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Original Article

In silico analysis to predict the carcinogenicity and mutagenicity of a group of triazole fungicides

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ABSTRACT

Background and Aims: Fungicides, particularly triazoles, are of global concern for pesticide contamination because of their widespread use. This study focuses on estimating the carcinogenicity and mutagenicity of 15 commonly used triazole fungicides.

Methods: *In silico* prediction tools such as ProTox-II, Toxtree, Lazar, and VEGA were used to predict mutagenicity and carcinogenicity.

Results: All compounds were predicted to be "non-mutagenic" by ProTox-II, Toxtree, and Lazar. However, the CONSENSUS of VEGA identified epoxiconazole and prothioconazole as "mutagenic." Regarding carcinogenicity predictions, ProTox-II indicated non-carcinogenicity for all compounds, whereas Toxtree and VEGA (ISS) raised structural alerts for 10 compounds. In addition, Lazar predicted carcinogenicity for tebuconazole, paclobutrazol, and penconazole. It is worth noting that the results exhibit variable reliability, emphasising the need for further investigation and validation.

Conclusion: *In silico* tools proved valuable for predicting the toxicity of triazole fungicides, emphasising the need for additional data. Although the study categorises compounds as non-mutagenic, some exhibit structural alerts for potential carcinogenicity. This strategic approach contributes to pesticide risk assessment by highlighting the role of computational models in advancing our understanding of the health impacts associated with pesticide exposure.

Keywords: Carcinogenicity, genotoxicity, *in silico*, mutagenicity, triazole fungicides

INTRODUCTION

Pesticide contamination in the environment and food is a major issue in global agriculture (Li et al., 2022). Fungicides, particularly azoles (triazoles, imidazoles), are widely used worldwide for pesticide control and to enhance agricultural productivity. Azoles, such as triazoles, are the most commonly used antifungals because of their broad-spectrum activity and high efficiency (Rjiba-Touati et al., 2022a). Numerous conazoles, a category of fungicides, are used in the management of fungal infections and the prevention of fungal proliferation in diverse crops. This leads to their introduction into the ecosystem, with the potential for accumulation in living organisms (Perdichizzi et al., 2014). Triazole fungicides, which are pivotal in hindering fungal ergosterol biosynthesis, play a crucial economic role in crops (Filipov & Lawrence, 2001). The prevalent use of triazole pesticides has generated concerns regarding environmental contamination and food safety (Li et al., 2022).

Alterations in agricultural methodologies and the adoption of intensive farming practices have notably elevated the use of pesticides (Camilo-Cotrim et al., 2022). In 2019, it was estimated that 2 million tonnes of pesticides were used globally annually (Sharma et al., 2019).

Because of the capacity of triazole compounds to impede oestrogen/androgen biosynthesis, extended exposure is under suspicion for potentially inducing diverse disorders in both humans and animals (Hamdi et al., 2022). Studies underscore the diverse cytotoxic and genotoxic impacts of these fungicides across different biological systems, emphasising the importance of understanding their potential risks in various environmental and health contexts (Ben Othmène et al., 2020; Hamdi et al., 2022; Macar, 2021; Rjiba-Touati et al., 2022a; Rjiba-Touati et al., 2022b). Kahle et al. (2008) showed that commonly used azole fungicides are widely available and are continuously released into the aquatic environment. Furthermore, higher octanol-water partition coefficients for propiconazole and tebuconazole (log Kow 3.7) indicate the bioaccumulation potential of these substances (Kahle et al., 2008). In addition, triazole fungicides can accumulate in aquatic organisms, leading to toxic effects on reproduction and embryonic development (Wang et al., 2023).

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Despite widespread use, toxicity assessments for most triazole fungicides are limited to legal regulations, and comprehensive data evaluating their biological effects are lacking. Data addressing potential adverse effects during development are even more limited (Filipov & Lawrence, 2001).

The rise in hazardous chemical release raises concerns about its impact on living organisms and ecosystems. Genotoxic and mutagenic effects, causing genetic damage and affecting future generations, are particularly worrisome. Consequently, genotoxicity and mutagenicity analyses are needed to ensure environmental quality (Leme & Marin-Morales, 2009).

In the computational domain, there are models that predict bacterial mutagenicity, which serve as an indirect indication of whether a construct acts as an electrophile or has the potential to transform into an electrophile (Benigni & Bossa, 2011). These models effectively anticipate the mutagenic potential of a structure, and their utilisation has gained acceptance as a substitute for the Ames test in assessing mutagenicity. The U.S. The Food and Drug Administration (FDA) Centre has introduced rodent carcinogenicity (Quantitative) Structure-Activity Relationship ((Q)SAR) models through the VEGA Hub, designed to predict the carcinogenic potential of chemicals in rodents (Tice et al., 2021).

This study aims to prioritise 15 triazole fungicides (Figure 1) based on *in silico* predictions, providing a foundation for future investigations. Given the increasing release of hazardous chemicals into the environment, understanding their potential genotoxic and mutagenic effects is crucial. This study explores computational models for predicting bacterial mutagenicity, offering insights into the potential carcinogenicity of these compounds.

MATERIALS AND METHODS

Chemical structures of the fungicides

The molecular structures of bromuconazole, diniconazole, epoxiconazole, fenbuconazole, flutriafol, hexaconazole, enilconazole/imazalil, metconazole, myclobutanil, paclobutrazol, penconazole, prothioconazole, tebuconazole, triticonazole, and uniconazole were inserted to run the analysis using the Simplified Molecular Input Line Entry Specification (SMILES) system, according to the PubChem canonical SMILES in (Table 1).

In silico predictions

Mutagenicity and Carcinogenicity

Mutagenicity is an important toxicological endpoint for chemical risk assessment. Structural alert (SA) refers to the molecular structure associated with adverse outcomes or toxicological endpoints. In the context of mutagenicity, SA includes molecular functions or substructures linked to the mutagenic activity of chemical compounds. Mutagenicity tests include in vitro tests such as the Ames test, primarily using bacterial and mammalian systems such as *Salmonella typhimurium*. For mutagenicity in this study, ProTox-II, Toxtree version 2.6.13, Lazar version 1.4.2, and VEGA version 1.2.3 mutagenicity (Ames) models (CEASAR, ISS, SarPy-IRFMN, and CONSENSUS) were used for prediction analyses. The ProTox-II web server includes molecular similarity and machine learning models for various toxicity endpoints. The prediction scheme of ProTox-II is classified according to different toxicity levels such as toxicological endpoints (such as mutagenicity, carcino-toxicity). Toxtree predicts various toxic hazards using structured rules that can predict toxic hazards by applying a decision tree approach. A decision tree for carcinogenicity and mutagenicity prediction by discriminant analysis and structural rules based on in vitro mutagenicity (Ames test) alerts by the ISS, published in the Benigni 2008 document. Vega is a prediction model covering human toxicity predictions, including mutagenicity and carcinogenicity models. Within the VEGA platform, the CAESAR and Benigni/Bossa computer models are implemented for mutagenicity and carcinogenicity (Benigni & Bossa, 2006; Benigni & Bossa, 2008; Mombelli & Devillers, 2010). Lazar predicts toxicological endpoints such as mutagenicity and mouse, rat, and rodent carcinogenicity. Lazar uses the random forest algorithm in R's Caret package to build local QSAR models. In all applications, canonical SMILES representations of the compounds are used as input. Toxtree indicates SA, whereas ProTox-II and VEGA models determine mutagenicity as positive/negative. The CONSENSUS approach categorises compounds as "possibly mutagenic" or "non-mutagenic" (Bhat and Chatterjee, 2021).

During the process of loading the chemical structures in SMILES format into the programmes we used in our study, the structures in each programme were carefully examined separately. No inconsistencies were detected in this study. To validate our approach, we entered and validated the canonical SMILES codes of the fungicides in Table 1 into the programmes. This validation process increases the reliability of our findings and demonstrates the accuracy of our study.

Carcinogenicity

Carcinogenicity, the potential to cause cancer, can result from genotoxic or non-genotoxic pathways. In this study, carcinogenicity predictions were made using the ProTox-II web server, Toxtree version 2.6.13, Lazar version 1.4.2, and VEGA version 1.2.3 carcinogenicity models (CAESAR and ISS). While ProTox-II and Lazar predict whether a compound is carcinogenic, CAESAR provides additional information. Toxtree has a decision tree for predicting carcinogenicity through discriminant analysis and structural rules based on those published in Benigni 2013.

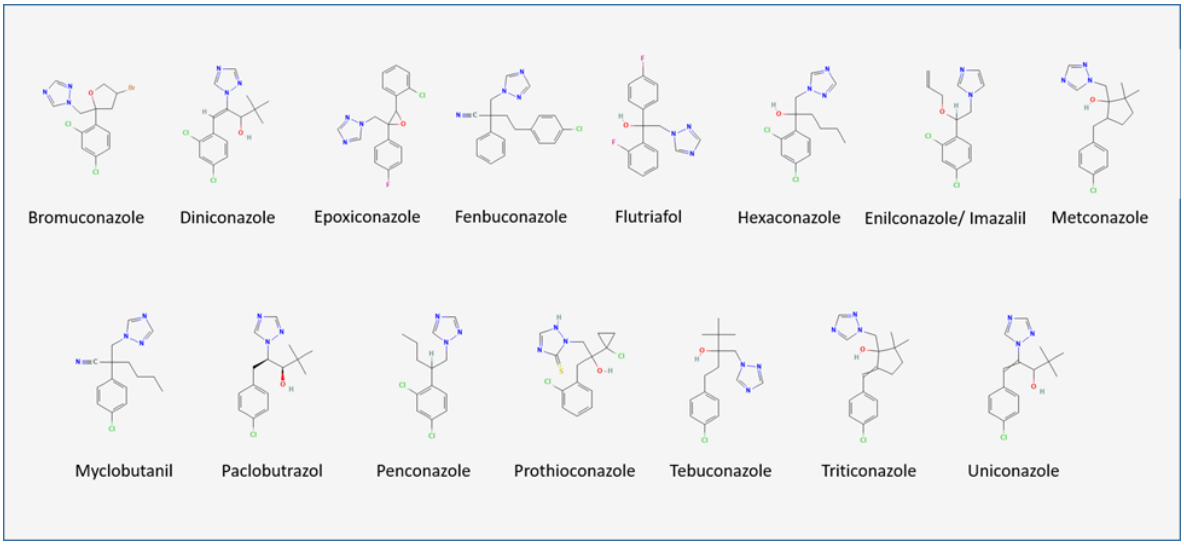

Figure 1. Chemical structures of a group of triazole fungicides.

Table 1. Chemical structures of a group of triazole fungicides.

Chemical Name	CAS number	Log Kow[1]	Molecular weight (g/mol)	Canonical SMILES
Bromuconazole	116255-48-2	3.24	377.1	(C3NCN(CC1(CC(CO1)BR)C2CCC(CC2CL)CL)N3
Diniconazole	83657-24-3	-	326.2	CC(C)(C)C(C(=CC1=C(C=C(C=C1)Cl)Cl)N2C=NC=N2)O
Epoxiconazole	106325-08-0	-	329.8	C1=CC=C(C(=C1)C2C(O2)(CN3C=NC=N3)C4=CC=C(C=C4)F)Cl
Fenbuconazole	114369-43-6	-	336.8	C1=CC=C(C=C1)C(CCC2=CC=C(C=C2)Cl)(CN3C=NC=N3)C#N
Flutriafol	76674-21-0	-	301.29	C1=CC=C(C(=C1)C(CN2C=NC=N2)(C3=CC=C(C=C3)F)O)F
Hexaconazole	79983-71-4	-	314.2	CCCCC(CN1C=NC=N1)(C2=C(C=C(C=C2)Cl)Cl)O
Enilconazole/imazalil	35554-44-0	3.82 (pH 9.2 buffer)	297.2	C=CCOC(CN1C=CN=C1)C2=C(C=C(C=C2)Cl)Cl
Metconazole	125116-23-6	-	319.8	CC1(CCC(C1(CN2C=NC=N2)O)CC3=CC=C(C=C3)Cl)C
Myclobutanil	88671-89-0	2.94	288.77	CCCCC(CN1C=NC=N1)(C#N)C2=CC=C(C=C2)Cl
Paclobutrazol	76738-62-0	-	293.79	C1CC1(C(CC2=CC=CC=C2Cl)(CN3C(=S)N=CN3)O)Cl
Penconazole	66246-88-6	-	284.18	CCCC(CN1C=NC=N1)C2=C(C=C(C=C2)Cl)Cl
Prothioconazole	178928-70-6	4.05 (unbuffered, 20 °C), 4.16 (pH 4), 3.82 (pH 7), 2.00 (pH 9)	344.3	SC1CC1(C(CC2=CC=CC=C2Cl)(CN3C(=S)N=CN3)O)Cl
Tebuconazole	107534-96-3	3.7	307.82	CC(C)(C)C(CCC1=CC=C(C=C1)Cl)(CN2C=NC=N2)O
Triticonazole	131983-72-7	3.29 at 20 °C	317.8	CC1(CCC(=CC2=CC=C(C=C2)Cl)C1(CN3C=NC=N3)O)C
Uniconazole	83657-22-1	-	291.77	CC(C)(C)C(C(=CC1=CC=C(C=C1)Cl)N2C=NC=N2)O

[1] Log Kow: Logarithm of the octanol-water partition coefficient.

Considering the results obtained from VEGA, a value greater than zero was considered consensus for the applicability domain of all models. For the overall assessment, consensus was accepted and the compound was labelled as "probably

carcinogenic" if two or more applications predicted positive carcinogenicity, whereas the compound was labelled as "not carcinogenic" if two or more applications predicted negative carcinogenicity (Bhat, & Chatterjee, 2021).

RESULTS

Mutagenicity

Based on the consensus of results from three prediction tools (ProTox-II, Toxtree, and Lazar), all compounds were classified as “non-mutagenic,” as shown in Tables 2, 3, and 4, respectively. These three prediction tools consistently demonstrated the absence of mutagenic potential in the evaluated compounds. However, in the predictions derived from VEGA, all compounds exhibited different levels of safety, ranging from low to moderate. According to the VEGA CONSENSUS model, consensus mutagenicity scores ranged from 0 to 0.3. Although VEGA detected mutagenicity in 2 (epoxiconazole and prothioconazole) out of 15 compounds (13.3%) according to the CONSENSUS model (Table 5). For epoxiconazole, one of the two compounds detected as mutagenic by VEGA, both the "mutagenic" and "non-mutagenic" consensus scores were equal (50%). This finding is due to the result of the Predicted Consensus Mutagen activity, where the model indicates mutagenicity with the same scores for both the mutagenic and non-mutagenic categories (both scores are 0.15).

Carcinogenicity

ProTox-II assessed all compounds as "non-carcinogenic" with probability values ranging from 0.56 to 0.62 (Table 2). According to SA, Toxtree predicted that all compounds fall into High-Class III. Toxtree and VEGA (ISS model) predicted SA for genotoxic carcinogenicity for 1 (epoxiconazole) out of 15 compounds and non-genotoxic carcinogenicity for 10 out of 15 compounds (66.7%). In 9 of 15 compounds (60%), the most frequently observed SA was predicted to be monohalogenated benzene. Again, n-alkyl carboxylic SA was observed in 26.7% of the compounds. (Table 3). Lazar predicted that fenbuconazole and penconazole are carcinogenic in rats and that paclobutrazol is carcinogenic in mice and rodents, but this model warned that this prediction "may be outside the domain of predictability with a similarity threshold < 0.5" (Table 4).

The VEGA results demonstrated varying levels of reliability, with approximately 26.7% of compounds exhibiting relatively lower reliability and approximately 20% displaying middle levels of reliability. Notably, for 8 out of 15 compounds (53,3%), the caesar result on VEGA indicated that "the predicted compound is outside the applicability domain of the model." The chemical compound under evaluation falls outside the range of compounds for which the model is considered reliable. (Table 5). In the context of predictive models, the applicability domain refers to the specific conditions or characteristics under which a model is expected to provide accurate and reliable predictions. Further investigation and validation, possibly using additional experimental data or domain-specific knowledge, are recommended to assess the reliability of the model’s prediction of carcinogenicity in this particular case.

DISCUSSION

Epidemiological studies consistently associate pesticide exposure with an elevated risk of cancer, as supported by various literature reviews highlighting a positive correlation between pesticide exposure and cancer development (Mostafalou & Abdollahi, 2017; Varghese et al., 2020). The assessment of the risk of specific chemical substances heavily relies on the availability of experimental toxicological data and adequate exposure information. Unfortunately, in numerous instances, such data are either insufficient or entirely unavailable, making a reliable risk assessment nearly unattainable. Over the past decades, (Q)SAR models have emerged as valuable tools for predicting toxic properties (Chen et al., 2022; Kianpour et al., 2021; Wang et al., 2022).

In this study, *in silico* tools were employed to predict the toxicity of several triazole fungicide compounds, with a primary focus on mutagenicity and carcinogenicity, which are considered the most crucial endpoints. According to our toxicity assessment, two compounds (epoxiconazole and prothioconazole) were found to be mutagenic based on VEGA’s *in silico* mutagenicity prediction (CONSENSUS). However, it is noteworthy that other *in silico* tools used in this study, including Toxtree, ProTox-II, and Lazar, classified all assessed compounds, including epoxiconazole and prothioconazole, as non-mutagenic. Additionally, using three *in silico* carcinogenicity prediction models (Toxtree and VEGA (ISS)), 10 compounds—diniconazole, epoxiconazole, fenbuconazolemetconazole, myclobutanil, paclobutrazol, prothioconazole, tebuconazole, triticonazole, and uniconazole—were indicated as potentially carcinogenic.

Holečková et al. (2013) reported that most of the experimental data suggest that the mutagenic properties of the pesticide are questionable. In addition, the genotoxic effects of commercial forms commonly used in agriculture are greater than the genotoxic effects of individual compounds. While conazoles are not considered classical mutagens because they do not give positive results in short-term mutagenicity tests such as Ames (Šiviková et al., 2018), a study has shown that stereoisomers of difenoconazole can cause liver injuries, mutagenicity and skin sensitisation (Gridan et al., 2019). Our study revealed that all compounds were classified as non-mutagenic. However, VEGA detected mutagenicity in two compounds, namely epoxiconazole and prothioconazole.

The results of our carcinogenicity study reflect some findings of previous studies. Epoxiconazole showed cytotoxic effects,

Table 2. ProTox-II mutagenicity and carcinogenicity predictions.

Triazole Fungicides	Octanol/water partition coefficient (logP)	Prediction of Carcinogenicity	Prediction of Mutagenicity	Predicted Toxicity Class	Prediction accuracy
Bromuconazole	3.66	Inactive	Inactive	4	100%
Diniconazole	3.99	Inactive	Inactive	4	100%
Epoxiconazole	3.74	Inactive	Inactive	4	68.07%
Fenbuconazole	4.03	Inactive	Inactive	4	100%
Flutriafol	2.49	Inactive	Inactive	3	100%
Hexaconazole	3.66	Inactive	Inactive	4	100%
Enilconazole/imazalil	4.13	Inactive	Inactive	3	100%
Metconazole	3.34	Inactive	Inactive	4	100%
Myclobutanil	3.58	Inactive	Inactive	4	100%
Paclobutrazol	3.12	Inactive	Inactive	4	100%
Penconazole	4.17	Inactive	Inactive	5	100%
Prothioconazole	3.34	Inactive	Inactive	4	100%
Tebuconazole	3.34	Inactive	Inactive	4	100%
Triticonazole	3.57	Inactive	Inactive	4	68.07%
Uniconazole	3.34	Inactive	Inactive	4	100%

Table 3. Toxtree mutagenicity and carcinogenicity predictions.

Trizole Fungicides	Genotoxic carcinogenicity	Non-genotoxic carcinogenicity	Mutagenicity by ISS
Bromuconazole	Negative	Negative	No
Diniconazole	Negative	SA for nongenotoxic carcinogenicity QSA41_nogen.substituted n-alkylcarboxylic acids	No
Epoxiconazole	SA for genotoxic carcinogenicity QSA7_gen. Epoxides and aziridines	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens)	No
Fenbuconazole	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens)	No
Flutriafol	Negative	Negative	No
Hexaconazole	Negative	Negative	No
Enilconazole/imazalil	Negative	Negative	No
Metconazole	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens)	No
Myclobutanil	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens)	No
Paclobutrazol	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens) QSA41_nogen.substituted n-alkylcarboxylic acids	No
Penconazole	Negative	Negative	No
Prothioconazole	Negative	SA for nongenotoxic carcinogenicity QSA17_nogen. Thiocarbonyl (Nongenotoxic carcinogens) QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens)	No
Tebuconazole	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens) QSA41_nogen.substituted n-alkylcarboxylic acids	No
Triticonazole	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens)	No
Uniconazole	Negative	SA for nongenotoxic carcinogenicity QSA31a_nogen. Halogenated benzene (Nongenotoxic carcinogens) QSA41_nogen.substituted n-alkylcarboxylic acids	No

Table 4. Lazar mutagenicity and carcinogenicity predictions.

Trizaole Fungicides	Carcinogenicity Prediction	Warning for Carcinogenicity	Mutagenicity Predictiton (*Salmonella typhimurium*)	Warning for Mutagenicity
Bromuconazole	(Mouse) Non-carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.
Diniconazole Flutriafol	(Mouse, rat) Cannot create prediction	Only one similar compound for threshold 0.2 in the training set (Threshold: 0.2).	Non-mutagenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.
Epoxiconazole	(Mouse) Non-carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.
Fenbuconazole	(Mouse) Non-carcinogenic (Rat) Carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similar to bioassay results
Hexaconazole Myclobutanil	(Mouse) non-Carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similar to bioassay results
Enilconazole/imazalil	(Rodent) Non-carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similar to bioassay results
Metconazole	(Mouse, rat) Cannot create prediction	Only one similar compound for threshold 0.2 in the training set (Threshold: 0.2).	Non-mutagenic	Similar to bioassay results
Paclobutrazol	(Mouse, rodent) Carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.
Penconazole	(Rat) Carcinogenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.	Non-mutagenic	Similar to bioassay results
Prothioconazole	(Rat, rodent) Cannot create prediction	Only one similar compound for threshold 0.2 in the training set (Threshold: 0.2).	Non-mutagenic	Similar to bioassay results
Tebuconazole	(Mouse, rat) Cannot create prediction	Only one similar compound for threshold 0.2 in the training set (Threshold: 0.2).	Non-mutagenic	Similar to bioassay results
Triticonazole Uniconazole	(Mouse, rat, rodent)	Could not find similar substances for threshold 0.2 with experimental data in the training dataset.	Non-mutagenic	Similarity threshold 0.2 < 0.5, prediction may be out of applicability domain.

induced DNA damage through a caspase-dependent pathway, triggered apoptosis, and caused oxidative stress in PC12 rat pheochromocytoma cells (Hamdi et al., 2022). Furthermore, only the VEGA (CEASAR) prediction tool identified bromuconazole as a carcinogen in our study. Previous studies have shown that bromuconazole causes genotoxic damage and organ damage in rat liver and kidney tissues, possibly associated with impaired oxidative stress in these organs, supporting findings from different studies (Rjiba-Touati et al., 2022b). Furthermore, our research revealed that tebuconazole and myclobutanil are predicted to be non-genotoxic carcinogens in line with the consistent results of Toxtree and VEGA (ISS). Tebuconazole has been associated with genotoxicity in adult Danio rerio (Castro et al., 2018) and poses a potential carcinogenic risk to humans (Liu et al., 2016). Myclobutanil is a potential carcinogen (Shellenberger and Briggs, 1986).

In summary, the computer models utilised in this study exhibit adequacy in predicting crucial toxicological endpoints for health safety, including mutagenicity and carcinogenicity; however, their predictability is acknowledged to be less than fully reliable. Despite this limitation, this study introduces a strategic approach to harness *in silico* prediction tools for prioritisation purposes, presenting a valuable concept for future investigations.

Table 5. VEGA mutagenicity and carcinogenicity prediction models (CEASAR, ISS, SarPy and CONSENSUS).

VEGA Prediction Models	Predicted Carcinogen activity: Carcinogen	Predicted Carcinogen activity: NON-Carcinogen	Predicted Mutagen activity: Mutagenic	Predicted Mutagen activity: NON-Mutagenic
CEASAR	Bromuconazole** Epoxiconazole* Flutriafol** Hexaconazole** Metconazole* Prothioconazole* Triticonazole*	Diniconazole* Fenbuconazole** Enilconazole/imazalil* Myclobutanil* Paclobutrazol* Penconazole* Tebuconazole* Uniconazole*	Epoxiconazole* (Suspect Mutagenic, SA7 Epoxides and aziridines)	Bromuconazole** Diniconazole* Fenbuconazole* Flutriafol** Hexaconazole** Enilconazole/imazalil** Metconazole** Myclobutanil* Paclobutrazol* Penconazole** Prothioconazole* Tebuconazole** Triticonazole* Uniconazole*
ISS	Diniconazole* (SA41 Substituted n-alkylcarboxylic acids) Epoxiconazole* (SA7 Epoxides and aziridines; SA31a Halogenated benzene (Nongenotoxic carcinogens) Fenbuconazole* (SA31a Halogenated benzene (Nongenotoxic carcinogens) Metconazole* (SA31a Halogenated benzene (Nongenotoxic carcinogens) Myclobutanil* (SA31a Halogenated benzene (Nongenotoxic carcinogens) Paclobutrazol* SA31a Halogenated benzene (Nongenotoxic carcinogens); SA41 Substituted n-alkylcarboxylic acids) Prothioconazole** (SA17 Thiocarbonyl (Nongenotoxic carcinogens); SA31a Halogenated benzene (Nongenotoxic carcinogens) Tebuconazole** (SA31a Halogenated benzene (Nongenotoxic carcinogens); SA41 Substituted n-alkylcarboxylic acids) Triticonazole**	Bromuconazole* Flutriafol* Hexaconazole* Enilconazole/imazalil* Penconazole**	Epoxiconazole*	Bromuconazole* Diniconazole* Fenbuconazole* Flutriafol* Hexaconazole* Enilconazole/imazalil* Metconazole* Myclobutanil* Paclobutrazol* Penconazole* Prothioconazole* Tebuconazole* Triticonazole* Uniconazole*

Table 5. Continued

	(SA31a Halogenated benzene (Nongenotoxic carcinogens) Uniconazole* (SA31a Halogenated benzene (Nongenotoxic carcinogens); SA41 Substituted n-alkylcarboxylic acids)		
SarPy		Bromuconazole* Epoxiconazole* Prothioconazole**	Diniconazole** Fenbuconazole** Flutriafol** Hexaconazole** Enilconazole/imazalil** Metconazole** Myclobutanil** Paclobutrazol** Penconazole** Tebuconazole** Triticonazole* Uniconazole**
CONSENSUS (based on 4 models)		Epoxiconazole Prothioconazole	Bromuconazole Diniconazole Fenbuconazole Flutriafol Hexaconazole Enilconazole/imazalil Metconazole Myclobutanil Paclobutrazol Penconazole Tebuconazole Triticonazole Uniconazole

The reliability levels are indicated as follows: *Low reliability; **Moderate reliability.

CONCLUSION

In conclusion, our study underscores the invaluable role of *in silico* tools, such as (Q)SAR models, in predicting the toxic properties of triazole fungicide compounds. The prioritisation strategy proposed in this study, which combines multiple predictive models and emphasises the need for additional data, provides a valuable framework for future investigations in the field of pesticide risk assessment. It is crucial to recognise the inherent limitations and uncertainties in the current predictive capabilities and to continuously refine these tools for improved accuracy. Overall, this study contributes to the ongoing efforts to bridge the gap between traditional toxicological assessments and the evolving landscape of computational approaches, offering a strategic pathway for advancing our understanding of the health impacts associated with pesticide exposure.

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