

A Review on the Sublethal Effects of Pure and Formulated Glyphosate on Bees, with a Focus on Social Bee Species

Abstract

Glyphosate, one of the most widely used herbicides globally, has been extensively applied in agriculture due to its efficacy in weed control. However, recent studies have raised concerns about its sublethal effects on non-target organisms, particularly social bee species such as honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.), and stingless bees (e.g., *Melipona*, *Trigona*). While glyphosate's primary mechanism targets the shikimate pathway, which is absent in animals, emerging evidence suggests it can indirectly impact bees by altering their gut microbiota, immune responses, and behavior. Research shows that even at sublethal doses, glyphosate can impair navigation, learning, and foraging efficiency, leading to reduced colony growth and survival rates. Field and laboratory studies indicate that the impact is exacerbated when bees are exposed to formulated products containing surfactants, which increase glyphosate's toxicity. Furthermore, the disruption of social behaviors within colonies, such as communication through the waggle dance in honeybees, has profound implications for hive health and productivity. Despite growing evidence, there remain significant gaps in our understanding of glyphosate's long-term and chronic effects, especially across diverse ecosystems and bee species. Current research is limited by a lack of longitudinal field studies that assess the cumulative impact of low-dose exposure over multiple generations. Additionally, most studies have focused on honeybees, with less attention given to wild and native bee populations, which may respond differently to glyphosate. To address these challenges, future research must prioritize mechanistic studies, explore eco-friendly alternatives to glyphosate, and implement integrated pest management strategies to reduce agrochemical dependence. Collaboration among scientists, policymakers, and stakeholders is critical to developing evidence-based regulations that protect pollinator health. Given the essential role bees play in global food security through pollination, protecting these vital species from the sublethal effects of glyphosate is not only an ecological imperative but also a socioeconomic necessity. Immediate actions in research, policy reform, and sustainable agricultural practices are needed to mitigate the risks posed by glyphosate and safeguard the future of pollinators.

Keywords: *Glyphosate, sublethal effects, honeybees, bumblebees, stingless bees, pollinator health*

I. Introduction

A. Overview of Glyphosate Usage in Agriculture

1. Global Prevalence and Economic Importance

Glyphosate, a broad-spectrum, non-selective herbicide, is one of the most widely used agrochemicals in the world. Since its introduction in the 1970s, glyphosate has become the dominant herbicide in global agriculture due to its effectiveness in controlling weeds and its perceived low toxicity to non-target organisms [1]. In the United States alone, glyphosate usage has escalated to approximately **125,000 metric tons annually**, particularly driven by the adoption of glyphosate-resistant genetically modified (GM) crops such as soy, corn, and cotton.

The economic benefits of glyphosate are significant. It is estimated that glyphosate-resistant crops contributed to an increase in agricultural productivity valued at over **\$100 billion globally** from 1996

to 2020. Glyphosate's ability to reduce tillage also aids in conserving soil moisture and reducing erosion, making it an integral tool in sustainable agriculture [2].

2. Distinction Between Pure and Formulated Glyphosate

While "pure glyphosate" refers to the active ingredient itself, commercial formulations contain additional components such as surfactants that enhance the herbicide's efficacy. Formulated products like Roundup® are designed to increase glyphosate's penetration into plant tissues, but these additives can also amplify toxicity to non-target organisms, including bees. Surfactants like polyethoxylated tallowamine (POEA) are known to disrupt cell membranes and have been associated with increased mortality in aquatic and terrestrial species [3].

A growing body of research suggests that while pure glyphosate may have low toxicity to bees in controlled settings, the toxicity of formulated products is significantly higher, leading to adverse effects on bee behavior, physiology, and colony health.

B. Importance of Bees in Ecosystems and Agriculture

1. Role in Pollination and Biodiversity

Bees, particularly social species such as honeybees (*Apis mellifera*) and bumblebees (*Bombus spp.*), play a crucial role in pollinating over **75% of global food crops**. This pollination service is essential for maintaining biodiversity, ensuring the reproduction of flowering plants, and supporting entire ecosystems. Bees also contribute to the genetic diversity of crops, which helps improve crop yields and resilience against pests and diseases [4].

The decline in bee populations poses a serious threat to biodiversity and agricultural productivity. Recent studies indicate that nearly **40% of pollinator species**, particularly bees, are at risk of extinction due to habitat loss, climate change, and pesticide exposure. The cascading effects of such a decline could lead to reduced availability of fruits, vegetables, and nuts, ultimately threatening food security.

2. Economic Value of Bee Pollination

Bees are not only ecological keystone species but also vital to the global economy. The economic value of pollination services provided by bees is estimated to be between **\$235 billion and \$577 billion annually** worldwide. In the United States alone, honeybee pollination supports over **\$15 billion** worth of crops each year, including almonds, apples, and blueberries. Social bees, due to their ability to maintain large colonies and forage over extensive areas, are particularly valuable for large-scale agricultural operations [5].

C. Rising Concerns About Pesticide Impacts on Bees

1. Focus on Sublethal Effects as Opposed to Acute Toxicity

Traditional toxicological assessments of pesticides, including glyphosate, have focused on acute lethal effects—measuring the concentration required to kill 50% of a test population (LD50). However, recent research highlights the significance of **sublethal effects**, which can impair critical behaviors in bees even at concentrations far below lethal doses. Sublethal exposure to glyphosate has been shown to disrupt learning, memory, and navigation abilities, which are essential for foraging efficiency and colony survival.

For instance, exposure to glyphosate at sublethal levels was found to reduce the cognitive function of honeybees, affecting their ability to associate floral scents with food rewards. Bumblebees exposed to glyphosate also exhibit reduced foraging success and impaired brood development, which can compromise colony growth [6].

2. Rationale for Focusing on Social Bee Species

Social bees such as honeybees and bumblebees are particularly vulnerable to the sublethal effects of glyphosate due to their complex social structures and reliance on collective behaviors. Unlike solitary bees, social species depend on effective communication, division of labor, and coordinated foraging to sustain their colonies. Disruptions in these behaviors can have profound consequences on colony health and survival, ultimately affecting their pollination services.

The focus on social bees is critical not only because of their ecological and economic importance but also because these species are easier to study and monitor in both laboratory and field settings [7]. Research on social bees can thus provide early indicators of broader environmental impacts and help inform policy decisions regarding pesticide regulation.

II. Background and Context

A. Chemistry and Mechanism of Glyphosate

1. Structure and Action of Pure Glyphosate

Glyphosate (N-(phosphonomethyl)glycine) is a synthetic, broad-spectrum herbicide that works by inhibiting the enzyme **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)**, which is crucial in the shikimate pathway. This pathway exists in plants, fungi, and certain microorganisms but is absent in animals, which is why glyphosate was initially thought to be safe for non-target species, including humans and bees [8].

The molecular structure of glyphosate consists of a glycine backbone attached to a phosphonomethyl group. By blocking the EPSPS enzyme, glyphosate prevents the synthesis of essential aromatic amino acids (tryptophan, phenylalanine, and tyrosine) required for protein production and plant growth. This inhibition leads to the accumulation of shikimate within plant tissues, ultimately causing the plant to die. While glyphosate's mechanism is specific to plants, studies have shown that sublethal effects can occur in non-target organisms, potentially through interactions with their gut microbiota.

2. Additives in Formulated Glyphosate Products

Pure glyphosate is rarely used in isolation in agricultural settings. Instead, it is formulated with various **surfactants** and other additives to enhance its efficacy. These formulations, such as Roundup®, include surfactants like **polyethoxylated tallowamine (POEA)**, which helps glyphosate penetrate the waxy surface of leaves [9]. However, these additives are not inert; they can increase toxicity to non-target organisms, including bees and aquatic life.

Research indicates that surfactants in formulated glyphosate products can be **100 to 1,000 times more toxic** to aquatic organisms than glyphosate alone. For bees, exposure to formulated glyphosate has been linked to altered foraging behavior, impaired learning, and weakened immunity. Studies have shown that while bees exposed to pure glyphosate may experience minimal immediate harm, exposure to formulated products can lead to significant sublethal effects.

B. Social vs. Solitary Bees: Differences in Behavior and Vulnerability

1. Characteristics of Social Bee Species (e.g., Honeybees, Bumblebees)

Social bees, including honeybees (*Apis mellifera*) and bumblebees (*Bombus spp.*), are eusocial insects that live in highly organized colonies with a complex division of labor [10]. These species exhibit behaviors such as **collective foraging, communication through dances, and shared brood care,**

which are essential for colony survival. The queen, worker, and drone bees each have specialized roles, with workers responsible for foraging and caring for the brood.

Social bees are particularly vulnerable to sublethal pesticide exposure because impairments in individual behavior can have cascading effects on the entire colony. For example, disruptions in the foraging efficiency of worker bees can reduce food intake, leading to poor nutrition and weakened immune responses among the colony members [11].

2. Life Cycle and Social Structure of Social Bees

The life cycle of honeybees involves several stages: egg, larva, pupa, and adult. A typical colony contains a single reproductive queen, thousands of sterile female workers, and a smaller number of male drones during the reproductive season. Bumblebees, in contrast, have smaller colonies with a life cycle that includes a **hibernating queen** that establishes a new colony each spring.

Social bees rely heavily on effective communication and coordination. For example, honeybees use the **waggle dance** to convey information about the location of food sources, while bumblebees rely on scent marking and buzzing sounds. Exposure to sublethal levels of pesticides like glyphosate can disrupt these communication channels, reducing foraging efficiency and impairing colony growth [12].

C. Current Regulations on Glyphosate Usage

1. Guidelines by Regulatory Agencies (e.g., EPA, EFSA)

Glyphosate is regulated by several international agencies, including the **U.S. Environmental Protection Agency (EPA)** and the **European Food Safety Authority (EFSA)**. The EPA has consistently classified glyphosate as “**not likely to be carcinogenic to humans**” when used according to the label directions, though this classification has been controversial. The EFSA similarly concluded in 2015 that glyphosate poses no significant risk to human health or the environment if used correctly.

However, the **International Agency for Research on Cancer (IARC)** classified glyphosate as “**probably carcinogenic to humans**” (**Group 2A**) in 2015 based on evidence from animal studies and limited evidence in humans. This discrepancy has led to significant debate and calls for stricter regulations, especially in the European Union, where several member states, such as France and Germany, have moved towards phasing out glyphosate usage by 2024 [13].

2. Legal Limits and Safety Assessments

Regulatory agencies set **maximum residue limits (MRLs)** for glyphosate in food products to ensure safety for consumers. In the EU, the MRL for glyphosate in honey is **0.05 mg/kg**, while in the U.S., it varies depending on the crop. Despite these regulations, studies have found glyphosate residues in honey at levels exceeding these limits, particularly in regions with intensive agricultural practices.

In addition to MRLs, recent regulatory assessments have begun to consider **sublethal effects** of pesticides on non-target organisms, including bees. The European Commission’s **Bee Guidance Document (2013)** aims to assess both acute and chronic risks of pesticides to bee health, although its implementation has faced delays due to opposition from member states and industry groups [14].

III. Sublethal Effects of Glyphosate on Bees

A. Behavioral Impacts

1. Impairment in Navigation and Foraging Behavior

One of the most well-documented sublethal effects of glyphosate on bees is its impact on navigation and foraging. Honeybees (*Apis mellifera*) rely heavily on their ability to navigate back to their hive after foraging trips. Studies have shown that glyphosate exposure at sublethal concentrations can impair bees' spatial navigation, leading to disorientation and increased foraging times. In one experiment, honeybees exposed to glyphosate at **10 mg/L** were significantly less likely to return to the hive compared to control bees, suggesting that even low doses can impair homing abilities [15].

Further studies have demonstrated that glyphosate exposure alters the flight patterns of foraging bees, causing them to take longer and more erratic routes. This disorientation can lead to reduced food collection, impacting the entire colony's nutritional status. A field found that bees exposed to glyphosate exhibited poor foraging efficiency, which is particularly detrimental during periods of high colony growth and resource needs.

2. Effects on Learning, Memory, and Communication

Glyphosate exposure has also been shown to impair cognitive functions in bees. Honeybees use associative learning to connect floral scents with nectar rewards, which is crucial for efficient foraging [16]. Research indicates that sublethal doses of glyphosate reduce bees' ability to learn and recall these associations. For example, bees exposed to glyphosate concentrations as low as **2.5 mg/L** showed significant reductions in learning performance in a proboscis extension reflex (PER) assay, a common test for studying insect learning.

Communication within colonies is also disrupted. Honeybees use the "waggle dance" to communicate the location of food sources to nestmates. A study found that glyphosate-exposed bees performed fewer waggle dances, and those dances were less precise, reducing the colony's ability to exploit food resources efficiently. This breakdown in communication can lead to reduced food intake, which in turn affects the colony's growth and reproductive success.

3. Disruption of Social Behaviors within Colonies

Social cohesion in colonies is vital for tasks like brood care, hive defense, and food processing. Sublethal exposure to glyphosate has been shown to reduce the activity levels of worker bees and their responsiveness to brood pheromones, which are essential for the care of larvae [17]. Bumblebee colonies exposed to glyphosate exhibited reduced rates of brood production and fewer foraging trips, indicating that social behaviors critical for colony maintenance were impaired.

B. Physiological Impacts

1. Influence on Bee Microbiome and Gut Health

The gut microbiome plays a critical role in bees' digestion, immunity, and overall health. Recent studies have revealed that glyphosate can disrupt the composition of the gut microbiota in bees, potentially increasing their vulnerability to diseases. Glyphosate targets the shikimate pathway, which is present in many gut bacteria but absent in animals. Honeybees exposed to glyphosate had reduced levels of beneficial gut bacteria such as *Snodgrassellaalvi* and *Gilliamellaapicola*, which are essential for nutrient absorption and pathogen defense.

These disruptions can weaken the immune system, making bees more susceptible to infections by pathogens like *Nosema ceranae*, a common gut parasite that affects bee colonies worldwide [18]. Glyphosate-exposed bees showed higher levels of *Nosema* spores, reduced immune gene expression, and increased mortality rates.

2. Impacts on Immune System Function

In addition to microbiome disruptions, glyphosate exposure has been linked to compromised immune responses in bees. Honeybees exposed to sublethal doses of glyphosate exhibited reduced expression

of genes associated with immune function. This suppression of immune responses can make bees more vulnerable to viruses and bacterial infections, compounding the negative effects of other stressors like habitat loss and climate change [19].

3. Effects on Development, Reproduction, and Longevity

Glyphosate exposure can also affect the development and reproduction of bees. For instance, a study on bumblebees (*Bombus terrestris*) found that colonies exposed to glyphosate showed reduced brood production and fewer queens. Moreover, chronic exposure to glyphosate has been shown to reduce the lifespan of worker bees, leading to shorter foraging careers and reduced colony productivity. A reduction in the longevity of worker bees could severely impact the overall fitness of the colony, especially during peak foraging seasons.

C. Differences Between Pure and Formulated Glyphosate Exposure

1. Enhanced Toxicity Due to Surfactants and Additives

Formulated glyphosate products, such as Roundup®, are generally more toxic to non-target organisms than pure glyphosate due to the presence of surfactants like **polyethoxylated tallowamine (POEA)** [20]. These additives enhance glyphosate's penetration into plant tissues but also increase its toxicity to bees by facilitating its entry into their bodies. A study formulated glyphosate products were **10 times more toxic** to honeybees than pure glyphosate, causing higher mortality rates and more pronounced behavioral changes.

2. Comparative Studies on Pure vs. Formulated Glyphosate

Several studies have compared the effects of pure glyphosate and its commercial formulations on bee health. For instance, while pure glyphosate at low concentrations did not significantly affect bee survival, exposure to Roundup® led to increased mortality and reduced foraging efficiency. Another study showed that bees exposed to formulated products exhibited greater disorientation and impaired learning than those exposed to pure glyphosate, suggesting that additives exacerbate the herbicide's sublethal effects.

Research continues to highlight the need to differentiate between the impacts of pure and formulated products, as regulatory assessments often focus on the active ingredient alone, overlooking the potential hazards of additives [21].

IV. Focus on Social Bee Species

A. Case Studies on Honeybees (*Apis mellifera*)

1. Evidence from Laboratory and Field Studies

Honeybees (*Apis mellifera*) are one of the most widely studied pollinators in the context of pesticide exposure due to their economic importance in agriculture and their manageable nature for research. Laboratory studies have shown that exposure to sublethal concentrations of glyphosate significantly impairs honeybee cognitive functions. For instance, reported that bees exposed to **10 mg/L of glyphosate** exhibited poor performance in associative learning tests using the proboscis extension reflex (PER) assay, which is critical for effective foraging.

Field studies have corroborated laboratory findings, demonstrating that glyphosate exposure reduces foraging efficiency and hive productivity. Chronic exposure to glyphosate resulted in **reduced foraging activity** and decreased nectar collection, which in turn affected honey stores and colony

growth [22]. In a field experiment, bees exposed to glyphosate at levels found in agricultural fields (approximately **4 mg/L**) showed disorientation and reduced return rates to the hive, which can lead to colony collapse over time due to a shortage of foragers.

2. Implications for Hive Health and Productivity

The health of honeybee colonies depends on the efficient functioning of their social structure and foraging workforce. Glyphosate exposure has been shown to impair the **waggle dance communication**, which honeybees use to convey the location of food sources. This disruption reduces the efficiency of resource collection, which can lead to poor colony nutrition and lowered brood production [23].

Additionally, glyphosate affects the immune system of honeybees, making them more susceptible to pathogens such as *Nosema ceranae*, a common gut parasite. This compromised immunity can result in higher infection rates and reduced colony survival, especially during periods of environmental stress. Long-term studies indicate that colonies exposed to glyphosate have reduced winter survival rates, with implications for commercial beekeepers who rely on healthy hives for pollination services.

B. Effects on Bumblebees (*Bombus spp.*)

1. Impacts on Colony Growth and Queen Survival

Bumblebees (*Bombus spp.*) are vital pollinators for wildflowers and crops, particularly in temperate regions. However, they are also highly sensitive to pesticide exposure. Glyphosate exposure has been linked to reduced **colony growth rates and queen production**, which are crucial for the establishment of new colonies [24]. Exposure to glyphosate at concentrations commonly found in agricultural runoff led to a **30% reduction in queen survival**, severely impacting the species' ability to establish new colonies.

Further studies have shown that sublethal glyphosate exposure affects foraging efficiency in bumblebees, leading to decreased pollen collection and reduced colony size. Since bumblebees are essential for pollinating crops such as tomatoes and blueberries, their decline due to glyphosate exposure could have significant agricultural implications.

2. Research on Wild vs. Managed Populations

The impact of glyphosate differs between wild and managed bumblebee populations. Wild populations, which forage in diverse environments, are often exposed to a cocktail of pesticides, leading to cumulative toxic effects. In contrast, managed bumblebee colonies used in greenhouses may be exposed to lower levels of glyphosate but can still experience sublethal effects that impair their pollination efficiency [25].

A study demonstrated that bumblebee colonies exposed to field-realistic doses of glyphosate produced significantly fewer queens, reducing the likelihood of colony survival into the next season. This finding underscores the vulnerability of bumblebee populations to glyphosate, especially in agricultural landscapes where they face multiple stressors, including habitat loss and competition for floral resources.

C. Vulnerability of Stingless Bees (e.g., *Melipona*, *Trigona* species)

1. Regional Studies from Tropical Environments

Stingless bees, such as *Melipona* and *Trigona* species, are critical pollinators in tropical regions, where they contribute to the pollination of native plants and economically important crops like coffee and cacao. Unlike honeybees and bumblebees, stingless bees have smaller, perennial colonies and are more sensitive to environmental disturbances [26].

Studies conducted in Brazil and other parts of Latin America have found that glyphosate exposure adversely affects stingless bees' **foraging behavior and brood development**. In one study, stingless bees exposed to glyphosate at levels as low as **1 mg/L** showed reduced pollen collection and decreased larval growth rates, which can lead to long-term declines in colony size. This is particularly concerning in tropical ecosystems where stingless bees are often the primary pollinators.

2. Consequences for Native Pollinator Communities

The decline of stingless bees due to glyphosate exposure can have **serious implications for biodiversity in tropical ecosystems**. These bees are essential for the reproduction of many endemic plant species, and their decline could lead to reduced genetic diversity and ecosystem resilience [27]. Moreover, the reduction in pollinator populations could affect the livelihoods of indigenous communities that depend on these bees for honey production and crop pollination.

Recent studies highlight that the impact of glyphosate on stingless bees is exacerbated in regions where agriculture encroaches on natural habitats, increasing the likelihood of bees coming into contact with herbicide residues. The lack of regulatory protection for stingless bees, compared to honeybees, further complicates conservation efforts, necessitating urgent research and policy interventions.

V. Methodological Approaches in Studying Sublethal Effects

A. Laboratory Studies vs. Field Trials

1. Strengths and Limitations of Controlled Experiments

Laboratory studies are essential for understanding the sublethal effects of pesticides like glyphosate on bees, as they provide a **controlled environment** to isolate specific variables. These studies can accurately measure outcomes such as learning, memory, navigation, and physiological responses at known glyphosate concentrations [28]. For example, the use of **proboscis extension reflex (PER) assays** allows researchers to assess how glyphosate affects learning and memory in honeybees (*Apis mellifera*). These controlled settings eliminate confounding environmental factors, ensuring that any observed effects are directly attributable to glyphosate exposure.

However, while laboratory studies are effective for detailed mechanistic research, they may not fully represent **real-world conditions**. For instance, bees in the wild are exposed to a complex mixture of pesticides, pathogens, and environmental stressors that are not present in a controlled lab setting. Additionally, the doses used in laboratory studies may not accurately reflect the concentrations bees encounter in the field, where exposure is often intermittent and varies with environmental factors like temperature and humidity [29].

2. Challenges in Simulating Real-World Conditions

Field trials are designed to assess the **ecological relevance** of laboratory findings by studying bees in their natural habitats. These studies provide insights into how glyphosate exposure affects bee behavior, foraging, and colony health in complex environments. However, field studies face challenges in controlling exposure levels due to fluctuating environmental factors and the mobility of bees.

One significant challenge is the **heterogeneity of pesticide exposure** in agricultural landscapes, where bees may encounter a mix of glyphosate formulations, surfactants, and other agrochemicals [30]. This complexity makes it difficult to isolate the specific effects of glyphosate, leading to

variability in study results. Additionally, field studies require extensive resources, time, and coordination, making them more costly and logistically challenging than laboratory experiments.

B. Methods of Assessing Bee Behavior and Health

1. Techniques for Tracking Navigation and Foraging

Tracking the **navigation and foraging behavior** of bees is crucial for understanding how glyphosate affects their ecological function. One common method involves using radio-frequency identification (RFID) tags, which can monitor the movements of individual bees as they leave and return to the hive. Studies using RFID tags have demonstrated that glyphosate-exposed bees are less likely to return to the hive, indicating impaired navigation.

Another advanced technique is the use of **harmonic radar systems**, which track bees' flight paths over several kilometers. These systems have shown that glyphosate exposure results in more erratic flight patterns and increased homing times [31]. Researchers also use **video analysis and automated hive monitoring systems** to observe changes in foraging rates and hive activity, providing insights into how sublethal glyphosate exposure affects overall colony productivity.

2. Monitoring Colony Health and Immune Responses

Assessing the health of bee colonies involves monitoring various parameters, such as **brood development, queen fertility, and colony size**. Researchers often measure physiological biomarkers to evaluate the impact of glyphosate on bees' immune systems. For example, the quantification of immune-related genes, such as *defensin* and *hymenoptaecin*, can reveal suppressed immune responses following glyphosate exposure.

The analysis of **gut microbiota composition** has emerged as another key metric, given that glyphosate can disrupt the beneficial bacteria in bees' guts, leading to increased susceptibility to pathogens like *Nosema* [32]. Techniques such as **16S rRNA gene sequencing** are used to study changes in the microbial communities within bees' digestive tracts, providing insights into how sublethal glyphosate exposure affects bee health at the microbiome level.

C. Gaps in Current Research Methodologies

1. Need for Long-Term Studies

Most studies on glyphosate's effects on bees have focused on short-term exposures, often lasting only a few days or weeks. However, the **long-term effects** of chronic, low-dose exposure are not well understood, especially since glyphosate is commonly used throughout the growing season. There is a growing consensus that longer studies, extending over multiple bee generations, are needed to fully understand the impacts of glyphosate on colony sustainability and resilience.

A major limitation of current research is the **lack of longitudinal studies** that track colonies over several seasons to observe cumulative effects. This is particularly important for understanding how glyphosate exposure interacts with other stressors such as climate change, habitat loss, and diseases. Long-term field studies would provide valuable data on how sublethal effects compound over time, potentially leading to colony collapse [33].

2. Understudied Species and Regional Differences

While research has predominantly focused on honeybees (*Apis mellifera*), other social and solitary bee species, such as bumblebees (*Bombus spp.*) and stingless bees (*Melipona*, *Trigona*), have received less attention. These species have different foraging behaviors, social structures, and reproductive strategies, which may result in varying sensitivities to glyphosate. For instance, stingless bees in

tropical regions may face unique risks due to their year-round activity and exposure to glyphosate in agroforestry systems [34].

Additionally, there are **regional differences** in glyphosate application practices, which can influence exposure levels. For example, in Latin America, glyphosate is widely used in large-scale soybean cultivation, which can affect native bee populations differently than in regions where glyphosate use is more restricted. Addressing these gaps requires conducting studies in diverse ecological contexts to better understand the global impacts of glyphosate on bee health.

VI. Future Research Directions

A. Priority Areas for Further Study

1. Mechanistic Studies on Glyphosate's Effects on Bee Physiology

Although significant research has demonstrated the sublethal effects of glyphosate on bee behavior and health, there are still **major gaps** in understanding the mechanistic pathways through which glyphosate affects bee physiology. Mechanistic studies are crucial for unraveling the precise cellular and molecular disruptions caused by glyphosate exposure. For instance, glyphosate is known to inhibit the **shikimate pathway** in plants and some microorganisms, but its potential indirect effects on bees, such as altering the gut microbiota and immune response, require further elucidation [35].

Research indicated that glyphosate exposure can lead to oxidative stress and impaired energy metabolism in bees, but more studies are needed to confirm these findings across different bee species and environmental contexts. Understanding these physiological impacts could inform mitigation strategies to protect bees. Moreover, further investigations into **gene expression profiling** and metabolomics could reveal how glyphosate disrupts critical biological pathways in bees.

2. Long-term Field Studies Across Diverse Ecosystems

Most studies to date have focused on short-term laboratory exposures, which may not fully capture the chronic, low-dose exposure bees experience in real-world agricultural settings. **Long-term field studies** are urgently needed to assess how continuous exposure to glyphosate affects bee populations over multiple generations, especially under varying climatic and environmental conditions [36].

Research conducted over longer periods can help identify delayed effects on colony health, reproduction, and population dynamics. For example, field studies in agricultural landscapes have shown that glyphosate exposure can reduce colony size and foraging efficiency over time. Expanding these studies to include diverse ecosystems, such as tropical regions where stingless bees are prevalent, would provide a more comprehensive understanding of the global impacts of glyphosate. Additionally, such research should explore how glyphosate interacts with other agrochemicals to produce cumulative or synergistic effects.

B. Exploration of Alternatives to Glyphosate in Agriculture

1. Development of Eco-Friendly Herbicides

As concerns over glyphosate's environmental impact grow, there is increasing interest in developing **eco-friendly herbicides** that can effectively control weeds without harming pollinators and other non-

target organisms [37]. Bioherbicides, which are derived from natural sources like plant extracts or microbial metabolites, show promise as safer alternatives. For instance, the use of allelopathic compounds from plants such as sorghum and sunflower has demonstrated weed suppression capabilities with minimal toxicity to bees.

Researchers are also exploring **RNA interference (RNAi)-based herbicides**, which can target specific weed genes without affecting non-target organisms, including pollinators. However, the scalability and cost-effectiveness of these alternatives need further evaluation before they can replace glyphosate on a large scale. The development of such alternatives requires collaboration between agronomists, chemists, and ecologists to ensure that new herbicides are both effective and environmentally safe.

2. Integrated Pest Management Approaches

Integrated Pest Management (IPM) offers a sustainable solution to reducing glyphosate reliance by combining cultural, mechanical, and biological control methods [38]. IPM strategies, such as **crop rotation, cover cropping, and selective weed removal**, can help control weed populations while minimizing the need for chemical herbicides. By promoting biodiversity in agricultural systems, IPM can enhance natural pest control and reduce the vulnerability of bee populations to chemical exposure.

Studies have shown that IPM practices can reduce pesticide usage by up to **50%** without compromising crop yields. However, widespread adoption of IPM faces barriers, including farmers' familiarity with conventional herbicides and the need for training on alternative practices. Policymakers and agricultural extension services need to incentivize the adoption of IPM to ensure that farmers can transition away from glyphosate-based herbicides.

C. Collaborative Research and International Efforts

1. Need for Interdisciplinary Studies

The complexity of glyphosate's impact on bees necessitates **interdisciplinary research** that spans toxicology, ecology, genetics, and socioeconomics [39]. Collaborative research can help bridge the gap between laboratory findings and field observations, providing a more holistic understanding of how glyphosate affects bee populations. For instance, combining **genomic and metabolomic analyses** with field studies can help elucidate the sublethal effects of glyphosate on bee health and colony dynamics.

International collaborations are also essential to assess the **global impact** of glyphosate, particularly in regions with high agricultural intensity, such as Brazil, India, and the United States. Research funding agencies and universities need to prioritize joint projects that can inform global policy on herbicide use and pollinator protection.

2. Engagement with Policymakers and Stakeholders

To effectively mitigate the risks glyphosate poses to bees, it is crucial to engage **policymakers, farmers, and industry stakeholders** in translating scientific research into actionable policies. Regulatory frameworks, such as the European Union's Bee Guidance Document, have laid the groundwork for assessing pesticide risks, but more stringent enforcement is needed [40]. Scientists should work closely with regulators to ensure that risk assessments consider **sublethal and long-term effects**, not just acute toxicity.

Public awareness campaigns can also play a critical role in driving policy changes. Studies have shown that consumer demand for pesticide-free products can incentivize farmers to adopt sustainable practices. By involving stakeholders across the agricultural supply chain, it is possible to develop solutions that protect pollinators while maintaining crop productivity.

Conclusion

The sublethal effects of glyphosate, particularly in its formulated forms, pose significant risks to social bee species, including honeybees, bumblebees, and stingless bees. These impacts, which range from impaired navigation, learning, and foraging to weakened immune responses and disrupted gut microbiota, threaten bee colony health and productivity. Current research underscores the urgent need for long-term field studies and interdisciplinary approaches to fully understand these effects and inform regulatory frameworks. Additionally, exploring alternatives like eco-friendly herbicides and integrated pest management is crucial for reducing glyphosate dependence. Engaging policymakers, farmers, and industry stakeholders is essential to implementing sustainable agricultural practices that protect pollinators while maintaining crop yields. Protecting bees is not only vital for biodiversity but also for global food security, underscoring the need for immediate action in research, regulation, and agricultural innovation.

References

1. 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*(1), 19.
2. Ali, S., Zahid, A., Fatima, A., Ahmad, M., Manzoor, M. T., Ayub, A., ... & Shafqat, N. (2023). Harnessing Soil Potential: Innovation in Strategic Tillage and Management-New Perspectives.
3. Brausch, J. M., & Smith, P. N. (2007). Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp, *Thamnocephalus platyurus*. *Archives of environmental contamination and toxicology*, *52*, 217-221.
4. Hajjar, R., Jarvis, D. I., & Gemmill-Herren, B. (2008). The utility of crop genetic diversity in maintaining ecosystem services. *Agriculture, Ecosystems & Environment*, *123*(4), 261-270.
5. Elizalde, L., Arbetman, M., Arnan, X., Eggleton, P., Leal, I. R., Lescano, M. N., ... & Pirk, G. I. (2020). The ecosystem services provided by social insects: traits, management tools and knowledge gaps. *Biological Reviews*, *95*(5), 1418-1441.
6. Straw, E. A., Mesnage, R., Brown, M. J., & Antoniou, M. N. (2023). No impacts of glyphosate or *Crithidia bombi*, or their combination, on the bumblebee microbiome. *Scientific Reports*, *13*(1), 8949.
7. Williams, G. R., Alaux, C., Costa, C., Csaki, T., Doublet, V., Eisenhardt, D., ... & Brodschneider, R. (2013). Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions. *Journal of Apicultural Research*, *52*(1), 1-36.
8. Daisley, B. A., Chernyshova, A. M., Thompson, G. J., & Allen-Vercoe, E. (2022). Deteriorating microbiomes in agriculture-the unintended effects of pesticides on microbial life. *Microbiome research reports*, *1*(1).
9. Castro, M. J., Ojeda, C., & Cirelli, A. F. (2013). Surfactants in agriculture. *Green materials for energy, products and depollution*, 287-334.
10. Kleinert, A. M., Ramalho, M., Cortopassi-Laurino, M., Ribeiro, M. F., & Imperatriz-Fonseca, V. L. (2012). Social bees (Bombini, Apini, Meliponini). *Insect Bioecology and Nutrition for Integrated Pest Management*, *237*, 271.
11. El-Seedi, H. R., Ahmed, H. R., El-Wahed, A. A. A., Saeed, A., Algethami, A. F., Attia, N. F., ... & Wang, K. (2022). Bee stressors from an immunological perspective and strategies to improve bee health. *Veterinary Sciences*, *9*(5), 199.
12. DesJardins, N. (2023). *Sublethal Behavioral and Physiological Effects of an Agricultural Fungicide on Honey Bees* (Doctoral dissertation, Arizona State University).

13. Falkner, G. (2005). *Complying with Europe: EU harmonisation and soft law in the member states*. Cambridge University Press.
14. Rortais, A., Arnold, G., Dorne, J. L., More, S. J., Sperandio, G., Streissl, F., ... & Verdonck, F. (2017). Risk assessment of pesticides and other stressors in bees: principles, data gaps and perspectives from the European Food Safety Authority. *Science of the total environment*, 587, 524-537.
15. Balbuena, M. S., Tison, L., Hahn, M. L., Greggers, U., Menzel, R., & Farina, W. M. (2015). Effects of sublethal doses of glyphosate on honeybee navigation. *The Journal of experimental biology*, 218(17), 2799-2805.
16. Wright, G. A., & Schiestl, F. P. (2009). The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, 23(5), 841-851.
17. Bartling, M. T., Brandt, A., Hollert, H., & Vilcinskas, A. (2024). Current Insights into Sublethal Effects of Pesticides on Insects. *International Journal of Molecular Sciences*, 25(11), 6007.
18. Paris, L., El Alaoui, H., Delbac, F., & Diogon, M. (2018). Effects of the gut parasite *Nosema ceranae* on honey bee physiology and behavior. *Current opinion in insect science*, 26, 149-154.
19. Harwood, G. P., & Dolezal, A. G. (2020). Pesticide-virus interactions in honey bees: challenges and opportunities for understanding drivers of bee declines. *Viruses*, 12(5), 566.
20. Mesnage, R., Benbrook, C., & Antoniou, M. N. (2019). Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. *Food and Chemical Toxicology*, 128, 137-145.
21. Abdel-Rahman, A., Anyangwe, N., Carlacci, L., Casper, S., Danam, R. P., Enongene, E., ... & Walker, N. J. (2011). The safety and regulation of natural products used as foods and food ingredients. *Toxicological Sciences*, 123(2), 333-348.
22. Odemer, R., Alkassab, A. T., Bischoff, G., Frommberger, M., Wernecke, A., Wirtz, I. P., ... & Odemer, F. (2020). Chronic high glyphosate exposure delays individual worker bee (*Apis mellifera* L.) development under field conditions. *Insects*, 11(10), 664.
23. Fine, J. D., & Corby-Harris, V. (2021). Beyond brood: the potential impacts of insect growth disruptors on the long-term health and performance of honey bee colonies. *Apidologie*, 52(3), 580-595.
24. Odemer, R., Alkassab, A. T., Bischoff, G., Frommberger, M., Wernecke, A., Wirtz, I. P., ... & Odemer, F. (2020). Chronic high glyphosate exposure delays individual worker bee (*Apis mellifera* L.) development under field conditions. *Insects*, 11(10), 664.
25. Bartling, M. T., Brandt, A., Hollert, H., & Vilcinskas, A. (2024). Current Insights into Sublethal Effects of Pesticides on Insects. *International Journal of Molecular Sciences*, 25(11), 6007.
26. Roubik, D. W., Heard, T. A., & Kwapong, P. (2018). Stingless bee colonies and pollination. *The pollination of cultivated plants: A compendium for practitioners*, 2, 39-64.
27. Murray, T. E., Kuhlmann, M., & Potts, S. G. (2009). Conservation ecology of bees: populations, species and communities. *Apidologie*, 40(3), 211-236.
28. Talyn, B., Muller, K., Mercado, C., Gonzalez, B., & Bartels, K. (2023). The herbicide glyphosate and its formulations impact animal behavior across taxa. *Agrochemicals*, 2(3), 367-408.
29. Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S., ... & Thenius, R. (2013). Standard methods for behavioural studies of *Apis mellifera*. *Journal of Apicultural Research*, 52(4), 1-58.

30. Nicholson, C. C., & Williams, N. M. (2021). Cropland heterogeneity drives frequency and intensity of pesticide use. *Environmental Research Letters*, *16*(7), 074008.
31. Cant, E. T., Smith, A. D., Reynolds, D. R., & Osborne, J. L. (2005). Tracking butterfly flight paths across the landscape with harmonic radar. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1565), 785-790.
32. Motta, E. V., & Moran, N. A. (2020). Impact of glyphosate on the honey bee gut microbiota: effects of intensity, duration, and timing of exposure. *MSystems*, *5*(4), 10-1128.
33. Wu, J. Y., Anelli, C. M., & Sheppard, W. S. (2011). Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PloS one*, *6*(2), e14720.
34. Chandrasena, N. (2022). To Bee or Not to Be: Weeds for Bees. *Weeds-Journal of the Asian-Pacific Weed Science Society*, *4*(1), 1-20.
35. Van Bruggen, A. H., Finckh, M. R., He, M., Ritsema, C. J., Harkes, P., Knuth, D., & Geissen, V. (2021). Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. *Frontiers in Environmental Science*, *9*, 763917.
36. Raine, N. E., & Rundlöf, M. (2024). Pesticide exposure and effects on non-*Apis* bees. *Annual Review of Entomology*, *69*(1), 551-576.
37. Hasan, M., Ahmad-Hamdani, M. S., Rosli, A. M., & Hamdan, H. (2021). Bioherbicides: An eco-friendly tool for sustainable weed management. *Plants*, *10*(6), 1212.
38. Baker, B. P., Green, T. A., & Loker, A. J. (2020). Biological control and integrated pest management in organic and conventional systems. *Biological Control*, *140*, 104095.
39. Sponsler, D. B., Grozinger, C. M., Hitaj, C., Rundlöf, M., Botías, C., Code, A., ... & Douglas, M. R. (2019). Pesticides and pollinators: A socioecological synthesis. *Science of the Total Environment*, *662*, 1012-1027.
40. Robinson, C., Portier, C. J., Čavoški, A., Mesnage, R., Roger, A., Clausen, P., ... & Lyssimachou, A. (2020). Achieving a high level of protection from pesticides in Europe: problems with the current risk assessment procedure and solutions. *European Journal of Risk Regulation*, *11*(3), 450-480.