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

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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. 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.

1. INTRODUCTION

A. 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 (Myers, et al. 2016). 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 (Ali et al. 2023).

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 (Brausch & Smith 2007). 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 (Hajjar et al. 2008). 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 (Elizalde et al. 2020).

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 (Fig. 1). 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 (Straw et al. 2023).

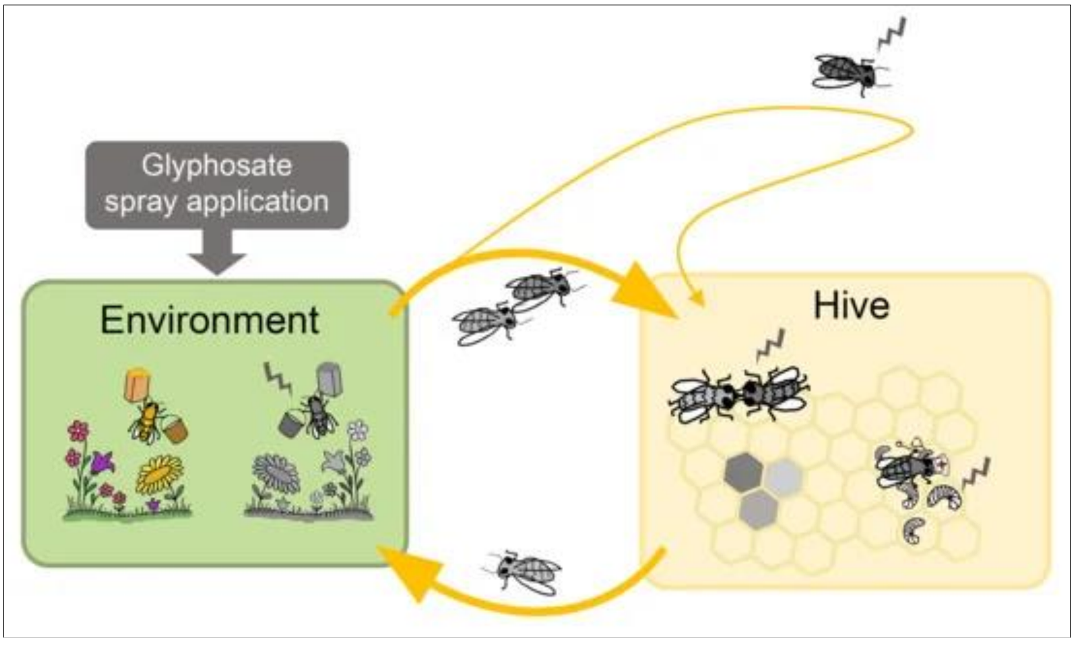

Fig. 1. Glyphosate effect (Source - MDPI)

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 (Williams et al. 2013). Research on social bees can thus provide early indicators of broader environmental impacts and help inform policy decisions regarding pesticide regulation.

2. GLYPHOSATE

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 (Daisley et al. 2022). 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 (Castro et al. 2013). 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 (Kleinert, et al. 2012). 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 (El-Seedi et al. 2022).

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 (DesJardins 2023).

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. 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 (Falkner 2005).

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 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 (Rortais et al. 2017).

3. 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 (Table 1). 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 (Balbuena et al. 2015). The 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 (Wright & Schiestl 2009). 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 (Bartling et al. 2024). 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 (Paris et al. 2018). 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

Table 1. Sublethal effects of glyphosate on bees

Aspect Affected	Impact	Mechanisms or Observations
Navigation and Foraging	Disruption in spatial memory and foraging efficiency.	Impairment of cognitive abilities linked to the gut microbiota -Difficulty in returning to hives
Gut Microbiota Composition	Alteration of the gut microbial balance, increasing vulnerability to pathogens.	Reduction in beneficial bacteria like *Snodgrassella alvi* and *Gilliamella apicola*
Larval Development	Delayed growth and abnormal development in bee larvae.	Residual glyphosate in pollen and nectar consumed by developing larvae
Immune Response	Weakening of the immune system, making bees more prone to diseases and pests.	Suppression of antimicrobial peptide production Synergistic effects with pesticides
Behavioral Changes	Altered social and individual behaviors, including reduced grooming and impaired communication.	Interference with neurotransmitter functions like acetylcholine
Reproductive Health	Decline in queen health and reproductive success of colonies.	Glyphosate residues in royal jelly affecting queen physiology
Longevity of Workers	Reduced lifespan of worker bees under chronic exposure to glyphosate.	Oxidative stress and cellular damage
Hive Productivity	Decrease in overall colony strength and productivity due to compounded sublethal effects.	Reduction in foraging efficiency and population density
Pesticide Synergism	Amplification of toxic effects when combined with other agrochemicals.	Interaction with neonicotinoids exacerbating toxicity
Pollination Services	Decline in pollination efficiency, affecting agricultural yields and ecosystem services.	Reduced flower visits and ineffective pollination

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 (Harwood et al. 2020).

3. Effects on Development, Reproduction, and Longevity

Glyphosate exposure can also affect the development and reproduction of bees. 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) (Mesnage et al. 2019). 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. 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 (Abdel-Rahman et al. 2011).

4. 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. 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 (Odemer et al. 2020). 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. 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 (Fine & Corby-Harris 2021). 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 (Odemer et al. 2020). 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 (Bartling et al. 2024). 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 (Roubik et al. 2018). 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 (Murray et al. 2009). 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.

5. 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 (Talyn et al. 2023). 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. Laboratory studies are effective for detailed mechanistic research, they may not fully represent real-world conditions. 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. 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 (Scheiner et al. 2013).

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. 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 (Nicholson & Williams 2021). This complexity makes it difficult to isolate the specific effects of glyphosate, leading to variability in study results. 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 (Cant et al. 2005). 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* (Motta & & Moran 2020). 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 (Wu et al. 2021).

2. Understudied Species and Regional Differences

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. Stingless bees in tropical regions may face unique risks due to their year-round activity and exposure to glyphosate in agroforestry systems (Chandrasena 2022). 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.

6. FUTURE

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. 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 (Van et al. 2021). 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 (Raine & Rundlöf 2024). 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. Such research should explore how glyphosate interacts with other agrochemicals to produce cumulative or synergistic effects.

B. 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 (Table 2) (Hasan et al. 2021). Bioherbicides, which are derived from natural sources like plant extracts or microbial metabolites, show promise as safer alternatives. 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. 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 (Baker et al. 2020 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.

Table 2. Alternatives to glyphosate in agriculture

Alternative Method/Technique	Description	Advantages	Challenges/Limitations	Examples of Applications
Mechanical Weed Control	Physical removal of weeds using tools or machinery like plows, harrows, or mowers.	Immediate results No chemical residues -Compatible with organic farming	Labor-intensive Not effective for all weed types High cost for equipment	Inter-row cultivation, rotary hoeing, flame weeding
Cover Cropping	Planting cover crops to suppress weed growth through competition or allelopathy.	Enhances soil health Reduces erosion Provides habitat for beneficial organisms	Limited weed control for persistent weeds Requires careful selection of cover crops	Rye, clover, vetch used in rotation systems
Mulching	Applying organic or synthetic materials on soil to inhibit weed growth by blocking sunlight.	Improves soil moisture retention Reduces soil temperature fluctuations	Expensive for large-scale application Labor-intensive installation	Straw mulch in vegetable gardens, plastic mulch in horticulture
Organic Herbicides	Use of natural compounds like acetic acid, clove oil, or pelargonic acid to kill weeds.	Environmentally friendly - Breaks down quickly in soil	Limited effectiveness on mature weeds Requires frequent application	Acetic acid sprays in small-scale farming systems
Biological Weed Control	Introduction of natural predators or pathogens to target specific weed species.	Sustainable and environmentally safe No chemical use	Slow process Requires specific conditions for efficacy	Use of insects like *Mogulones cruciger* for houndstongue control
Crop Rotation	Alternating crops to disrupt weed cycles and	Enhances soil fertility Reduces pest	Requires planning and knowledge Less effective for	Cereal-legume rotations,

Alternative Method/Technique	Description	Advantages	Challenges/Limitations	Examples of Applications
	reduce specific weed populations.	and disease incidence	generalist weeds	maize-soybean rotations
Precision Agriculture	Use of technologies like drones and sensors to identify and target weed infestations selectively.	Reduces herbicide usage Minimizes environmental impact	High initial investment Requires technical expertise	Weed mapping and spraying using drones equipped with sensors
Cultural Practices	Adjusting sowing times, row spacing, and water management to reduce weed competitiveness.	Cost-effective Can be integrated with other practices	Limited effectiveness as a standalone solution	Early sowing of competitive crops like wheat or barley
Integrated Weed Management	Combining multiple weed control strategies for a holistic approach.	Reduces reliance on single methods Long-term weed control	Requires coordination and knowledge High initial setup cost	Combining mechanical weeding with cover cropping
Laser Weeding	Targeted weed removal using lasers to damage weed cells and tissues.	No chemical residues Highly precise	Expensive technology Limited field coverage at a time	Commercial trials in vegetable production systems

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 (Sponsler et al. 2019). Collaborative research can help bridge the gap between laboratory findings and field observations, providing a more holistic understanding of how glyphosate affects bee populations. 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 (Robinson et al. 2020). 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.

7. 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. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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