al., 2023, 2024).

Changes in soil moisture strongly influence organic matter dynamics by affecting water availability and microbial activity. Drought and altered precipitation regimes can modify microbial growth, carbon use efficiency (CUE), and decomposition rates, thereby affecting soil carbon cycling. Microbial responses largely determine whether organic carbon is retained in biomass or released to the atmosphere. Consequently, under climate crisis conditions, soil organic matter decomposition and storage are regulated by complex interactions among ecosystem characteristics, soil properties, carbon stocks, and environmental conditions, resulting in highly variable responses across regions and climates (He et al., 2024).

The interaction between vegetation cover and soil moisture is a key mechanism influencing soil stability and ecosystem functioning. A study in a high-latitude grassland ecosystem found that spatial variations in soil moisture and vegetation water stress were closely linked to erosion patterns. Areas with water-stressed vegetation were more susceptible to soil erosion, resulting

in greater spatial variability in erosion risk. These findings indicate that the conservation of organic matter-rich topsoil depends not only on precipitation but also on vegetation responses to water stress and local soil moisture conditions. Therefore, interactions among vegetation dynamics, soil moisture, and erosion processes are critical for maintaining soil resilience under changing climatic conditions (Cutler et al., 2023).

6.2 Soil Erosion and Desertification

One of the most evident impacts of the climate crisis on soils is the acceleration of water and wind erosion. Assessments suggest that erosion risk will increase in many regions during the twenty-first century, particularly in semi-arid and Mediterranean climates. More frequent and intense rainfall events enhance surface runoff and water erosion, while drought-induced declines in vegetation cover reduce soil protection and increase susceptibility to erosion. Consequently, climate change is expected to intensify water erosion and simultaneously elevate wind erosion risk in dry regions where vegetation becomes sparse and soils are less protected (Eekhout & de Vente, 2022).

Climate change–driven erosion and soil degradation can substantially reduce soil quality through the loss of organic matter-rich topsoil. This process decreases soil organic carbon stocks and weakens soil structure, limiting essential soil functions over time. A study in mountain grassland ecosystems showed that simulated climate change accelerated both organic carbon losses and topsoil structural degradation. Reductions in large macroaggregates weakened the physical protection of organic carbon and reduced soil structural stability (Garcia-Franco et al., 2024). These findings indicate that climate change threatens not only soil carbon storage but also the structural mechanisms that stabilize and protect soil carbon, increasing the vulnerability of soils to further degradation.

Desertification is a multifaceted land degradation process involving not only soil erosion but also declines in soil quality, vegetation cover, and ecosystem functioning. Liu et al. (2024) showed that increasing desertification severity in semi-arid alpine regions significantly reduced soil health stability and was associated with losses of soil organic matter and biological functions. The study demonstrated that desertification extends beyond vegetation loss, disrupting nutrient cycling, weakening soil biological processes, and reducing ecosystem resilience. Therefore, climate change–driven aridification and rising temperatures may lead to long-term declines in soil productivity and ecosystem functioning, particularly in semi-arid and dry sub-humid regions that are already close to critical environmental thresholds (Liu et al., 2024).

Large-scale observational studies show that declining precipitation and rising temperatures can substantially accelerate desertification. Research in the arid regions of China demonstrated that drought and heat stress promote

the expansion of barren lands, increasing land degradation risk, particularly in arid and semi-arid environments. Huang and Zhai (2023) further showed that desertification is driven not only by land-use change but also by climate-related factors such as warming and increasing water deficits. These findings emphasize that desertification is a climate-sensitive process shaped by the interaction of environmental and anthropogenic drivers, with reduced water availability and rising temperatures acting as major accelerators of land degradation (Huang & Zhai, 2023).

6.3 Soil Salinization and Land Degradation

The link between climate change and soil salinity is becoming increasingly evident, particularly in arid, semi-arid, and coastal agricultural regions. Rising temperatures increase evapotranspiration, promoting salt accumulation in the soil surface and root zone, while altered precipitation patterns and reduced water availability limit salt leaching. In coastal areas, sea-level rise and saltwater intrusion further increase salinization risks. Corwin (2021) emphasized that climate change enhances soil salinity through multiple pathways, with the greatest vulnerability occurring in regions where water resources are already limited (Corwin, 2021).

A comprehensive review suggests that climate change may not only worsen existing salinity problems but also expose currently unaffected agricultural lands to future salinization. Rising temperatures, altered precipitation patterns, increasing pressure on water resources, and sea-level rise have been identified as major drivers of salinity development. The impacts of salinity extend beyond plant osmotic stress and ion toxicity; sodium accumulation can disperse soil aggregates, reduce infiltration and hydraulic conductivity, and degrade soil structure. Therefore, soil salinity should be viewed as a multidimensional land degradation process affecting the physical, chemical, and biological functions of soils under changing climatic conditions (Mukhopadhyay et al., 2021).

Recent modeling studies indicate that climate change–driven aridification and rising evapotranspiration may significantly increase soil salinization risks, particularly in water-limited regions. Kramer et al. (2025) showed that higher evapotranspiration promotes salt accumulation in soils and increases salinity-related land degradation. The study also reported that salinity can impair soil hydraulic properties and overall soil functioning. These findings suggest that climate change may accelerate secondary salinization in irrigated areas, leading to soil structural deterioration, reduced functionality, and lower agricultural productivity over time (Kramer et al., 2025).

Soil degradation affects not only the physical and chemical properties of soils but also their biological functions. Increasing aridification and desertification can alter habitats that support soil organisms, weakening key

ecosystem processes. Zheng et al. (2024) showed that desertification primarily affects soil fauna indirectly by reducing habitat complexity and the diversity of available energy resources. As desertification intensifies, the conditions required to sustain soil biota progressively deteriorate, reducing ecosystem resilience and impairing the biological processes essential for long-term soil health and productivity (Zheng et al., 2024).

From a soil-system perspective, the climate crisis triggers multiple interconnected degradation processes. First, rising temperatures and altered precipitation regimes disrupt soil moisture dynamics, affecting organic matter decomposition, microbial activity, and carbon sequestration, thereby weakening soil physical, chemical, and biological functions. Second, more frequent droughts, extreme rainfall events, and vegetation degradation increase erosion and desertification risks, accelerating topsoil loss and reducing soil productivity. Third, increasing evapotranspiration, water scarcity, and changing hydrological conditions elevate the risks of salinity and sodicity, further intensifying soil degradation. Importantly, these processes do not act independently but are connected through reinforcing feedbacks. Declining soil organic carbon reduces water-holding capacity, while lower soil moisture increases vulnerability to erosion and salinization. Rising salinity further impairs aggregate stability, hydraulic properties, and biological activity, accelerating soil degradation. Therefore, the climate crisis should be viewed as an integrated land degradation process affecting soil structure, carbon cycling, water regulation, and biological functioning, with important consequences for agricultural sustainability and ecosystem resilience (Corwin, 2021; Eekhout & de Vente, 2022; Kramer et al., 2025; McDermid et al., 2022).

7. RISING ATMOSPHERIC CO_2 CONCENTRATIONS AND PLANT RESPONSES

Rising atmospheric CO_2 concentrations are both a major driver of the climate crisis and an important factor influencing crop production. As the primary substrate for photosynthesis, elevated CO_2 can affect plant growth, biomass accumulation, yield formation, and water-use efficiency. However, plant responses vary considerably among species and depend on growing conditions and interactions with other environmental factors. Therefore, the effects of elevated CO_2 on crop performance are determined by complex physiological, ecological, and management-related interactions (Toreti et al., 2020).

One of the most important effects of rising atmospheric CO_2 is the stimulation of photosynthetic carbon assimilation. Elevated CO_2 generally enhances photosynthesis in C_3 plants, increasing biomass production and often improving yield through greater carbon fixation and reduced photorespiration. However, the magnitude of this CO_2 fertilization effect varies among species

and depends on environmental conditions, management practices, and plant physiological characteristics. Consequently, the benefits of elevated CO_2 are not expressed uniformly across all crops and production systems (da Silva Fortirer et al., 2023; Toreti et al., 2020).

Elevated atmospheric CO_2 also influences plant water-use characteristics. Many species exhibit reduced transpiration while maintaining or increasing photosynthetic carbon uptake, resulting in improved water-use efficiency. This response is largely driven by stomatal adjustments that reduce water loss without substantially limiting carbon assimilation. A recent meta-analysis reported significant increases in water-use efficiency under elevated CO_2, particularly in C_3 crops, although the magnitude of the response varied among crop types and environmental conditions. These findings suggest that elevated CO_2 may partially alleviate water limitation by enabling more efficient use of available water (Mokhtar et al., 2025).

The effects of elevated CO_2 extend beyond leaf physiology to root growth and architecture. Increased carbon assimilation can enhance root biomass, improve water and nutrient acquisition, and contribute to belowground carbon sequestration. These changes may increase plant resilience under resource-limited conditions and influence soil–plant interactions. However, root responses vary substantially among species, and the mechanisms involved remain only partly understood. Consequently, predicting belowground responses to rising atmospheric CO_2 remains a significant challenge in plant and ecosystem research (Bach & Gojon, 2023).

Although elevated CO_2 can stimulate plant growth and biomass production, these benefits do not necessarily improve crop quality. Studies have shown that higher atmospheric CO_2 concentrations may reduce the levels of proteins, mineral nutrients, and certain vitamins in many crop species. This is particularly concerning for staple crops such as wheat, where declines in protein and micronutrient content may threaten food security and nutritional adequacy, especially in populations dependent on cereal-based diets. These findings emphasize that the impacts of rising CO_2 should be assessed not only in terms of yield but also in relation to food quality and nutritional value (Ekele et al., 2025).

The relationship between yield gains and nutritional quality under elevated CO_2 has become a major research focus. Studies on wheat show that higher atmospheric CO_2 can alter grain composition, often reducing protein and essential nutrient concentrations. These findings indicate that increases in crop productivity may be accompanied by declines in nutritional quality, creating a trade-off between quantity and quality. Therefore, assessments of agricultural performance under climate change should consider not only yield responses but also changes in the nutritional composition of harvested

products, which are critical for food security and human nutrition (Ekele et al., 2025; Hay et al., 2022).

The effects of elevated atmospheric CO_2 cannot be evaluated independently of other climate change factors. Potential benefits of CO_2 fertilization are often limited by rising temperatures, water scarcity, and nutrient constraints. Many studies show that the positive effects of elevated CO_2 on crop growth and yield can be greatly reduced under heat and drought stress, and may even disappear under severe conditions. Therefore, future crop performance will depend not only on increasing CO_2 concentrations but also on their interactions with other environmental stressors. Understanding these interactions is essential for accurately projecting agricultural productivity under future climate scenarios (Toreti et al., 2020).

In conclusion, rising atmospheric CO_2 can enhance photosynthesis, biomass production, root development, and water-use efficiency. However, these responses vary among species and production systems, and potential productivity gains may be accompanied by declines in crop quality and nutritional value. Therefore, the impacts of elevated CO_2 should be evaluated not only in terms of yield but also with respect to product quality, nutritional composition, and interactions with other climate-related stressors. Such an integrated perspective is essential for assessing the future implications of CO_2 enrichment for agricultural productivity, food quality, and food security under climate change (Bach & Gojon, 2023; Ekele et al., 2025; Mokhtar et al., 2025; Toreti et al., 2020).

8. PLANT DISEASES, INSECT PESTS, AND WEED DYNAMICS

The climate crisis is significantly altering the distribution, severity, and impacts of plant diseases in agricultural systems. Rising temperatures, changing precipitation patterns, elevated atmospheric CO_2, and more frequent extreme events affect pathogen life cycles, host–pathogen interactions, and disease pressure. As a result, some pathogens are expanding into new regions, infection risks are increasing, and novel disease challenges are emerging. These changes have important consequences for crop productivity, disease management, and the sustainability of food production under changing climatic conditions (Singh et al., 2023).

8.1 Plant Diseases

Plant diseases are among the biotic stresses most sensitive to climate change. Because temperature and moisture directly affect pathogen survival, reproduction, infection, and dispersal, climatic shifts can substantially alter disease epidemiology. Studies show that global warming has expanded the ranges of many fungal, bacterial, and viral pathogens, prolonged favorable infection periods, and increased disease incidence in numerous agricultural regions. Climate change may also modify host–pathogen interactions and ac-

celerate pathogen adaptation, increasing the risk of new variants and more severe disease outbreaks. Consequently, disease pressure is expected to intensify in agricultural systems, creating growing challenges for crop protection and food security (Singh et al., 2023).

Climate-related stressors affect not only pathogens but also host plants. Drought, heat waves, flooding, and extreme temperature fluctuations can alter plant physiology and defense mechanisms, influencing susceptibility to disease. These changes may increase host vulnerability while creating more favorable conditions for pathogen infection, survival, and spread. Consequently, climate change simultaneously modifies interactions among the environment, host plants, and pathogens, often leading to shifts in disease dynamics and increased disease risk (Singh et al., 2023).

Global modeling studies suggest that climate change may substantially alter plant pathogen infection risks. Rising temperatures are expected to increase climatic suitability for many pathogens in higher-latitude regions, raising the likelihood of disease outbreaks, while some lower-latitude areas may become less favorable for certain pathogens. As a result, disease risks are likely to be redistributed geographically. Studies further show that changes in pathogen infection risk are closely associated with crop yield patterns, indicating that climate-driven shifts in disease pressure may have important implications for agricultural productivity and food security (Chaloner et al., 2021).

8.2 Insect Pest Dynamics

The climate crisis is significantly affecting the biology and ecology of agricultural pests. As ectothermic organisms, insects respond strongly to temperature increases, which can accelerate development, enhance reproduction, and increase the number of generations produced within a growing season. These changes promote faster population growth and enable pests to reach damaging levels more rapidly. In addition, milder winters improve overwintering survival, leading to higher population densities and greater pest pressure. Consequently, climate change is expected to increase the complexity and severity of pest management challenges in agricultural systems (Subedi et al., 2023).

Rising temperatures are reshaping the geographic distribution of insect pests, enabling many species to expand toward higher latitudes and elevations. This increases the risk of pest outbreaks in regions where they were previously of limited economic importance. Climate change can also disrupt the synchrony between pests and their natural enemies, reducing the effectiveness of biological control and favoring pest proliferation. Consequently, global warming is expected to increase pest-related yield losses by enhancing pest survival, reproduction, and dispersal across diverse agroecosystems (Eigenbrode & Adhikari, 2023; Subedi et al., 2023).

8.3 Weed Dynamics

Weeds possess high genetic diversity and phenotypic plasticity, allowing them to adapt readily to changing climatic conditions. Elevated CO_2 can increase photosynthesis, biomass production, and seed output in many weed species, enhancing their competition for water, light, and nutrients. As a result, weed interference with crop growth and associated yield losses may increase. Evidence suggests that many C_3 weed species benefit disproportionately from rising CO_2 concentrations, potentially gaining a competitive advantage over cultivated crops and intensifying weed management challenges under future climate conditions (V. Kumar et al., 2023).

Climate change can alter weed community composition and the dominance of species within agroecosystems. Changes in temperature, CO_2 concentration, and precipitation patterns may favor certain weeds, reshaping population dynamics and reducing the effectiveness of existing management strategies. In addition, environmental changes can affect herbicide uptake, translocation, and metabolism, potentially decreasing weed control efficacy. These shifts indicate that weed management practices will need to adapt to maintain effective control and minimize crop yield losses under future climate conditions (V. Kumar et al., 2023).

In conclusion, the climate crisis is intensifying biotic stresses in crop production by altering the distribution, abundance, and damage potential of plant diseases, pests, and weeds. The expansion of pathogens into new regions, increasing pest populations, and greater weed competitiveness threaten crop yield, product quality, and production stability. As climatic conditions continue to change, these challenges are likely to become more severe and complex. Therefore, sustainable crop production will depend on climate-informed monitoring systems, early-warning tools, resistant crop varieties, and strengthened integrated management strategies for pests, diseases, and weeds. These approaches are essential for reducing climate-related biological risks and enhancing agricultural resilience (V. Kumar et al., 2023; Singh et al., 2023; Subedi et al., 2023).

9. CONCLUSION

The climate crisis has emerged as one of the most significant threats to crop production systems worldwide. Rising temperatures, altered precipitation regimes, increasing drought severity, more frequent extreme climate events, and elevated atmospheric CO_2 concentrations are reshaping the environmental conditions under which agricultural production occurs. These changes affect not only plant growth and productivity but also crop quality, soil health, water resources, and the overall functioning of agroecosystems.

Current evidence demonstrates that the impacts of climate change on

crop production cannot be explained by any single environmental factor. Rather, they result from complex interactions among temperature, water availability, soil conditions, atmospheric CO_2 concentrations, and biotic stressors such as diseases, pests, and weeds. While elevated CO_2 may enhance photosynthesis, biomass production, and water-use efficiency, these benefits are often constrained by heat stress, drought, nutrient limitations, and declines in crop nutritional quality. At the same time, soil degradation processes including organic matter loss, erosion, desertification, and salinization together with increasing biotic pressures, further threaten the long-term sustainability of agricultural systems.

Ensuring future food security will require comprehensive adaptation strategies that enhance both productivity and resilience. Priority actions include the development of heat- and drought-tolerant crop varieties, improved water-use efficiency through advanced irrigation technologies, conservation-oriented soil management, climate-smart agricultural practices, and strengthened monitoring and early-warning systems. Integrating climate risks into agricultural planning and decision-making will be essential for reducing vulnerability and improving adaptive capacity.

Overall, the future sustainability of crop production will depend on the successful transformation of agricultural system toward greater resilience, resource-use efficiency, and ecological sustainability. The development and effective implementation of science-based adaptation and mitigation strategies will be critical for maintaining agricultural productivity, sustaining ecosystem services, and ensuring global food security under increasingly uncertain climatic conditions.

REFERENCES

(IPCC), I. P. on C. C. (2023a). *Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. https://doi.org/10.1017/9781009157896

(IPCC), I. P. on C. C. (2023b). *Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. https://doi.org/DOI: 10.1017/9781009325844

Abebaw, S. E. (2025). A Global Review of the Impacts of Climate Change and Variability on Agricultural Productivity and Farmers' Adaptation Strategies. *Food Science & Nutrition*, *13*(5), e70260. https://doi.org/10.1002/fsn3.70260

Adireddy, R. G., Anapalli, S. S., Reddy, K. N., Mubvumba, P., & George, J. (2024). Possible Impacts of Elevated CO2 and Temperature on Growth and Development of Grain Legumes. In *Environments* (Vol. 11, Issue 12, p. 273). https://doi.org/10.3390/environments11120273

Alotaibi, M. (2023). Climate change, its impact on crop production, challenges, and possible solutions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *51*(1 SE-Review Articles), 13020. https://doi.org/10.15835/nbha51113020

Amthor, J. S. (2025). After photosynthesis, what then: Importance of respiration to crop growth and yield. *Field Crops Research*, *321*, 109638. https://doi.org/10.1016/j.fcr.2024.109638

Bach, L., & Gojon, A. (2023). Root system growth and development responses to elevated CO2: underlying signalling mechanisms and role in improving plant CO2 capture and soil C storage. *Biochemical Journal*, *480*(11), 753–771. https://doi.org/10.1042/BCJ20220245

Berger, A., Cakpo, B., Suire, C., Pagan, L., Roques, S., Vial, R., Aguilar, G., Terrier, N., & Granier, C. (2025). Single versus recurrent heat stress: A case study on the impact of a stress at flowering on the sorghum response to a subsequent post-flowering stress. *Environmental and Experimental Botany*, *235*, 106162. https://doi.org/10.1016/j.envexpbot.2025.106162

Bista, M. K., Kodadinne Narayana, N., Chakravaram, A., Pieralisi, B., Dhillon, J., Reddy, K. R., & Bheemanahalli, R. (2025). Intensifying heat stress impacts cotton flowering and boll development efficiency. *BMC Plant Biology*, *25*(1), 984. https://doi.org/10.1186/s12870-025-06934-8

Bundy, L. R., Gensini, V. A., & Broeke, M. S. Van Den. (2023). Tropical cyclone impacts on crop condition ratings and yield in the Coastal Southern United States. *Agricultural and Forest Meteorology*, *340*, 109599. https://doi.org/10.1016/j.agrformet.2023.109599

Chaloner, T. M., Gurr, S. J., & Bebber, D. P. (2021). Plant pathogen infection risk tracks global crop yields under climate change. *Nature Climate Change*, *11*(8), 710–

715. https://doi.org/10.1038/s41558-021-01104-8

Chen, Y., Han, M., Yuan, X., Zhou, H., Zhao, X., Schimel, J. P., & Zhu, B. (2023). Long-term warming reduces surface soil organic carbon by reducing mineral-associated carbon rather than "free" particulate carbon. *Soil Biology and Biochemistry*, *177*, 108905. https://doi.org/10.1016/j.soilbio.2022.108905

Chen, Y., Qin, W., Zhang, Q., Wang, X., Feng, J., Han, M., Hou, Y., Zhao, H., Zhang, Z., He, J.-S., Torn, M. S., & Zhu, B. (2024). Whole-soil warming leads to substantial soil carbon emission in an alpine grassland. *Nature Communications*, *15*(1), 4489. https://doi.org/10.1038/s41467-024-48736-w

Cohen, I., Zandalinas, S. I., Huck, C., Fritschi, F. B., & Mittler, R. (2021). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum*, *171*(1), 66–76. https://doi.org/10.1111/ppl.13203

Corwin, D. L. (2021). Climate change impacts on soil salinity in agricultural areas. *European Journal of Soil Science*, *72*(2), 842–862. https://doi.org/10.1111/ejss.13010

Cutler, N. A., Kodl, G., Streeter, R. T., Thompson, P. I. J., & Dugmore, A. J. (2023). Soil moisture, stressed vegetation and the spatial structure of soil erosion in a high latitude rangeland. *European Journal of Soil Science*, *74*(4), 1–15. https://doi.org/10.1111/ejss.13393

da Silva Fortirer, J., Grandis, A., Pagliuso, D., de Toledo Castanho, C., & Buckeridge, M. S. (2023). Meta-analysis of the responses of tree and herb to elevated CO2 in Brazil. *Scientific Reports*, *13*(1), 15832. https://doi.org/10.1038/s41598-023-40783-5

Dąbrowski, P., Jełowicki, Ł., Jaszczuk, Z. M., Kryvoviaz, O., & Kalaji, H. M. (2024). Photosynthetic Performance and Yield Losses of Winter Rapeseed (Brassica napus L. var. napus) Caused by Simulated Hail. In *Plants* (Vol. 13, Issue 13, p. 1785). https://doi.org/10.3390/plants13131785

Deng, S., Lu, C., Chen, H., Song, X., Chen, T., Yang, N., & Fan, Y. (2025). Rainfall seasonality changes and underlying climatic causes in global land monsoon regions. *Atmospheric Research*, *326*, 108288. https://doi.org/10.1016/j.atmosres.2025.108288

Dietrich, A. S., Carini, V., Vico, G., Bommarco, R., & Hansson, H. (2025). Changing the understanding of crop production: Integrating ecosystem services into the production function. *Ecological Economics*, *230*, 108526. https://doi.org/10.1016/j.ecolecon.2025.108526

Dietz, K.-J., Zörb, C., & Geilfus, C.-M. (2021). Drought and crop yield. *Plant Biology*, *23*(6), 881–893. https://doi.org/10.1111/plb.13304

Disasa, K. N., Yan, H., Zhang, J., Wang, G., Zhang, C., Zhang, D., Wang, B., & Hao, B. (2026). Comprehensive review of drought characteristics and intensification under climate change: implications for agriculture and water resources. *Journal of Hydrology*, *664*, 134571. https://doi.org/10.1016/j.jhydrol.2025.134571

Eekhout, J. P. C., & de Vente, J. (2022). Global impact of climate change on soil erosion and potential for adaptation through soil conservation. *Earth-Science Reviews*, *226*, 103921. https://doi.org/10.1016/j.earscirev.2022.103921

Eigenbrode, S. D., & Adhikari, S. (2023). Climate change and managing insect pests and beneficials in agricultural systems. *Agronomy Journal*, *115*(5), 2194–2215. https://doi.org/10.1002/agj2.21399

Ekele, J. U., Webster, R., Perez de Heredia, F., Lane, K. E., Fadel, A., & Symonds, R. C. (2025). Current impacts of elevated CO(2) on crop nutritional quality: a review using wheat as a case study. *Stress Biology*, *5*(1), 34. https://doi.org/10.1007/s44154-025-00217-w

Farah, A. A., Mohamed, M. A., Musse, O. S. H., & Nor, B. A. (2025). The multifaceted impact of climate change on agricultural productivity: a systematic literature review of SCOPUS-indexed studies (2015–2024). *Discover Sustainability*, *6*(1), 397. https://doi.org/10.1007/s43621-025-01229-2

Franco-Navarro, J. D., Padilla, Y. G., Álvarez, S., Calatayud, Á., Colmenero-Flores, J. M., Gómez-Bellot, M. J., Hernández, J. A., Martínez-Alcalá, I., Penella, C., Pérez-Pérez, J. G., Sánchez-Blanco, M. J., Tasa, M., & Acosta-Motos, J. R. (2025). Advancements in Water-Saving Strategies and Crop Adaptation to Drought: A Comprehensive Review. *Physiologia Plantarum*, *177*(4). https://doi.org/10.1111/ppl.70332

Gangana Gowdra, V. M., Lalitha, B. S., Halli, H. M., Senthamil, E., Negi, P., Jayadeva, H. M., Basavaraj, P. S., Harisha, C. B., Boraiah, K. M., Adavi, S. B., Suresha, P. G., Nargund, R., Mohite, G., & Reddy, K. S. (2025). Root growth, yield and stress tolerance of soybean to transient waterlogging under different climatic regimes. *Scientific Reports*, *15*(1), 6968. https://doi.org/10.1038/s41598-025-91780-9

Garcia-Franco, N., Wiesmeier, M., Buness, V., Berauer, B. J., Schuchardt, M. A., Jentsch, A., Schlingmann, M., Andrade-Linares, D., Wolf, B., Kiese, R., Dannenmann, M., & Kögel-Knabner, I. (2024). Rapid loss of organic carbon and soil structure in mountainous grassland topsoils induced by simulated climate change. *Geoderma*, *442*, 116807. https://doi.org/10.1016/j.geoderma.2024.116807

Guo, W., Dai, H., Qian, J., Tan, J., Xu, Z., & Guo, Y. (2024). An assessment of the relationship between spring frost indicators and global crop yield losses. *Science of The Total Environment*, *954*, 176560. https://doi.org/10.1016/j.scitotenv.2024.176560

Haghpanah, M., Hashemipetroudi, S., Arzani, A., & Araniti, F. (2024). Drought Tolerance in Plants: Physiological and Molecular Responses. In *Plants* (Vol. 13, Issue 21, p. 2962). https://doi.org/10.3390/plants13212962

Hay, W. T., Anderson, J. A., McCormick, S. P., Hojilla-Evangelista, M. P., Selling, G. W., Utt, K. D., Bowman, M. J., Doll, K. M., Ascherl, K. L., Berhow, M. A., & Vaughan, M. M. (2022). Fusarium head blight resistance exacerbates nutritional loss of wheat grain at elevated CO2. *Scientific Reports*, *12*(1), 15. https://doi.org/10.1038/s41598-021-03890-9

He, X., Abs, E., Allison, S. D., Tao, F., Huang, Y., Manzoni, S., Abramoff, R., Bruni, E., Bowring, S. P. K., Chakrawal, A., Ciais, P., Elsgaard, L., Friedlingstein, P., Georgiou, K., Hugelius, G., Holm, L. B., Li, W., Luo, Y., Marmasse, G., … Goll, D. S. (2024). Emerging multiscale insights on microbial carbon use efficiency in the land carbon cycle. *Nature Communications*, *15*(1), 8010. https://doi.org/10.1038/s41467-024-52160-5

Hu, T., Zhang, X., Khanal, S., Wilson, R., Leng, G., Toman, E. M., Wang, X., Li, Y., & Zhao, K. (2024). Climate change impacts on crop yields: A review of empirical findings, statistical crop models, and machine learning methods. *Environmental Modelling & Software*, *179*, 106119. https://doi.org/10.1016/j.envsoft.2024.106119

Huang, M.-T., & Zhai, P.-M. (2023). Desertification dynamics in China's drylands under climate change. *Advances in Climate Change Research*, *14*(3), 429–436. https://doi.org/10.1016/j.accre.2023.05.001

Hultgren, A., Carleton, T., Delgado, M., Gergel, D. R., Greenstone, M., Houser, T., Hsiang, S., Jina, A., Kopp, R. E., Malevich, S. B., McCusker, K. E., Mayer, T., Nath, I., Rising, J., Rode, A., & Yuan, J. (2025). Impacts of climate change on global agriculture accounting for adaptation. *Nature*, *642*(8068), 644–652. https://doi.org/10.1038/s41586-025-09085-w

Janni, M., Maestri, E., Gullì, M., Marmiroli, M., & Marmiroli, N. (2024). Plant responses to climate change, how global warming may impact on food security: a critical review. *Frontiers in Plant Science*, *Volume 14*. https://doi.org/10.3389/fpls.2023.1297569

Jha, U. C., Nath, C. P., Paul, P. J., Nayyar, H., Kumar, N., Dixit, G. P., Sen, S., Kumar, Y., & Prasad, P. V. V. (2025). Decoding the heat stress resilience in Chickpea (Cicer arietinum L.): multi-trait analysis for genotypic adaptation. *Scientific Reports*, *15*(1), 25055. https://doi.org/10.1038/s41598-025-07573-7

Kramer, I., Peleg, N., & Mau, Y. (2025). Climate change shifts risk of soil salinity and land degradation in water-scarce regions. *Agricultural Water Management*, *307*, 109223. https://doi.org/10.1016/j.agwat.2024.109223

Kumar, A., Muthuramalingam, P., Kumar, R., Tiwari, S., Verma, L., Park, S., & Shin, H. (2025). Adapting Crops to Rising Temperatures: Understanding Heat Stress and Plant Resilience Mechanisms. In *International Journal of Molecular Sciences* (Vol. 26, Issue 21, p. 10426). https://doi.org/10.3390/ijms262110426

Kumar, V., Kumari, A., Price, A. J., Bana, R. S., Singh, V., & Bamboriya, S. D. (2023). Impact of Futuristic Climate Variables on Weed Biology and Herbicidal Efficacy: A Review. In *Agronomy* (Vol. 13, Issue 2, p. 559). https://doi.org/10.3390/agronomy13020559

Lakatos, L., & Csabai, K. E. (2025). Extreme weather risks for European agriculture (1981–2020): A quantitative review using the E3CI. *Science of The Total Environment*, *1003*, 180641. https://doi.org/10.1016/j.scitotenv.2025.180641

Liaqat, W., Barutçular, C., Farooq, M. U., Ahmad, H., Jan, M. F., Ahmad, Z., Nawaz,

H., & Li, M. (2022). Climate change in relation to agriculture: A review. *Spanish Journal of Agricultural Research*, *20*(2 SE-Agricultural environment and ecology), e03R01. https://doi.org/10.5424/sjar/2022202-17742

Liu, Z., Si, J., He, X., Jia, B., Zhou, D., Wang, C., Zhu, X., Qin, J., Ndayambaza, B., & Bai, X. (2024). The impact of desertification on soil health stability in Semi-Arid alpine Regions: A case study of the Qilian Mountains in the northeastern Tibetan Plateau, China. *Ecological Indicators*, *163*, 112098. https://doi.org/10.1016/j.ecolind.2024.112098

McDermid, S. S., Weng, E., Puma, M., Cook, B., Hengl, T., Sanderman, J., De Lannoy, G. J. M., & Aleinov, I. (2022). Soil Carbon Losses Reduce Soil Moisture in Global Climate Model Simulations. *Earth Interactions*, *26*(1), 195–208. https://doi.org/10.1175/EI-D-22-0003.1

Meng, H., Qian, L., & Duan, K. (2024). Evaluating the impacts of flooding on crop yields by different meteorological indices: A regional case study in the middle-lower reach of the Yangtze River, China. *Ecological Indicators*, *162*, 112068. https://doi.org/10.1016/j.ecolind.2024.112068

Mohan, V. R., MacDonald, M. T., & Abbey, Lord. (2025). Impact of Water Deficit Stress on Brassica Crops: Growth and Yield, Physiological and Biochemical Responses. In *Plants* (Vol. 14, Issue 13, p. 1942). https://doi.org/10.3390/plants14131942

Mokhtar, A., He, H., Attaher, S., Salem, A., & Alam, M. (2025). Optimizing water-use efficiency under elevated CO_2: A meta-analysis of crop type, soil modulation, and enrichment methods. *Agricultural Water Management*, *309*, 109312. https://doi.org/10.1016/j.agwat.2025.109312

Mukhopadhyay, R., Sarkar, B., Jat, H. S., Sharma, P. C., & Bolan, N. S. (2021). Soil salinity under climate change: Challenges for sustainable agriculture and food security. *Journal of Environmental Management*, *280*, 111736. https://doi.org/10.1016/j.jenvman.2020.111736

Nóia Júnior, R. de S., Asseng, S., Müller, C., Deswarte, J.-C., Cohan, J.-P., & Martre, P. (2025). Negative impacts of climate change on crop yields are underestimated. *Trends in Plant Science*, *30*(11), 1262–1273. https://doi.org/10.1016/j.tplants.2025.05.002

Portmann, R., Schmid, T., Villiger, L., Bresch, D. N., & Calanca, P. (2024). Modelling crop hail damage footprints with single-polarization radar: the roles of spatial resolution, hail intensity, and cropland density. *Natural Hazards and Earth System Sciences*, *24*(7), 2541–2558. https://doi.org/10.5194/nhess-24-2541-2024

Proctor, J., Zeppetello, L. V., Chan, D., & Huybers, P. (2025). Climate change increases the interannual variance of summer crop yields globally through changes in temperature and water supply. *Science Advances*, *11*(36), eady3575. https://doi.org/10.1126/sciadv.ady3575

Qi, B., Yang, S., Li, D., Qin, D., Zheng, X., Hu, J., Zhou, X., & Liu, H. (2025). Effects of Drainage Technology on Waterlogging Reduction and Rice Yield in Mid-

Lower Reaches of Yangtze River. In *Agronomy* (Vol. 15, Issue 4, p. 905). https://doi.org/10.3390/agronomy15040905

Qiao, M., Hong, C., Jiao, Y., Hou, S., & Gao, H. (2024). Impacts of Drought on Photosynthesis in Major Food Crops and the Related Mechanisms of Plant Responses to Drought. In *Plants* (Vol. 13, Issue 13, p. 1808). https://doi.org/10.3390/plants13131808

Ren, S., Wang, T., Guenet, B., Liu, D., Cao, Y., Ding, J., Smith, P., & Piao, S. (2024). Projected soil carbon loss with warming in constrained Earth system models. *Nature Communications*, *15*(1), 102. https://doi.org/10.1038/s41467-023-44433-2

Richetti, J., Sadras, V. O., He, D., Leske, B., Hu, P., Beletse, Y., Cossani, C. M., Nguyen, H., Zheng, B., Deery, D. M., Dreccer, M. F., Whish, J., & Lilley, J. (2025). Challenges in modelling the impact of frost and heat stress on the yield of cool-season annual grain crops. *Frontiers in Plant Science*, *Volume 16*. https://doi.org/10.3389/fpls.2025.1613432

Saleem, N., & Smirnova, J. (2026). The impact of climate change on agricultural production: a comparative analysis of three Global South regions. *Eurasian Economic Review*. https://doi.org/10.1007/s40822-025-00355-6

Samat, A. T., Soltabayeva, A., Bekturova, A., Zhanassova, K., Auganova, D., Masalimov, Z., Srivastava, S., Satkanov, M., & Kurmanbayeva, A. (2025). Plant responses to heat stress and advances in mitigation strategies. *Frontiers in Plant Science*, *Volume 16*. https://doi.org/10.3389/fpls.2025.1638213

Sher, A., Noor, M. A., Li, H. X., Nasir, B., Manzoor, M. A., Hussain, S., Zhang, J., Riaz, M. W., & Hussain, S. (2024). Heat stress effects on legumes: Challenges, management strategies and future insights. *Plant Stress*, *13*, 100537. https://doi.org/10.1016/j.stress.2024.100537

Singh, B. K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J. E., Liu, H., & Trivedi, P. (2023). Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology*, *21*(10), 640–656. https://doi.org/10.1038/s41579-023-00900-7

Subedi, B., Poudel, A., & Aryal, S. (2023). The impact of climate change on insect pest biology and ecology: Implications for pest management strategies, crop production, and food security. *Journal of Agriculture and Food Research*, *14*, 100733. https://doi.org/10.1016/j.jafr.2023.100733

Sun, Z.-X., Zhang, C.-C., Dong, J.-L., & Jiang, Y.-Y. (2024). Climate Change and Agriculture—Sustainable Plant Production. In *Agronomy* (Vol. 14, Issue 6, p. 1236). https://doi.org/10.3390/agronomy14061236

Teixeira, A. C., Bakon, M., Lopes, D., Cunha, A., & Sousa, J. J. (2025). A systematic review on soil moisture estimation using remote sensing data for agricultural applications. *Science of Remote Sensing*, *12*, 100328. https://doi.org/10.1016/j.srs.2025.100328

Timothy M. Lenton, Hans Joachim Schellnhuber, Johan Rockström, Owen Gaffney, Stefan Rahmstorf, Katherine Richardson, & Will Steffen. (2019). Climate tip-

ping points —too risky to bet against. *Nature*, *575*, 592–595.

Toreti, A., Deryng, D., Tubiello, F. N., Müller, C., Kimball, B. A., Moser, G., Boote, K., Asseng, S., Pugh, T. A. M., Vanuytrecht, E., Pleijel, H., Webber, H., Durand, J.-L., Dentener, F., Ceglar, A., Wang, X., Badeck, F., Lecerf, R., Wall, G. W., … Rosenzweig, C. (2020). Narrowing uncertainties in the effects of elevated CO2 on crops. *Nature Food*, *1*(12), 775–782. https://doi.org/10.1038/s43016-020-00195-4

Tran, B.-L., Tseng, W.-C., & Chen, C.-C. (2025). Climate change impacts on crop yields across temperature rise thresholds and climate zones. *Scientific Reports*, *15*(1), 23424. https://doi.org/10.1038/s41598-025-07405-8

Ullah, N., Collins, B., Christopher, J. T., Frederiks, T., & Chenu, K. (2024). Pre- and post-flowering impacts of natural heatwaves on yield components in wheat. *Field Crops Research*, *316*(March), 109489. https://doi.org/10.1016/j.fcr.2024.109489

Wang, T., Sun, S., Yin, Y., Zhao, J., Tang, Y., Wang, Y., Gao, F., & Luan, X. (2024). Status of crop water use efficiency evaluation methods: A review. *Agricultural and Forest Meteorology*, *349*, 109961. https://doi.org/10.1016/j.agrformet.2024.109961

Xu, Y., Li, T., Xu, M., Tan, L., & Shen, S. (2024). Assessing Climate Change Effects on Winter Wheat Production in the 3H Plain: Insights from Bias-Corrected CMIP6 Projections. In *Agriculture* (Vol. 14, Issue 3, p. 469). https://doi.org/10.3390/agriculture14030469

Yan, Z., Liu, Y., Gao, S., Yang, H., Feng, D., Gao, K., Ming, B., Wang, K., Hou, P., Li, S., & Xie, R. (2025). Water management mitigates drought stress effects on maize leaf senescence and post-anthesis dry matter accumulation. *Agricultural Water Management*, *318*, 109709. https://doi.org/10.1016/j.agwat.2025.109709

Yang, Y., Tilman, D., Jin, Z., Smith, P., Barrett, C. B., Zhu, Y.-G., Burney, J., D'Odorico, P., Fantke, P., Fargione, J., Finlay, J. C., Rulli, M. C., Sloat, L., Jan van Groenigen, K., West, P. C., Ziska, L., Michalak, A. M., Team, the C.-A., Lobell, D. B., … Zhuang, M. (2024). Climate change exacerbates the environmental impacts of agriculture. *Science*, *385*(6713), eadn3747. https://doi.org/10.1126/science.adn3747

Yuan, X., Li, S., Chen, J., Yu, H., Yang, T., Wang, C., Huang, S., Chen, H., & Ao, X. (2024). Impacts of Global Climate Change on Agricultural Production: A Comprehensive Review. In *Agronomy* (Vol. 14, Issue 7, p. 1360). https://doi.org/10.3390/agronomy14071360

Zhang, Y., Chen, X., Geng, S., & Zhang, X. (2025). A review of soil waterlogging impacts, mechanisms, and adaptive strategies. *Frontiers in Plant Science*, *16*, 1545912. https://doi.org/10.3389/fpls.2025.1545912

Zheng, C., Li, Y., Wang, X., Wang, L., Duan, Y., Chen, Y., & Lu, J. (2024). Desertification indirectly affects soil fauna by reducing complexity of soil habitats and diversity of energy sources. *Science of The Total Environment*, *954*, 176509. https://doi.org/10.1016/j.scitotenv.2024.176509

Chapter 3

AI MEETS DOCKING: TOWARD A NEW ERA IN VIRTUAL SCREENING FOR DRUG DEVELOPMENT

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1. Introduction

The accelerating demands of drug discovery have underscored the critical need for rapid, accurate, and cost-effective approaches to identify potential therapeutic candidates (Tiwari et al., 2023). Traditional wet-lab screening methods, while experimentally robust, are often time-consuming, resource-intensive, and constrained by limited chemical space (Petzold and Mukhopadhyay, 2025). In this context, molecular docking has emerged as a pivotal in silico strategy in structure-based drug design, enabling the prediction of ligand-target interactions by estimating binding affinities and orientations (Panday and Ghosh, 2019). Over the past decade, the evolution of docking algorithms has contributed substantially to virtual screening workflows, yet challenges such as receptor flexibility, scoring function accuracy, and hit reproducibility have persisted (Zhang et al., 2024).

Recent advancements in artificial intelligence (AI) particularly machine learning (ML) and deep learning (DL) have transformed the molecular docking landscape (Gupta et al., 2021). By enabling pattern recognition, dynamic prediction models, and automated feature extraction from vast biochemical datasets, AI-enhanced docking systems now offer improved precision, scalability, and adaptability over classical approaches (Junaid, 2025; Fadahunsi et al., 2024). These methods not only accelerate virtual screening pipelines but also enable data-driven hypothesis generation, redefining how candidate molecules are ranked and selected (Albani et al., 2024). Compared to conventional docking protocols that rely on rigid scoring functions, AI models can learn complex, nonlinear relationships between molecular structures and biological activities, increasing the reliability of binding predictions (Choudhury et al., 2022).

This narrative review aims to explore the emerging role of AI in molecular docking within the context of virtual drug screening. Using the PICO framework as a guiding structure, we focus on: (P) the biomedical challenges in modern drug discovery, (I) the integration of AI technologies into docking workflows, (C) comparisons with classical docking and other computational approaches, and (O) the measurable outcomes in terms of docking accuracy, screening efficiency, and translational potential. By synthesizing recent developments, highlighting current limitations, and discussing prospective innovations, this review provides a comprehensive overview of how AI is ushering in a new era of intelligent molecular docking.

2. Database Search and Methodology

This narrative review was conducted through a structured and comprehensive search of the scientific literature to investigate the integration of AI in molecular docking and its applications in virtual drug screening. The review further aimed to explore the comparative advantages of AI-enhanced

docking over traditional methods, the accuracy of predictive models, and translational implications in drug discovery pipelines.

A systematic literature search was performed across major scientific databases, including PubMed, Web of Science, and Scopus (Elsevier). The search covered publications between January 2011 and April 2025 and included peer-reviewed research articles, high-impact narrative and systematic reviews, in silico docking case studies, benchmark analyses, and translational research on AI-guided drug design. Only articles published in English in peer-reviewed journals were considered.

The following search terms were used in various combinations, utilizing Boolean operators where appropriate: "molecular docking AND artificial intelligence", "AI-guided virtual screening", "machine learning AND structure-based drug design", "deep learning AND ligand binding prediction", "neural networks AND protein-ligand interaction", "AI-powered scoring functions AND docking accuracy", "structure-based drug discovery AND AI integration", "graph neural networks AND drug screening".

Inclusion criteria:

· Studies evaluating AI/ML-based methods in molecular docking or virtual screening.

· Research comparing AI-guided docking approaches with classical or rule-based methods.

· Articles involving drug discovery applications, predictive performance evaluations, or experimental validation of AI-assisted docking predictions.

· Studies utilizing public compound libraries (e.g., ZINC, ChEMBL) or target-specific datasets.

Exclusion criteria:

· Non-peer-reviewed sources such as preprints, conference abstracts, or editorials.

· Articles that did not involve AI or did not focus on docking or virtual screening.

· Studies with insufficient methodological detail or lacking docking performance assessment.

Additionally, manual reference screening was conducted on landmark reviews and highly cited original research papers to identify relevant studies not captured in the initial queries. Emphasis was placed on methodological transparency, reproducibility, and translational potential in drug discovery applications. This approach ensured a coherent synthesis of current advances, critical challenges, and emerging directions in the field of AI-enhanced

molecular docking.

3. Theoretical Background of Molecular Docking

3.1. Principles of Molecular Docking

Molecular docking is a computational approach designed to predict the preferred orientation of one molecule, typically a small ligand, when bound to a second molecule, usually a macromolecular target such as a protein, in order to form a stable complex (Mohanty and Mohanty, 2023). The core principle underlying docking methodologies is that the binding affinity between two molecules can be approximated through conformational sampling combined with scoring functions that estimate the free energy of binding (Meli et al., 2022).

Docking simulations generally fall into two major categories. In rigid docking, both the ligand and the receptor are treated as inflexible structures (Asim, 2024). Although this approach allows for faster computational processing, it does so at the expense of biological realism, often overlooking important dynamic interactions (Ouma et al., 2024). In contrast, flexible docking introduces conformational variability either in the ligand, the receptor, or both, better mimicking in vivo molecular dynamics but requiring significantly greater computational resources (Lee et al., 2025).

A typical docking workflow begins with the preparation of both the ligand and the target, including processes such as protonation state assignment, geometric optimization, and energy minimization (Bender et al., 2021). This is followed by the execution of the docking algorithm, which systematically searches for favorable conformations and orientations of the ligand relative to the target binding site (Yang et al., 2022). Finally, generated binding poses are evaluated and ranked using scoring functions based on predicted energetic criteria, guiding the selection of the most likely biologically relevant interactions (de Angelo et al., 2025).

3.2. Scoring Functions

Scoring functions are mathematical models used to predict the binding affinity between the ligand and its target (Hasan et al., 2022). They fall into three major categories (Zhang et al., 2025):

Force field-based (e.g., AutoDock); Estimate interaction energy using molecular mechanics.

Empirical (e.g., GlideScore); Combine physics-based terms with regression-fitted parameters.

Knowledge-based (e.g., X-Score); Derived from statistical analysis of known protein-ligand complexes.

Despite their utility, classical scoring functions often oversimplify biological reality, leading to false positives and low reproducibility. These limitations have opened avenues for AI-based alternatives (Mohanan, 2025).

3.3. AI Integration into Docking Frameworks

AI has introduced a paradigm shift in docking methodologies by learning from massive chemical and biological datasets (Niazi, 2022). ML algorithms are now employed to predict docking scores with higher accuracy, recognize active and inactive ligands, and optimize binding poses beyond rigid assumptions (Priya et al., 2022). DL models, such as convolutional neural networks (CNNs) and graph neural networks (GNNs), have demonstrated remarkable performance in predicting binding affinities directly from 3D molecular representations or SMILES notations (Liyaqat et al., 2025). These approaches enable real-time feedback, greater generalizability across diverse biological targets, and seamless integration into high-throughput virtual screening workflows (Nag et al., 2022).

In practice, the AI-enhanced docking process typically begins with ligand and target preparation, followed by predictive modeling of binding poses and affinity scoring using DL techniques (Durojaye et al., 2025). Subsequent in silico filtering refines candidate selection prior to experimental validation through wet-lab assays (Elgawish et al., 2025). Post-validation rescoring and prioritization further optimize the identification of promising therapeutic compounds. This streamlined workflow exemplifies the growing role of AI in augmenting traditional structure-based drug discovery pipelines. The typical AI-enhanced docking and validation workflow is summarized in Figure 2.

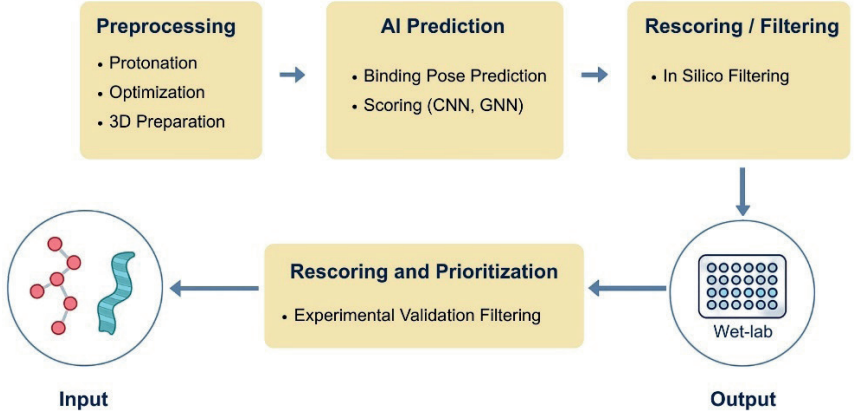

Figure 2. *AI-enhanced molecular docking workflow.*

**The figure depicts the full pipeline of AI-driven molecular docking, from preprocessing and predictive modeling to wet-lab experimental validation and final rescoring. This figure was created by the authors.*

3.4. Emerging Hybrid Methods

Several platforms now combine AI with traditional docking algorithms, either by re-scoring generated poses or guiding the conformational search space. These hybrid pipelines e.g., DeepDock, AtomNet, and DeepDocking are pushing the limits of virtual screening accuracy and speed, especially in large chemical libraries (Zhang et al., 2024). The evolution of molecular docking techniques from rigid scoring approaches to hybrid AI-driven methods is summarized in Figure 1.

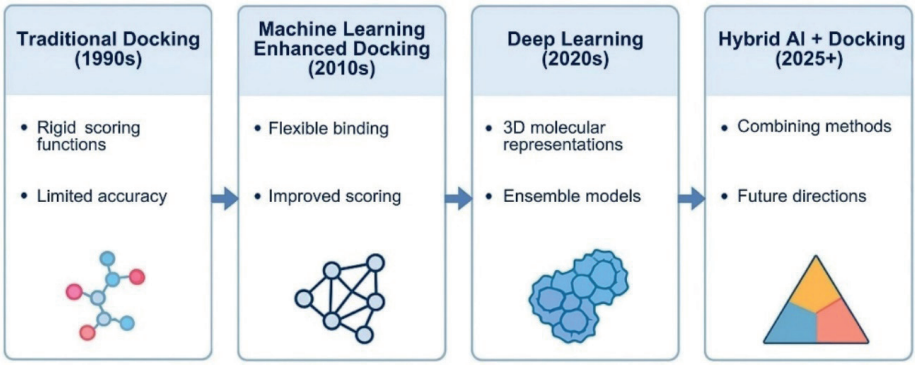

Figure 1. *Timeline illustrating the evolution of molecular docking techniques, transitioning from traditional rigid scoring methods to hybrid AI and quantum computing approaches.*

**Timeline illustrating the technological evolution of molecular docking, starting from traditional rigid scoring functions in the 1990s, advancing through machine learning-enhanced flexible binding models in the 2010s, deep learning-driven 3D molecular representations in the 2020s, and progressing toward hybrid AI and quantum computing-integrated docking approaches projected for 2025 and beyond. This figure was created by the authors.*

4. Tools and Platforms for AI-Enhanced Molecular Docking

Over the past two decades, a wide array of computational tools has been developed to support molecular docking in virtual drug screening workflows. These tools vary in their algorithmic approaches, scoring functions, interface flexibility, and the extent to which they incorporate AI. This section outlines the key platforms in use today, highlighting both traditional and AI-powered docking systems.

4.1. Traditional Docking Software

Several established docking programs have long served as critical components of structure-based drug design workflows (Das et al., 2025). Among the most widely utilized are AutoDock and its successor, AutoDock

Vina, both of which are open-source platforms tailored for small-molecule docking applications (Che et al., 2023). AutoDock Vina enhances docking efficiency by employing a gradient-based local optimization algorithm, improving both speed and accuracy relative to earlier methods (Zayed, 2025).

GOLD (Genetic Optimization for Ligand Docking) represents another prominent tool in the field, offering a commercial docking solution that leverages genetic algorithms to enable flexible ligand and receptor docking as well as diverse scoring strategies (Mursal et al., 2024). Similarly, Glide, part of the Schrödinger Suite, has become a GOLD standard in pharmaceutical industry pipelines, renowned for its high precision and empirically derived scoring functions (Rustagi et al., 2025).

Web-based platforms such as SwissDock and PatchDock provide more accessible alternatives, designed for rapid, low-resource docking simulations without sacrificing usability (Kihn et al., 2024). These tools have democratized molecular docking by enabling broader access to virtual screening technologies (Tarabara et al., 2022).

Despite their extensive contributions, traditional docking platforms generally rely on rigid scoring functions and predefined molecular feature sets (Blanes-Mira et al., 2022). As a result, they often face challenges when dealing with novel targets, cryptic binding sites, or highly flexible molecular systems, thereby limiting their adaptability in complex drug discovery scenarios.

4.2. AI-Integrated Docking Platforms

The integration of AI into molecular docking workflows has ushered in a new generation of intelligent docking systems (Gangwal and Lavecchia, 2025). These platforms leverage ML and DL models to improve pose prediction, scoring accuracy, and screening efficiency across extensive chemical libraries (Manan et al., 2025).

Among the notable advancements, DeepDock employs 3D (CNNs) to predict ligand binding poses with high spatial resolution, enabling more accurate docking simulations (Vittorio et al., 2024). AtomNet, one of the pioneering DL-based platforms, revolutionized affinity prediction by treating molecular interactions as image recognition problems, using CNNs to extract and learn structural features critical for binding (Wang et al., 2025).

GNINA builds upon the AutoDock framework by integrating neural networks to enhance pose scoring through learned molecular feature representations, resulting in improved ranking of candidate compounds (McNutt et al., 2021). DeepDocking offers a scalable solution for virtual screening of ultra-large compound libraries by pre-filtering candidates using ML models before applying traditional docking procedures, significantly accelerating the discovery process (Ma et al., 2021).

MolPal further extends AI-driven screening capabilities by combining active learning techniques with docking scores, iteratively refining compound libraries to prioritize high-potential candidates and optimize hit rates over successive rounds (Sadybekov and Katritch, 2023)

Together, these AI-enhanced platforms represent a major advancement over traditional docking methods, addressing limitations in flexibility, scoring consistency, and scalability while expanding the horizons of structure-based drug discovery. A comparative overview of commonly used traditional and AI-enhanced docking platforms, highlighting their strengths and typical applications, is presented in Table 1

Table 1. *Comparative features of traditional and AI-integrated molecular docking platforms.*

Platform	AI-Based	Strengths	Common Use
AutoDock Vina	-	Fast, widely validated	Academic research
Glide	-	High precision, commercial pipelines	Pharma & biotech
AtomNet	+	CNN-based affinity prediction	Lead optimization
GNINA	+	Neural-network-based scoring	Academic + AI-focused labs
DeepDocking	+	Large-scale screening	Big data virtual screening

5. Applications in Drug Discovery

The integration of AI into molecular docking workflows has significantly expanded the scope and efficiency of virtual screening in drug discovery (Rakshit et al., 2022). By enabling high-throughput, structure-based prediction of ligand-receptor interactions, AI-enhanced docking systems have been successfully applied across a range of therapeutic domains, including oncology, neurological disorders, and antimicrobial research (Yingngam, 2024; Garg et al., 2024; Malla and Banka, 2025).

5.1. Oncology: Targeting Kinases and Oncoproteins

In cancer research, molecular docking augmented with DL has been instrumental in discovering novel kinase inhibitors and optimizing lead compounds (Elgawish et al., 2025). AI-based models trained on kinase-ligand complexes have demonstrated improved specificity in identifying binding pockets and predicting activity, particularly for targets such as EGFR and CDK family proteins, and have also been applied to mutated kinases such as BRAF (Mendolia et al., 2022). Hybrid approaches that integrate ML-based scoring with traditional docking platforms (e.g., Glide, AutoDock, GOLD) have been reported to improve pose ranking and selectivity profiling in cancer

drug discovery, particularly in lung cancer applications (Ahmad et al., 2025).

5.2. Neurological Disorders: Structure-Based Repurposing

AI-enhanced docking has been leveraged to identify potential neuroprotective agents in conditions such as Alzheimer's and Parkinson's diseases (Vicidomini et al., 2024). Virtual screening of existing drug libraries guided by neural network-based binding predictions has uncovered candidates that modulate critical enzymes such as Acetylcholinesterase (AChE), Monoamine Oxidase B (MAO-B) and Glycogen Synthase Kinase 3 Beta (GSK-3β) (Cheong et al., 2022). These insights have accelerated preclinical testing and supported the repositioning of known compounds (Pan et al., 2022).

5.3. Antibacterial and Antifungal Agents

The growing threat of antimicrobial resistance has driven the application of AI-based docking in the identification of novel antibiotics and antifungal agents (Talat and Khan, 2023). Predictive models have facilitated the screening of natural product libraries and synthetic scaffolds targeting bacterial enzymes such as DNA gyrase, β-lactamase, and Dihydrofolate Reductase (DHFR) (Parvaiz et al., 2021; Dasí-Delgado et al., 2025; Soni and Pratap, 2022). These efforts have contributed to the discovery of non-traditional inhibitors with unique mechanisms of action.

5.4. Rare and Neglected Diseases

AI-assisted virtual screening has also found utility in addressing rare and neglected diseases, where traditional drug discovery efforts are often limited due to low commercial interest (Gangwal and Lavecchia, 2024). Docking studies driven by ML algorithms have led to the identification of promising compounds for diseases such as leishmaniasis, Chagas disease, and cystic fibrosis, offering new hope in areas with unmet clinical need (Altamura et al., 2022; Chauhan et al., 2024).

6. Challenges and Limitations

Despite its growing impact and undeniable promise, the integration of AI into molecular docking is not without critical challenges. These limitations span computational constraints, algorithmic generalizability, data quality, and the translational fidelity of predicted results (Jiang et al., 2017; Tiwari et al., 2023).

6.1. Quality and Bias in Training Data

The accuracy of AI models is inherently dependent on the quality and diversity of the training data (Gong et al., 2023). Many ML algorithms used in molecular docking are trained on benchmark datasets derived from crystallized protein-ligand complexes. These datasets often suffer from sampling bias, overrepresentation of well-studied targets, and limited

chemical diversity, which restricts the models' generalizability to novel targets or scaffolds (Siebenmorgen et al., 2024). This can lead to false positives or false negatives, especially when extrapolating to underexplored chemical spaces (Montoya et al., 2025).

6.2. Scoring Function Limitations and Interpretability

While DL -based scoring functions outperform traditional empirical methods in some contexts, their black-box nature often hampers interpretability (Doğan et al., 2021). Unlike classical scoring models, AI systems do not readily offer mechanistic insights into why a specific pose or compound is favored, which can reduce confidence in decision-making—particularly in regulatory or translational settings (Tursunalieva et al., 2024).

6.3. Protein Flexibility and Binding Site Dynamics

Most docking algorithms, even AI-enhanced ones, still struggle to fully capture protein flexibility and conformational changes upon ligand binding (induced fit) (Son et al., 2024). Although some hybrid frameworks integrate molecular dynamics simulations, this approach significantly increases computational cost and complexity (Joshi and Deshmukh, 2021). As a result, static snapshots of protein structures may not reflect biologically relevant conformations, limiting the predictive power of docking results (Harmalkar and Gray, 2021).

6.4. Computational Cost and Scalability

Although AI models promise speed and automation, training deep neural networks and deploying them for large-scale virtual screening remains computationally expensive (Gentile et al., 2022). Access to high-performance computing infrastructure is often necessary for efficient execution, particularly in projects involving ultra-large chemical libraries. This presents a barrier for smaller research institutions and academic laboratories (Ungerer and Carpenter, 2018).

6.5. Limited Experimental Validation

Despite impressive in silico results, relatively few AI-predicted ligands undergo thorough experimental validation (Ferguson and RSci, 2025). The persistent shortage of prospective wet-lab validations restricts the ability to rigorously evaluate the translational potential of AI-enhanced docking, thereby constraining its clinical relevance and commercial viability (Alzhikeyeva et al., 2025).

Addressing these limitations requires a multi-pronged approach involving curated datasets, hybrid modeling strategies, cross-validation with experimental methods, and increased transparency in AI architectures. Only through such efforts can the field fully realize the potential of intelligent

docking systems in modern drug discovery.

7. Future Perspectives

As AI continues to evolve, its intersection with molecular docking is poised to redefine the paradigms of virtual drug screening (Bhatia et al., 2024). Looking ahead, several technological and conceptual innovations are likely to expand the applicability, accuracy, and translational potential of AI-enhanced docking systems.

7.1. Integration with AlphaFold and Protein Structure Prediction

The advent of highly accurate protein structure prediction tools such as AlphaFold and RoseTTAFold offers unprecedented opportunities for structure-based drug discovery (Chang et al., 2024). When combined with AI-driven docking algorithms, these tools enable reliable virtual screening against previously undruggable or structurally unresolved targets (Abramson et al., 2024; Krishna et al., 2024). Future docking frameworks are expected to incorporate dynamic ensembles of predicted protein conformations, thereby addressing long-standing limitations related to receptor flexibility and cryptic binding sites.

7.2. Toward Explainable AI in Molecular Docking

One of the critical needs moving forward is the development of interpretable and explainable AI models. These models should not only predict binding affinities or pose rankings but also provide mechanistic insights into key molecular interactions. Such transparency will be crucial for regulatory approval, hypothesis generation, and adoption in clinical settings. Emerging efforts in explainable DL and attention-based architectures may bridge this gap.

7.3. AI-Driven Polypharmacology and Multi-Target Screening

Future docking platforms are likely to shift from a single target focus toward polypharmacological modeling, where AI systems predict the behavior of ligands across multiple targets. This could accelerate the discovery of multi-target therapeutics for complex diseases such as cancer, neurodegeneration, and metabolic disorders, where simultaneous modulation of several pathways is desirable.

7.4. Quantum Computing and Next-Generation Algorithms

As quantum computing matures, it holds the promise to revolutionize conformational sampling, energy calculations, and molecular interaction modeling (Lappala, 2024). Hybrid quantum-classical algorithms may significantly reduce the computational burden of docking simulations and improve binding energy predictions beyond the current capabilities of classical AI models.

7.5. Personalized Docking and Precision Medicine

The future of molecular docking may align more closely with precision medicine through personalized docking models trained on patient-specific mutations, expression profiles, and proteomic landscapes. AI could facilitate real-time therapeutic profiling by predicting the best drug-target interactions for individual patients, bringing virtual screening from the bench to the bedside.

Once considered a supplementary technique, AI-powered molecular docking now stands at the forefront of rational drug design (Martin et al., 2023; Cosmas et al., 2020). Its future lies in intelligent integration with structural biology, transparent model architectures, and personalized healthcare applications. Continued interdisciplinary collaboration among computational scientists, biologists, and clinicians will be essential to realize this transformative potential.

8. Conclusion

AI has revolutionized molecular docking by enabling more accurate, scalable, and dynamic approaches to virtual drug screening. The integration of ML and DL into docking workflows has addressed longstanding limitations such as rigid scoring functions, inadequate handling of protein flexibility, and limited predictive accuracy in novel chemical spaces. Through platforms like AtomNet, DeepDock, and GNINA, AI has accelerated lead identification and optimized screening efficiency across therapeutic domains ranging from cancer and neurodegeneration to antimicrobial resistance and rare diseases.

Yet, the field continues to face important challenges. These include the interpretability of AI models, dependency on biased training data, and the need for broader experimental validation to ensure clinical relevance. As AI algorithms grow more complex, it is essential to balance predictive power with transparency and reproducibility.

Looking forward, the convergence of AI-powered docking with tools like AlphaFold, advancements in explainable AI, and the rise of personalized docking models are likely to reshape structure-based drug design. To fully realize this potential, ongoing interdisciplinary collaboration and investment in data quality and model validation will be key. In doing so, AI-driven molecular docking may become not just a computational asset, but a transformative engine in precision medicine.

REFERENCES

Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., ... & Jumper, J. M. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*, *630*(8016), 493-500. https://doi.org/10.1038/s41586-024-07487-w

Ahmad, S., Shah, S. N. A., Parveen, R., & Raza, K. (2025). Machine learning for genomic profiling and drug discovery in personalised lung cancer therapeutics. *Journal of Drug Targeting*, 1-20. https://doi.org/10.1080/1061186X.2025.2530656

Albani, F. G., Alghamdi, S. S., Almutairi, M. M., & Alqahtani, T. (2025). Artificial Intelligence-Driven Innovations in Oncology Drug Discovery: Transforming Traditional Pipelines and Enhancing Drug Design. *Drug Design, Development and Therapy*, 5685-5707. https://doi.org/10.2147/DDDT.S509769

Altamura, F., Rajesh, R., Catta-Preta, C. M., Moretti, N. S., & Cestari, I. (2022). The current drug discovery landscape for trypanosomiasis and leishmaniasis: Challenges and strategies to identify drug targets. *Drug development research*, *83*(2), 225-252. DOI: 10.1002/ddr.21664

Alzhikeyeva, A., Yazici, A., & Sultankulov, B. (2025). Enzyme Engineering and Its Applications in Cancer Therapies: A Review of Machine Learning Approaches. doi:10.20944/preprints202507.1406.v1

Asim, A. (2024). Approaches to Backbone Flexibility in Protein–Protein Docking. In *Protein-Protein Docking: Methods and Protocols* (pp. 45-68). New York, NY: Springer US.https://doi.org/10.1007/978-1-0716-3985-6_4

Bender, B. J., Gahbauer, S., Luttens, A., Lyu, J., Webb, C. M., Stein, R. M., ... & Shoichet, B. K. (2021). A practical guide to large-scale docking. *Nature protocols*, *16*(10), 4799-4832.https://doi.org/10.1038/s41596-021-00597-z

Bhatia, N., Khan, M. M. U., & Arora, S. (2024). The role of artificial intelligence in revolutionizing pharmacological research. *Current Pharmacology Reports*, *10*(6), 323-329.https://doi.org/10.1007/s40495-024-00367-x

Blanes-Mira, C., Fernández-Aguado, P., de Andrés-López, J., Fernández-Carvajal, A., Ferrer-Montiel, A., & Fernández-Ballester, G. (2022). Comprehensive survey of consensus docking for high-throughput virtual screening. *Molecules*, *28*(1), 175. https://doi.org/10.3390/molecules28010175

Chang, L., Mondal, A., Singh, B., Martínez-Noa, Y., & Perez, A. (2024). Revolutionizing peptide-based drug discovery: advances in the post-AlphaFold era. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, *14*(1), e1693.DOI: 10.1002/wcms.1693

Chauhan, R., Jena, M., & Kaur, H. (2024, December). Machine Learning-Enhanced Drug Repurposing Strategies for Cystic Fibrosis. In *International Conference on Intelligent Computing and Advances in Communication* (pp. 129-140). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-96-4071-3_11

Che, X., Liu, Q., & Zhang, L. (2023). An accurate and universal protein-small molecule batch docking solution using Autodock Vina. *Results in Engineering*, *19*, 101335. https://doi.org/10.1016/j.rineng.2023.101335

Cheong, S. L., Tiew, J. K., Fong, Y. H., Leong, H. W., Chan, Y. M., Chan, Z. L., & Kong, E. W. J. (2022). Current pharmacotherapy and multi-target approaches for Alzheimer's disease. *Pharmaceuticals*, *15*(12), 1560. https://doi.org/10.3390/ph15121560

Choudhury, C., Murugan, N. A., & Priyakumar, U. D. (2022). Structure-based drug repurposing: Traditional and advanced AI/ML-aided methods. *Drug discovery today*, *27*(7), 1847-1861. https://doi.org/10.1016/j.drudis.2022.03.006

Cosmas, S., Durojaye, O. A., Joshua, P. E., Ogidigo, J. O., Difa, C. A., & Nwachukwu, J. N. (2020). Comparative in-silico parmacokinetics and molecular docking study on gedunin isolated from Azadirachta indica, its modified derivatives and selected antifolate drugs as potential dihydrofolate reductase inhibitors of Plasmodium falciparum. *International Journal of Computational Biology and Drug Design*, *13*(3), 237-254.https://doi.org/10.1504/IJCBDD.2020.107888

Das, I. J., Bhatta, K., Sarangi, I., & Samal, H. B. (2025). Innovative computational approaches in drug discovery and design. In *Advances in Pharmacology* (Vol. 103, pp. 1-22). Academic Press.https://doi.org/10.1016/bs.apha.2025.01.006

Dasí-Delgado, P., Andreu, C., & del Olmo, M. L. (2025). Strategies Used for the Discovery of New Microbial Metabolites with Antibiotic Activity. *Molecules*, *30*(13), 2868. https://doi.org/10.3390/molecules30132868

de Angelo, R. M., de Sousa, D. S., da Silva, A. P., Chiari, L. P., da Silva, A. B., & Honorio, K. M. (2025). Molecular Docking: State-of-the-Art Scoring Functions and Search Algorithms. In *Computer-Aided and Machine Learning-Driven Drug Design: From Theory to Applications* (pp. 163-198). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-76718-0_7

Doğan, T., Akhan Güzelcan, E., Baumann, M., Koyas, A., Atas, H., Baxendale, I. R., ... & Cetin-Atalay, R. (2021). Protein domain-based prediction of drug/compound–target interactions and experimental validation on LIM kinases. *PLoS Computational Biology*, *17*(11), e1009171.https://doi.org/10.1371/journal.pcbi.1009171

Durojaye, O. A., Bellah, S. F., Uzoeto, H. O., Okoro, N. O., Cosmas, S., Ajima, J. N., ... & Odiba, A. S. (2025). Harnessing AI-driven reverse docking in drug discovery: a comprehensive review of opportunities, challenges, and emerging trends. *Journal of Molecular Modeling*, *31*(9), 256.https://doi.org/10.1007/s00894-025-06480-y

Elgawish, M. S., Almatary, A. M., Zaitone, S. A., & Salem, M. S. (2025). Leveraging artificial intelligence and machine learning in kinase inhibitor development: advances, challenges, and future prospects. *RSC Medicinal Chemistry*. DOI: 10.1039/d5md00494b

Fadahunsi, A. A., Uzoeto, H. O., Okoro, N. O., Cosmas, S., Durojaye, O. A., & Odiba,

A. S. (2024). Revolutionizing drug discovery: an AI-powered transformation of molecular docking. *Medicinal Chemistry Research*, *33*(12), 2187-2203. https://doi.org/10.1007/s00044-024-03253-9

Ferguson, D., & RSci, M. R. S. B. (2025). AI-Driven Integrative Design of ADAMTS Partial Agonists within a Multi-Hallmark Therapeutic Framework for Neurodegenerative Disorders.DOI:10.26434/chemrxiv-2025-4n2nb-v4

Gangwal, A., & Lavecchia, A. (2024). AI-driven drug discovery for rare diseases. *Journal of Chemical Information and Modeling*, *65*(5), 2214-2231.https://doi.org/10.1021/acs.jcim.4c01966

Gangwal, A., & Lavecchia, A. (2025). Artificial intelligence in natural product drug discovery: current applications and future perspectives. *Journal of medicinal chemistry*, *68*(4), 3948-3969.https://doi.org/10.1021/acs.jmedchem.4c01257

Garg, P., Singhal, G., Kulkarni, P., Horne, D., Salgia, R., & Singhal, S. S. (2024). Artificial intelligence–driven computational approaches in the development of anticancer drugs. *Cancers*, *16*(22), 3884. https://doi.org/10.3390/cancers16223884

Gentile, F., Yaacoub, J. C., Gleave, J., Fernandez, M., Ton, A. T., Ban, F., ... & Cherkasov, A. (2022). Artificial intelligence–enabled virtual screening of ultra-large chemical libraries with deep docking. *Nature Protocols*, *17*(3), 672-697.https://doi.org/10.1038/s41596-021-00659-2

Gong, Y., Liu, G., Xue, Y., Li, R., & Meng, L. (2023). A survey on dataset quality in machine learning. *Information and Software Technology*, *162*, 107268.https://doi.org/10.1016/j.infsof.2023.107268

Gupta, R., Srivastava, D., Sahu, M., Tiwari, S., Ambasta, R. K., & Kumar, P. (2021). Artificial intelligence to deep learning: machine intelligence approach for drug discovery. *Molecular diversity*, *25*(3), 1315-1360.https://doi.org/10.1007/s11030-021-10217-3

Harmalkar, A., & Gray, J. J. (2021). Advances to tackle backbone flexibility in protein docking. *Current opinion in structural biology*, *67*, 178-186. https://doi.org/10.1016/j.sbi.2020.11.011

Hasan, M. R., Alsaiari, A. A., Fakhurji, B. Z., Molla, M. H. R., Asseri, A. H., Sumon, M. A. A., ... & Kim, B. (2022). Application of mathematical modeling and computational tools in the modern drug design and development process. *Molecules*, *27*(13), 4169. https://doi.org/10.3390/molecules27134169

Jiang, F., Jiang, Y., Zhi, H., Dong, Y., Li, H., Ma, S., ... & Wang, Y. (2017). Artificial intelligence in healthcare: past, present and future. *Stroke and vascular neurology*, *2*(4).https://doi.org/10.1136/svn-2017-000101

Joshi, S. Y., & Deshmukh, S. A. (2021). A review of advancements in coarse-grained molecular dynamics simulations. *Molecular Simulation*, *47*(10-11), 786-803. https://doi.org/10.1080/08927022.2020.1828583

Junaid, M. A. L. (2025). Artificial intelligence driven innovations in biochemistry: A review of emerging research frontiers. *Biomolecules and Biomedicine*, *25*(4),

739. doi: 10.17305/bb.2024.11537

Kihn, K. C., Purdy, O., Lowe, V., Slachtova, L., Smith, A. K., Shapiro, P., & Deredge, D. J. (2024). Integration of Hydrogen–Deuterium Exchange Mass Spectrometry with Molecular Dynamics Simulations and Ensemble Reweighting Enables High Resolution Protein–Ligand Modeling. *Journal of the American Society for Mass Spectrometry*, *35*(11), 2714-2728.https://doi.org/10.1021/jasms.4c00202

Krishna, R., Wang, J., Ahern, W., Sturmfels, P., Venkatesh, P., Kalvet, I., ... & Baker, D. (2024). Generalized biomolecular modeling and design with RoseTTAFold All-Atom. *Science*, *384*(6693), eadl2528. DOI: 10.1126/science.adl2528

Lappala, A. (2024). The next revolution in computational simulations: Harnessing AI and quantum computing in molecular dynamics. *Current Opinion in Structural Biology*, *89*, 102919.https://doi.org/10.1016/j.sbi.2024.102919

Lee, J., Hao Nguyen, C., & Mamitsuka, H. (2025). Beyond rigid docking: deep learning approaches for fully flexible protein–ligand interactions. *Briefings in Bioinformatics*, *26*(5), bbaf454. https://doi.org/10.1093/bib/bbaf454

Liyaqat, T., Ahmad, T., & Saxena, C. (2025). Advancements in Molecular Property Prediction: A Survey of Single and Multimodal Approaches: T. Liyaqat et al. *Archives of Computational Methods in Engineering*, 1-31. https://doi.org/10.1007/s11831-025-10317-5

Ma, W., Xie, Q., Zhang, J., Li, S., Xu, Y., Deng, X., & Zhang, W. (2021). Deep Learning Model of Dock by Dock Process Significantly Accelerate the Process of Docking-based Virtual Screening. *arXiv preprint arXiv:2110.10918*.https://doi.org/10.48550/arXiv.2110.10918

Malla, A. M., & Banka, A. A. (2025). AI-Powered Revolution in Infectious Disease Management: From Early Diagnostics to Drug Discovery. In *Artificial Intelligence in Human Health and Diseases* (pp. 221-236). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-96-8176-1_12

Manan, A., Baek, E., Ilyas, S., & Lee, D. (2025). Digital Alchemy: The Rise of Machine and Deep Learning in Small-Molecule Drug Discovery. *International Journal of Molecular Sciences*, *26*(14), 6807. https://doi.org/10.3390/ijms26146807

Martin, R. L., Heifetz, A., Bodkin, M. J., & Townsend-Nicholson, A. (2023). High-throughput structure-based drug design (HT-SBDD) using drug docking, fragment molecular orbital calculations, and molecular dynamic techniques. *High Performance Computing for Drug Discovery and Biomedicine*, 293-306.https://doi.org/10.1007/978-1-0716-3449-3_13

McNutt, A. T., Francoeur, P., Aggarwal, R., Masuda, T., Meli, R., Ragoza, M., ... & Koes, D. R. (2021). GNINA 1.0: molecular docking with deep learning. *Journal of cheminformatics*, *13*(1), 43.https://doi.org/10.1186/s13321-021-00522-2

Meli, R., Morris, G. M., & Biggin, P. C. (2022). Scoring functions for protein-ligand binding affinity prediction using structure-based deep learning: a review. *Frontiers in bioinformatics*, *2*, 885983. https://doi.org/10.3389/fbinf.2022.885983

Mendolia, I., Contino, S., De Simone, G., Perricone, U., & Pirrone, R. (2022). EMBER—Embedding multiple molecular fingerprints for virtual screening. *International Journal of Molecular Sciences*, *23*(4), 2156. https://doi.org/10.3390/ijms23042156

Mohanan, P. V. (Ed.). (2025). *Artificial Intelligence and Biological Sciences*. CRC Press, Taylor & Francis Group. DOI: 10.1201/9781003492726

Mohanty, M., & Mohanty, P. S. (2023). Molecular docking in organic, inorganic, and hybrid systems: a tutorial review. *Monatshefte für Chemie-Chemical Monthly*, *154*(7), 683-707.https://doi.org/10.1007/s00706-023-03076-1

Montoya, A. L., Hogendorf, A. S., Tingey, S., Kuberan, A., Yuen, L. H., Schüler, H., & Franzini, R. M. (2025). Widespread false negatives in DNA-encoded library data: how linker effects impair machine learning-based lead prediction. *Chemical Science*.DOI: 10.1039/D5SC00844A

Mursal, M., Ahmad, M., Hussain, S., & Khan, M. F. (2024). Navigating the computational seas: a comprehensive overview of molecular docking software in drug discovery. *Unravelling Molecular Docking-From Theory to Practice*.DOI: 10.5772/intechopen.1004802

Nag, S., Baidya, A. T., Mandal, A., Mathew, A. T., Das, B., Devi, B., & Kumar, R. (2022). Deep learning tools for advancing drug discovery and development. *3 Biotech*, *12*(5), 110. https://doi.org/10.1007/s13205-022-03165-8

Niazi, S. K. (2022). Molecular Biosimilarity—An AI-Driven Paradigm Shift. *International journal of molecular sciences*, *23*(18), 10690.https://doi.org/10.3390/ijms231810690

Ouma, R. B., Ngari, S. M., & Kibet, J. K. (2024). A review of the current trends in computational approaches in drug design and metabolism. *Discover Public Health*, *21*(1), 108. https://doi.org/10.1186/s12982-024-00229-3

Pan, X., Lin, X., Cao, D., Zeng, X., Yu, P. S., He, L., ... & Cheng, F. (2022). Deep learning for drug repurposing: Methods, databases, and applications. *Wiley interdisciplinary reviews: Computational molecular science*, *12*(4), e1597. DOI: 10.1002/wcms.

Panday, S. K., & Ghosh, I. (2019). In silico structure-based prediction of receptor–ligand binding affinity: current progress and challenges. *Structural Bioinformatics: Applications in Preclinical Drug Discovery Process*, 109-175. https://doi.org/10.1007/978-3-030-05282-9_5

Parvaiz, N., Ahmad, F., Yu, W., MacKerell Jr, A. D., & Azam, S. S. (2021). Discovery of beta-lactamase CMY-10 inhibitors for combination therapy against multi-drug resistant Enterobacteriaceae. *PLoS One*, *16*(1), e0244967. https://doi.org/10.1371/journal.pone.0244967

Petzold, C. J., & Mukhopadhyay, A. (2025). From bench to biofactory: high-throughput technologies and automated workflows to accelerate biomanufacturing. *Current Opinion in Biotechnology*, *94*, 103320. https://doi.org/10.1016/j.copbio.2025.103320

Priya, S., Tripathi, G., Singh, D. B., Jain, P., & Kumar, A. (2022). Machine learning approaches and their applications in drug discovery and design. *Chemical Biology & Drug Design*, *100*(1), 136-153. https://doi.org/10.1111/cbdd.14057

Rakshit, G., Murtuja, S., Kumar, B. K., Murugesan, S., & Jayaprakash, V. (2022). Structure-based drug design (SBDD). In *Computer aided drug design (CADD): from ligand-based methods to structure-based approaches* (pp. 181-229). Elsevier. https://doi.org/10.1016/B978-0-323-90608-1.00003-4

Rustagi, V., Gupta, S. R., Singh, A., & Singh, I. K. (2025). Beyond trial and error: Leveraging advanced software for Therapeutic discovery. *Chemical Biology Letters*, *12*(1), 1251-1251. DOI:10.62110/sciencein.cbl.2025.v12.1251

Sadybekov, A. V., & Katritch, V. (2023). Computational approaches streamlining drug discovery. *Nature*, *616*(7958), 673-685.https://doi.org/10.1038/s41586-023-05905-z

Siebenmorgen, T., Menezes, F., Benassou, S., Merdivan, E., Didi, K., Mourão, A. S. D., ... & Popowicz, G. M. (2024). MISATO: machine learning dataset of protein–ligand complexes for structure-based drug discovery. *Nature computational science*, *4*(5), 367-378. https://doi.org/10.1038/s43588-024-00627-2

Son, A., Park, J., Kim, W., Yoon, Y., Lee, S., Park, Y., & Kim, H. (2024). Revolutionizing molecular design for innovative therapeutic applications through artificial intelligence. *Molecules*, *29*(19), 4626.https://doi.org/10.3390/molecules29194626

Soni, M., & Pratap, J. V. (2022). Development of novel anti-leishmanials: the case for structure-based approaches. *Pathogens*, *11*(8), 950. https://doi.org/10.3390/pathogens11080950

Talat, A., & Khan, A. U. (2023). Artificial intelligence as a smart approach to develop antimicrobial drug molecules: A paradigm to combat drug-resistant infections. *Drug discovery today*, *28*(4), 103491.https://doi.org/10.1016/j.drudis.2023.103491

Tarabara, U., Zhytniakivska, O., Vus, K., Trusova, V., & Gorbenko, G. (2022). Multiple Docking of Fluorescent Dyes to Fibrillar Insulin. *East European Journal of Physics*, (3), 115-120. https://doi.org/10.26565/2312-4334-2022-3-15

Tiwari, P. C., Pal, R., Chaudhary, M. J., & Nath, R. (2023). Artificial intelligence revolutionizing drug development: Exploring opportunities and challenges. *Drug Development Research*, *84*(8), 1652-1663.https://doi.org/10.1002/ddr.22115

Tursunalieva, A., Alexander, D. L., Dunne, R., Li, J., Riera, L., & Zhao, Y. (2024). Making sense of machine learning: a review of interpretation techniques and their applications. *Applied Sciences*, *14*(2), 496. https://doi.org/10.3390/app14020496

Ungerer, T., & Carpenter, P. (2018). Eurolab-4-HPC long-term vision on high-performance computing. *arXiv preprint arXiv:1807.04521*. https://doi.org/10.48550/arXiv.1807.04521

Vicidomini, C., Fontanella, F., D'Alessandro, T., & Roviello, G. N. (2024). A survey on computational methods in drug discovery for neurodegenerative diseases. *Biomolecules*, *14*(10), 1330.https://doi.org/10.3390/biom14101330

Vittorio, S., Lunghini, F., Morerio, P., Gadioli, D., Orlandini, S., Silva, P., ... & Beccari, A. R. (2024). Addressing docking pose selection with structure-based deep learning: recent advances, challenges and opportunities. *Computational and Structural Biotechnology Journal*, *23*, 2141-2151.https://doi.org/10.1016/j.csbj.2024.05.024

Wang, X. Y., Chen, Y., Li, Y. F., Wei, C. Y., Liu, M. Y., Yuan, C. X., ... & Li, X. T. (2025). Advancing active compound discovery for novel drug targets: insights from AI-driven approaches. *Acta Pharmacologica Sinica*, 1-12. https://doi.org/10.1038/s41401-025-01591-x

Yang, C., Chen, E. A., & Zhang, Y. (2022). Protein–ligand docking in the machine-learning era. *Molecules*, *27*(14), 4568. https://doi.org/10.3390/molecules27144568

Yingngam, B. (2024). AI-Driven Drug Discovery and Repurposing for Neurodegenerative Disorders. In *Machine Learning for Neurodegenerative Disorders* (pp. 80-124). CRC Press. https://doi.org/10.1201/9781032661025

Zayed, A. O. H. (2025). Optimizing protein-ligand docking through mac https://doi.org/10.1007/s44371-025-00246-4

Zhang, K., Fan, Z., Wu, Q., Liu, J., & Huang, S. Y. (2025). Improved Prediction of Drug–Protein Interactions through Physics-Based Few-Shot Learning. *Journal of Chemical Information and Modeling*. https://doi.org/10.1021/acs.jcim.5c00427

Zhang, X., Shen, C., Zhang, H., Kang, Y., Hsieh, C. Y., & Hou, T. (2024). Advancing ligand docking through deep learning: challenges and prospects in virtual screening. *Accounts of chemical research*, *57*(10), 1500-1509.https://doi.org/10.1021/acs.accounts.4c00093

Chapter 4

MICROGLIA-MEDIATED NEUROINFLAMMATION: CELLULAR AND MOLECULAR MECHANISMS, PATHOPHYSIOLOGICAL CONSEQUENCES, AND THERAPEUTIC PERSPECTIVES

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1. INTRODUCTION

Neuroinflammation is seen as the process of inflammation happening in the brain and spinal cord. The process happens when cytokines, chemokines, reactive oxygen species (ROS), and other secondary messengers are made. These processes are controlled by different types of support cells in the central nervous system (CNS), like microglia, astrocytes, endothelial cells, and also immune cells from outside the CNS (DiSabato et al., 2016). The main job of microglia, which are the immune cells in the central nervous system, is to keep the immune system balanced and protect the neurons from damage. Neuroinflammation starts the process of microglial activation and changes in their function, which can be seen in several diseases like Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and prion-related neurodegenerative diseases (Schetters et al., 2018). Kim and de Vellis (2005) state that interleukins, interferons, tumor necrosis factors, colony-stimulating factors, and transforming growth factors produced by microglia play roles in both regulating inflammation, and in development, growth, and homeostasis. Oxidative stress is a hallmark of most neurodegenerative diseases. It is characterized by too much of a number of harmful chemicals that are known as reactive oxygen species. In normal physiological situations, reactive oxygen species (ROS) help maintain a redox balance in the cells, but an excess of ROS can damage lipids, proteins and DNA, leading to the loss of cellular function and cell death (Teleanu et al., 2022). Oxidative stress is an early indicator of damage in chronic neurodegenerative diseases such as Alzheimer's disease, and is known to contribute to the chronic loss of brain cells and deterioration of synaptic connections (Moreira et al., 2005). At present, the forward progression of neuroinflammation is regulated by the interactions among microglia, astrocytes, and the peripheral immune cells within the central nervous system. Chronic activation of microglial cells and their interaction with T lymphocytes and astrocytes make the continuous release of cytokines, proteases, and other oxidative stress-mediated factors, which aggravates and sustains the chronic inflammation in the brain, as observed in multiple sclerosis, stroke, Alzheimer's disease, and Parkinson's disease (Shi and Yong, 2025). A vicious cycle exists between brain inflammation and brain cell death because activated brain-resident immune cells and infiltrating peripheral immune cells continuously produce harmful factors. As a result, the modulation of immune responses by agents capable of reaching the brain becomes a promising research field for the treatment of brain diseases characterized by cell loss (Shi and Yong, 2025).

Neurodegenerative diseases are a group of heterogeneous disorders that characteristically involve the progressive loss of nerve cells in the central and/or peripheral nervous system. Due to the limited capacity of neurons for self-repair, their loss leads to structural damage and dysfunction of the

brain networks, resulting in varying degrees of impairment of memory, cognition, behavior, sensorimotor and other functions (Wilson et al., 2023). In this context, elucidating the molecular mechanisms underlying brain inflammation mediated by microglia and their links to neurodegenerative diseases is paramount for developing novel therapeutic strategies.

2. MICROGLIA and NEUROINFLAMMATION

2.1 General Characteristics of Microglia

The central nervous system (CNS) contains a special type of immune cells, called microglia cells. These cells stem from the myeloid cell lineage and enter the CNS and multiply during embryonic development. Microglia cells represent nearly 5–20% of the whole CNS glial cell population. Microglia cells play important part in homeostasis maintenance of the CNS, including physical defense, work out and pathogenesis, embryonic development, and tissue restoration (Brück, 2020). Being intrinsic macrophages of the brain, microglia, with a variety of surface receptors, can sense the external environment and detect the external agents, damaged and dying cells, and misfolded proteins through a series of receptors such as toll-like receptors (TLRs) and pattern recognition receptors (PRRs) (Schetters et al., 2018). Except for the immunological functions, the microglia cells participate in the synaptic pruning, removal of apoptotic cells and debris, and modulation of neuronal activity, which is crucial for the plasticity maintenance of the CNS. Rather than representing a single activated state, microglia exhibit diverse functional phenotypes depending on the microenvironment in which they operate. Under physiological conditions, microglia display neuroprotective properties, whereas during infections, acute injuries, and chronic neurodegenerative processes they may acquire pro-inflammatory characteristics and contribute to the progression of neuroinflammation (Giunti et al., 2014).

2.2 Microglial Activation and Neuroinflammation

During central nervous system (CNS) injury and various neurological disorders, microglia undergo a complex series of cellular responses collectively referred to as “activation” (Gao et al., 2023). Neurodegeneration-associated alterations in the microenvironment trigger microglial activation and excessive inflammatory responses (Karabag et al., 2023). In this process, pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) play essential roles. In particular, IL-1β amplifies inflammatory responses by stimulating the release of other pro-inflammatory mediators (Barrientos et al., 2015). Reactive oxygen species (ROS) generated during microglial activation are normally counterbalanced by endogenous antioxidant defense systems; however, excessive ROS production leads to the development of oxidative stress (Simpson and Oliver, 2020). Although ROS produced by microglia constitute an important

defense mechanism against pathogens, excessive ROS generation may induce cellular damage through multiple pathways, including lipid peroxidation, protein and DNA damage, mitochondrial dysfunction, and caspase activation (Simpson and Oliver, 2020; Kaymak and Aydın, 2021). Persistent exposure to inflammatory mediators and oxidative stress disrupts synaptic transmission and neuronal plasticity, ultimately resulting in cognitive dysfunction and impairment of neuronal network integrity (Henstridge et al., 2016; Nugent et al., 2023). Therefore, chronic microglial activation and the resulting neuroinflammatory responses are considered among the major pathophysiological mechanisms underlying neurodegenerative diseases.

3. MOLECULAR MECHANISMS

3.1 NF-κB Signaling Pathway

The transcription factor known as nuclear factor-kappa B, or NF-κB, is a group of proteins that help the body manage inflammation and protect against infections.They do this by controlling the genes that produce certain molecules, such as cytokines, chemokines, proteins involved in the body's reaction to damage, molecules that help cells stay connected, and enzymes that can be activated when needed (Babkina et al., 2021). NF-κB signaling occurs via two primary routes, the canonical pathway and the non-canonical pathway. The usual process begins with various signals, such as those from cytokine receptors, pattern recognition receptors (PRRs), some parts of the tumor necrosis factor receptor (TNFR) family, and T- and B-cell receptors. In the usual process, the IKK complex leads to a chemical change called phosphorylation, which makes the blocking protein IκBα break down, allowing parts of NF-κB, like the p50/RelA and p50/c-Rel pairs, to enter the nucleus, as found by Liu and their team in 2017. Unlike the main NF-κB pathway, the non-canonical pathway gets activated by certain receptors in the TNFR family, including LTβR, BAFFR, CD40, and RANK. The non-canonical NF-κB pathway works differently from the canonical pathway because it uses a protein called p100, which is part of the NF-κB2 protein, instead of breaking down the IκBα protein. When NIK and IKKα become active, they break down the p100 protein into p52.Then, p52 joins RelB and enters the cell's nucleus, according to Liu and other researchers in 2017. The usual NF-κB pathway is important for the body's first line of defense against infections and also helps in building up long-term immunity.But there's also another NF-κB pathway that works together with the usual one, and it helps manage some specific functions of the immune system that adapt over time (Liu et al., 2017).

In acute inflammation, the NF-κB protein becomes very active when it is activated by molecules like PAMPs and DAMPs, as well as by some inflammatory signals such as TNF-α and IL-1β. When NF-κB becomes active, the body starts making more of several substances that cause inflammation,

such as IL-1, IL-6, IL-12, TNF-α, chemokines, cell adhesion molecules, and other inflammatory proteins like inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2.These substances help bring in immune cells, fight off harmful germs, and start the process of healing damaged tissues (Mao et al., 2025). If the NF-κB signaling pathway is activated too much or not in the right way, it can cause a continuous release of chemicals that trigger inflammation, which may lead to a long-lasting and ongoing inflammation issue. Continuous activation of NF-κB can occur due to factors such as oxidative stress, imbalances in the body's metabolism, autoimmune reactions, or exposure to environmental factors.This ongoing activation is one of the proposed ways that neurodegenerative diseases might begin, as mentioned in Mao et al., 2025.

3.2 NLRP3 Inflammasome

NLRP3 is a kind of protein that helps detect harmful signals in microglia and astrocytes. When cells experience stress or get damaged, NLRP3 joins with other proteins to create a group called an inflammasome. This group plays a role in starting the process of neuroinflammation. The NLRP3 inflammasome is a group of proteins found inside cells that help manage inflammation and are involved in removing damaged cells and aiding the body in fixing tissues when they are active in the brain (Xu et al., 2025). The NLRP3 inflammasome can be activated by different signals.For example, when the P2X7 receptor causes potassium to exit the cell, when calcium flows into the cell from inside, when reactive oxygen species are produced due to stress in mitochondria, when DNA or cardiolipin from mitochondria leaks out, or when lysosomal cathepsin B is released after the lysosome breaks (He et al., 2025). Inflammasome activation takes place in two parts. The first part is called priming, and the second part is called activation. During the priming phase, toll-like receptors (TLRs) pick up signals from harmful microorganisms and injured cells, which sets off the NF-κB signaling pathway. Once NF-κB becomes active, it travels to the nucleus of the cell, attaches to the DNA, and begins producing copies of the genes for NLRP3, pro-IL-1β, and pro-IL-18 (He et al., 2025). If the body isn't able to control the inflammasome properly, it can cause neuroinflammation.This type of inflammation can harm neurons and is involved in the development of several diseases that affect the central nervous system, such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, and stroke (Xu et al., 2025).

3.3 TLR4 and MAPK Signaling Pathways

Toll-like receptor 4 (TLR4) is a type of protein that is found in the cell membrane. It has three parts: one part is on the outside of the cell, one part is embedded in the cell membrane, and one part is inside the cell. The part outside the cell that helps in recognizing specific molecules has repeated

sections called leucine-rich repeats. Every one of these sections is comprised of 20 to 29 building blocks known as amino acids. These having been arranged are a set of specific locations, as a result of which the accession of ligands can be detected subsequently. Lipopolysaccharide or LPS is an LPS binding TLR4, which interaction with this receptor has been comprehensively studied (Kim et al., 2023). Gallium does not bind with TLR4 directly, and therefore uses a companion protein known as myeloid differentiation factor-2 (MD-2) to link to the TLR4 receptor. When the MD-2, TLR4 and LPS complex binds together, they initiate the process of signaling to upstream events. There are other bacterial, viral and fungal proteins which can also activate TLR4 in a similar fashion to LPS. When cellular injury takes place, various substances are released into the vicinity. Among them are fragments of the cell's outer layer, such as the extracellular matrix and other constituents within the cell, such as high-mobility group box 1 (HMGB1), DNA binding proteins and heat shock proteins (HSPs). Some damage-associated molecular patterns (DAMPs) are known to start immune responses through TLR4, but exactly how they do this is still not fully understood (Kim et al., 2023).

Inside the cell, the TLR4 signaling process uses two different routes: one that relies on MyD88 and another that doesn't need MyD88. When the TIR domain inside the cell joins together, it brings in two helper proteins called MyD88 and TIRAP, which is also known as MAL-like protein. This process starts a signal by making certain enzymes called IRAKs become activated through a process called phosphorylation. When IRAKs are activated, they get a phosphate group added, which then helps them start a process by activating TRAF6.This happens through TAK1-binding proteins called TAB2 and TAB3. When TAK1 is turned on, it helps activate several proteins called MAPKs, like JNK, p38, ERK 1/2, and the IKK complex.These then help activate certain proteins inside the cell, such as NF-κB and AP-1, and lead to the production of inflammatory proteins called cytokines (Ageeva et al., 2024). MyD88-independent signaling happens when the TLR4-ligand complex is taken inside the cell into endosomes. This process needs the TIR domain to interact with TRIF and TRAM adaptors. TBK1 and IKKε become active through TRAF3, which is necessary for moving IRF3 into the nucleus, leading to the production of type I interferons (IFNs) (Kim et al., 2023). In microglial cells, when the TLR4 pathway activates the PI3K/Akt pathway, it increases the production of pro-inflammatory substances. In this situation, microglial cells act in a harmful way toward neurons. They produce harmful substances like reactive oxygen species, certain enzymes, nitric oxide, and inflammatory proteins such as IL-6, IL-1β, and TNF-α.These substances attract more glial cells and make the damage to neurons worse (Biswas, 2023).

3.4 JAK/STAT Signaling Pathway

The JAK/STAT pathway is an important process that allows signals from cytokines and growth factors to move into cells. The JAK/STAT signaling pathways help control genes that play a role in causing inflammation, creating glial scars, and handling immune responses (Ageeva et al., 2024). The JAK/STAT signaling pathway plays an important role in helping myeloid and lymphoid cells develop and control their functions. When the JAK/STAT pathway is disrupted, it leads to myeloid cells and T cells becoming more harmful, and this also helps in the start and worsening of neuroinflammatory diseases (Jain et al., 2021). The NF-κB, NLRP3, TLR4/MAPK, and JAK/STAT pathways work together and control the neuroinflammatory response caused by microglia. When certain signaling pathways don't work properly, it helps keep chronic inflammation going and also makes neurodegenerative diseases get worse.

4. ASSOCIATION with NEURODEGENERATIVE DISEASES

4.1 Alzheimer's Disease

Alzheimer's disease is the most common reason for dementia, causing about 70% of all dementia cases, and it is a big problem for public health because it has a large effect on society and the economy. Neuropathologically, Alzheimer's disease is marked by the buildup of faulty and clumped amyloid-beta (Aβ) peptides outside nerve cells and the development of tangles inside nerve cells made up of a protein called tau that has been overly modified with phosphate groups. Alois Alzheimer first noticed changes in astroglial and microglial cells, but for a long time, these changes were seen as secondary and not very important for understanding the disease (Heneka et al., 2025). New research has shown that microglia linked to disease and their changed signaling paths play a key role in Alzheimer's disease. In the year AD, microglia react to certain molecules from pathogens and damaged cells by changing into a type of cell that causes more inflammation, which makes the disease get worse faster. On the other hand, microglia can help protect the brain by releasing anti-inflammatory chemicals like interleukin (IL)-10 and transforming growth factor β (TGF-β).They also clean up harmful substances such as Aβ and damaged nerve cells when they receive signals to do so. Studies have helped us learn more about how microglia become activated and cause brain inflammation when they respond to many types of signaling molecules like cytokines, chemokines, phospholipases, and others. More and more research shows that the buildup of Aβ is closely linked to inflammation in the brain. In brain tissue from people with Alzheimer's disease, activated microglia surround both the Aβ plaques that form outside brain cells and the neurons that have neurofibrillary tangles inside them. Aβ has been shown to trigger microglia, causing them to release inflammatory

substances like IL-1β, IL-6, TNF-α, and certain chemokine ligands (CCL2, CCL4, and CCL11).These substances then help bring more microglia and astrocytes to the areas where Aβ is accumulating. Microglia can clear Aβ by utilizing various surface receptors such as CD14, TLR2, TLR4, α6β1 integrin, CD47 and CD36 scavenger receptors. The large accumulation of Aβ in Alzheimer's disease (AD) brains results from failure of clearance by brain resident microglia. Studies with post mortem Alzheimer's disease brain tissue discovered reduced Aβ uptake by microglia that are surrounding Aβ plaques in Alzheimer's disease brain tissues in comparison with microglia in healthy brain (Onyango et al., 2021). The brain resident microglia phenotypically respond to 'danger' signals such as PAMPs and DAMPs from infected or dead cells. This change leads them to release harmful chemicals, such as TNF-α, IL-1β, IL-6, nitric oxide, and reactive oxygen species. This happens because several internal signal pathways, including NF-κB, MAPKs, ERK, JNK, p38, and the NLRP3 inflammasome, get activated (García-Domínguez, 2025). In people with Alzheimer's disease, high levels of inflammation markers have been found in both the brain's outer layer and the fluid around the brain, showing that the brain keeps having inflammation happening over time. Besides that, studies linking genes related to Alzheimer's disease risk with the body's natural defense system and findings from after death have shown that neuroinflammation plays a key role in the development of Alzheimer's disease (Zhang et al., 2024).

At the early stage of AD, activated microglia can ameliorate Aβ accumulation by phagocytosis, shielding barriers around plaques, and degradation of Aβ, to return to the normal state. However, the over-activation of inflammatory pathways and the down-regulation of anti-inflammatory cascades severely affect the phagocytic and degradative functions of microglia. Therefore, an irreversible self-amplifying positive feedback loop is formed, which continuously activates microglia, promotes progressive neuronal injury, and increases plaque deposition, further accelerating the progression of the disease (Valiukas et al., 2025).

4.2 Parkinson's Disease

Parkinson's disease is a lasting condition that impacts the brain and happens because of the gradual loss of certain nerve cells that produce dopamine.These cells are located in a part of the brain known as the substantia nigra.When these cells are damaged, it causes issues with movement, such as slow movements, muscle stiffness, shaking, and trouble with balance.This information is based on Dhariwal et al., 2025. Different things, like when α-synuclein folds incorrectly, issues with mitochondria, and changes in genes connected to the immune system, could all contribute to starting long-term brain inflammation in Parkinson's disease. Other than the loss of neurons that make dopamine, there are also several bad changes happening in the

substantia nigra pars compacta.These include the buildup of alpha-synuclein, problems with the body's ability to break down proteins, and issues with mitochondria, which are the energy centers of cells.These changes create an environment that promotes inflammation in the SNpc and encourage other cell types to come and become active (Isik et al., 2023). Studies have shown that in people with Parkinson's disease, there is more activation of microglia, which are a type of brain cell involved in the immune response (Tansey et al., 2022). In the early stages of the disease, microglia start to become active and take part in inflammation in certain areas of the brain that are more affected (Tansey et al., 2022). Parkinson's disease mainly happens when the nigrostriatal pathway doesn't work properly.This happens because the neurons that make dopamine in the substantia nigra pars compacta start to die, and there's not enough dopamine signaling in the striatum (Troncoso-Escudero et al., 2018).

4.3 Multiple Sclerosis

Multiple sclerosis is a long-term inflammatory condition that mainly affects the central nervous system. It is marked by damage to the myelin sheath, which is the protective covering of nerves, and can also lead to some loss of the nerve fibers themselves. Multiple sclerosis affects both gray and white matter in the brain, such as the cerebral cortex, basal ganglia, brainstem, and white matter, as well as the spinal cord (Höftberger and Lassmann, 2017). Demyelination happens when astrocytes become active during active injury and also occurs when there are gliotic scars in areas where the injury is not active anymore. In areas where the myelin is breaking down, activated microglia and macrophages produce an enzyme called NADPH oxidase, which helps cause oxidative damage to tissues (Höftberger and Lassmann, 2017). Microglia become very active around the edges of areas where the myelin is damaged, and they are also clearly seen in the region around the plaques. Sometimes, they can even be found in parts of the white matter that look normal. You can see groups of active microglia and lymphocytes around some axons that are starting to break down, which is common in multiple sclerosis plaques, as noted by Lassmann in 2018. Over the years, scientists have studied a lot about how microglia and macrophages contribute to the development of multiple sclerosis. At first, these cells were thought to just be helpers that cause inflammation, making tissue damage worse by presenting antigens using major histocompatibility complex, creating harmful oxygen and nitrogen substances, releasing pro-inflammatory signaling molecules, and eating up damaged cells (Muzio et al., 2021).

4.4 Microglia-Mediated Neuroinflammation in Other Neurological Disorders

Microglia-caused brain inflammation has been found in not only Alzheimer's disease, Parkinson's disease, and multiple sclerosis, but also in many other brain conditions such as amyotrophic lateral sclerosis, Huntington's disease, epilepsy, stroke, traumatic brain injury, spinal cord injury, and several other similar disorders (Subhramanyam et al., 2019). Under usual conditions, microglia help keep the brain balanced and healthy.But when the brain faces harmful changes, like infections, lack of blood flow, injuries, or the start of diseases that damage nerves, microglia change their behavior and become active (Subhramanyam et al., 2019). Microglia detect danger signals from harmful or damage-related molecules using toll-like receptors (TLRs) and other types of receptors that help the body respond to threats in the brain and spinal cord. Microglial activation can be beneficial or detrimental under neuroinflammatory conditions. The excessive release of some of the noxious substances like pro-inflammatory cytokines, reactive oxygen species and other toxic products can maintain chronically injured neurons (Kraft and Harry, 2011). The research studies have revealed that microglia, the resident immune cells of the brain, modulate their functions based on the signals that they receive from their surrounding environment.If these cells remain active for a sustained period of time it can result in chronic inflammation and brain cell injury which is closely related to several neurodegenerative diseases (Fołta et al., 2025). These diseases may differ in terms of symptoms and way of injury, but they do have common features resulting in damage. Some of them are prolonged activation of microglia, increased oxidative stress and prone inflammatory response of the body. We need to learn more about how microglia lead to brain inflammation so we can discover new treatments for diseases that impact the nervous system.

5. CURRENT APPROACHES and THERAPEUTIC STRATEGIES

5.1 Therapeutic Targeting of Neuroinflammation

Cytokines and chemokines play a key role in controlling how the immune system responds outside the body's main organs. In addition, they help the immune and nervous systems work together (Ramesh et al., 2013). Some chemicals in the nervous system help control how the brain develops, how inflammation happens in the nerves, and how nerve cells communicate with each other (Ramesh et al., 2013). When microglia become activated, certain health issues can lead to damage in neurons and other brain cells. This happens because the activated microglia produce various harmful substances like cytokines, chemokines, glutamate, and reactive oxygen species, which cause inflammation and harm. They also help move parts of cells across the blood-brain barrier, which happens when the body's immune

system responds and cells move toward certain signals. In cases of chronic inflammation, the positive feedback loop comprised of effector cells sustains the immune response over long periods, and it ultimately leads to neuronal damage (Ramesh et al., 2013). Therefore, the development of therapies that target the modulation of neuroinflammation is nowadays an essential strategy to ameliorate several neurological disorders, including Parkinson's disease, Alzheimer's disease, multiple sclerosis and other similar pathologies, as these have a highly complex etiology and progression. This complex understanding of neuroinflammation has spawned numerous pharmacological therapies able to modulate both molecular and cellular processes that underpin inflammation. These treatments comprise commonly used drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), steroids, monoclonal antibodies, and novel therapies in development, such as the modulation of microglial cells and anti-inflammatory drugs (García-Domínguez, 2025). Many NSAIDs have emerged as an alternative to traditional drugs used to control inflammation. Despite their ability to inhibit cyclooxygenase (COX), these molecules also influence the synthesis of inflammatory molecules in a COX-independent manner. NSAIDs stop the process where NF-κB moves into the cell nucleus and activate it, which helps reduce inflammation. This effect, along with blocking prostaglandin production, helps repair damage in nerve cells. In addition to NSAIDs, several other known anti-inflammatory compounds, such as the corticosteroids dexamethasone and methylprednisolone, also show potent anti-inflammatory activity because they act through glucocorticoid receptors (García-Domínguez, 2025).

5.2 Therapeutic Modulation of Microglia

The nervous system and immune system communicate with each other, resulting in a bidirectional interaction between the two systems, in which each system has profound effects on the other (Perry and Teeling, 2013). Microglia are highly sensitive to virtually all injury or disease of the central nervous system and become activated in response to suitable stimuli (Perry and Teeling, 2013). Neuron-microglia interactions have two major outcomes: (1) during acute or chronic neurodegenerative processes, neuronal loss releases microglia from negative tonic inhibitory signals derived from neurons, which results in continuous microglial activation (Perry and Teeling, 2013). (2) Neuron-microglia interactions also enhance tissue repair and regenerative functions. Colony-stimulating factor-1 (CSF-1) is critical for microglial activation, survival, and functional maintenance.

The common receptor for IL-34 and CSF-1, known as CSF1R, is required for signaling by both cytokines. Pharmacological inhibition of CSF1R is known to attenuate microglial cell numbers, although the potential unintentional effect on peripheral immune cells remains a concern. Microglia can release circulating pro-inflammatory mediators, but they also play important roles in

tissue repair and regeneration. So, using molecular methods to turn harmful microglia-like cells into more protective ones has been proposed as a possible treatment, especially for diseases like multiple sclerosis, as mentioned in Muzio et al., 2021.

5.3 Experimental Therapies and Emerging Perspectives

5.3.1 Novel Therapeutic Approaches in Alzheimer's Disease

In recent years, scientists have looked into different methods to slow or stop Alzheimer's disease, including using nanoparticles, plant-based chemicals, and small molecule drugs. The blood-brain barrier is very strict and helps keep the central nervous system balanced, but it makes it hard to get medicine to the brain. So, using nanoparticle-based platforms is seen as a promising way to send drugs to the right places in the body. PEG-PLGA nanoparticles that have been modified with ligands that target Aβ have been used to deliver curcumin and help lower the amount of amyloid plaques. In addition, nanoparticles targeted at hyperphosphorylated tau protein allow the selective delivery of therapeutic agents to the affected regions in an attempt to reduce systemic side effects and improve therapeutic efficacy (Dhariwal et al., 2025).

5.3.2 Novel Therapeutic Approaches in Parkinson's Disease

Current therapeutic approaches for Parkinson's disease (PD) are focused on motor symptom control and are not able to stop disease progression. Rapid drug metabolism, low bioavailability, short half-life, and poor brain penetration are major challenges of current therapies for PD. Nanotechnology has been more recently explored to overcome many of these difficulties, including protection of therapeutic agents against degradation, targeted delivery, and reduction of drug dosage. Gold-doped titanium oxide (TiO) nanotubes have been used for the detection of α-synuclein on the basis of photoelectrical techniques. Moreover, gold nanoparticles conjugated with nerve growth factor have been found to suppress α-synuclein aggregation. Curcumin nanomaterials improve dopamine levels, enhance blood-brain barrier penetration, inhibit α-synuclein aggregation, and improve mitochondrial function. On the other hand, liposomal preparations show the advantage of delivering more controlled and sustained dopamine release and have been shown to have beneficial effects in experimental paradigms of Parkinson's disease (Dhariwal et al., 2025).

5.3.3 Novel Therapeutic Approaches in Multiple Sclerosis

Therapeutic strategies to treat multiple sclerosis (MS) include symptomatic nutraceutical remedies and conventional pharmacological drugs. All current therapeutic approaches directly or indirectly aim at controlling symptoms and preserving the patient's quality of life. More recently, nanotechnology-

based systems have been proposed for drug delivery and can improve the therapeutic approaches to treat MS. For instance, porous and water-soluble corticosteroid implants in poly(ethylene glycol) liposomes were demonstrated in a murine model of neuroinflammation to selectively accumulate at inflammatory sites and/or delay the onset of inflammation. Liposomes containing myelin basic protein (MBP) peptides were also used to circumvent disease by a mannosylated liposome. Liposome formulations have been also delivered to the brain through the nasal cavity. As another therapeutic alternative, non-oligomeric gold nanocrystal suspension CNM-Au8® was proposed to favor neuroprotection and remyelination in the brain (Dhariwal et al., 2025). Another experimental method uses nanostructures that mimic natural structures, made from polycyclic aromatic hydrocarbons (PAHs) and single-walled carbon nanotubes (SWNTs).These structures might help find specific disease markers in the breath of people with MS. However, most of these methods still need more testing in big preclinical and clinical studies.

6. CONCLUSION and FUTURE PERSPECTIVES

Neuroinflammation is a complex response in the cells of the central nervous system that happens when the body's balance is thrown off. It is considered one of the main reasons that cause and make worse neurodegenerative diseases. Microglia are the immune cells that are naturally present in the central nervous system, and they help keep the tissue healthy and in balance. When cells in the brain get hurt, microglia — which are a type of brain cell — become active as part of the body's natural way to keep things balanced. This activation causes the release of harmful things like pro-inflammatory cytokines and chemokines, reactive oxygen species, proteases, and other toxic materials. These harmful substances help start and keep going the process of brain cell damage and diseases. If these processes keep happening out of control and over a long time, it can cause problems in the brain cells, loss of connections between cells, and gradual damage to nerve cells, which can eventually lead to diseases that damage the nervous system. New research shows that microglia-driven inflammation is not just a result of brain cell damage but also plays an active role in causing it. Also, there is strong evidence that in the pathways related to brain inflammation, certain processes like NF-κB, the NLRP3 inflammasome, TLR4/MAPK, and JAK/STAT signaling play a major role in activating microglia. These processes could be important targets for treatments that reduce brain inflammation, and might offer new ways to treat the harmful effects of neurodegenerative diseases. Having a better grasp of these signaling networks is important for creating new treatment options. Alzheimer's disease, Parkinson's disease, and multiple sclerosis each have different signs and changes in the body, but scientists are now learning more about the shared causes and processes that affect all three. Chronic activation of microglial cells, increased oxidative stress, problems with mitochondria,

and the activation of pathways that cause inflammation are common issues that need more attention. These could be important areas to focus on when creating better treatments that protect the brain from specific diseases and more general damage.

Inflammation is a common pathological phenomenon of many brain diseases in which nerve cells undergo degeneration. Anti-inflammatory drugs are a class of broad-spectrum medicines and do not target one single disease. Researchers are paying greater attention to them as they help the development of these diseases. Anti-inflammatory drugs are often recommended as a good treatment for symptoms of inflammatory diseases, as they can reduce or control the body's inflammatory responses. Traditional anti-inflammatory and immunomodulatory drugs have some evidence that they can reduce inflammation in the nervous system. However, the penetration of these target substances into the brain remains a major challenge due to the blood-brain barrier and the complex neuroinflammatory processes in the central nervous system, which makes it difficult to treat and prevent neuroinflammation. Therefore, CSF1R inhibitors, inflammasome inhibitors, and certain modulators of signaling pathways appear to be handsome and new options for reducing neuroinflammation and because not all substances are able to reach the brain and control inflammation in the nervous system. New technologies based on nanotechnology, targeting, and biocompatible nanoparticles have been developed to help drugs reach the brain more safely and efficiently. These strategies are effectively used in treating nervous system diseases. Importantly, therapies targeting the Aβ and tau proteins in Alzheimer's disease, nanostructures that contribute to prevent α-synuclein aggregation in Parkinson's disease, and nanosystems designed to facilitate remyelination in multiple sclerosis are promising for real-world studies and applications. Recent studies indicate that microglia can't simply be classified as either pro-inflammatory or anti-inflammatory. Microglia expressed different activity levels according to the type of disease and environmental signals. Recent advances in single-cell omics, transcriptomic research, and the use of bioinformatics for artificial intelligence are expected to improve our understanding of the different types of microglia and may help identify disease targets and personalized treatment plans.

In conclusion, a thorough knowledge of the molecular mechanisms of microglia-mediated neuroinflammation is crucial for clarifying the pathogenesis of neurodegenerative diseases and identifying novel therapeutic targets. Multidisciplinary research, integrating advances in molecular biology, neuroscience, immunology and nanotechnology, will potentially lead to more innovative and sustainable interventions for neurodegenerative diseases.

REFERENCES

Ageeva, T., Rizvanov, A., & Mukhamedshina, Y. (2024). NF-κB and JAK/STAT signaling pathways as crucial regulators of neuroinflammation and astrocyte modulation in spinal cord injury. *Cells, 13*(7), 581.

Babkina, I. I., Sergeeva, S. P., & Gorbacheva, L. R. (2021). The role of NF-κB in neuroinflammation. *Neurochemical Journal, 15*(2), 114–128.

Barrientos, R. M., Kitt, M. M., Watkins, L. R., & Maier, S. F. (2015). Neuroinflammation in the normal aging hippocampus. *Neuroscience, 309*, 84–99.

Biswas, K. (2023). Microglia mediated neuroinflammation in neurodegenerative diseases: A review on the cell signaling pathways involved in microglial activation. *Journal of Neuroimmunology, 383*, 578180.

Brück, M. D. L. (2020). Homöostase im Gehirn: Die differentielle Aktivität der Mikroglia (Doctoral dissertation, Christian-Albrechts-Universität zu Kiel, Medical Faculty).

Dhariwal, R., Jain, M., Mir, Y. R., Singh, A., Jain, B., Kumar, P., Tariq, M., Verma, D., Deshmukh, K., Yadav, V. K., & Malik, T. (2025). Targeted drug delivery in neurodegenerative diseases: The role of nanotechnology. *Frontiers in Medicine, 12*, 1522223.

DiSabato, D. J., Quan, N., & Godbout, J. P. (2016). Neuroinflammation: The devil is in the details. *Journal of Neurochemistry, 139*, 136–153.

Fołta, J., Rzepka, Z., & Wrześniok, D. (2025). The role of inflammation in neurodegenerative diseases: Parkinson's disease, Alzheimer's disease, and multiple sclerosis. *International Journal of Molecular Sciences, 26*(11), 5177.

Gao, C., Jiang, J., Tan, Y., & Chen, S. (2023). Microglia in neurodegenerative diseases: Mechanism and potential therapeutic targets. *Signal Transduction and Targeted Therapy, 8*(1), 359.

García-Domínguez, M. (2025). Neuroinflammation: Mechanisms, dual roles, and therapeutic strategies in neurological disorders. *Current Issues in Molecular Biology, 47*(6), 417.

Giunti, D., Parodi, B., Cordano, C., Uccelli, A., & Kerlero de Rosbo, N. (2014). Can we switch microglia's phenotype to foster neuroprotection? Focus on multiple sclerosis. *Immunology, 141*, 328–339.

He, K., Yu, X., Xia, L., Xie, Y., Qi, E., Wan, L., Hua, X., & Jing, C. (2025). A new perspective on the regulation of neuroinflammation in intracerebral hemorrhage: Mechanisms of NLRP3 inflammasome activation and therapeutic strategies. *Frontiers in Immunology, 16*, 1526786.

Heneka, M. T., van der Flier, W. M., Jessen, F., et al. (2025). Neuroinflammation in Alzheimer disease. *Nature Reviews Immunology, 25*, 321–352.

Henstridge, C. M., Pickett, E., & Spires-Jones, T. L. (2016). Synaptic pathology: A shared mechanism in neurological disease. *Ageing Research Reviews, 28*, 72–84.

Höftberger, R., & Lassmann, H. (2017). Inflammatory demyelinating diseases of the central nervous system. *Handbook of Clinical Neurology, 145*, 263–283.

Isik, S., Yeman Kiyak, B., Akbayir, R., Seyhali, R., & Arpaci, T. (2023). Microglia mediated neuroinflammation in Parkinson's disease. *Cells, 12*(7), 1012.

Jain, M., Singh, M. K., Shyam, H., Mishra, A., Kumar, S., Kumar, A., & Kushwaha, J. (2021). Role of JAK/STAT in neuroinflammation and its association with neurological disorders. *Annals of Neurosciences, 28*(3–4), 191–200.

Karabag, D., Scheiblich, H., Griep, A., Santarelli, F., Schwartz, S., Heneka, M. T., & Ising, C. (2023). Characterizing microglial senescence: Tau as a key player. *Journal of Neurochemistry, 166*, 517–533.

Kaymak, G., & Aydın, H. (2021). Nörodejeneratif hastalıklarda oksidatif stresin rolü. *Osmangazi Tıp Dergisi, 43*(6), 696–704.

Kim, H. J., Kim, H., Lee, J. H., & Hwangbo, C. (2023). Toll-like receptor 4 (TLR4): New insight immune and aging. *Immunity & Ageing, 20*(1), 67.

Kim, S. U., & de Vellis, J. (2005). Microglia in health and disease. *Journal of Neuroscience Research, 81*(3), 302–313.

Lassmann, H. (2018). Multiple sclerosis pathology. *Cold Spring Harbor Perspectives in Medicine, 8*(3), a028936.

Liu, T., Zhang, L., Joo, D., et al. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy, 2*, 17023.

Mao, H., Zhao, X., & Sun, S. C. (2025). NF-κB in inflammation and cancer. *Cellular & Molecular Immunology, 22*(8), 811–839.

Moreira, P. I., Smith, M. A., Zhu, X., Nunomura, A., Castellani, R. J., & Perry, G. (2005). Oxidative stress and neurodegeneration. *Annals of the New York Academy of Sciences, 1043*, 545–552.

Muzio, L., Viotti, A., & Martino, G. (2021). Microglia in neuroinflammation and neurodegeneration: From understanding to therapy. *Frontiers in Neuroscience, 15*, 742065.

Nugent, F. S., Li, K. W., & Chen, L. (2023). Editorial: Synaptic plasticity and dysfunction, friend or foe? *Frontiers in Synaptic Neuroscience, 15*, 1204605.

Onyango, I. G., Jauregui, G. V., Čarná, M., Bennett, J. P., Jr., & Stokin, G. B. (2021). Neuroinflammation in Alzheimer's disease. *Biomedicines, 9*(5), 524.

Perry, V. H., & Teeling, J. (2013). Microglia and macrophages of the central nervous system: The contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Seminars in Immunopathology, 35*(5), 601–612.

Ramesh, G., MacLean, A. G., & Philipp, M. T. (2013). Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators of Inflammation, 2013*, 480739.

Schetters, S. T. T., Gomez-Nicola, D., Garcia-Vallejo, J. J., & Van Kooyk, Y. (2018). Neuroinflammation: Microglia and T cells get ready to tango. *Frontiers in Immunology, 8*, 1905.

Shi, F. D., & Yong, V. W. (2025). Neuroinflammation across neurological diseases. *Science, 388*(6753), eadx0043.

Simpson, D. S. A., & Oliver, P. L. (2020). ROS generation in microglia: Understanding oxidative stress and inflammation in neurodegenerative disease. *Antioxidants, 9*(8), 743.

Subhramanyam, C. S., Wang, C., Hu, Q., & Dheen, S. T. (2019). Microglia-mediated neuroinflammation in neurodegenerative diseases. *Seminars in Cell & Developmental Biology, 94*, 112–120.

Tansey, M. G., Wallings, R. L., Houser, M. C., Herrick, M. K., Keating, C. E., & Joers, V. (2022). Inflammation and immune dysfunction in Parkinson disease. *Nature Reviews Immunology, 22*(11), 657–673.

Teleanu, D. M., Niculescu, A. G., Lungu, I. I., Radu, C. I., Vladâcenco, O., Roza, E., Costăchescu, B., Grumezescu, A. M., & Teleanu, R. I. (2022). An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. *International Journal of Molecular Sciences, 23*(11), 5938.

Troncoso-Escudero, P., Parra, A., Nassif, M., & Vidal, R. L. (2018). Outside in: Unraveling the role of neuroinflammation in the progression of Parkinson's disease. *Frontiers in Neurology, 9*, 860.

Valiukas, Z., Tangalakis, K., Apostolopoulos, V., & Feehan, J. (2025). Microglial activation states and their implications for Alzheimer's disease. *The Journal of Prevention of Alzheimer's Disease, 12*(1), 100013.

Wilson, D. M., Cookson, M. R., Van Den Bosch, L., Zetterberg, H., Holtzman, D. M., & Dewachter, I. (2023). Hallmarks of neurodegenerative diseases. *Cell, 186*(4), 693–714.

Xu, W., Huang, Y., & Zhou, R. (2025). NLRP3 inflammasome in neuroinflammation and central nervous system diseases. *Cellular & Molecular Immunology, 22*, 341–355.

Zhang, S., Gao, Z., Feng, L., & Li, M. (2024). Prevention and treatment strategies for Alzheimer's disease: Focusing on microglia and astrocytes in neuroinflammation. *Journal of Inflammation Research, 17*, 7235–7259.

Chapter 5

MYXOMYCETES (MYXOGASTRIA); GENUS *Angioridium* GREV. (*Angioridium sinuosum* (BULL.) GREV.) IN TÜRKIYE

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Introduction:

Myxomycetes, (plasmodial slime molds, true slime molds or mxogastrids), is a free-living protists, a group of amoeboid eukaryotes, placed phylum Amoebozoa of the kingdom Protozoa. According to the current classification, Myxomycetes form the phylum Eumycetozoa together with the classes Dictyosteliomycetes and Protosteliomycetes within Amoebozoa (Adl et al., 2019; Baba and Akgül, 2024). Myxomycetes, like macrofungi, are capable of forming both macro and very small micro-sized fruiting bodies. Therefore, they are very valuable organisms for scientists and researchers working on protista diversity and ecology (Azirakhmet et al., 2026). Myxomycetes are very important group of organisms in morphological and molecular studies, phylogenetic relationships, protista systematics and soil and environmental microbial ecology studies (Urich et al., 2008).

Myxomycetes are fungus-like organisms that have an assimilation stage (vegetative), which is a free-living, multinucleated, motile mass of protoplasm called a plasmodium, and a sporulation stage (generative), which typically consists of a mass of spores that are surrounded by a simple or complex membrane or carried in a rigid, cell-free spore coat (Chimankar, 2022). Myxomycete species can form myxoamoebas, myxoflagellates, and multinucleated plasmodiums in their vegetative stages, both microscopic and macroscopic. The resistance forms that myxomycetes develop in extreme conditions during their vegetative stages; cysts and sclerotia are very different formations. They have a complex life cycle containing sporophores that produce endospore type spores within a membranous structure called the peridium during the generative stage (García-Martín et al., 2023).

Myxomycetes feed on bacteria, yeasts, nematodes and other microorganisms and play a crucial role in balancing soil microbial communities (Gao et al., 2022). They are particularly abundant in decaying bark, wood and dead or living leaves and plant materials in forest areas (Stephenson and Stempen, 1994). There are 1218 species worldwide (Lado 2005–2026) and 312 species in Türkiye (Baba et al., 2025).

Materials and Methods:

Lister (1925) classified myxomycetes into Lamprosporales (non-purple-brown or purplish-grey spores) and Amaurosporales (purple-brown or purplish-grey spores) based on their distinctive spore colors and pigments. This concept has recently been updated with the recognition of two main clades: Lucisporidia (bright-spored) and Fuscisporida (dark-spored). Lucisporidia has 34% of myxomycete and into two orders Liceida and Trichiida. Fuscisporida myxomycetes orders Physarida and Stemonitida (Fiore-Donno et al., 2010b).

Within the dark spore-bearing clade, the order Physarida is distinguished by exhibiting distinct crystalline or amorphous calcareous deposits in different parts of its fruiting bodies (Keller et al., 2022). Studies on the formation, development and morphological characteristics of species in the Physarida order and other orders have been conducted since ancient times. Molecular systematic studies on myxomycetes, have only recently begun and are relatively new compared to other eukaryotes. Recent advancements in modern molecular biology techniques have led to significant progress in the molecular classification and identification of myxomycetes. The application of new molecular methods has enabled the acquisition of crucial information about phylogenetic relationships at the molecular level. These developments have helped clarify the taxonomic status of many previously obscure groups. A phylogenetic analysis combining detailed morphological research with three commonly studied genes (nSSU, EF-1α, and COI) has been performed. The classification of the order Physarida recognizes three families: Didymiaceae, Elaeomyxaceae and Physaraceae. Members of these families exhibit calcium deposits in different parts of their fruit bodies. However, they differ in the following respects: 1) the type of capillitium; 2) the shape of the peridial calcium deposits; 3) the chemical composition of the calcium compounds; and 4) the manner in which these deposits are excreted when fruit formation begins. Traditionally, different morphological characteristics are used to distinguish between families, genera, and species. Historically, lime knots have been a significant morphological feature distinguishing the Didymiaceae and Physaraceae families (García-Martín et al., 2023; Li et al., 2024).

The order Physarida is the largest group of myxomycetes and has got 491 species (Lado 2005–2026). Didymiaceae has got 6 genus (*Diachea* 19, *Diderma* 94, *Didymium* 102, *Neodiderma* 6, *Polyschismium* 14, *Trabrooksia* 1 species) and 236 species. Elaeomyxaceae has got 1 genus (*Elaeomyxa*) and 4 species. Physaraceae has got 16 genus (*Aethaliopsis* 1, *Angioridium* 1, *Badhamia* 40, *Badhamiopsis* 7, *Claustria* 1, *Craterium* 21, *Erionema* 1, *Fuligo* 8, *Kelleromyxa* 1, *Leocarpus* 1, *Lignydium* 1, *Nannengaella* 14, *Physarella* 1, *Physarina* 3, *Physarum* 149, *Willkommlangea* 1) and 251 species (Lado 2005–2026).

The fruiting bodies of the Physaraceae family are sessile or stalked, with a sporangium, plasmodiocarp or aethaloid. Containing calcareous deposits in some parts and never containing oil or wax. The peridium is usually a thin membrane covered with granular calcareous deposits. Its outer layer is covered with small, rounded calcareous granules. The stalk is sometimes present or absent, rarely extending as a columella within the sporangium to a specific region of the sporotheca. The columella is rarely present, sometimes appearing as a calcareous pseudo-columella. The capillitium is a network of transparent tubes connecting calcareous nodes. It sometimes contains branched threads or

simple and unbranched peridial projections. It forms a complex network that branches repeatedly in all directions. It consists of thin tubes that anastomose, their ends attached to the sporangium wall on all sides. Calcareous deposits of varying shapes and sizes are found at the corners of the network. The physaroid capillitium structure, consisting of large calcareous nodes connected by non-calcareous tubes, is the characteristic capillitium structure of the Physaraceae family. Spores globose, very rarely ellipsoidal, violaceous (Baba and Tamer, 2008; Baba and Sevindik, 2020b).

In recent years, numerous molecular studies have been conducted on Myxomycetes, providing in-depth information about the phylogenetic relationships between taxa. Genera and species previously placed in other taxa have been reclassified within the Physarida taxon, and their positions at the family and genus level have been changed. The species *Angioridium sinuosum* (Bull.) Grev. was previously a species belonging to the genus *Physarum* and was known as *Physarum bivalve* Pers. As a result of morphological and molecular studies by García-Martín et al. (2023), this species was removed from the genus *Physarum* and reclassified as *Angioridium sinuosum* (Bull.) Grev. as the sole species of the genus *Angioridium*.

Results:

The genus *Physarum*, the largest genus of Myxomycetes with 149 species (Lado 2005-2026), is characterized by the presence of calcium in the capillitium, a network of finely interwoven tubes between spores in the sporotheca; the nodes are often filled with calcium granules. In addition, the peridium and stalk may also contain calcium (Baba et al., 2012c; Renato et al., 2019).

The genus *Angioridium* Grev. was first described by Greville (1828). The type species consisted of a plasmodiocarp with a longitudinal cleft and laterally strongly flattened. The genus *Angioridium* was initially proposed as a single species genus containing the species *Angioridium sinuosum*. Over time, the genus and species were revised to be synonymous and named *Physarum bivalve* and with different names. In the most recent phylogenetic revision, the genus was re-established by García-Martín et al. (2023) within the genus *Angioridium*, the species *Angioridium sinuosum* is considered a single species genus.

Taxonomic category of *Angioridium* Grev.:

Angioridium Grev., Scott. crypt. fl. 6(62):pl. 310 (1827) [See García-Martín, Zamora & Lado, Persoonia 51:116. 2023];

The genus *Angioridium* Grev. is a genus of plasmodial slime molds (Myxomycetes/true slime molds). It belongs to the kingdom Amoebozoa, class Myxomycetes (or Myxogastrea) and order Physarida and Physaraceae family.

Angioridium sinuosum is the known only species in this genus. *Angioridium* Grev. is a monotypic genus Lado (2005-2026).

Morphological features of *Angioridium Grev*.:

Description: In *Angioridium*, granular or crystalline calcareous deposits (calcium carbonate) may be found in the peridium, stem, capillitium or all of fruiting bodies. Sporophores are plasmodiocarpous, sessile, cylindrical or laterally strongly flattened and compressed. The peridium is white to gray in color, bilayered and calcareous. Both layers are distant from each other and usually separate distinctly upon opening. The capillitium is always present and has a reticular structure. It consists of numerous white calcareous nodules, transparent filaments, or tubes connecting the nodules. Columella present or absent. Spores black, dark purple or purplish brown, free. Plasmodium of the phaneroplasmodium type (Ashworth and Dee, 1975; García-Martín et al., 2023).

According to Lado (2005-2026) there are 1 species all over the world and there are 1 species *Angioridium Grev*. in Türkiye; *Angioridium sinuosum* (Bull.) Grev. (Baba and Sevindik, 2023).

***Angioridium sinuosum* (Bull.) Grev., Scott. crypt. fl. 6(62):pl. 310 (1827) [García-Martín, Zamora & Lado, Persoonia 51:116. 2023];**

Synonyms: *Reticularia sinuosa* Bull., Herb. France 10(109-120):pl. 446, fig. 3 (1790),

Physarum sinuosum (Bull.) Weinm., in Fries, Syst. mycol. 3(1):145 (1829),

Physarum sinuosum (Bull.) Rostaf., Sluzowce monogr. 112 (1874),

Lignydium sinuosum (Bull.) Kuntze, Revis. gen. pl. 3(3):490 (1898),

Physarum bivalve Pers., Ann. Bot. (Usteri) 15:5 (1795),

Diderma valvatum Fr., Syst. mycol. 3(1):109 (1829),

Carcerina valvata (Fr.) Fr., Summa veg. Scand. 451 (1849).

Description: Sporocarps are scattered or clustered plasmodiocarps, mixed with a small number of sessile sporangia. Plasmodiocarps are short or long, simple or branched, segmented, laterally quite flattened, with an upper slit similar to those of bivalve mollusks, light brown to yellowish-brown in color, 0.3 to 13 mm long, 0.2 to 1 mm high. Sporangia appear stalk-like on a small, constricted base. Scattered and crowded, sessile or long, curved or branched, constricted-based plasmodiocarps are formed; the upper dorsal side is flattened and eventually longitudinally divided, crowded, laterally flattened, parallelogram-shaped, sometimes curved sporangia and plasmodiocarps (some branched), white, gray or yellowish, sometimes with a constricted base.

The hypothallus is inconspicuous. The peridium is two-layered structure; the outer layer is usually thick and calcareous, containing more or less dense deposits of lime, mostly white, with a light lemon-yellow color at the base of some sporangia, flat or reticulated; light yellowish towards the top, darker towards the base, opens from the upper slit, after opening resembles a bivalve mollusk; the inner layer is wrinkled and colorless, membranous, iridescent, usually adherent to the outer layer, thin and translucent, grayish-white, sprinkled with lime, opens irregularly, but remains intact for a longer time. Opening occurs from a pre-formed longitudinal slit, ± regular (Figure 1). Columella are absent. Capillitium is abundant, consisting of numerous white, usually branching, large or small calcareous nodes and connected by relatively short translucent threads, composed of calcareous nodes and non-calcareous internodes; Nodes are numerous, white, spherical, semi-spherical, irregular, smooth; The internodes are short, thin, tube-shaped, delicate, transparent, attached to the nodes, and form a network. The spore mass is black. Spores are brown, dull purple, dark purplish-brown under light, finely spiny/wart-like, 8-11 µm in diameter, spherical, and the warts are in the form of a small curved line (Figure 2). Plasmodium grey, pallid or yellowish (Poulain et al., 2011; Chimankar, 2022; García-Martín et al., 2023).

Figure 1: Plasmodiocarpous structure of Angioridium sinuosum (Bull.) Grev.

Strongly laterally flattened, compressed, convoluted plasmodiocarps, double peridium; pre-formed longitudinal cleft, thin and smooth spines, lighter colored spores 8-10 µm in diameter are features that distinguish the species *Angioridium sinuosum*. The opened sporangia of *Angioridium sinuosum* resemble those of a mollusk, making it easily distinguishable from others. The species can vary from simple to branched plasmodiocarp to sporangium-forming.

It is closely related to *Physarum laevisporum* Agnihothr. However, the latter is characterized by smaller spores, cylindrical, slightly laterally flattened, yellowish-brown or grayish-brown fruiting, and quite large nodes up to 100 µm with irregular opening. It is comparable to *Physarum echinosporum* Lister;

This species is characterized by its rather flattened, chalky white fruiting body, double peridiatum, and larger, dark, and highly warty (or spiny) spores measuring 7–11 µm in diameter. The spiny warts are arranged in irregular lines, forming a very incomplete and highly irregular reticulate structure (Baba et al., 2021a; Chimankar, 2022).

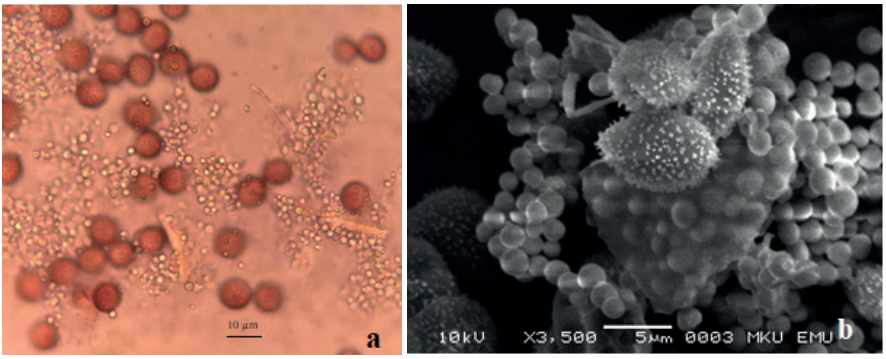

Figure 2: Spores and capillitium of Angioridium sinuosum: a: Light microscope, b: Electron microscope.

Distribution of *Angioridium sinuosum* (Bull.) Grev. **in Türkiye:**

Nizip/Gaziantep (Baba et al., 2021a).

Conclusion:

Angioridium is a recognized genus of plasmodial slime molds (myxomycetes) belonging to the family Physaraceae and the order Physarida. In previous literature, the name *Angioridium sinuosum* was known as *Physarum bivalve* within the genus *Physarum*, which contained multiple species. However, recent multiple genetic revisions of *Physarida* have re-established the genus to represent a specific, well-defined group of dark-spored myxomycetes. Today, the genus *Angioridium* contains only one universally accepted species: *Angioridium sinuosum* in Türkiye.

REFERENCES:

Adl SM, Bass D, Lane CE, et al. (2019). Revisions to the classification, nomenclature, and diversity of Eukaryotes. Journal of Eukaryotic Microbiology 66 (1): 4-119. doi: 10.1111/jeu.12691.

Ashworth JM, Dee J. (1975). The biology of the slime moulds. London: Arnold.

Azirakhmet Z, Zemlyanskaya I, Novozhilov YK, Shchepin O, Nursafina A, Bersimbaev R and Schnittler M (2026). A Checklist of Myxomycetes from Kazakhstan Asian Journal of Mycology, 9(1): 239–263. Doi 10.5943/ajom/9/1/6.

Baba H, Tamer AÜ (2008). A New Myxomycetes Genus and Three Species Record for Turkish mycoflora. The Herb Journal of Systematic Botany, 15(2), 81-86.

Baba H, Gelen M, Zümre M (2012c). A new Physarum (Myxomycetes) record from Hatay-Turkey. The Herb Journal of Sytematic Botany, 19(2): 125–131.

Baba H, Sevindik M (2020b) Mycetozoa of Turkey. Nobel scientific Works, 1st edition, Ankara. book barcode no: 9786257296021, ISBN 978-625-7296-02-1, E-ISBN978-625-7296-03-8, Number of Pages 308.

Baba H, Gündoğdu F, Sevindik M (2021a). Myxomycetes biodiversity in Gaziantep Province (Turkey) with four new records. Phytotaxa 478(1): 105–118.

Baba H, Sevindik M (2023). Myxomycetes (Myxogastria) of Türkiye: A checklist 2023, Mycopath Review Article 21(1): 53-67.

Baba H, Akgül H (2024). Miksomisetlerde (Mxogastrea) Fruktifikasyon Yapısı ve Morfolojisi II: Hipotallus. Fen Bilimleri ve Matematik Alanında Araştırmalar ve Değerlendirmeler. Sayfa 42-55. www.gecekitapligi.com

Baba H, Narin S, Sevindik M (2025). New Myxomycetes (Myxogastria) records from Türkiye: A new genus and two species from Hatay. Biology Bulletin, 52:48. doi: 10.1134/S1062359024612229.

Chimankar NV (2022). Genus *Physarum* Pers. From Manudevi forest Dıst. Jalgaon, Maharashtra (Indıa). International journal of Advance and Applied Research (IJAAR), 9:4 20-26.

Fiore-Donno AM, Nikolaev SI, Nelson M, Pawlowski J, Cavalier-Smith T and Baldauf SL (2010b). Deep phylogeny and evolution of slime moulds (Mycetozoa). Protist, 161: 55-70.

Gao Y, Song H, Zhou F, Chen S, He G, Yan J, Sun Q, Long H, Zhai Z, Hu D and Hu H (2022). Community of soil-inhabiting myxomycetes shares similar assembly mechanisms with fungi, and is affected by bacterial community in subtropical forests of China. Soil Biology and Biochemistry Volume 175, https://doi.org/10.1016/j.soilbio.2022.108854

García-Martín J, Zamora JC, Lado C (2023). Multigene phylogeny of the order Physarales (Myxomycetes, Amoebozoa): shedding light on the dark-spored clade. Persoonia - Molecular Phylogeny and Evolution of Fungi 51, 89–124.

Keller HW, Everhart SE, Kilgore CM. (2022). The Myxomycetes: introduction, basic biology, life cycles, genetics, and reproduction. In: Myxomycetes: 1–45. Academic Press.

Lado C (2005-2026). An online nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC. Madrid, Spain. Last updated June 16, 2026. http://www.nomen. eumycetozoa.com.

Li X, Hu J, Tuo Y, Li Y, Dai D, Sossah FL, Liu M, Wang J, Song J, Zhang B, Li X and Li Y (2024). Catalogue of fungi in China 4: Didymiaceae and Physaraceae (Myxomycetes) Mycology. Nov 7;16(1):124-157. doi: 10.1080/21501203.2024.2410508. eCollection 2025.

Lister G (1925). A monograph of the Mycetozoa, 3rd ed. Witherby, London. p.296.

Poulain M, Meyer M and Bozonnet J (2011). Les Myxomycètes. Fédération mycologique et botanique Dauphiné-Savoie. Sevrier. Pl. 290, p. 427.

Renato C, deHaan M, Meyer M, Bonkowski M and Fiore-Donno AM (2019). Phylogeny of Physarida (Amoebozoa, Myxogastria) Based on the Small-Subunit Ribosomal RNA Gene, Redefinition of Physarum pusillum s. str. and Reinstatement of P. gravidum Morgan. Journal of Eukaryotic Microbiology ISSN 1066-5234, 67; 327-336.

Stephenson SL, Stempen H (1994). Myxomycetes: A Handbook of Slime Molds. Timber Press.

Urich T, Lanzén A, Qi J, Huson DH, Schleper C and Schuster SC (2008). Simultaneous assessment of soil microbial community structure and function through analysis of the meta ranscriptome. PLoS ONE 3(6), e2527.