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Review

The Future of Azoles in Agriculture—Balancing Effectiveness and Toxicity

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Abstract

Azole compounds are extensively utilized in plant protection products for managing pests and diseases in both agriculture and horticulture. Moreover, azoles are the most extensively used class of fungicides worldwide. In addition to being effective against human pathogenic fungi, they are used in the food and agricultural industries to prevent and control fungal infections in crops. Unfortunately, the extensive use of azoles and subsequent overexposure have led to undesirable effects on ecosystems and non-target aquatic and terrestrial organisms. In the last decade alone, the European Union (EU) has prohibited numerous pesticides, many of which are based on azoles. Numerous azoles, especially triazoles, pyrazoles, imidazoles, and oxazoles, are still approved as active ingredients in plant protection products in the EU due to their excellent activity and minimal environmental and health impacts. However, for some, the expiry date is as close as March 2026. A computational approach for estimating their effectiveness against harmful and non-target organisms in soil, as well as detailed research into the molecular mechanism of action, is used for further evaluation of the compounds. This review provides an overview of azole pesticides and a summary of recent knowledge addressing their toxicity, future prospects, methods, and strategies to overcome their limitations.

Keywords: azoles; pesticides; toxicity; aquatic organisms; terrestrial organisms; advancements

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1. Introduction

Azoles are a broad group of heterocyclic compounds characterized by a five-membered ring containing at least one nitrogen atom, while other heteroatoms could be sulfur or oxygen atoms, or NH as well. The ring system also contains two double bonds. While triazoles are the most common compounds in this group, it also includes pyrazoles, imidazoles, tetrazoles, thiadiazoles, and oxadiazoles (Figure 1). Not all of these parent motifs are found in naturally occurring compounds, but they are ubiquitous because of their extensive use. Mainly known as potent antifungal agents, azoles are used in plant protection products to control fungal diseases, as drugs to treat fungal infections in humans and animals, as biocidal agents in wood preservatives, intermediates and dyes in industrial applications, and in cosmetic products [1]. Although incomplete, the data collected from multiple European Union and European Economic Area (EU/EEA)

Member States indicated that, in total, approximately 120,000 tonnes of azoles were reported to have been sold on the EU/EEA market between 2010 and 2021 for purposes other than human medicine. The majority of those are used as plant protection products (PPPs) [2].

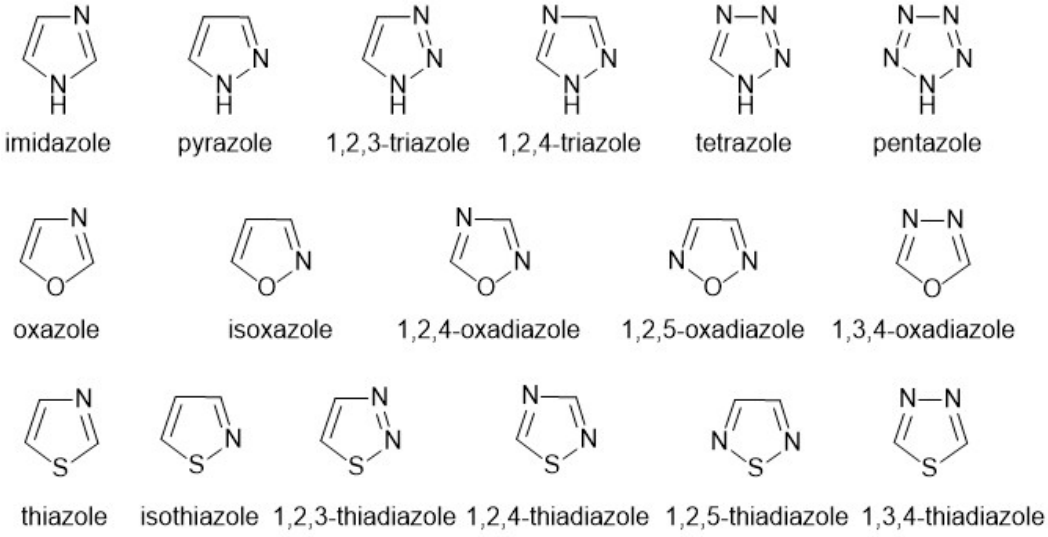

Figure 1. Common heterocycles belonging to the azole compound class.

Azoles are widely used in European Union (EU) agriculture, with their usage steadily rising from the mid-1970s. Currently, it is estimated that just under half of the EU's cereal and grapevine acreage is treated with azole fungicides each year [3]. Azoles act by inhibiting CYP51 (lanosterol 14α-demethylase), a crucial enzyme in the sterol biosynthesis pathway of fungi. However, this inhibitory effect is not limited to fungi and has been noted in various mammalian cytochrome P450-dependent processes, which may lead to potential interactions with the metabolism of other drugs [4–7].

A multitude of issues associated with ecotoxicity and human toxicity have resulted from the improper application of pesticides, incorrect selection, and overuse on agricultural areas. Azoles are also included in this drawback, as their excessive use had a substantial effect on non-target organisms, particularly aquatic organisms [8,9]. Human exposure to agricultural azoles can occur through the ingestion of contaminated foods, drinking water, and animal products due to their bioaccumulation and persistence in the environment. Subsequently, azole resistance has also become more common in the target pest organisms, and there is increasing evidence of multiple resistance mechanisms in triazole-resistant strains and cross-resistance to medical azoles [10–12].

Azoles and problems accompanying their overuse are known and reviewed subjects, with significantly more articles covering the effect on aquatic environments. This review provides an overview of agricultural azoles, with a particular focus on the current knowledge regarding their overall environmental toxicity and associated regulations. The aim was to encompass all pesticides bearing an azole ring, not just the triazoles as the most commonly reviewed class, and to synthesize the most current research regarding both aquatic and terrestrial toxicity. A special emphasis was put on computational methods, as they are an almost indispensable tool today. An extensive literature search was conducted using the PubMed, ScienceDirect, and Scopus databases with keywords "azoles", "conazoles", "pesticides", and "toxicity". In addressing future prospects, methods, and strategies to mitigate the drawbacks of azoles, new keyword combinations were incorporated into the literature search, including "computational methods", "QSAR", "hybrid" (compounds), "ionic liquids", and "nano" (pesticides/formulations).

As this topic is of great interest, more than a thousand articles remained after excluding doubles as a first step. Articles were then selected based on the following inclusion criteria: they addressed only agricultural azoles and their effects on the environment and non-target animal organisms, and they were original, peer-reviewed articles with a publication date within the last 10 years. Finally, after full-text examination, articles without a detailed explanation of azole effects and those with similar information but with an earlier publication date were excluded, leaving 106 articles. A few articles from earlier periods containing significant information were also included, as well as several reviews and references from the extracted articles.

2. Azoles in Agriculture

Pesticides, whether natural or synthetic, are compounds applied to prevent, control, and eliminate insects, weeds, and other pests that hinder plant growth. They are classified according to their mechanism of action, chemical structure, hazards, and application [13]. Most azoles fall under the category of fungicides and are further characterized by their mechanism of action as demethylase inhibitors [4]. Oftentimes, the term conazole fungicides is used, but it only refers to imidazole and triazole fungicides [14]. Azoles, especially triazoles, are also important agents for the regulation of plant growth, where they play a vital role in the regulation of physiological and biochemical processes that lead to better crop yields. Their application modulates processes that help mitigate the negative effect of abiotic stress, including drought, salinity, and cold and heat stress [15].

Imazalil, one of the first azole pesticides, was registered in the late 1970s for use as a seed treatment, particularly for cereals and seed potatoes. During the late 1970s and early 1980s, several other azoles, such as triadimefon and propiconazole, were introduced to the market for controlling leaf diseases in cereal and fruit production. In the early 1980s, prochloraz quickly gained popularity in Europe, addressing the growing resistance to benzimidazole at that time. Over the years, new azoles emerged, including tebuconazole, difenoconazole, epoxiconazole, bromuconazole, and prothioconazole [16].

2.1. Azole Fungicides

Azoles are routinely used to treat diseases caused by phytopathogenic fungi, including eyespot disease in barley and wheat, powdery mildew on grapes, leaf blotch in wheat, leaf spot in sugar beet, apple scab, and mold on citrus fruit. Azoles disrupt fungal cell membranes by altering sterol composition. They achieve this by inhibiting the sterol 14α-demethylase, CYP51, from the cytochrome P450 enzyme family, crucial in the ergosterol biosynthetic pathway and necessary for proper fungal membrane fluidity and permeability [17]. Fungal membranes depend on ergosterol for fluidity and stability; its absence compromises the membrane's structural integrity. The inhibition of CYP51 also causes the fungal cell membrane to accumulate 14α-methylated sterols, resulting in the disruption of the normal membrane composition. This stops membrane transport mechanisms from working properly, which prevents the pathogen from further damaging the host [18].

Unfortunately, some fungal pathogens developed resistance to azole antifungals' primary mechanism of action [19]. The review by Rosam et al. [20] explores the diverse resistance mechanisms, particularly focusing on amino acid substitutions in sterol 14α-demethylase, that impact the binding of azole inhibitors. It further discusses correlation with azole resistance phenotypes in various human, animal, and plant pathogenic fungi, emphasizing the urgency for new antifungal strategies [20–22].

Azole fungicides have been extensively utilized either as standalone products or in combination with other active ingredients to expand their range of control or reduce the likelihood of resistance development. Although they have been reported to offer nu-

merous advantages across various crops and industries, this category of fungicides has been under surveillance in recent years due to their widespread and often excessive use, as well as their potential environmental and health impacts. Table 1 shows an overview of azole fungicides registered in the Pesticide Properties Database [23] that are still in use in the EU or have been withdrawn in the recent years.

Table 1. Azole-based fungicides listed in the Pesticide Properties DataBase [23]. (Designated colors: EU-approved, green; approval expiration date within the next two years, light green; pending approval, blue; not approved, red; never authorized, gray; novel and under development, orange). n.a. (not applicable)

Name	Azole Type	EC Regulation 1107/2009 Status	Expiration Date
Amisulbrom	triazole	approved	15 September 2026
Bixafen	pyrazole	approved	31 October 2027
Bromuconazole	triazole	approved	30 April 2027
Cyazofamid	imidazole	approved	31 July 2036
Difenoconazole	triazole	approved	15 March 2026
Fluxapyroxad	pyrazole	approved	31 October 2027
Hymexazol	oxazole	approved	31 August 2026
Imazalil	Imidazole	approved	31 May 2027
Mefentrifluconazole	triazole	approved	20 March 2029
Metconazole	triazole	approved	31 August 2031
Oxathiapiprolin	oxazole, pyrazole, thiazole	approved	3 March 2027
Paclobutrazole	triazole	approved	31 August 2026
Penconazole	triazole	approved	15 October 2026
Prothioconazole	triazole	approved	31 March 2027
Pyraclostrobin	pyrazole	approved	15 September 2026
Sedaxane	pyrazole	approved	31 October 2027
Tebuconazole	triazole	approved	15 August 2026
Tetraconazole	triazole	approved	31 March 2027
Thiabendazole	benzimidazole, thiazole	approved	31 March 2032
Triticonazole	triazole	approved	31 January 2027
Fluindapyr	pyrazole	pending approval	n.a.
Inpyrfluxam	pyrazole	pending approval	n.a.
Metyltetraprole	pyrazole, tetrazole	pending approval	n.a.
Isoflucypram	pyrazole	pending approval	n.a.
Pydiflumetofen	pyrazole	pending approval	n.a.
Azaconazole	triazole	not approved	n.a.
Bitertanol	triazole	not approved	29 August 2013
Cyproconazole	triazole	not approved	31 May 2021
Diniconazole	triazole	not approved	n.a.
Epoxiconazole	triazole	not approved	30 April 2020
Etridiazole	thiadiazole	not approved	31 May 2021
Famoxadone	oxazole	not approved	9 September 2021
Fenbuconazole	triazole	not approved	30 April 2021
Fenpyrazamine	pyrazolium	not approved	15 January 2025
Fluquinconazole	triazole	not approved	31 December 2021
Flusilazole	triazole	not approved	30 June 2008
Flutriafol	triazole	not approved	31 May 2021
Furametpyr	pyrazole	not approved	n.a.
Hexaconazole	triazole	not approved	n.a.
Ipconazole	triazole	not approved	31 May 2023

Myclobutanil	triazole	not approved	31 May 2021
Octhilinone	thiazole	not approved	n.a.
Oxadixyl	oxazole	not approved	n.a.
Penthiopyrad	pyrazole	not approved	31 October 2025
Prochloraz	Imidazole	not approved	31 December 2021
Propiconazole	triazole	not approved	19 December 2018
Pyraoxystrobin	pyrazole	not approved	n.a.
Pyrisoxazole	isoxazole	not approved	n.a.
Thifluzamide	thiazole	not approved	n.a.
Triadimenol	triazole	not approved	31 August 2019
Triazoxide	imidazole	not approved	30 September 2021
Tricyclazole	triazolobenzothiazole	not approved	n.a.
Triflumizole	Imidazole	not approved	30 June 2020
Vinclozolin	oxazole	not approved	authorizations withdrawn 01/01/07
Isopyrazam	pyrazole	not approved	8 June 2022
Penflufen	pyrazole	not approved	31 January 2024
Ethaboxam	thiazole	never notified and authorized in the EU	n.a.
Imibenconazole	triazole	never notified and authorized in the EU	n.a.
Isotianil	thiazole	never notified and authorized in the EU	n.a.
Oxpoconazole	Imidazole	never notified and authorized in the EU	n.a.
Simeconazole	triazole	never notified and authorized in the EU	n.a.
Fenopyramid	pyrazole	novel, not approved	n.a.
Flubeneteram	pyrazole	novel, not approved	n.a.

2.2. Azole Herbicides and Plant Growth Regulators

Apart from their fungicidal effect, azoles also exhibit growth-regulating properties. Certain azoles disrupt the production of gibberellins, plant hormones responsible for controlling stem growth, seed germination, dormancy, flowering, the development of flowers, and the aging of leaves and fruits. Paclobutrazol was introduced in 2006 for use as a plant growth regulator in ornamentals, mangoes, cotton, corn, chili, and grapes [1].

There are several herbicides that are not classified as azoles, but contain azole moieties. Halosulfuron is a post-emergence herbicide used to control annual broadleaved weeds that contains a pyrazole moiety, while amitrole is a triazole herbicide that is used to control a wide range of perennial grasses and broadleaved weeds [24]. Azole herbicides and plant growth regulators with their approval status within EU are listed in Table 2.

Table 2. Azole-based herbicides and plant growth regulators listed in the Pesticide Properties DataBase [23]. (Designated colors: EU-approved, green; approval expiration date within the next two years, light green; not approved, red; never authorized, gray; novel and under development, orange). n.a. (not applicable)

Name	Azole Type	EC Regulation 1107/2009 Status	Expiration Date
Carfentrazone-ethyl	triazolone	approved	31 July 2033
Halosulfuron	pyrazole	approved	15 November 2026
Isoxaben	isoxazole	approved	31 January 2027
Isoxaflutole	isoxazole	approved	31 July 2034
Metazachlor	pyrazole	approved	31 October 2026
Paclobutrazole	triazole	approved	31 August 2026
Pyraflufen-ethyl	pyrazole	approved	31 March 2031
Amitrole	triazole	not approved	30 June 2016
Azimsulfuron	pyrazole, tetrazole	not approved	31 December 2021

Difenzoquat	pyrazole	not approved	n.a.
Flupoxam	triazole	not approved	n.a.
Methiozolin	isoxazole	not approved	n.a.
Pyraclonil	pyrazole	not approved	n.a.
Pyrazosulfuron-ethyl	pyrazole	not approved	n.a.
Thidiazuron	thiadiazole	not approved	n.a.
Topramezone	isoxazole, pyrazole	not approved	Provisional authorisations ended on 30 April 2015
Amicarbazone	triazole	never notified and authorized in the EU	n.a.
Cafenstrole	triazole	never notified and authorized in the EU	n.a.
Fentrazamide	tetrazolinone	never notified and authorized in the EU	n.a.
Pyroxasulfone	oxazole, pyrazole	never notified and authorized in the EU	n.a.
Pyroxasulfone	pyrazole	never notified and authorized in the EU	n.a.
Uniconazole	triazole	never notified and authorized in the EU	n.a.
Fenpyrazone	pyrazole	novel, not approved	n.a.
Metazosulfuron	pyrazole	novel, not approved	n.a.
Prochlorosulfone	pyrazole, oxadiazolone	novel, not approved	n.a.
Tripyrasulfone	pyrazole	novel, not approved	n.a.
Cyclopyranil	pyrazole	under development	n.a.

2.3. Azole Insecticides

There are several insecticides containing azole moieties listed in the Pesticide Properties Database (Table 3). Tebufenpyrad is a pyrazole acaricide and insecticide widely used in greenhouses. It acts as a mitochondrial complex I electron transport inhibitor in mites. Thiamethoxam is a broad-spectrum, systemic insecticide containing a thiazole moiety. Aside from being used to control a range of common pest, it is also a biostimulant because it was found to enhance root mass and overall plant strength [24].

Table 3. Azole-based insecticides listed in the Pesticide Properties DataBase [23]. (Designated colors: EU-approved, green; approval expiration date within the next two years, light green; pending approval, blue; not approved, red; never authorized, gray; novel and under development, orange). n.a. (not applicable)

Name	Azole Type	EC Regulation 1107/2009 Status	Expiration Date
Chlorantraniliprole	pyrazole	approved	31 May 2027
Cyantraniliprole	pyrazole	approved	14 September 2026
Etoxazole	oxazoline	approved	31 January 2028
Fenpyroximate	pyrazole	approved	15 June 2026
Tebufenpyrad	pyrazole	approved	31 January 2027
Dimpropyridaz (Trinconazole)	pyrazole	pending approval	n.a.
Azocyclotin	triazole	not approved	n.a.
Clothianidin	thiazole	not approved	31 January 2019
Cyclaniliprole	pyrazole	not approved	application withdrawn on 3 October 2016
Fipronil	pyrazole	not approved	n.a.
Flufiprole	pyrazole	not approved	n.a.
Isazofos	triazole	not approved	n.a.
Pyflubumide	pyrazole	not approved	n.a.
Tetraniliprole	pyrazole, tetrazole	not approved	n.a.
Thiamethoxam	thiazole	not approved	30 April 2019
Tiorantraniliprole	pyrazole	not approved	n.a.

Triazamate	carbamoyltriazole	not approved	n.a.
Cyenopyrafen	pyrazole	never notified and authorized in the EU	n.a.
Ethiprole	pyrazole	never notified and authorized in the EU	n.a.
Tolfenpyrad	pyrazole	never notified and authorized in the EU	n.a.
Fluchlordiniliprole	pyrazole	novel, not approved	n.a.
Pioxaniliprole	pyrazole	novel, not approved	n.a.
Pyrafluprole	pyrazole	under development	n.a.

3. Environmental Toxicity of Azole Pesticides

Excessive and uncontrolled pesticide use has led to significant environmental, agricultural, and aquatic pollution. Acute and chronic health effects associated with agricultural pesticides represent major public health concerns. Due to the non-specific mode of action of pesticides, non-target organisms, including humans, are frequently affected [25]. After the initial application, pesticides may dissolve, volatilize, permeate, or undergo degradation through photodegradation and hydrolysis. They can also be taken up, absorbed, and metabolized by crops, animals, and microorganisms. Therefore, many transformation products are generated during pesticide degradation and metabolism, as shown with tebuconazole [26]. Pesticide residues present in terrestrial environments can be transported to aquatic systems via runoff, precipitation, leaching, and wastewater, thereby posing risks to aquatic organisms. Notably, certain pesticide transformation products exhibit a greater toxicity, mobility, and persistence than their parent compounds, resulting in increased health hazards [27]. The usual mechanisms by which pesticides cause harm are often due to accumulation over time and involve processes like bioconcentration. Many sources describe the severe adverse effects of pesticides on natural systems, plants, aquatic animals (including neurotoxicity and histological changes), and human health, citing issues like genetic damage, cancer, and respiratory disorders such as asthma and allergies [28–30].

Triazole pesticides tend to have an asymmetrically substituted C-atom and thus consist of enantiomers, of which often the more active one is used. Their non-target and environmental toxicity behavior should be observed in relation to enantioselectivity, as has been shown for propiconazole [31], tebuconazole [32,33], myclobutanil [32], hexaconazole [34], flutriafol [35], and flufiprole [36].

The majority of azole-based pesticides are fungicide agents which, according to the Fungicide Resistance Action Committee (FRAC) belong to the Mode of Action group G—Sterol Biosynthesis in Membranes, more specifically, Group 3—Demethylation inhibitors. They are considered broad-spectrum fungicides [37]. Other azole pesticides are scattered throughout different fungicide, herbicide, and insecticide groups by the corresponding committees [37–39]. To better understand the toxicity of azole pesticides, a comparison between several azoles and other commonly used non-azole pesticides has been shown in Table 4. The majority of approved azole pesticides pose a moderate risk to the environment according to the Pesticide Properties DataBase categorization [23].

Table 4. Comparison of several toxicological endpoints (bioconcentration factor BCF and toxicity to non-target animal organisms) for azole pesticides (bold) and commonly used non-azole pesticides. Data were taken from the Pesticide Properties DataBase (values considered highly toxic or of high concern are written in italic) [23].

Substance	BCF	Fish Acute LC_{50}	Rat Acute Oral LD_{50}	Earthworm Acute LC_{50}	Honeybee Contact Acute LD_{50}
Imazalil	56.3	1.48	227	271	39
Tebuconazole	78	4.4	1700	1381	>200
Hymexazol	0.49	>100	1600	281.9	>100

Metconazole	*105.1*	2.1	595	>500	>100
Mancozeb	3.2	*0.074*	>5000	>299.1	>85.3
Azoxystrobin	* low risk	0.47	>5000	283	>200
Metalaxyl	7	0.96	>669	>1000	200
Copper(II) hydroxide	* low risk	*0.017*	489	>677	>44.46
Isoxaflutole	11	>1.7	>5000	>500	>100
Metazachlor	* low risk	8.5	3480	500	>100
Paclobutrazole	44	23.6	1336	>500	>40
Triclopyr	0.77	117	630	>521	>100
Fluroxypyr	62.1	14.3	>2000	>64.8	>180
Glyphosate	0.5	>100	>2000	>5600	>200
Chloranthraniliprole	15	>1.09	>5000	>1000	>4.0
Etoxazole	*2900*	2.8	>5000	>1000	>200
Tebufenpyrad	*953*	*0.023*	>202	20.5	6.7
Clothianidin	* low risk	>104.2	>500	13.21	*0.044*
Lambda-cyhalothrin	*4982*	*0.00021*	*56*	>500	*0.038*
Deltamethrin	*1400*	*0.00015*	*87*	>645	*0.0015*
Acetamiprid	* low risk	>100	146	*9*	8.09
Malathion	*103*	*0.018*	1778	306	*0.16*

* Only computational estimation is given. BCF (l kg^{-1}); freshwater fish acute 96 h LC_{50} (mg l^{-1}); rat acute oral LD_{50} (mg kg^{-1}); earthworm acute 14-day LC_{50} (mg kg^{-1} dw soil); bee contact acute LD_{50} (worst case from 24, 48, and 72 h values—μg bee^{-1}).

3.1. EU Regulatory Aspects Concerning Azole Pesticides

In the European Union, the Pesticides Unit of the European Food Safety Authority (EFSA) is tasked with conducting peer reviews of risk assessments for active substances in plant protection products, working alongside EU Member States. The EFSA Panel on Plant Protection Products and their Residues offers guidance in areas such as toxicology, ecotoxicology, and the fate and behavior of these substances [40]. Within the EU, Regulation (EC) No 1107/2009 governs the market placement of plant protection products [41]. This regulation sets the criteria these products must meet for EU approval, including considerations of toxicological effects, environmental impact, persistence, and bioaccumulation, thereby safeguarding human and animal health as well as the environment. While this regulation provides a framework for pesticide approval and use, it is complemented by Directive 2009/128/EC, which establishes a framework for the sustainable use of pesticides [42]. Approximately 70 active substances have been banned in Europe under Regulation (EC) 1107/2009, accounting for 7% of the substances evaluated through the European Union's review program, which operated from 1993 to 2009. Currently, 65 approved substances are identified as candidates for substitution. Although these substances meet the safety requirements for humans and the environment as defined by Regulation (EC) 1107/2009, they may be replaced due to inherent hazardous properties. Most substances in this category are characterized by environmental persistence, bioaccumulation, or toxicity to humans. Out of 37 azoles' active substances in this database, only 12 are still approved, although for some the expiry date is as close as March 2026 [41,43].

Unlike Regulation (EC) No 1107/2009, which applies directly to all Member States, Directive 2009/128/EC requires national implementation, allowing countries to determine how best to achieve its objectives. EU-wide, the monitoring of pesticide residues in soil is not required, but such monitoring is mandatory for water under the Water Framework Directive 2000/60/EC [44]. The first EU Watch List in 2015 was compiled to monitor substances (pharmaceuticals, hormones, and pesticides) in the EU water basins. Those

compounds showing a relevant risk were listed as priority pollutants. The third Watch List (Decision 2020/1161) included azole pesticides, which have been maintained in the following Watch List (Decision 2022/1307). The latest Watch list (Decision 2025/439) ceased monitoring the pesticides imazalil, ipconazole, metconazole, penconazole, prochloraz, tebuconazole, and tetraconazole. However, s new group of azole fungicides (bromuconazole, climbazole, cyazofamid, difenoconazole, epoxiconazole, itraconazole, ketoconazole, mefentrifluconazole, propiconazole, triticonazole) and the insecticide etoxazole were included, noting that they could have additive effects, therefore should be analyzed together [45]. A qualitative survey from Portugal revealed thirty-nine systemic active antifungals used in agriculture, of which only 51% were "approved for use" concerning placing plant protection products on the market in the EU. Five identified compounds are on the EU Watch List: azoxystrobin, famoxadone, imazalil, tebuconazole, and tetraconazole. For azoles, the occurrence in surface water ranged from a residual (10%) to a moderate detection rate for two compounds (31%—propiconazole and tebuconazole) [46].

The Land Use/Cover Area frame Survey (LUCAS) provides data on soil pesticide concentrations across the European Union. LUCAS pesticide monitoring began with a pilot study in 2015, followed by a comprehensive sampling campaign in 2018 that assessed 118 substances at 3473 predominantly agricultural sites. In 2018, 14% of the monitored sites were at risk of adverse effects on soil organisms from pesticide mixtures. One of the risk drivers was azole fungicide epoxiconazole, still approved for use during the survey period [47]. In over 5000 soil samples from across Europe, collected between 2015 and 2022, pesticide contamination trends were studied. It revealed widespread soil pollution from both currently approved pesticides (Boscalid and epoxiconazole) and banned substances (p,p'-DDT and Atrazine) [48]. This shows that both past and current farming practices continue to affect the environment.

The zero-pollution action plan is a key element of the EU's efforts to cut pollution, safeguard the environment, and improve the health and well-being of its citizens as part of the European Green Deal. It envisions that, by 2050, the EU will have reduced pollution to a level that no longer poses a threat to human health and natural ecosystems. To achieve this, several targets were set, among which are "Target 4b: Reduce the use and risk of chemical pesticides by 50%" and "Target 4c: Reduce the use of the more hazardous chemical pesticides by 50%". Compared to the set baseline (2015–2017 data), there is a 46% reduction progress for target 4b and 25% for target 4c [49].

3.2. Toxicity to Aquatic Organisms

Azole pesticides' uncontrolled use determines their accumulation in aquatic habitats, endangering many non-target organisms [50]. Imazalil and triadimefon, for instance, were shown to negatively affect the early development of the marine ascidian *Phallusia mammillata* [51]. The review by Hou et al. covers the occurrence, environmental behavior, toxicity, and ecological risk of triadimefon, noting its ubiquitous presence in surface waters, often with limited concentration data. Triadimefon degradation and metabolism are stereoselective, which complicates risk assessment [52]. The effect of mixtures of different azoles and other pesticides should also be taken into consideration [53].

Assessments conducted by the European Topic Centre on Inland, Coastal and Marine Water have confirmed the presence of azole fungicides, specifically tebuconazole, propiconazole, and epoxiconazole, in various lakes, rivers, and coastal seas across Europe [54]. A recent study presented an extensive survey of pesticide and transformation product residues in 75 arable soil samples from the Czech Republic, representing Central European agricultural land. The authors analyzed 53 parent pesticides and 15 transformation products, finding multiple pesticide residues in nearly all soils, often exceeding

established thresholds. The study highlighted conazole fungicides as one of the most frequent and abundant contaminants, with their occurrence strongly correlated to their persistence and hydrophobicity rather than simply the amount applied [55].

Lee et al. [56] used zebrafish as a model to study the effects of ipconazole. The study found that, even at low concentrations, ipconazole exposure disrupts mitochondrial homeostasis, increases oxidative stress, and specifically impairs the development of GABAergic inhibitory neurons in the central nervous system. These molecular changes resulted in reduced locomotor activity and suggested that ipconazole may cause an uncoordinated neural network due to neurotransmission imbalance [56]. The adverse effects of three common triazole fungicides (cyproconazole, paclobutrazol, and triadimenol) on developing zebrafish embryos and larvae were investigated by Thrikawala et al. [57]. It was found that exposure to these triazoles causes skeletal malformations in bone and cartilage structures. Mechanistically, these triazoles repress gene expression for osteogenesis and chondrogenesis (bone and cartilage formation), while simultaneously inducing adipogenesis (fat cell formation) both in vitro and in vivo [57]. A chiral bioaccumulation behavior investigation of tebuconazole in zebrafish showed the preferential accumulation and slower degradation of (−)-*R*-tebuconazole [58].

Multiple assays on non-target organisms, including aquatic plants (*Spirodela polyrhiza*), freshwater invertebrates (*Daphnia magna* and *Thamnocephalus platyurus*), marine fish (*Chelon auratus*), and various bacteria and yeast strains, were conducted to determine the ecotoxicological risk of tebuconazole. This fungicide was highly toxic across most tested non-target species, with the marine life more sensitive than the freshwater species. Furthermore, tebuconazole showed a selective toxic effect on microorganisms, strongly inhibiting Gram-positive bacteria and yeast [59,60]. Boros et al. [61] evaluated the aquatic toxicity of several triazole fungicides like metconazole and tetraconazole, performing growth inhibition assays on the freshwater plant *Lemna minor*. The findings categorize the tested fungicides by their toxicity levels and suggest that they likely affect the plants at a molecular level by disrupting photosynthesis and cellular detoxification processes [61].

The study by Saha et al. [62] investigated the toxicological impact of difenoconazole on the freshwater worm *Tubifex tubifex*, a non-target aquatic organism. Acute toxicity and the effects of sublethal exposure on the worms' health were examined. Difenoconazole was found to reduce worm survival, disrupt normal behavior, and cause significant cellular stress by altering oxidative stress enzymes and inducing histological alterations, suggesting long-term population consequences for aquatic ecosystems [62].

Data on the most commonly observed aquatic toxicity endpoints for several above-mentioned azoles, as well as a few still approved in the EU, are given in Table 5. The thresholds given for each endpoint, as explained by the Pesticide Properties DataBase, are consistent with the regulatory thresholds used in both the UK and EU [23]. Overall, azoles have been categorized as moderately to highly toxic on aquatic organisms.

Table 5. Half maximal effective concentration (EC_{50}), half maximal lethal concentration (LC_{50}), and "No Observed Effect Concentration" (NOEC) of some azole pesticides for common aquatic ecotoxicological endpoints. Values categorized as highly toxic by the Pesticide Properties DataBase are written in italics (less than 0.1 for acute toxicity on invertebrates and fish; less than 0.01 for acute toxicity on algae and chronic toxicity) [23].

Azole	Invertebrates (*Daphnia magna*)		Fish (*Oncorhynchus mykiss*)		Algae (*Raphidocelis subcapitata*)
	Acute 48 h EC_{50} (mg l^{-1})	Chronic 21-Day NOEC (mg l^{-1})	Acute 96 h LC_{50} (mg l^{-1})	Chronic 21-Day NOEC (mg l^{-1})	Acute EC_{50} (Growth Rate, mg l^{-1})
Azimsulfuron	378	5.4	154	6.3	0.012

Cyproconazole	>22	0.023	19	0.65	0.66 [b]
Difenoconazole	0.77	*0.0056*	1.1	0.023	0.032 [c]
Epoxyconazole	>3.13	0.63	>0.92	0.01 *	>10.69
Etoxazole	*0.0071*	*0.0002*	2.8	0.015	>10
Imazalil	3.5	-	1.48	0.043	1.2
Ipconazole	1.7	0.13	>1.5	>0.76	>2.2
Isoxaben	>1.3	0.69	>1.1	0.87	25.9
Metazachlor	33	0.1	8.5	2.15	0.0318
Metconazole	4.2	0.16	2.1	1.14 *	2.2
Propiconazole	10.2	0.31	2.6 [a]	0.068	9
Pyraclostrobin	*0.016*	*0.004*	*0.0062*	*0.0045*	0.843
Tebuconazole	2.79	0.01	4.4	0.01	5.3 [c]
Tebufenpyrad	*0.046*	0.02	*0.023*	*0.00245*	>0.068
Thiabendazole	0.81	0.042	0.55	0.012	9
Triadimefon/Triadimenol	7.16/51	0.1/0.1	4.08/21.3	0.017/3.13	2.01/38

* Chronic 28-day NOEC; [a] *Leiostomus xanthurus*; [b] *Chlorella vulgaris*; [c] *Desmodesmus subspicatus*.

3.3. Toxicity to Terrestrial Organisms and Humans

The effects of pesticides in terrestrial ecosystems are important because soil organisms drive relevant processes for crop production, such as nutrient cycling, and the activities of individual species of microorganisms may be greatly affected [63]. The fungicide tetraconazole induces several specific structural and functional changes in soil bacterial communities, with the magnitude and type of impact often dependent on the soil's agricultural management history [64]. Observations regarding tebuconazole made within a lab-to-field study concluded that tebuconazole and its transformation product impact the soil bacterial community diversity and composition, potentially because of the close interactions between fungal and bacterial species in soil [65]. Fungicides imazalil, fenarimol, penconazole, and tebuconazole were found to inhibit the growth of the biocontrol agent *Bacillus velezensis* strain isolated from the tomato rhizosphere [66].

Modern testing for pesticide effects on terrestrial microorganisms falls behind aquatic toxicity testing. The potential of a pesticide's toxicity on the soil microbiota is usually dependent on its chemical structure, mechanism of action, and the target organisms, dose rates, and mode of application. Karpouzas et al. [67] summarized research on soil microbial indicators, specifically pointing to ammonia-oxidizing microorganisms and arbuscular mycorrhizal fungi as the most relevant bioindicator groups due to their critical roles in ecosystem services. However, in choosing the right bioindicators, they should meet several criteria: they must have a key ecological role and show a high sensitivity and a clear ecotoxicological response to pesticides compared to other species in the same group, their life cycle must be well understood, and standardized tests for determining their response must be available [67].

Azoles have been shown to affect specific soil enzymes. In studies concerning triticonazole and myclobutanil, experimental and computational methods were employed to analyze the impact on the activity of soil enzymes. These fungicides are chiral triazoles, and their stereoisomers showed distinct patterns of interaction with soil enzymes. The environmental toxicity of (*R*)-triticonazole is lower than that of (*S*)-triticonazole; however, both were able to fit into the catalytic sites of several dehydrogenases [68,69]. The enantiomers of myclobutanil were docked into the active sites of dehydrogenase, phosphatase, and protease, with somewhat higher interacting energies for the (*S*)-myclobutanil (less active against target organisms and toxic to non-target organisms) [70].

Invertebrates in soil are exposed to pesticides that reach the soil through spraying, seed coating, fumigation, leaching, or tillage practices. The question about the terrestrial toxicity of azoles goes back to the last decades of the past century, when the ecotoxicity of the fungicide imazalil, its sulfate salt, and a transformation product on earthworms was assessed. A 48 h contact test and 14-day artificial soil test following The Organization for Economic Co-operation and Development (OECD) guidelines were conducted, and it was determined that the lethal concentrations far exceeded the concentrations expected from normal use. Therefore, imazalil was marked as not harmful to earthworms in the soil. The low residue levels and bioconcentration factors in surviving worms suggested its low bioavailability due to the substance's strong sorption to soil [71]. However, in a more recent study, the effect of imazalil on earthworms (*Eisenia andrei*) was more closely examined. Acute contact, avoidance, and chronic soil tests to evaluate several endpoints, including biomass changes, reproduction, immune system cell effects, and oxidative stress, were performed. It was found that, while imazalil did not cause immediate death, the earthworms did not avoid contaminated soil, leading to a prolonged exposure and cumulative cellular damage, overall compromising earthworm health [72]. The mixtures of pesticides in soil may change and even amplify each other's toxicity. The results from single pesticide exposure studies may not reveal the real toxicological responses of earthworms [73]. Toxicological effects of commercial fungicides containing tebuconazole, prothioconazole, and cyproconazole on *Eisenia foetida* were investigated. The formulations composed of two active ingredients induced DNA alteration, indicating genotoxicity. Moreover, an imbalance of reactive oxygen species was observed, indicating a potential detrimental effect on the immune system [74].

A recent large-scale study on pesticide residue contamination in European wheat fields was conducted across nine European pedoclimatic zones. It analyzed over 600 compounds in 188 fields under both conventional and organic farming systems. It showed that pesticide residues are ubiquitous, with significantly higher concentrations and numbers of compounds in conventional fields, though organic fields were not pesticide-free due to the persistence of banned substances and environmental transfer. The study included an ecological risk assessment, identifying fungicides like epoxiconazole and boscalid as presenting the highest risks to soil organisms. Although epoxiconazole has not been approved for agricultural use since 2020, it is still detected in the environment due to its high persistence. The fungicide tebuconazole did not present a high risk level in any soil, but exhibited a low risk level in a significant number of fields [75].

When assessing the terrestrial and non-target toxicity of pesticides, one of the main concerns is their effect on insects, especially honeybees. Honeybees are exposed to insecticides, fungicides, and herbicides and have long been recognized as useful indicators of environmental pollution [76]. For instance, tebuconazole was found to have detrimental effects on honeybee brain health, specifically redox homeostasis and fatty acid profile. Acute sublethal exposure causes oxidative stress and metabolic alterations, potentially contributing to colony collapse disorder [77]. Iwasaki and Hogendoorn [78] focused on the impacts of non-insecticide pesticides on bees. They conducted a systematic literature review of studies on fungicides, herbicides, and adjuvant surfactants, summarizing their individual effects and their interactive effects when combined with other chemicals, including insecticides. While fungicides alone are often minimally impactful, combinations, particularly with insecticides, frequently result in harmful synergistic effects on bee health. Synergism in this concept can be described as two or more substances exhibiting a significantly larger effect together when compared to the sum of their individual effects [79]. Synergistic effects can also manifest between pesticide exposure and poor nutrition or some diseases, leading to reduced bee survival, decreased food consumption, and lower energy levels [80]. The negative effects of various pesticides in mixtures with azole

fungicides on pollinators is clearly of great concern [81–83], and a combination involving neonicotinoids or pyrethroids with azole fungicides seems to be the most problematic. Fenbuconazole was found to enhance the toxicity of neonicotinoid pesticides, specifically imidacloprid and acetamiprid. Pyrethroid and triazole/imidazole fungicide combinations have been shown to have consistent negative interactive outcomes [78]. These mixtures can exert sublethal effects that do not immediately impact survival rates. The reason lies in the ability of azoles to inhibit cytochrome P450 monooxygenase in honeybees and wild bees, disrupting their detoxification process. Cytochrome P450 enzymes in bees are crucial for detoxifying both natural phytochemicals and various insecticides; therefore, this combination can have detrimental effects [84]. This synergistic effect of azoles is evaluated in a study by Haas and Nauen [6], in which they developed a fluorescence-based high-throughput in vitro assay utilizing honeybee cytochrome P450 enzymes. This method allowed for the mechanistic prediction of synergistic toxicity when insecticides are combined with other common agricultural chemicals like azole fungicides. A strong correlation between the in vitro inhibition of these enzymes by fungicides and the enhanced acute toxicity was observed in live honeybees when exposed to the chemical mixtures [6]. When combined with the fungicide propiconazole, the insecticide chlorantraniliprole exhibited a significantly higher toxicity to both adult and immature bees, drastically reducing larval emergence and increasing adult toxicity by more than sevenfold [85].

The widespread presence of azole pesticides also affects wildlife and birds. Fritsch et al. [86] document the ubiquitous contamination of wild small mammals in French agricultural landscapes with complex mixtures of both banned and currently used pesticides. Serra et al. [87] focused on the in vitro effects of eight specific triazole fungicides on male chicken reproductive functions, demonstrating that most of these compounds impaired testicular steroidogenesis (testosterone and lactate production) and compromised sperm quality. This was further discussed in the study by Napierkowska et al. [88], through the specific reproductive toxicity of tebuconazole in chicken spermatozoa, revealing that it functions as a calcium channel blocker that modulates sperm motility, lipid peroxidation, and apoptosis. Several azole fungicides are also discussed in relation to their ability to induce or inhibit hepatic cytochrome P450 enzymes in birds [5,89].

Airborne pesticides contribute to environmental contamination and pose serious acute and chronic health risks to humans, particularly agricultural workers and those in nearby communities. Pesticides in the air can be in all forms (solid, liquid, and gaseous). Their entrance into the atmosphere happens through different routes, including drift and evaporation during aerial spraying, volatilization from crops and agricultural soils, wind erosion of contaminated soils, and emissions from manufacturing and disposal processes. Difenoconazole, epoxiconazole, propiconazole, and tebuconazole can be detected in the outdoor air [90,91]. Some sources discuss the hepatotoxicity and mechanisms of action of azole fungicides, focusing on how they affect the liver, particularly in rodents. Heise et al. [92] presented an in vivo feeding study in rats investigating the hepatotoxic combination effects of three azole fungicides (cyproconazole, epoxiconazole, and prochloraz), finding that mixtures often caused more pronounced effects on liver weight and gene expression than individual substances. To others, azole hepatotoxicity can be attributed to the activation of xeno-sensing nuclear receptors, which are implicated in adverse outcomes like hepatocellular hypertrophy, fatty changes, and tumor development. Significant species differences in nuclear receptor activation between rodents and humans, however, complicate the assessment of human risks from azole exposure [93,94]. Kadić et al. [95] looked into the effects of triazole fungicides, cyproconazole, epoxiconazole, and prochloraz, on the thyroid hormone system in rats. They observed a decrease in serum thyroxine and a compensatory increase in the thyroid-stimulating

hormone, along with follicular hypertrophy and hyperplasia in the thyroid gland. A mechanistic analysis showed that the primary mode of action was the induction of hepatic enzyme activity, which sped up the elimination of thyroid hormones [95]. Azole interference with mammalian CYP enzymes, specifically the disruption of steroid hormone synthesis and signaling, is particularly concerning during pregnancy, as fetal exposure to azoles can potentially lead to reproductive disorders in offspring. This is reviewed in the work by Draskau and Svingen [7], where the authors examine evidence from human and animal studies, noting how an exposure to certain azoles may cause adverse effects such as shorter anogenital distance and genital malformations in males.

3.4. Biodegradation Concerns

Cai et al. [96] focused their investigation on the biodegradation of climbazole and fluconazole in aerobic activated sludge. They investigated the removal mechanisms, finding that the imidazole fungicide climbazole was quickly degraded due to its adsorption to solid sludge. In contrast, the triazole fungicide fluconazole showed limited removal. They concluded that the persistence and biodegradability of azole fungicides in activated sludge systems are primarily determined by their chemical structure (specifically the type of azole ring) and related physicochemical properties. The main biotransformation routes observed for climbazole were azole ring loss and hydroxylation occurring at the active moiety/side chain. For triazole fungicides, there is typically no change in the azole ring, and hydroxylation only occurs at other active moieties. The presence of electron-withdrawing groups was found to deactivate the aromatic ring in certain positions for attack by oxygenase, leading to a lower biodegradation rate. For instance, the fluorine atom in fluconazole withdraws electrons from the aromatic ring, making it more difficult for fluconazole to react with peroxidases, resulting in a lower oxidation ability and greater resistance to microbial degradation. Their findings therefore highlight a significant environmental concern regarding the persistence of fluconazole and the need to find efficient ways for its removal from the environment [96].

Pacholak et al. [97] examined the environmental persistence, degradation, and toxicity of four common azole fungicides: fluconazole, clotrimazole, climbazole, and epoxiconazole. Their removal using biological degradation against chemical degradation methods was compared. The investigation showed the limited effectiveness of biodegradation, especially for fluconazole, clotrimazole, and epoxiconazole, and the superior performance of the Fenton reaction coupled with UV irradiation, which achieved a near-complete removal of clotrimazole, climbazole, and epoxiconazole. They also identified transformation products resulting from chemical degradation and assessed the varying levels of cytotoxicity of the parent compounds and their transformation products toward two Gram-negative bacterial strains. Clotrimazole exhibited the highest cytotoxicity among the parent compounds, while fluconazole exhibited the lowest initial cytotoxicity. The transformation products of epoxiconazole and fluconazole showed an increased cytotoxicity when compared to their parent compounds for both bacterial strains. Based on the experiments evaluating the removal of azole fungicides by activated sludge, clotrimazole exhibited the most significant biosorption (38.8 ± 1.7% of the total amount introduced to the culture adsorbed onto the sewage biomass) [97].

3.5. The Role of Computational Methods in Toxicity Estimation

Quantitative Structure–Activity Relationship QSAR represents a computational technique whose result is a mathematical model that connects quantitative structural properties of compounds represented by numerical variables (molecular descriptors) with chemical or biological activity. Each compound is quantitatively or qualitatively described by a set of molecular descriptors, and these are mathematically correlated with

an activity as an endpoint of interest. The performance of the compound thus becomes a function of one or more structural properties. QSAR helps to reduce the time of environmental risk assessments of pesticides, along with the experimental costs, material, energy, and space required to perform experimental tests. In addition, it helps to stimulate the research of new potential pesticides and enables the assessment of environmental risks in a prognostic way. This fact makes it possible to screen a large number of candidates for pesticides on the market and to find the most optimal ones [98]. The QSAR methodology has been applied in this field for several decades, and the approach has been accepted in several legislative frameworks on pesticides, including the important European Regulation on pesticides no. 1107/2009 on the registration, evaluation, authorization, and restriction of chemicals (REACH) [99]. Thus, many QSAR models are widely used today and are implemented in different software for the estimation of properties and toxicity endpoints [100–103].

One of the applications of QSAR is utilizing lipophylicity/hydrophobicity as key parameters to predict the environmental behavior of pesticides, specifically in relation to soil adsorption, bioaccumulation, and aquatic toxicity [104]. The QSAR models are well established in the prediction of toxicity and data gap filling. In order to enhance regulatory confidence and the acceptance of QSAR toxicity predictions, it is important to improve the description, transparency, and usability of such models. This is summarized in the work by Cronin et al. [105], where, besides the existing OECD principles for QSAR model validation, additional criteria were proposed, intended to bring awareness to the potential areas of uncertainty, variability, and bias in a model, to provide an opportunity to reduce them, and to make an informed decision on their impact and suitability for the intended purpose. The need for this is quite evident, as can be seen from the review by Weyrich et al. [106] where they evaluated computational models designed to predict reproductive and developmental toxicity, focusing specifically on a case study involving pesticides. They tested the predictive performance of several prominent QSAR models, including VEGA, OECD (Q)SAR Toolbox, Leadscope Model Applier, and CASE Ultra, against a database of 315 pesticides with known toxicity classifications. A large percentage of pesticides fell outside the domain of these models, resulting in low prediction accuracies [106].

The study by Abbod et al. [107] presents a quantitative structure–toxicity relationship (QSTR) study focused on developing models for the acute toxicity (acute oral LD_{50} in rats) of 45 different sterol biosynthesis inhibitors. The azoles used in the study are mostly imidazole and triazole pesticides, found in both the training set (36 compounds) and the test set (9 compounds). Several modeling techniques were used, including genetic algorithm multiple linear regression (GA-MLR), support vector regression (SVR), and artificial neural networks (ANN), to build models that predict their toxicity. The ANN model demonstrated the highest predictive accuracy, with a correlation coefficient value of 0.8, outperforming the MLR and SVR models. The developed MLR model, however, exhibited a wide range of applicability and could accurately predict the acute toxicity of sterol biosynthesis inhibitors within relevant thresholds; therefore, it can serve as a checkpoint to evaluate the potential hazards associated with their future applications [107].

Gottardi et al. [108] investigated whether the harmful effects of azole fungicides can be accurately predicted in aquatic invertebrates using in silico and in vitro models. In their study, they explored if the inhibition of xenobiotic-metabolizing cytochrome P450s by azoles could be extrapolated between rat livers (which served as a common model) and two non-target aquatic species: the insect *Chironomus riparius* and the snail *Lymnaea stagnalis*. The study included 18 azole fungicides, 5 imidazoles, and 13 triazoles, selected based on their application in either agriculture or medicine. The inhibition measurements were conducted using all 18 selected azoles for rat liver microsomes, but only the most

potent ones for *C. riparius* and *L. stagnalis*. The P450 structures used for computational models were primarily human isoforms involved in xenobiotic metabolism (CYP3A4, CYP2C9, CYP2D6) and the fungal CYP51. The findings showed a strong correlation between in silico predictions and the measured inhibition in rat livers, and to a lesser extent in *C. riparius*, but significantly failed to predict the effect in *L. stagnalis*, probably due to the differences in their enzymatic susceptibility to the investigated fungicides [108].

Research by Yang et al. [109] details the modeling of pesticide toxicity on the Sheepshead minnow using the QSAR approach. The authors developed highly robust predictive models for both specific types of pesticides (insecticides, herbicides, and fungicides) and a general combined type to assess their acute ecotoxicity. The total fungicide dataset comprised 78 compounds, mainly azoles, amides, and strobilurins. The key findings indicated that molecular lipophilicity tended to increase toxicity, while factors like polarity and hydrophilicity generally decreased it [109].

Galimberti et al. [110] developed and validated MLR SAR models for several aquatic organisms, designed to predict the aquatic toxicity of various pesticides, considering it crucial for filling data gaps. The study on pesticide ecotoxicity used a dataset of 70 pesticide active substances, including several azoles. The study also generated a prioritization list of potentially hazardous compounds for the aquatic environment. Azoles were mostly grouped in the medium or low concern category [110].

Skanes et al. [111] used in silico toxicity assessments with software like Toxtree (v3.1.0.1851) and the Toxicity Estimation Software Tool (TEST) (v5.1.2) to predict the potential risks of several pesticides and their transformation products to human health, including carcinogenicity, mutagenicity, and acute oral toxicity. The study identified dozens of transformation products, noting that some, particularly those derived from boscalid and fenbuconazole, were predicted to be significantly more orally toxic than their parent compounds, suggesting a need for further testing [111].

4. Directions for Novel Azole Pesticides

Pesticide use within the European Union is shaped by a wide range of factors. Directive 2009/128/EC seeks to promote the sustainable use of pesticides by minimizing risks and adverse effects on human health and the environment. It also encourages the adoption of Integrated Pest Management and alternative methods, including non-chemical options. Key actions include training users, advisors, and distributors of pesticides, inspecting pesticide application equipment, prohibiting aerial spraying, restricting pesticide use in sensitive areas, and increasing information and awareness regarding pesticide risks. [42] However, there are not enough replacement options for the wide-scale combatting of agricultural pests, especially fungi.

4.1. New Pesticides Using Computational Methods

Major advances in cheminformatics have improved the discovery of bioactive molecules. They have advanced the experiments from the laboratory to a virtual environment, where countless molecules can be rapidly investigated. The review by Djombou-Feunang et al. [112] explores how cheminformatics and artificial intelligence (AI) are integrated into the agrochemical discovery pipeline. It discusses how these technologies address challenges like pest resistance and stricter regulations by accelerating the development of novel, sustainable active molecules. The reviewed applications include predictive modeling (QSAR/QSPR), molecular design using deep generative models, and the use of AI for enhancing testing, data analysis, and the elucidation of metabolic pathways. The authors, however, emphasize that the field still faces challenges related to data scarcity and the generalizability of models [112].

According to Lewer et al. [113], the current computational methods in predictive toxicology lack the necessary multifaceted approach needed for system-based chemical development. They introduce a structure-to-process design framework for developing pesticides that are both safer and environmentally degradable. This approach integrates predictions for minimal ecotoxicity and tunable photodegradation properties by analyzing mechanistically derived physicochemical and electronic parameters, and can be used to screen existing molecules to identify candidates with desirable characteristics [113]. A different approach was described by An et al. [114]. They investigated a drug repurposing strategy aimed at finding new fungicide leads from existing approved drugs. For this, they screened 600 commercially available drugs for activity against six major phytopathogenic fungi, seeking alternatives due to increasing fungal resistance to current agrochemicals. Out of the 46 azole drugs screened against plant pathogens, 16 demonstrated optimal activity against at least one pathogen. Econazole, isoconazole nitrate, and clotrimazole specifically showed excellent activity against plant pathogenic fungi and were noted for their low toxicity [114].

4.2. Hybrid Compounds as Better Pesticides

Hybrid compounds combine two or more bioactive moieties into a single molecule, acting in synergy to enhance the activity or exhibiting multiple activities. This approach has been thoroughly investigated and used in drug discovery; however, the same principle can be applied to pesticides. Several studies describe novel hybrid compounds as pesticides, mostly focusing on triazole hybrids [115–120].

Sui et al. [121] designed, synthesized, and evaluated 19 novel trialkylamine derivatives containing a triazole moiety. These compounds were specifically developed as ergosterol biosynthesis inhibitors to combat various phytopathogenic fungi. The combination of the 1,2,4-triazole structure with a tertiary amine group significantly improved the antifungal activity against six tested fungal strains [121]. In a study by Tesh et al. [122], a novel isopropanol–triazole fungicide, mefentrifluconazole, is described. It was identified by in silico and in vitro screening focused on finding azole derivatives with a high fungal CYP51 inhibition and low human CYP19 (aromatase) inhibition. The extensive toxicity testing, including prenatal developmental and two-generation reproduction studies in rats and rabbits, showed that mefentrifluconazole was non-genotoxic, non-carcinogenic, and lacked adverse effects indicative of endocrine disruption at systemically toxic doses [122].

The insecticidal and acaricidal activities of new β-naphthol derivatives containing benzothiazolylamino and various heteroaryl groups towards a variety of agricultural pests were evaluated in a study by Shang et al. [123]. Several derivatives containing pyrazole and 1,2,3-triazole moieties showed promising results against the spider mite, oriental armyworm, and diamondback moth. Zheng et al. [124] introduced and modified pyrazole, imidazole, and imidazolium salt moieties into gallic acid, creating three derivatives with enhanced fungicidal properties against *Fusarium graminearum*. The introduction of an imidazolium salt structure showed the best growth inhibition of *F. graminearum*, with an EC50 of 0.42 mg/mL and an inhibition rate of 73.5% [124]. Nishio et al. [125] described an azole carboxamide compound called iptriazopyrid as a novel herbicide, designed to inhibit 4-hydroxyphenylpyruvate dioxygenase, an enzyme critical for plant survival. The study found that iptriazopyrid is highly phytotoxic to the weed *Echinochloa crusgalli* at lower concentrations and exhibits a strong selectivity for rice crops, in which it was rapidly degraded [125].

4.3. Advancement in Ionic Liquid-Based Pesticides

Ionic liquids (ILs) are considered as environmentally friendly "compounds" with melting points below 100 °C, some remaining in the liquid state at room temperature. They have a low toxicity, low vapor pressure, high thermal stability, and exceptional solubility in organic and inorganic chemicals. The tunability of ILs allows for the customization of their properties for specific applications [126]. ILs derived from miconazole are reported to be the first ionic liquids derived from a bioactive molecule, offering complex and functionally diverse side chains [127]. In a more recent study, the fungicides thiabendazole and imazalil were converted into protic ILs by pairing them with the hydrophobic docusate anion. The purpose of this modification was to enhance the fungicides' rain persistence, penetration into potato tissue, and overall activity. Initial in vitro testing demonstrated that the fungicidal activity was not only retained, but was also enhanced against several potato pathogens [128].

A review article by Wang et al. [129] summarizes the advancement of the applications of ILs in agriculture, from extraction and smart pesticide delivery systems to the development of ionic liquid-based pesticides. Their biodegradability and lower toxicity were one of the main drives to investigate them as potential eco-friendly alternatives. A wide variety of combinations of anions and cations in pesticides offer many opportunities for pesticide development, and progress has been made in the field of herbicides [130], plant growth regulators [131], and microbial control [129].

ILs with dual functionalities have been developed for agricultural applications by combining several bioactive ions. These include dicationic triazolium fungicidal ILs that also exhibit herbicidal properties. A homologous series of ten dicationic ILs uses the commercial fungicide tebuconazole and various herbicidal acids like 2-methyl-4-chlorophenoxyacetic acid (MCPA) and Dicamba. They generally showed both improved fungicidal and herbicidal activities [132]. Newly developed ILs derived from ammonium and triazolium were investigated as potential wood preservatives. The cation and anion structure of the ILs significantly influences their effectiveness, and combining them with tebuconazole produces a synergistic effect that improves protection, penetration depth, and resistance to leaching [133].

A research conducted by Montalbán et al. [134] describes an investigation into the aquatic toxicity of twenty-nine different ionic liquids (ILs), primarily focusing on imidazolium-, pyridinium-, and ammonium-based structures. The study's main objective was to establish how variations in the cation core, alkyl chain length, functionalization, and anion type influence the ILs' toxicity. It was found that IL toxicity increases as the alkyl chain length increases, no matter which anion is present. This is attributed to the fact that longer alkyl chains increase the cation's lipophilicity, which in turn facilitates the entrance of the IL through the cell membrane. Furthermore, imidazolium-based ILs exhibited a lower toxicity than similar pyridinium-based ILs [134].

4.4. Nanopesticides

Nanopesticides (NPs) are considered potential substitutes for conventional pesticides, primarily because they address the numerous drawbacks associated with traditional pesticides due to their nanoscale properties. They can be defined as agricultural formulations on the nano scale, ranging up to 100 nm in size. The nanocarriers can be constructed from polymers, lipids, or inorganic materials, and serve as protective carriers for pesticides. NPs offer a high uptake with reduced dosage requirements, with the efficiency attributed to their small size, high penetration ability into plant tissues or insect cuticles, and high surface area. NPs also have an improved water solubility, stability, and regulated release of active ingredients [135,136]. They are shown to be up to ten times more toxic to pests than their traditional analogs, while having a reduced environmental contamination by up to 30% [137]. However, their ability to retain their properties, reac-

tivity, and particle size in the environment can also make them toxic to the non-target species [138].

Wang et al. [139] discuss the use of nano-enabled pesticides for sustainable agriculture. They compared the performance and environmental impact of nanopesticides to their conventional counterparts, finding that they offer a significantly higher efficacy against target organisms and lower toxicity toward non-target organisms. Two main types of nanopesticides are described: metal-based and those using nanocarriers to encapsulate active ingredients. The overall performance of NPs against target pests was 31.5% higher, showing promising results even in field trials. The toxicity of NPs toward non-target organisms was 43.1% lower [139].

The effectiveness of nano-azoles has been tested in several studies, yielding ketoconazole nanoemulsion [140], the nanodispersion of hexaconazole [141], cross-linked lignin nanocarriers with tebuconazole [142], cyazofamid encapsulated in polylactic-co-glycolic acid [143], and nano metal–organic frameworks as carriers for hexaconazole [144], tebuconazole [145–147], and diniconazole [148,149]. The fungicide hymexazol was encapsulated using a thermosensitive poly-dopamine-graphene oxide nanocarrier. The bioactivity of the prepared nano-hymexazol was investigated against *Fusarium oxysporum*. sp. *cucumebrium* Owen. It showed a similar inhibition activity with a standard hymexazol solution. Furthermore, it demonstrated a temperature-controlled release of hymexazol, potentially enhancing crop growth by releasing the active ingredient during warmer periods [150]. In the study by Gao et al. [151], the inclusion complex of beta-cyclodextrin and difenoconazole was synthesized with the aim to enhance its water solubility and antifungal activity against *Gibberella* sp. With this approach, they successfully improved both its solubility and fungicidal efficacy.

Jiang et al. [152] constructed a nano-delivery system for imidaclothiz using a star polymer, which spontaneously loads the pesticide through hydrophobic associations, resulting in smaller, spherical particles. It was prepared by simply mixing and incubating at room temperature for 15 min. This carrier significantly promoted the plant uptake of imidaclothiz and improved activity against green peach aphids, both in the laboratory and the field. It also decreased pesticide residue in tobacco plants seven days after treatment due to the faster degradation of the nanoscale complex, demonstrating a potential pathway toward more sustainable agriculture. Furthermore, the nano-imidaclothiz complex had minimal negative effects on tobacco plants and only a slight synergistic toxicity toward predatory lady beetles [152].

Even though azole-based NPs have many good aspects, and thus seem to be a good substitute for conventional azole pesticides, there are several concerns that need to be taken into account. First is finding the right carrier that ensures formulation stability over time and under various pH and temperature variations. This can increase the time and costs of investigation. However, the greatest concern still lies in the potential negative impact of such formulations, since their improved properties regarding toxicity and availability might have unintended consequences [136]. Before using azole-based NPs, an extensive study should be performed, encompassing not only standard testing reserved for new pesticide candidates, but also a detailed comparison of effects on both target and non-target organisms in regard to the conventional azole analogs.

5. Conclusions

The balance between the efficiency and toxic effects of azole pesticides has been steadily progressing toward the latter in the past few decades. Since it is a global problem, many regulations have been put in place to limit their usage, with the EU's being among the strictest. Even though many azole-based compounds have now been withdrawn from the market, the situation has not been greatly improved, and pesticide res-

idues continue to pollute the environment. The summary has shown an increase in chemoinformatic approaches for the design of more effective molecules, preliminary screening for toxicity and environmental effects, and elucidation of the underlying mechanisms of action.

For over five decades, azoles have been important in controlling plant diseases and reducing crop losses around the world. They are integrated into modern farming techniques and are likely to remain so, since better alternatives are still lacking. Because of this, researchers are working to create sustainable ways to manage azole pesticide issues and find alternative solutions. Computational tools have played a big role by filling gaps in experimental data and quickly assessing toxicity. The approaches discussed here show promising progress, and, with a better education for users, there is hope for reducing the environmental impact of azole pesticides by discovering, developing, and using new, effective azoles responsibly. The further development of azoles as environmentally and health-safe pesticides will improve agricultural production, leading to higher production and profitability for farmers and lower food prices, while also contributing to food security. In addition, the export of surplus agricultural products can boost a country's economy and improve the quality of life of its citizens.

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