GLYPHOSATE

GLYPHOSATE BASED HERBICIDES & THEIR IMPACT ON BEES' HEALTH

Many people assume that herbicides, since they are designed to kill plants, are not toxic for bees. This, however, is far from the truth.

In this summary we provide scientific findings that reveal that glyphosate-based herbicides (GBH) are toxic to bees in many ways. Bees are exposed to this chemical either by direct contact or by ingestion via food and contaminated water.

Our research shows that the herbicide:

- **disrupts the gut microbiome and makes the bees more susceptible to disease**
- **disturbs development of bee brood (the eggs, larvae and pupae of the bees)**
- **can negatively affect thermoregulation of the bumblebee colonies**
- **has a negative impact on the reproduction of wild bees and honeybees**
- **disrupts their foraging abilities and navigation**
- **has a negative impact on bees' learning ability and memory**

Interaction with other chemicals (cocktail effect) and also other ingredients (e.g. co-formulants) used in glyphosate-based herbicides can significantly increase toxicity. All these negative effects in combination significantly weaken the colony and its survival.

Furthermore, a large number of studies confirm the unavoidable and unacceptable risks of glyphosate to the environment, especially to aquatic species and to soil biota. These ecotoxicological threats suggest that glyphosate does not meet the approval criteria of the EU Pesticides Regulation (EC) 1107/2009, which aims to ensure that pesticide active substances and products placed on the market do not adversely affect human or animal health or the environment, including honey bees. Therefore, in light of the scientific evidence re-authorization of glyphosate in the EU should not be granted for this reason alone.

Glyphosate has raised concerns as it has been linked to different negative effects on human health. Most importantly, the International Agency for Research on Cancer classified glyphosate in 2015 as "probably carcinogenic to humans". Scientific research suggests that human exposure to glyphosate may also cause endocrine disruption, Parkinson's disease and microbiome alterations that can lead to the suppression of the immune system. Therefore the use of glyphosate should be banned due to its impact on humans as well as the environment. Here, we focus on the impact of glyphosate on bee-health, which is less known to the general public and policy-makers.

Pesticide Action Network Europe

Widespread use of glyphosate puts bees' health at risk

Glyphosate is the active ingredient of the most widely used pesticide (herbicide) products in the World. Glyphosate-based herbicides (GBH) are being used widely to combat weeds in agriculture, towns and cities, peoples' homes and gardens and even in nature-protected areas. As a consequence exposure to glyphosate is widespread for the environment, which is impossible for bees to avoid.

The use of glyphosate particularly as a pre-harvest crop desiccant, leads not only to a high contamination of the crops but also to the contamination of wild plant nectar and pollen (Zioga et al. 2022), therefore non-target flowering plants become a significant exposure route for bees too. Exposed foraging bees then bring back the herbicide into their hives. Glyphosate has been detected in approximately 33% of analysed honey samples (Ledoux et al. 2020).

Glyphosate is a broad spectrum herbicide that is toxic to all plants as well as certain bacteria. Its widespread use results in its drift and runoff to non-crop areas where it can impact plant biomass production, seed germination of various plant species and diversity of soil microorganisms (Zaller and Brühl, 2019). This gradually impacts biodiversity, food web interactions and ecosystem function, which consequently impacts bee colonies since they are a crucial component for the function of the ecosystem.

Nevertheless, glyphosate appears to also be directly toxic to bees. A 2021 meta-analytical review (Battisti et al.), based on 16 studies, showed that exposure to GBH products has significant negative impacts on most bee species, including wild and solitary bees. Another 2021 review by Tan et al. demonstrated the different risks that glyphosate exposure poses to bees, which include negative effects on their behaviour, growth and development, metabolic processes and immune defence systems. These risks are presented below.

Environmental relevant concentrations and direct toxicity

Farina et al. collected information on environmentally relevant GBH exposure. Studies found glyphosate concentrations in water bodies close to agricultural areas from a few micrograms to 1.7 mg/L. *"For the worst case scenario in small water bodies (ponds or puddles), a median expected environmental concentration of 3.49 mg/L was calculated"*. Herbert et al. suggested 1.4 to 7.6 (active ingredient) mg/L glyphosate as environmentally relevant concentrations, measured in natural environments.

El Agrebi et al. found that more than 90% of the beebread samples collected in Belgian apiaries were contaminated with glyphosate. The substance was detected in beeswax too, but no transfer from wax to honey was detected.

Direct toxicity of glyphosate herbicides had been demonstrated by Abraham et al. in a laboratory experiment when honey bees (Apis mellifera) and Hypotrigona bees died after contact with plants freshly sprayed with the GBH product "Sunphosate 360SL" at the manufacturer's recommended concentration (10 ml/L). The mortality increased along with the concentration of the herbicide used.

Gut microbiota and pathogens

Glyphosate is known to be toxic or interact with different strains of bacteria including gut bacteria that regulate the immune system, which makes the herbicide harmful to different species, including bees. The gut microbiota of bees plays a key role in stress tolerance and disease resistance. Therefore, disruption of the gut bacteria weakens immunity, exacerbates pesticide toxicity, and reduces host's nutritional status (Daisley et al. 2020). This makes bees more vulnerable to a variety of negative health impacts including decreased resistance to harmful pathogens which can result in weakening or complete loss of bee colonies.

Exposure to glyphosate makes bees more susceptible to pathogens. Motta et al. (2018) demonstrated that treatment with glyphosate concentrations similar to those found in the environment (5-10 mg/L) cause the reduction of dominant gut microbiota species. A similar study from Motta et al. in 2020 proved that both oral and topical exposure to GBH below recommended dose (0.1% Roundup formulation) affected the abundance of beneficial bacteria in the honey bee gut in a dose-dependent way. According to both studies, the exposure of the herbicide caused higher mortality of worker bees when exposed to the pathogen *Serratia marcescens*. A 2022 study by Motta et al. found that glyphosate exposure (0.1, 1 or 10 mM for 5 days) decreased the expression of antimicrobial peptides: apidaecin, defensin, melanization and hymenoptaecin, in honey bees. The authors concluded that glyphosate can reduce the abundance of beneficial gut bacteria and lead to dysregulation of their immune system. In relation to bumble bees, a recent study (Motta and Moran, 2023) found that 5-days exposure to either glyphosate or a glyphosate-based formulation at field relevant concentrations (0.01 mM to 1 mM) reduced the relative abundance of the beneficial gut bacterium Snodgressela. Although this change became less significant at 3 and 7 days post-exposure, at day 7 there was a decreased survival rate in bees exposed to 1 mM glyphosate formulation.

Blot et al. (2019) highlighted that glyphosate (1.5 mM in their sugar syrup) itself and not its metabolite AMPA, induced a significant change in the honeybee gut microbiota.

Research in 2018 (Dai et al.) found that higher 20 mg/L concentrations of glyphosate significantly changed species, diversity and richness of all core honey bee intestinal bacteria. In the in vitro study brood survival decreased in 4 or 20 mg/L, larval weight decreased in 0.8 or 4 mg/L glyphosate treatments.

Glyphosate

Development and reproduction

Graffigna et al. (2021) found a significant negative effect of exposure to GBH Roundup® ControlMax in the reproductive success of solitary wild bees in their South American study. Trap nests were sprayed with the GBH once with the manufacturer-recommended concentration (8 g GLY-CF/L distilled water), and once with double the concentration and the "control" with distilled water. GBH exposure halved the probability of finding breeding cells and completed larvae development decreased to one-fourth. The survival of the immature stages in the control nests was 52%, while the nests treated had a survival of 14% for the normal concentration and around 4% for the double concentration.

In a laboratory experiment by Vázquez et al. a honey bee brood fed with a glyphosate-contaminated food source (1.25-5.0 mg/L of food) caused delayed larvae development and reduced weight.

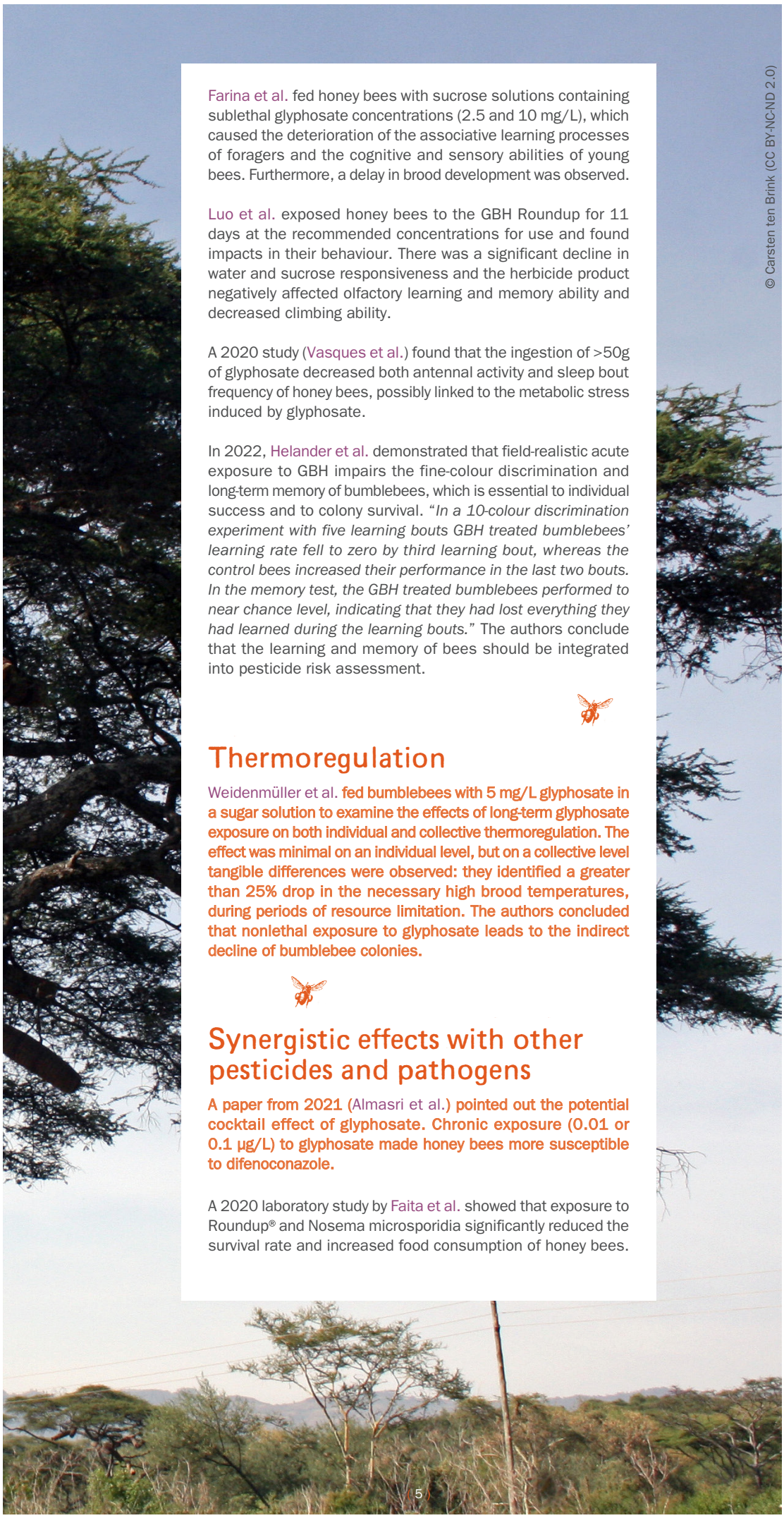
Wang et al. (2022) investigated whether oral exposure to glyphosate (7.12 mg/L) affects the regulation of honey bee metabolites in 2, 5, and 10 days using metabolomics analysis. The study demonstrated that although no significant behavioural differences were observed in honey bees under sub-lethal doses of glyphosate, metabolomic level perturbation due to changes in amino acids and carbohydrates was observed under short-term exposure when met with other environmental stressors or long-term exposure.

Movement, navigation, behaviour, neurotoxicity

Several studies have pointed out the neurotoxic properties of glyphosate in recent years (Costas-Ferreira and Faro, 2022). According to a 2019 study (Zgurzynski and Lushington) the neurotoxic properties of GBHs are significantly greater in bees than in humans. In 2013, Boily et al. demonstrated that glyphosate exposure decreases the activity of the neurotransmitter acetylcholinesterase in honey bees.

According to Balbuena et al. a sublethal dose of glyphosate (2.5, 5 and 10 mg/L corresponding to 0.125, 0.250 and 0.500 µg per animal) can cause disruption to honeybee navigation - exposed bees spent more time performing homeward flights than control species.

Herbert et al. found that glyphosate concentration (0 to 3.7 mg/L) below that recommended for aquatic and terrestrial weed control or those measured in natural environments (1.4 to 7.6 mg/L), reduced honeybees' sensitivity to nectar and elemental learning and short-term memory retention.



Farina et al. fed honey bees with sucrose solutions containing sublethal glyphosate concentrations (2.5 and 10 mg/L), which caused the deterioration of the associative learning processes of foragers and the cognitive and sensory abilities of young bees. Furthermore, a delay in brood development was observed.

Luo et al. exposed honey bees to the GBH Roundup for 11 days at the recommended concentrations for use and found impacts in their behaviour. There was a significant decline in water and sucrose responsiveness and the herbicide product negatively affected olfactory learning and memory ability and decreased climbing ability.

A 2020 study (Vasques et al.) found that the ingestion of >50g of glyphosate decreased both antennal activity and sleep bout frequency of honey bees, possibly linked to the metabolic stress induced by glyphosate.

In 2022, Helander et al. demonstrated that field-realistic acute exposure to GBH impairs the fine-colour discrimination and long-term memory of bumblebees, which is essential to individual success and to colony survival. "*In a 10-colour discrimination experiment with five learning bouts GBH treated bumblebees' learning rate fell to zero by third learning bout, whereas the control bees increased their performance in the last two bouts. In the memory test, the GBH treated bumblebees performed to near chance level, indicating that they had lost everything they had learned during the learning bouts.*" The authors conclude that the learning and memory of bees should be integrated into pesticide risk assessment.

Thermoregulation

Weidenmüller et al. **fed bumblebees with 5 mg/L glyphosate in a sugar solution to examine the effects of long-term glyphosate exposure on both individual and collective thermoregulation. The effect was minimal on an individual level, but on a collective level tangible differences were observed: they identified a greater than 25% drop in the necessary high brood temperatures, during periods of resource limitation. The authors concluded that nonlethal exposure to glyphosate leads to the indirect decline of bumblebee colonies.**

Synergistic effects with other pesticides and pathogens

A paper from 2021 (Almasri et al.) pointed out the potential cocktail effect of glyphosate. Chronic exposure (0.01 or 0.1 µg/L) to glyphosate made honey bees more susceptible to difenoconazole.

A 2020 laboratory study by Faita et al. showed that exposure to Roundup® and Nosema microsporidia significantly reduced the survival rate and increased food consumption of honey bees.

Effects of co-formulants, surfactants

Glyphosate's herbicide action is not effective to plants without the addition of co-formulants in the product formulation. Glyphosate-based herbicides contain chemical surfactants to adhere to the plant surface and help glyphosate break through its protective outer layer (membrane), facilitating its faster absorption into the interior of the cells where it exerts its toxic action. However, this also increases the toxic risks for non-target species exposed to GBHs. Currently, the EU pesticide authorisation process does not address the combined toxic effect of all the ingredients of the formulated products properly. The risk to human health and the environment by GBHs is systematically underestimated, as the additional risks of added toxic co-formulants remain under the radar. According to studies, formulated GBHs, have, in most cases, a greater toxicity than the active ingredient alone (Defarge et al., 2018; Mesnage et al., 2019; Vanlaeys et al., 2018)

In 2000 Goodwin and Mc Brydie demonstrated the negative effects of surfactants on honey bee survival. A Brazilian study from 2022 by da Silva et al. found that ingestion of GBH caused "*differences in digestive cell ultrastructure, impaired midgut digestive cell cohesion by disorganizing the smooth septate junctions between cells*" in Brazilian stingless bees. The authors concluded that this was probably caused by the POE-tallowamine (POAE) adjuvant. Due to its high toxicity, in 2016 the EU agreed on a ban on POE-tallowamine as a co-formulant in GBH products.

A study by Straw et al. (2021) suggests that surfactants, or other co-formulants in GBH products, could cause death by incapacitating the gas exchange system of bumble bees. Dose-dependent mortality caused by Roundup® Ready-To-Use confirmed its acute toxicity. There was a significant difference in the observed mortality between different Roundup products; the surfactants also had different modes of toxicity.

POAE is often replaced in GBH products by surfactant Tween 20 (Polysorbate 20). This surfactant is used in the selected "representative formulated product" MON 52276 submitted by the producers for the ongoing EU-level re-authorisation procedure of glyphosate's licence. A study by Pehlevan, B., & Kovanci (2016) indicates that Tween 20 is already so toxic to insects that it could be used as an insecticide.

Conclusion and legal recommendations

Exposure of bees to glyphosate is widespread. Glyphosate can be directly toxic to bees at concentrations that are often found in the field but adverse effects have also been observed at lower, non lethal concentrations. Exposure of bees to very low and environmentally-relevant concentrations disrupts their foraging abilities, which weakens the colony's potential to survive. The interaction of glyphosate with bee gut microbiota also makes colonies much more vulnerable to disease and other harmful effects. Glyphosate impacts normal development in bees and reproduction success and has subtle effects on their nervous system affecting bees' behaviour at very low concentrations. Finally, glyphosate products contain co-formulants that can be toxic to bees as well, the toxicity of which is not properly assessed neither alone nor in combination with the other ingredients of glyphosate-based formulations.

Therefore, based on independent literature we can conclude that the current extensive use of GBH herbicides poses a significant risk to bee health. According to the EU pesticides Law (Regulation 1107/2009), which is underpinned by the precautionary principle, pesticide active substances and products placed on the EU market must not adversely affect human or animal health or the environment. More specifically, active substances shall be approved only if they "*result in a negligible exposure of honeybees*" and have "*no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour*" (Article 4.1; Annex II 3.8.3.). Furthermore, both active substances and pesticide products should have no unacceptable effects on the environment, paying attention to their "*impact on non-target species, including on the ongoing behaviour of those species*" and "*impact on biodiversity and the ecosystem*" (Article 4.4).

Leaving aside the International Agency for Research on Cancer's conclusion that glyphosate probably causes cancer in humans, the impact of GBH on bee health and the different unavoidable ecotoxicological dangers resulting from its use clearly show that the approval criteria of Regulation (EC) 1107/2009 are not satisfied for glyphosate, which means that re-authorization must not be granted to this substance in the EU, once its market licence expires in December 2023.

Pesticide Action Network Europe

Written by Gergely Simon March 2023
Pesticide Action Network Europe
67 Rue de la Pacification 1000 Brussels Belgium www.pan-europe.info

References

- Abraham et al., Commercially formulated glyphosate can kill non-target pollinator bees under laboratory conditions, Entomol. Exp. Appl. 166 (2018) 695–702, https://doi.org/10.1111/eea.12694.
- El Agrebi et al., Honeybee and consumer's exposure and risk characterisation to glyphosate-based herbicide (GBH) and its degradation product (AMPA): Residues in beebread, wax, and honey, Science of The Total Environment, Volume 704,135312, ISSN 0048-9697,(2020) https://doi.org/10.1016/j.scitotenv.2019.135312.
- Almasri et al., Toxicological status changes the susceptibility of the honey bee *Apis mellifera* to a single fungicidal spray application, Environ. Sci. Pollut. Res. 28 (2021) 42807–42820, https://doi.org/10.1007/s11356-021-13747-3.
- Balbuena et al., Effects of sublethal doses of glyphosate on honeybee navigation, J. Exp. Biol. 218 (2015) 2799–805, https://doi.org/10.1242/jeb.117291.
- Battisti et al., Is glyphosate toxic to bees? A meta-analytical review, Sci. Total Environ. 767 (2021) 145397, https://doi.org/10.1016/j.scitotenv.2021.145397.
- Blot et al., Glyphosate, but not its metabolite AMPA, balters the honeybee gut microbiota, PLoS ONE 14 (2019) e0215466, https://doi.org/10.1371/journal.pone.0215466.
- Boily et al., Acetylcholinesterase in honey bees (Apis mellifera) exposed to neonicotinoids, atrazine and glyphosate: laboratory and field experiments, *Environ Sci Pollut Res* 20, 5603–5614 (2013). https://doi.org/10.1007/s11356-013-1568-2
- van Bruggen et al., Indirect Effects of the Herbicide Glyphosate on Plant, Animal and Human Health Through its Effects on Microbial Communities.Frontiers in Environmental Science 9:2021 doi: 10.3389/fenvs.2021.763917
- Costas-Ferreira and Faro, Toxic effects of glyphosate on the nervous system: a systematic review, Int. J. Mol. Sci. 23 (2022) 4605, https://doi.org/10.3390/ijms23094605.
- Dai et al., The Herbicide Glyphosate Negatively Affects Midgut Bacterial Communities and Survival of Honey Bee during Larvae Reared in Vitro, *J. Agric. Food Chem.* (2018), 66, 29, 7786–7793, https://doi.org/10.1021/acs.jafc.8b02212
- Daisley et al., Missing Microbes in Bees: How Systematic Depletion of Key Symbionts Erodes Immunity, Trends in Microbiology (2020) VOLUME 28, ISSUE 12, P1010-1021, https://doi.org/10.1016/j.tim.2020.06.006
- Defarge et al., Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides, Toxicol. Rep. 5 (2018) 156–163, https://doi.org/10.1016/j.toxrep.2017.12.025.
- Faita et al., Glyphosate-based herbicides and *Nosema* sp. microsporidia reduce honey bee (*Apis mellifera* L.) survivability under laboratory conditions, J. Apic. Res. 59 (2020) 332–342, https://doi.org/10.1080/00218839.2020.1736782.
- Farina et al., Effects of the herbicide glyphosate on honey bee sensory and cognitive abilities: Individual impairments with implications for the hive, Insects, 10 (2019) PMID: 31635293, https://doi.org/10.3390/insects10100354.
- Goodwin and McBrydie, Effect of Surfactants on Honey Bees, *New Zealand Plant Protection* 53 (August 1, 2000): 230–234. (2023) https://doi.org/10.30843/nzpp.2000.53.3694
- Graffigna et al., Glyphosate commercial formulation negatively affects the reproductive success of solitary wild bees in a Pampean agroecosystem, Apidologie, 52 (2021) 272–281, https://doi.org/10.1007/s13592-020-00816-8.
- Helander et al., Field-realistic acute exposure to glyphosate-based herbicide impairs fine-color discrimination in bumblebees, Sci Total Environ, (2023) 857(Pt 1):159298. doi: 10.1016/j.scitotenv.2022.159298.
- Herbert et al., Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour, J. Exp. Biol. 217 (2014) 3457–3464, https://doi.org/10.1242/jeb.109520.
- Ledoux at al., Penetration of glyphosate into the food supply and the incidental impact on the honey supply and bees, Food Control (2020) 109: 106859, https://doi.org/10.1016/j.foodcont.2019.106859
- Lesseur et al., Maternal urinary levels of glyphosate during pregnancy and anogenital distance in newborns in a US multicenter pregnancy cohort. Environ Pollut. 2021 doi: 10.1016/j.envpol.2021.117002
- Luo et al., Effects of a commercially formulated glyphosate solutions at recommended concentrations on honeybee (*Apis mellifera* L.) behaviours, Sci. Rep. 11 (2021) 2115, https://doi.org/10.1038/s41598-020-80445-4.
- Mesnage et al., Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides, Food Chem. Toxicol. 128 (2019) 137–145, https://doi.org/10.1016/j.fct.2019.03.053.
- Motta et al., Glyphosate perturbs the gut microbiota of honey bees, J. Appl. Biol. Sci. 115 (2018) 10305–10310, https://doi.org/10.1073/pnas.1803880115.
- Motta et al., Impact of glyphosate on the honey bee gut microbiota: effects of intensity, duration, and timing of exposure, mSystems 5 (2020) https://doi.org/10.1128/msystems.00268-20.
- Motta et al., Glyphosate induces immune dysregulation in honey bees, Anim. Microbiome 4 (2022) 16, https://doi.org/10.1186/s42523-022-00165-0.
- Motta and Moran, The effects of glyphosate, pure or in herbicide formulation, on bumble bees and their gut microbial communities, Sci Total Environ. 2023 Feb 8;162102. doi: 10.1016/j.scitotenv.2023.162102
- Pehlevan and Kovancı, Laboratory evaluation of Tween 20 for potential use in control of *Cacopsylla pyri* L. eggs and nymphs (Homoptera: Psyllidae), Journal of Biological & Environmental Sciences (2016) 29:39-43
- da Silva et al., Glyphosate-Based Herbicide Causes Cellular Alterations to Gut Epithelium of the Neotropical Stingless Bee Melipona quadrifasciata quadrifasciata (Hymenoptera: Meliponini), Neotrop Entomol. 2022 Dec;51(6):860-868. doi: 10.1007/s13744-022-01001-5
- Straw et al., Roundup causes high levels of mortality following contact exposure in bumble bees, J. Appl. Ecol. 58 (2021) 1167–1176, https://doi.org/10.1111/1365-2664.13867.
- Tan et al., Effects of glyphosate exposure on honeybees, Environ. Toxicol. Pharmacol. 90 (2022) 103792, https://doi.org/10.1016/j.etap.2021.103792.
- Vanlaeys et al., Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells, Toxicol. In Vitro 52 (2018) 14–22, https://doi.org/10.1016/j.tiv.2018.01.002.
- Vázquez et al., Sleep in honey bees is affected by the herbicide glyphosate, Sci. Rep. 10 (2020) 10516, https://doi.org/10.1038/s41598-020-67477-6.
- Vázquez et al., Glyphosate affects the larval development of honey bees depending on the susceptibility of colonies, PLoS ONE 13 (2018) e0205074, https://doi.org/10.1371/journal.pone.0205074.
- Wang et al., Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism & Related Disorders 17(6):P486-487 (2011) https://doi.org/10.1016/j.parkreldis.2011.02.003
- Wang et al., Metabolomic analysis of honey bee (*Apis mellifera* L.) response to glyphosate exposure, Mol. Omics 2022 (2022b) https://doi.org/10.1039/D2MO00046F.
- Weidenmüller et al., Glyphosate impairs collective thermoregulation in bumblebees, Science 376 (2022) 1122–1126, https://doi.org/10.1126/science.abf7482.
- Zaller and Brühl, Editorial: Non-target Effects of Pesticides on Organisms Inhabiting Agroecosystems (2019)Frontiers in Environmental Science DOI:10.3389/fenvs.2019.00075
- Zioga et al., Glyphosate used as desiccant contaminates plant pollen and nectar of non-target plant species, Heliyon, (2022) https://doi.org/10.1016/j.heliyon.2022.e12179
- Zgurzynski and Lushington, Glyphosate Impact on Apis mellifera Navigation: A Combined Behavioral and Chemin-formatics Study". EC Pharmacology and Toxicology 7.8 (2019): 806-824.

Pesticide Action Network Europe