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REVIEW

Pharmaceutical pollutants and bee health: Exposure pathways and occurrence studies

[version 1; peer review: 1 approved, 2 not approved]

Devsankar Sunilkumar[1], Laura Grácio[2], Marta Leite[1,3], Andreia Freitas[1,3]

[1]Instituto Nacional de Investigacao Agraria e Veterinaria, Vairão, Vila do Conde, 4485-655, Portugal
[2]Universidade do Porto Faculdade de Ciencias, Porto, Porto District, Portugal
[3]REQUIMTE LAQV Porto, Porto, Porto District, Portugal

v1 **First published:** 30 Mar 2026, **6**:85
https://doi.org/10.12688/openreseurope.21872.1
Latest published: 30 Mar 2026, **6**:85
https://doi.org/10.12688/openreseurope.21872.1

Abstract

The increase in livestock and agricultural production has led to the widespread use of veterinary drugs, including antibiotics, antiparasitic, nonsteroidal anti-inflammatory drugs (NSAIDs), hormones, and pesticides, to accelerate productivity and control disease. After excretion, these compounds remain unmetabolized and can contaminate the surrounding environment through uncontrolled use of manure, wastewater, and direct treatment practices. This review focuses on the multiple environmental exposure pathways through which pharmaceuticals and agrochemicals may contaminate ecosystems, particularly emphasizing the impact that this problem may have on the health of pollinators. For example, honeybees can be exposed to pharmaceutical residues through contaminated soil, water, nectar, and pollen, which often leads to acute toxicity and sublethal effects on reproduction, immune function, and behavior. The persistence of these residues in the environment exacerbates antimicrobial resistance, which poses a global threat. In addition, this study reveals different methods of extraction and detection of pharmaceutical residues from honey, encompassing advanced analytical techniques such as liquid chromatography coupled with mass spectrometry detection. This review highlights the chronic and synergistic effects of pharmaceuticals on bee health and emphasizes risk assessment strategies to mitigate the ecological impact of pharmaceuticals on livestock production.

Keywords

Pharmaceutical pollution; Honeybee health; Honey contamination; One Health; Environmental exposure pathways

Open Peer Review

Approval Status ✗ ✓ ✗

	1	2	3
version 1 30 Mar 2026	✗ view	✓ view	✗ view

1. **Dani Sukkar**, Université de Lorraine, Yutz, France
2. **Jelena ĆIRIĆ**, Institute of Meat Hygiene and Technology, Belgrade, Serbia
3. **Ben A. Woodcock**, UK Centre for Ecology & Hydrology, Wallingford, UK

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Corresponding author: Andreia Freitas (andreia.freitas@iniav.pt)

Author roles: Sunilkumar D: Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Grácio L**: Investigation, Methodology, Writing – Original Draft Preparation; **Leite M**: Conceptualization, Investigation, Writing – Review & Editing; **Freitas A**: Conceptualization, Formal Analysis, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from the European Union's Framework Programme for Research & Innovation as part of the COST Action CA22105 - BEekeeping products valorization and biomonitoring for the SAFEty of BEEs and HONEY (BeSafeBeeHoney), as supported by the COST Association (European Cooperation in Science and Technology). supporting this work'. This work was also supported by the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and by the project PRR-C05-i03-I-000190-LA2.2- "RumiRes LA 2.2"
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Sunilkumar D, Grácio L, Leite M and Freitas A. **Pharmaceutical pollutants and bee health: Exposure pathways and occurrence studies [version 1; peer review: 1 approved, 2 not approved]** Open Research Europe 2026, **6**:85 https://doi.org/10.12688/openreseurope.21872.1

First published: 30 Mar 2026, **6**:85 https://doi.org/10.12688/openreseurope.21872.1

Introduction

Animal-derived products such as meat, milk, eggs, and honey are vital sources of protein and energy in the human food system. The global demand for these products has intensified the need to increase food production, including livestock and aquaculture production (Wilkinson *et al.*, 2017). Veterinary medicines are pharmaceutical compounds used for therapeutic efficiency and as growth stimulants in animal production (Khalifa *et al.*, 2024). The Food and Drug Administration (FDA) defines drug residue as a compound that is present in the edible tissue after the use of a drug or its metabolite, or any other substances formed in food (Pratiwi *et al.*, 2023). The intensification of animal production has led to a consequent increase in the use of veterinary medicines, either to treat infections and prevent diseases, or even illegally, to promote animal growth. Veterinary drugs are essential for maintaining animal health and improving productivity; however, they can enter the environment through the application of manure, animal excreta, treatments against ectoparasites, or through aquaculture practices (Topp *et al.*, 2018). A wide range of compounds are often used, such as antibiotics, anthelmintics, hormones, and anti-inflammatory drugs, among others (Delgado *et al.*, 2023), and their occurrence in food or the environment can have an impact on global health. Small molecules are not biodegradable and may persist in different environmental compartments after excretion. Most medicines are designed to have a prolonged effect for a longer period of time. This stability transforms their occurrence into their persistence in the environment (Berkner & Thierbach, 2014).

The One Health Perspective approach states that human health is closely correlated with the combination of animal and environmental health. Environmental pollutants, such as pharmaceuticals, which are widely used in livestock production and in human medicine, can represent a real threat to wild animals and, more specifically, to natural pollinators such as honeybees. The toxic effects of constant exposure to veterinary drugs and pesticides used in food production can also affect survival (Mahefarisoa *et al.*, 2021). The cumulative exposure to residual levels of pharmaceutical medicines can affect the metabolism of both humans and animals, and the continuous discharges of antibiotics into the environment can promote the development of antimicrobial-resistant microorganisms, which is of global level concern. Antimicrobial resistance (AMR) refers to pathogens, such as bacteria, viruses, and fungi, that no longer respond to antimicrobials. It mainly occurs due to the irrational use of medicines, failure to follow the drug withdrawal period, poor infection prevention and control, pollution, and poor waste and wastewater management. According to global statistics, AMR is directly responsible for 39 million deaths and contributes to more than 169 million deaths between 2025 and 2050 (Larsson & Flach, 2022).

Several studies have shown that veterinary medicines administered to animals are normally excreted at 90 per cent of the dose in urine and feces and thus discharged into sewage plants, which eventually reach surface water. Animal waste is used as manure in crop fields as a natural fertilizer, which leads to the indirect transfer of drug residues to the food chain (Delgado *et al.*, 2023). The release of active pharmaceuticals, mainly due to the excessive use of veterinary medicines in the environment, (Wilkinson *et al.*, 2017), is incomparable to human medicine, which causes the accumulation of pharmaceutical residues in soil, water, plants, and sediments, even though the quantities that are used on animals are much higher, leading to an increase in the impact that it will cause (Gworek *et al.*, 2021).

In the "One Health" approach, and considering the effects that this situation may have in all ecosystems, the persistent contamination of the environment can be one of the main factors causing the decline of pollinators. Bees are assumed to be the most important pollinators in agricultural and natural ecosystems. However, other insects such as beetles, flies, and butterflies are also involved in pollination (Rader *et al.*, 2016). According to the global statistics, bees can pollinate approximately 1500 crops globally, contributing to 15–30% of global food production. (V.S., A., 2024). Honeybees are more prone to environmental contamination because of their natural behavior of gathering nectar and pollen from a diverse range of flowering and nonflowering plants. Bees can encounter multiple unidentified pathways of exposure to chemicals from livestock farms, manure and slurry, animal excreta, urine, surface water, soil, air, and plants (Mahefarisoa *et al.*, 2021).

There is evidence that veterinary medicines can accumulate in hives (Venable *et al.*, 2014) and cause adverse effects on bee health, such as acute toxicity and sublethal effects including weakened immune function, reduced memory and learning capability, compromised reproductive function, and disrupted social behavior.

However, although pharmaceutical pollution of environmental matrices is increasing, its impacts and specific risks on bees are not completely understood. There are knowledge gaps related to the concentration of veterinary drugs on different bee-related matrices (pollen, nectar, and hive matrices), antagonistic or synergetic effects with different chemical contaminants, and chronic effects of low-dose exposure (Di Noi *et al.*, 2021). Identifying these knowledge gaps is essential for the development of strategic risk assessment plans to protect bees and other pollinators. Therefore, this review focuses on a comprehensive overview of environmental contamination by veterinary drugs in animal production and their potential exposure pathway to bee health.

Sources and pathways of contamination

Veterinary drugs can enter the environment through multiple pathways, either directly or indirectly, thereby creating potential risks to the environment, animals, and humans. Several sources should be considered for direct contamination.

Runoff from Livestock Farms: The Waste originating from livestock farms is the major source of direct contamination (Delgado *et al.*, 2023). Most drugs administered to humans and animals after metabolism are transformed into different chemically active structures, which ultimately reach water through agricultural wastes, aquaculture, and sewage treatment plants (Joshua *et al.*, 2022). The concentration of pharmaceuticals in different environmental matrices depends on various factors,

such as livestock farm management practices, animal density, and drug administration behaviors in farms (Delgado *et al.*, 2023).

- Use of manure as a natural fertilizer: Manure obtained from livestock farms can comprise a cocktail of different contaminants, such as antimicrobial, antiparasitic, and hormones, among other possible products applied to animals. These pharmaceutical residues eventually accumulate in the soil and affect soil microbes, plant uptake, and groundwater quality (Jaga *et al.*, 2022).

- Direct application of medications to beehives: Depending on the country and purpose of use, medications can be applied to beehives to control the colonies from pests and diseases. Beekeepers use diverse xenobiotics against Varroa mites, which pose a significant threat to their health. Certain insecticides can cause acute toxicity in bees; for example, imidacloprid is a highly toxic insecticide for honeybees, according to the U.S. Environmental Protection Agency (EPA) (Manjon *et al.*, 2018). Therefore, the use of antibiotics is often prohibited.

In terms of indirect contamination also several routes can be expected and here summarized:

- Disposal of pharmaceutical compounds used in human medicine can eventually result in the occurrence of these contaminants in wastewater treatment plans (WWTP). Because WWTP are not well designed to remove pharmaceuticals from water, effluents can contain detectable residues of pharmaceuticals (Delgado *et al.*, 2023);

- Uptake by Plants: Pharmaceuticals and other agrochemicals present in treated wastewater used for irrigation, especially during drought time, can contaminate plants. This assimilation can cause pharmaceutical compounds to accumulate in plant tissues, nectar, and pollen, leading to exposure of bees and other pollinators. Although there are limited data regarding the uptake of pharmaceuticals by plants, some studies have shown that plants can absorb pharmaceuticals from contaminated water and soil (Mastro *et al.*, 2025). The pharmaceutical uptake by plants depends on several factors, such as the specificity of the compounds, soil texture and behavior, and plant species (Carter *et al.*, 2014). Some studies have revealed that medicines are biotransformed into other metabolites by flowering plants, which may be equally or more hazardous. For instance, Obregon *et al.* (2024) found that anthelmintic medicines administered to bovines undergo biotransformation and can be converted into other compounds by flowering plants.

- Atmospheric deposition of pharmaceutical compounds: There is evidence that volatile and semi-volatile contaminants, such as polycyclic aromatic hydrocarbons (PAHs), originating from incomplete combustion or natural sources, such as volcanic activity and forest fires, exert toxic effects on both the environment and human health (Jiménez *et al.*, 2015). In contrast, the atmospheric deposition of veterinary pharmaceuticals remains a relatively understudied topic despite its potential as a significant pathway for environmental contamination.

Understanding contamination pathways is essential for developing and implementing effective strategies to eradicate environmental contamination caused by veterinary medicines, thus protecting ecosystems and human and animal health, and especially to develop measures to protect essential pollinators such as honeybees. Table 1 shows some examples of previous studies correlating the occurrence of contaminants in hive products with the main impact on bee health, along with possible exposure pathways.

Risks posed by veterinary pharmaceuticals to bees and other pollinators

Veterinary pharmaceutical medicines play a crucial role in maintaining animal health, welfare, and productivity in intensive animal production systems. The presence of veterinary medicines, either by direct application or indirect contamination, can cause potentially harmful consequences, affecting microbial health in the gut. Studies have revealed that honeybees are an important reservoir of antibiotic resistance genes that affect reproduction, immune system, and bee development (Baffoni *et al.*, 2021). For instance, oxytetracycline is a widely employed antibiotic used for the control of bacterial diseases in European Foulbrood (causative agent Streptococcus pluton) and American foulbrood (causative agent Bacillus larvae). Although EU regulations prohibit the use of antibiotics in apiaries, the use of lincomycin, oxytetracycline, and tylosin has been approved for the treatment of honeybees in other countries. Studies conducted by Baffoni *et al.* (2021) elucidated that tylosin treatment causes a drastic decline in bifidobacteria and lactobacilli found in the rectum but enhances the growth of Frischella spp., Gilliamella, and Snodgrassella in the ileum. The sulfonamides also showed some changes; they increased in Snodgrassella spp. and Bombilactobacillus spp., and declined in Frischella and Bartonella species. Honeybees were found to be a reservoir of tetracycline-resistant genes, as confirmed using qPCR techniques. However, some studies provide evidence that antibiotics, such as sulfonamides, promote the decline of species, such as Frischella and Bartonella, while tylosin decreases Bifidobacteria and Lactobacilli (Baffoni *et al.*, 2021). These imbalances in the gut microbiota due to different factors, such as exposure to chemicals, diet, and season, called dysbiosis, have a negative impact on the bee population (Baffoni *et al.*, 2021). Antibiotic treatment plays a pivotal role in affecting the size and composition of the honey gut microbiome and enhances the risk of infection from widespread opportunistic pathogens (Raymann *et al.*, 2017). Healthy gut microbiota is essential for maintaining proper immune function, nutrition acquisition, and resistance to pathogenic organisms in bees. Dysbiosis can weaken immune function and make bees more prone to attacks by pathogens. (Raymann *et al.*, 2017).

Varroosis (a destructive parasitic disease) is a major threat to honeybees worldwide. To address this problem, insecticides or acaricides, such as amitraz, are often used. Amitraz and its metabolites were found to be potent neurotoxicants, making amitraz more cytotoxic than its metabolites (Giorgini *et al.*, 2023). Bees can also be exposed to fungicides, such as chlorothalonil, and the insecticide chlorpyrifos through

Table 1. Occurrence of contaminants in hive products, probable exposure pathways and possible health effects on bees.

Contaminants	Matrix	Exposure Pathway	Detected Concentration	Impact on Bees	Reference
Carbendazim	Honey, Pollen & Bees	Atmospheric pollutants, contaminated food sources of food such as pollen, nectar or water	0.08 to 4.28 mg/kg	Declines foraging activity, mortality, impairs reproduction & Limits pollination efficiency	Lambert *et al.* (2013)
Amitraz	Honey & Bees wax	Environmental contamination & Hive treatment	----	Declines honey production, diminished colony growth & decreased memory and learning ability	Savarino *et al.* (2020)
Streptomycin Sulfanilde & Tetracyclines	Honey	from livestock farms, water,	----	Antimicrobial resistance	Mărgăoan *et al.* (2024)
Chlorpyrifos	Bee pollen, Honey & Bee Bread	Surface residues, oral/ contact exposure	Detected in 30% of samples	Acetyl cholinesterase inhibition-Affecting the honeybee brain & behavioral impairment	Liu *et al.* (2022)
Fenpropathrin (pyrethroid)	Honeybee	Crop raiding	0.3 to 81.5 ng/g	Decreased homing ability & survival, acute and chronic toxicities	Obregon *et al.* (2024)
Avermectin/Ivermecetin	Bee bread	Biotransformation from ivermectin to abamectin by flowering plants- (absorbed animal waste of ivermectin by cattle)	9.6–1,856 µg/kg	Adaptive response	Savarino *et al.* (2020)

contaminated pollen and nectar. These two chemicals are the most commonly found pesticides in bee wax-derived samples. (Walsh *et al.*, 2021).

Even though in some cases there is improper use of chemicals in hives, veterinary drugs should only be administered to honeybee colonies under the guidance of a veterinary practitioner or apiculture specialist. Alternative treatment methods include the growth of bioactive plants containing secondary metabolites such as sesquiterpene lactones or condensed tannins. These metabolites act as antiparasitic agents to avoid the administration of fungicides or herbicides. (Rodríguez-Hernández *et al.*, 2023).

In the following sections, the main classes of medicines that can cause adverse health effects on pollinators are summarized. Apart from these compounds, it may also be considered that possible cross-contamination of the environment can lead to the presence of other groups of compounds used in urbanized regions, mainly human medicines.

Antibiotics

The excessive use of antibiotics and antimicrobial resistance are currently trending topics that compromise both human and animal health. These compounds can be used in apiculture practices to treat and prevent different bee diseases, such as European and American foulbroods, Varroa mites, bee acariasis, and Nosema. This may lead to a rise in concerns about honey contamination (food safety), contribution to the increase in antimicrobial resistance, and other environmental implications from a "One Health" perspective (Moumeni *et al.*, 2025). A recent study revealed the presence of antibiotics such as chloramphenicol and oxytetracycline residues in untreated and treated hives. Situations like this raise concerns about the environmental dissemination of contaminants and/or cross-contamination from sources other than the direct use of medicines. These studies and the detection of residues of medicines in hive products make honeybees act as vectors for transferring antibiotic-resistant genes (Almutairi *et al.*, 2025) and a possible contributor to pollution bioindicators.

Antiparasitic agents

Antiparasitic resistance will be an upcoming threat in the future, elucidated the emergence of resistance from key classes of similar to what is currently being discussed regarding antimicrobial antiparasitic benzimidazoles, which are extensively used by resistance. A recent study from the northern German research group livestock farmers for the treatment and prevention of infections caused by parasites. (Ehnert *et al.*, 2025). The global rise in the population has led to a higher demand for food production and, consequently, to avoid losses due to health problems in animals, there is also an increase in the use of veterinary medicines (Barmpouni *et al.*, 2025), revealing how antiparasitic compounds such as albendazole and ivermectin can persist in animal tissues, leading to drug residues in the food chain and environment (Barmpouni *et al.*, 2025). In Table 2 we can observe Analytical methodologies

Table 2. Analytical methodologies for detection of different compounds collected from hives products.

Target Compounds	Sample (g)	Extraction Method	Appropriate LC Column	Mobile Phase	Ionization	MS Mode	Mass Analyzer	LOD / CCβ (µg/kg)	References
Nitrofurans (Honey)	1.00 ± 0.02	Magnetic SPE	Acclaim 120 C18 (2.1×100 mm, 3 µm)	0.5% FA–H_2O / ACN:MeOH (50:50)	ESI+ and ESI–	MRM	SCIEX Triple Quad 5500	0.1–0.3	Jayasinghe *et al.* (2021)
Aminoglycosides (11) (Honey, milk and pork)	2.0	MIP-SPE	HILIC (50×2.1 mm, 3.5 µm)	175 mM ammonium formate + 0.3% FA / MeOH + 0.3% FA	ESI+	MRM	Sciex 3200 QTRAP	2–30	Ma *et al.* (2016)
β-Lactams, Macrolides, Sulfonamides, etc. (Honey)	1.0	Oasis HLB SPE	Synergi Hydro-RP (150×2 mm, 4 µm)	0.1% FA / MeOH	HESI	Full scan + DIA	Thermo Q-Exactive Orbitrap	0.03–4.80 ng g^{-1}	Chen *et al.* (2021)
Multi-class antibiotics (Honey)	2.0	Dispersive SPE	Zorbax SB C18 (100×2.1 mm, 3.5 µm)	H_2O + 100 mM HFBA / ACN	ESI+	MRM	Agilent 6410 Triple Quad	0.73–3.58	Zhang *et al.* (2016)
Multi-residue antibiotics (Honey)	5.0 ± 0.01	SPE (Strata-X)	Obelisc R (2.1×150 mm, 5 µm)	1% FA / ACN	ESI+	Full Scan	Bruker Q-TOF	13.4–47.9 ng g^{-1}	Liu *et al.* (2022)
Pesticides + Antibiotics (Honey)	1.0	Oasis HLB SPE	Synergi Hydro-RP (150×2 mm, 4 µm)	0.1% FA in MeOH	HESI	DIA	Thermo Q-Exactive Plus	0.73–3.58	Wang *et al.* (2023)
Perchlorate (Honey)	2.0	Freezing-assisted LLE	Torus Waters (2.1×100 mm, 1.7 µm)	20 mM ammonium acetate / ACN	ESI–	MRM	AB Sciex 6500+	0.05	Wang *et al.* (2023)
Tylosin A & Desmycosin (Honey)	2.00 ± 0.05	LLE	Kinetex HILIC (100×2.1 mm, 1.7 µm)	40 mM ammonium formate / ACN + FA	ESI+	MRM	Sciex 3500 Triple Quad	0.3–0.4	Li *et al.* (2019)
Nitrofurans Chloramphenicol, Florfenicol (Honey)	3.0	LLE	Zorbax Eclipse XDB-C18 (50×4.6 mm, 1.8 µm)	0.1% FA / ACN + ammonium acetate	APCI+ / ESI–	MRM	Agilent 1260 + Sciex 5500	0.19–2.35	Zhao & Sun (2017)
Penicillin G, Dihydrostreptomycin, Enrofloxacin (Honey)	10 mL	Micro-LLE	Zorbax SB-Aq / Eclipse Plus C18	ACN / 0.1% FA–H_2O	ESI+	MRM	Waters Quattro Triple Quad	0.55–0.79 ng g^{-1}	Kim *et al.* (2021)
Quinolones & Nitroimidazoles (Honey)	3.0	QuEChERS	XTerra RP18 (2.1×150 mm, 5 µm)	ACN / 10 mM ammonium formate + 0.5% FA	ESI+	MRM	Waters Micro Quattro	0.83–1.58	Alvarenga *et al.* (2018)
Quinolones (Honey)	5.0 ± 0.01	QuEChERS	Waters C18 (2.1×50 mm, 1.7 µm)	0.5 mM ammonium formate + 0.005% FA / MeOH	ESI+	SRM	Waters Xevo TQ	0.61–0.69	Patel & Singh (2021)
Sulfonamides, Quinolones, Tetracyclines, etc. (Honey and royal jelly)	2.0	QuEChERS	Poroshell 120 EC-C18 (100×2.1 mm, 2.7 µm)	2 mM ammonium formate + 0.1% FA / MeOH	ESI+	dMRM	Agilent 6460 Triple Quad	0.14–2.91 ng g^{-1}	Garcia & Torres (2016)

Target Compounds	Sample (g)	Extraction Method	Appropriate LC Column	Mobile Phase	Ionization	MS Mode	Mass Analyzer	LOD / CCβ (µg/kg)	References
Sulfonamides, Quinolones, Macrolides (Honey)	2.0	QuEChERS	ACQUITY (100×2.1 mm, 1.7 µm)	MeOH / 0.2% FA–H_2O	ESI+	MRM	Waters Xevo TQ-XS	0.05–1.02	Nguyen & Hoang (2021)
Sulfonamides Tetracyclines, Macrolides (Honey)	2.0 ± 0.05	QuEChERS	Acquity (2.1×150 mm, 1.8 µm)	0.1% FA / MeOH	AJS ESI	MRM	Agilent 6470 Triple Quad	0.1–2.5	Silva & Costa (2023)
Pesticides (96) (Honey)	5.0	QuEChERS	C18 (2.1×150 mm, 1.8 µm)	5 mM ammonium formate + 0.1% FA / ACN	ESI+	dMRM	Agilent 6490 Triple Quad	0.5–1	Zhang & Chen (2016)
Cephalosporins, Quinolones, Macrolides, etc. (Honey)	2.0 ± 0.05	QuEChERS	ACQUITY C18 (100×2.1 mm, 1.8 µm)	0.1% FA–H_2O / ACN	ESI+	TOF	Shimadzu Nexera UHPLC + AB Sciex 5600+	0.05–0.64	Mendes & Oliveira (2025)

for detection of different compounds collected from hives products.

Anti-inflammatory medicines

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used around the globe in veterinary and human medicine worldwide. NSAIDs are prevalent as parent compounds or metabolites in the environment. However, there are no efficient wastewater treatment methods for the removal of these chemical contaminants from aquatic environments (Izadi *et al.*, 2020) Common NSAIDs such as naproxen, diclofenac and ibuprofen are present in various aquatic resources such as groundwater, sea water, surface water, and sludge (Lakshmi *et al.*, 2024). Coming from direct human and veterinary uses. Bees and other pollinators are directly exposed to contaminated water bodies, flowers, pollen, and soil, and continuous contact with NSAIDs can affect their immune system, foraging ability, and colony vitality. Therefore, a thorough study of NSAID occurrences and their behavior in the environment is vital to elucidate the potential exposure pathways and threats to the bee population and to develop mitigation strategies against pharmaceutical pollution (Izadi *et al.*, 2020).

Analytical methodologies for determination of pharmaceutical residues in honey

Honey is a natural product that is prone to contamination from various sources in the environment (Puścion-Jakubik *et al.*, 2020). Honey comprises 300 distinct compounds produced from Apis mellifera being the main component simple sugars, approximately 80–83%. It is composed of proteins, vitamins, microelements, and organic compounds (Lewiński *et al.*, 2023). Two international regulatory bodies, the Codex Alimentarius (CA) and the European Council Directive, have set standards for honey quality control (Zammit Young & Blundell, 2023). The complex composition and requirements to fulfill the standard regulations pose a significant challenge in developing an analytical method for the determination and quantification of pharmaceuticals in honey. This session aims to provide an overview of different analytical strategies to determine contaminants such as pharmaceuticals in honey, from sample preparation methods to analytical detection techniques to ensure the quality and safety of honey before reaching consumers.

An optimized and targeted sample preparation technique is vital for isolating the target analyte and obtaining results with minimal matrix interference. The new trends in sample preparation include less time, solvent, and sample volume and are more automated, achieving results in less time (Rodrigues *et al.*, 2024).

Regarding the detection techniques, Liquid Chromatography coupled with Mass Spectrometry detection (LC-MS), particularly triple quadruple detectors (LC-MS/MS), (Kivrak *et al.*, 2016), are widely employed for the detection and quantification of pharmaceutical residues from biological matrices including honey. (Gün *et al.*, 2024). This analytical approach offers the possibility of developing high-throughput and fast multidetection screening and confirmatory methods (Er Demirhan & Demirhan, 2022). High resolution Mass Spectrometry (HRMS) allows the possibility to do both targeted and un-targeted analysis (qualitative and quantitative analysis) (Bandini & Spisso, 2021), being the Orbitrap and the time-of-flight mass spectrometer the examples of those technologies (Khalifa *et al.*, 2024). LCMS/MS is often considered the gold standard for the detection and quantification of pharmaceutical residues in honey owing to its high sensitivity and specificity. (Cavaliere *et al.*, 2019). Although other approaches can be used at lower associated costs and speed, the specificity of mass spectrometry

and the possibility of multi-detection analysis overcome these characteristics. Despite this, examples in the literature can be found with different methods: a High Fundamental Frequency Quartz Crystal Microbalance with Dissipation (HFF-QCMD) has been developed for detecting pharmaceutical and agro-chemical residues in honey (Calero *et al.*, 2022); aptamer-based biosensors have also been employed to isolate and identify streptomycin, an aminoglycoside, in honey samples (Kong *et al.*, 2024); enzyme-linked immunosorbent assays (ELISA) are commonly used for screening tetracycline residues in honey (Yanovych *et al.*, 2023) 43 along with microbiological screening methods for detecting various drug residues.

Sample preparation strategies for honey analysis

Although LC–MS/MS is a powerful analytical technique for unequivocally detecting and quantifying multiple pharmaceutical residues, matrix effects in honey can significantly affect sensitivity and selectivity. Therefore, it is essential to select and optimize the appropriate sample pretreatment and extraction methods. Currently, the focus is on minimizing the sample volume and processing time while maintaining high extraction efficiency (Leite *et al.*, 2020). Different extraction methods are often employed for the extraction of pharmaceutical residues from honey and other hive products, such as solid-phase extraction (SPE), dispersive solid-phase extraction (d-SPE), Liquid-Liquid Extraction (LLE), Quick Easy Cheap Effective Rugged Safe (QuEChERS), and simple dilution (Rodrigues *et al.*, 2024)34. SPE and d-SPE are the most conventional extraction methods, using different solid-phase cartridges, such as C18, Strata X, and Oasis HLB. These methods offer high selectivity by eliminating unwanted analytes and matrices. SPE compensates for many limitations of LLE, such as requiring less solvent volume, more selectivity, and easy operation, and can be automated and integrated with other sample pretreatment techniques (Ścigalski & Kosobucki, 2020). The main limitation of this technique is the relatively long sample preparation time. In addition, there are some variations in SPE, such as the Matrix Solid Phase Dispersion (MSPD), Solid Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE) (Ścigalski & Kosobucki, 2020).

Liquid-Liquid Extraction (LLE) can also present different possibilities, such as dispersive liquid-liquid microextraction (DLLME), hollow fiber liquid-phase microextraction (HF-LPME), salting-out liquid-liquid extraction (SALLE), aqueous two-phase system extraction (ATPS), single drop microextraction (SDME), liquid-phase microextraction (LPME), and sugaring-out assisted liquid-liquid extraction (SULLE) (Khatibi *et al.*, 2022). Because honey is mainly composed of different sugars, a SULLE strategy is important for eliminating the main sugars and allowing the extraction and purification of diverse target pharmaceutical compounds. Freeze-assisted sugaring-out LLE is another approach developed by Fei *et al.* (2024) for the extraction of perchlorate from honey. Ultrasonicate-assisted Dispersive Liquid-Liquid Microextraction is a modification made by DLLME to improve extraction efficiency and minimize equilibrium time. A deep-eutectic-solvent-based LLME was developed by Uddin *et al.* (2025) for the determination of antibiotics in honey.

QuEChERS has been used for residue analysis of pesticides, antibiotics, hormones, mycotoxins, organic pollutants, and polycyclic aromatic hydrocarbons in environmental and food-derived matrices (Kim *et al.*, 2021). Honey analysis is usually carried out using acetonitrile with a modified QuEChERS method (Baša Česnik *et al.*, 2019).

Multi-detection of pharmaceutical veterinary medicines in hive products

Beeswax, pollen, propolis, and royal jelly are the most widely consumed bee-derived products. These substances are produced by bees and collected from the hive; therefore, residues of pharmaceutical medicines can appear in any of them and from multiple sources. To monitor the occurrence of these compounds, either to ensure consumer safety or to understand their effects on the health of bee colonies, it is important to develop efficient analytical methods. The use of multi-detection and multi-class detection methods for pharmaceutical veterinary medicines in hive products offers several significant advantages for both analytical performance and food safety assurance. This approach facilitates the concurrent identification of multiple drug classes, including antibiotics, anti-inflammatory agents, and antiparasitic compounds, in a single analysis. This enhancement in efficiency results in a reduction in the laboratory workload. This approach has been shown to reduce both sample and solvent consumption, leading to a decrease in costs and environmental impact. Furthermore, the use of modern techniques such as LC-MS/MS and GC-MS/MS ensures high levels of sensitivity, selectivity, and accuracy, even when analyzing complex matrices such as honey, wax, or propolis. The utilization of multi-detection methods in conjunction with comprehensive screening facilitates the expeditious identification of contamination sources and augmentation of regulatory control, thereby ensuring that bee-derived products meet the requisite safety standards. Moreover, integration of this analytical approach with emerging technologies, including molecular imprinting, biosensors, and artificial intelligence, has the potential to enhance the speed, automation, and data interpretation of the process. Multidetection has been demonstrated to be a cost-effective, time-efficient, and sustainable solution that supports the principles of One Health by safeguarding human, animal, and environmental health. (Lewiński *et al.*, 2023).

A compilation of recent studies demonstrates that advanced sample preparation and chromatographic techniques are at the core of this strategy. The main advantages of multi-detection techniques for pharmaceutical residues in hive products include their high coverage range, enabling the simultaneous identification of multiple drug classes and contaminants within a single sample preparation and chromatographic analytical run. These methods require only small sample amounts (ranging from 1 to 5 g) and still reach low analytical concentration thresholds; use of advanced extraction sorbents, such as molecularly imprinted polymers (MIP), solid-phase extraction (SPE), and QuEChERS-based methods, which enhance selectivity and method robustness, reduce matrix effects, and improve accuracy. Finally, multi-detection methods provide sufficient data to support both the targeted quantification of

known compounds and non-targeted screening for emerging or unknown contaminants. This characteristic makes them powerful and reliable tools for ensuring the safety and quality of hive products.

Emerging technologies for screening pharmaceutical contaminants in honey

Molecular Imprinting Technology is one of the newest innovative advances in sample preparation. Molecularly Imprinted Polymers (MIPs) are synthetic sorbents capable of producing specific binding sites that selectively capture target analytes. These polymers are produced through the interaction of target molecules (such as veterinary drugs) with functional monomers, either by covalent or noncovalent bonding, leading to the formation of polymeric networks that mimic the structural and chemical characteristics of the analyte. MIPs have demonstrated higher selectivity and extraction efficiency than conventional (Yanovych *et al.*, 2023). The application of MIP-based Solid Phase Extraction (SPE) coupled with advanced chromatographic systems, such as LCMS/MS, has proven to be successful for the extraction of veterinary medicines from honey and related matrices. This cutting-edge technology can also be integrated into microchip systems to create miniaturized analytical platforms.

Portable biosensors are another rapid tool for multiresidue detection in environmental and animal-derived products, including honey. In addition to traditional chromatographic, electrochemical, ELISA, and spectroscopic methods, biosensors offer a practical analytical option close to the field. Recent developments have incorporated advanced nanomaterials such as carbon nanotubes, gold nanoparticles, and graphene oxides to enhance their sensitivity and specificity (Elbasiony & Alharthi, 2024). Despite their potential, portable biosensors still face technical limitations, including minimal sample volumes, degradation of biological components, and integration challenges between sample preparation and data-processing systems. Nevertheless, increasing environmental contamination and population growth are driving the demand for portable and miniaturized options such as biosensors capable of detecting residual contaminants in food systems (Jafarzadeh *et al.*, 2024).

Artificial Intelligence (AI) and Machine Learning (ML) are emerging as innovative tools for honey analysis. Honey adulteration and contamination are significant concerns, and the complexity of hive products is a significant challenge. Advanced computational methods have been successfully applied to detect contaminants and ensure product authenticity (Ahmed, 2024). The integration of ML algorithms with spectral and chromatographic techniques, such as infrared (IR), nuclear magnetic resonance (NMR), Fourier-transform infrared (FTIR), Raman spectroscopy, and high-resolution mass spectrometry (HRMS), provides new and unlimited possibilities for multiresidue detection and pattern recognition (Tang *et al.*, 2025). Recent advances by Sánchez *et al.* combined the least absolute shrinkage and selection operator (LASSO) with support vector regression (SVR) and ultra-fast gas chromatography (UF-GC) to demonstrate how machine learning can enhance analytical performance and interpretation accuracy.

New advanced technologies mark a significant step forward in the monitoring and quality control of honey. By combining high selectivity and sensitivity from molecular imprinting, portability and rapid response of biosensors, and predictive power of artificial intelligence, future analytical systems will be more efficient, sustainable, and accessible. Such progress will not only strengthen food safety and consumer confidence, but also support environmental monitoring and regulatory compliance, ultimately contributing to a more resilient and transparent apicultural sector. The integration of new advanced technologies represents an innovative approach for improving efficient monitoring and quality control of honey. By combining high selectivity and sensitivity from molecular imprinting, portability and rapid response of biosensors, and predictive power of artificial intelligence, future analytical systems will be more efficient and accessible. (Gusarov & Gusarova, 2023). Such progress will not only strengthen food safety and consumer confidence but also support environmental monitoring and regulatory compliance, ultimately contributing to a more resilient apicultural sector.

Conclusion

The overall use of veterinary drugs in animal production and agriculture is essential for managing animal health and productivity, which poses a significant threat to the environment and pollinator health. This review highlights the mapping of different exposure pathways for pharmaceuticals entering the ecosystem through runoff from livestock farms, manure application, direct hive treatments, and atmospheric deposition. Honeybees are key pollinators and are prone to the acute and sublethal effects of diverse pharmaceuticals and agrochemicals, resulting in microbial dysbiosis, immune suppression, reproductive dysfunction, and behavioral impairments. The synergistic effect between veterinary drugs and agrochemicals can contribute to the accelerated increase in AMR, which is a global threat. There are significant knowledge gaps regarding the environmental exposure of bees, particularly to veterinary drugs and agrochemicals that persist in the air after administration and low dose, chronic effects, and interactions among diverse contaminants. This requires interdisciplinary research and the implementation of one health approach to reduce risks. The continuous discovery and studies to archive new methodologies are fundamental, as honey and other hives products are one of the multiple examples in the nature of complex matrixes that are being polluted not only, but mainly by veterinary drugs, creating a general prejudice in the environment and animal and human health. Despite all efforts to avoid the occurrence of contaminants, minimize risks, and avoid undesirable cross-contamination, it is impossible to have a clean system, which makes information, detection, and legislation about these drugs and how to use them even more important, giving farmers, bee keepers, and society all the support they might need to prevent and to take care of this One Health that covers every human being and their quality of life.

Ethics and consent

Ethical approval and consent were not required.

Data availability

No data associated with this article.

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Version 1

Reviewer Report 14 May 2026

https://doi.org/10.21956/openreseurope.23661.r72450

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✗ **Ben A. Woodcock**
UK Centre for Ecology & Hydrology, Wallingford, England, UK

Overall I found this interesting (and defiantly timely work)- but I felt was more of a summary of some reported effects on honeybees + analytical approaches. I have made suggestions below about how this needs to be expanded to actually define known toxicity endpoints for at least major pharmaceutical contaminants relative to reported environmental concentrations (ideally showing how this regionally varies). I think just listing some studies that show some effects is too limited and does not significantly expand on current knowledge (even if you may ultimately - as I suspect - show that defined toxicity endpoints are largely absent, this sets you up for defining future research needs). This is a paper with value - just needs some more specifics on the detail front for me.

For a review this also quite selective - its not really following a formalized structure as in PRISMA or something like that. I feel that this is probably overkill here, but even so i am not getting a feel for the depth of research that you did for this review to support conclusions and so at times it feels a little bit arbitrary what statements are made and how that impacts on your conclusions. I would consider tightening this aspect of the paper up.

Abstract
- Suggest ' For example, honeybees can be exposed to pharmaceutical residues present in contaminated soil, water, nectar, and pollen, which may result in sublethal or acute toxicity effects on reproduction, immune function, and behavior endpoints'.
- line ' The persistence of these residues in the environment exacerbates antimicrobial resistance, which poses a global threat.' Whats that got to do with bees? Seems out of context of an abstract unless you state why this is relevant.
- suggest ' We also review different methods of extraction and detection of pharmaceutical residues from honey....'

Keywords
- this seems fine

Introduction
- I can see this is linked more with veterinary pharmaceuticals contamination - but see Nightingale 2026 https://doi.org/10.1021/acs.jafc.5c10414 for honey pharmaceutical residues from human origin resulting from sewage sludge spreading on agricultural land.
- line ' Small molecules are not biodegradable and may persist in different environmental compartments after excretion' needs a reference.
- Line 'The cumulative exposure to residual levels of pharmaceutical medicines can affect the metabolism of both humans and animals, and the continuous discharges of antibiotics into the environment can promote the development of antimicrobial-resistant microorganisms, which is of global level concern.' needs a reference (just to say this would have been easier with line number - but not your fault as i think this is just their formatting.)
- for the AMR stuff i think you need to give this context for its relevance to bees? Are you saying this can impact them - if not why mention it. Is the argument that bees a re a bio monitor for AMR - which is fine, but make that clear.
- Line 'In the “One Health” approach, and considering the effects that this situation may have in all ecosystems, the persistent contamination of the environment can be one of the main factors causing the decline of pollinators' needs a citation. same point for ling 'Honeybees are more prone to environmental contamination because of their natural behavior of gathering nectar and pollen from a diverse range of flowering and nonflowering plants.'
GENERAL POINT - you have a tendency to make statements without backing up with a reference, your not saying anything crazy, but it needs to be supported. Check throughout for this issue.

Sources and pathways of contamination
- See nightingale reference given above - relevant for this section as its a potentially large contamination route linked to the use of sewage sludge as a soil conditioner. You consider sewage water for irrigation, but not this contamination route.
- for this section I wonder if a schematic of exposure routes would be useful- see Kiljanek, T., et al , 2016. Pesticide Poisoning of Honeybees: A Review of Symptoms, Incident Classification, and Causes of Poisoning. Journal of Apicultural Science 60, 24 - 25. which has a good example. Might help to sell the paper.

Risks posed by veterinary pharmaceuticals to bees and other pollinators
- So I think one thing that really interested me was your reference to defining acute toxicity and also sublethal effect. Specifically when you said this I was expecting details of LD50 or LDD50 equivalents for pharmaceuticals. If all you are doing is a review of 'effects found' i don't think that is the same thing, and you need to reconsider the wording in the abstract. Especially when you say acute and risk the suggestion is that you will able to infer that residues exceeding a level of exposure relative to a toxicity endpoint (like LD50) will cause mortality. You don't seem to be doing this - presumably due to data paucity. Even so this needs to be more transparent in terms of the scope of this work.
- In relation to this comment I think It would be useful to capture reported concentrations (minim levels) for different media (bees, honey, pollen, nectar) and relate these to reported to acute toxicity effects (and maybe in a separate table chronic or sublethal effects). You could do this for particularly widely reported pharmaceuticals, or high use pharmaceuticals like antihelminthic drugs for example. You could then relate this to reported field level concentrations in honey / pollen/ etc. I think this is crucial really to show scope of evidence.
- I think reporting (if you can find it) regional variation in pharmaceutical exposure for the different mediums - either by country, or larger region.

-building on this I think you may need a section then saying what the current data limitations are (e.g. there are limited LD50 or LDD%0 data for safe exposure to honeybees) and what the implications may be for this. Use this as a basis for research priorities

Analytical methodologies for determination of pharmaceutical residues in honey
- see comment above, hopefully other reviewers will have expertise in this to comment on.

Conclusions
- Again you talk about acute effects, but never really define these or give details of where these sit in the review.
- the bit on synergisms linked to AMR comes out the blue. This needs developing. I like the idea of talking about synergisms, and how pharmaceuticals may interact with other known stressors (e.g. altering gut microbiota impacting on disease) or more direct synergisms linked to detoxification pathways of actives. Arguably a section on this would be valuable. Generally this i underdeveloped here. Again no references.
- See earlier points about the toxicity endpoint side of this - how more research may be needed to define what environmental concertation's mean in terms of mortality or sublethal effects for key compounds in a consistent manner (as you get for pesticides, and also in terms of their availability for pesticides).

Is the topic of the review discussed comprehensively in the context of the current literature?
No

Are all factual statements correct and adequately supported by citations?
No

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 22 April 2026

https://doi.org/10.21956/openreseurope.23661.r72449

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Jelena ĆIRIĆ
Institute of Meat Hygiene and Technology, Belgrade, Serbia

This review paper addresses a highly relevant and important topic comprehensively and systematically, linking the intensification of livestock and agricultural production with increasing environmental risks.

A particular strength of the manuscript lies in its clear presentation of the pathways through which pharmaceutical and agrochemical residues contaminate the environment, as well as its focus on their impact on pollinator health, an issue of great contemporary significance.

The authors successfully integrate data on both acute and sublethal effects on bees, including impacts on reproduction, immune function, and behavior, while adequately considering the broader problem of antimicrobial resistance.

An additional merit of the paper is its overview of modern analytical methods for detecting residues in honey, which enhances the practical and scientific relevance of the manuscript. The conclusions are well founded, balanced, and consistent with the current state of the literature.

Overall, the paper represents a valuable contribution to the fields of ecotoxicology, food safety, and pollinator protection. I believe that the manuscript is suitable for indexing in a journal. Certainly, the results are important to scientific literature.

My recommendation is to index the paper.
Sincerely,

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental Science; Ecotoxicology; Veterinary Pharmacology; Food Safety; Pollinator Health; Agricultural Sustainability.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 14 April 2026

https://doi.org/10.21956/openreseurope.23661.r71948

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Dani Sukkar
Université de Lorraine, Yutz, France

The authors have presented a review on the subject of the pharmaceutical pollutants and bee health. Though the topic is interesting there are several major concerns.

The introduction focuses mainly on pharmaceutical products, their importance and their use. In comparison, the mention of bee health appears secondary and under-explained.

Sources and pathways of contamination:
The authors categorize the direct and indirect routes of exposure, however, they mention manure as the first route but then speak about indirect routes which could be confusing for readers.
In the section that states “Certain insecticides can cause…. Therefore, the use of antibiotics is often prohibited’?
This statement is either misplaced, or the authors mistook imidacloprid for an antibiotic.

Risks posed by veterinary pharmaceuticals to bees and other pollinators:
Species' Latin names should always be italicized.
In the second paragraph concerning varroosis the authors state that “Amitraz and its metabolites were found to be potent neurotoxicants, making amitraz more cytotoxic than its metabolites”. On what basis did the authors come to this conclusion? In fact, even if amitraz itself is neurotoxic, its main toxicity lies in its metabolites and that is one of the main reasons that its singular effect appeared more in mites than in bees which do not metabolize amitraz as efficiently.

Table 1:
The table is disorganized.
The authors should categorize the contaminants (pesticides, antibiotics, etc..). In addition, a single study is not sufficient to have a comprehensive idea on the routes of exposure or effects on bees. The detected concentrations could be relative to certain conditions or countries. The use of pesticides for example varies widely between countries and could affect the detection. there are many studies for each contaminant that show effects on various health parameters but the authors only chose a single reference for each. The authors should use several studies on those contaminants to find the ranges and totality of the observed effect and know the limitations of the observations.

What is the point of Table 2 stating the methodologies of detection? This would be better placed in a manuscript concerning standard methods than a review about the effects on bee health.

Overall,

The manuscript lacks structure as the ideas are not well organized and moving forward the authors change focus from effects of contaminants to analytical methodologies which are not relevant to the main topic.
The synergy of contaminant co-exposures is mentioned but not at all demonstrated int he review as well. I believe that is a major point of concern on the topic.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Partly

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Ecotoxicology, pesticides, bee health, invertebrate immunology, pollinators, insects

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.