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PREDICTIVE ECOTOXICOLOGICAL STUDY OF FUNGICIDES DERIVED FROM LUOTONIN A

ESTUDO PREDITIVO ECOTOXICOLÓGICO DE FUNGICIDAS DERIVADOS DA LUOTONINA A

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Abstract

The fungus *Botrytis cinerea* is a pathogen that causes gray mold on greenhouse crops, affecting the quality of the products and causing the death of the plants. Control with fungicides has been limited due to the high resistance of the fungus and as a result of the continuous application of these substances, environmental impacts occur. New fungicides are therefore needed that eliminate *B. cinerea* but are less toxic to the ecosystem. In this sense, new antifungal compounds

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have been derived from luotonin A, which have shown greater efficacy against *B. cinerea*. This study aims to evaluate *in silico* the consequences of fungicides derived from luotonin A (10a, 10m, 10l, 10r, and 10s) on the ecosystem, analyzing their environmental properties, ecotoxicity, and toxicological safety. The molecules were analyzed using ECOSAR® and JANUS® software, which made it possible to analyze toxicity in aquatic organisms and persistence in water, soil, and sediment. The GUSAR© and GraphPAD Prism® tools were also used to measure toxicity in different exposure routes in rats and to compare doses between routes, using the one-way ANOVA statistical test. The results indicated that compound 10s showed low toxicity to fish and *Daphnia magna* in both the acute and chronic tests. In green algae, in the acute evaluation 10s showed low inhibition of biomass growth, while in the chronic test, fungicides 10l, 10r, and 10s showed low inhibitory effects. In the toxicity assessment on rats, all the compounds proved to be harmful to health when ingested. Given these results, further *in silico* and *in vivo* studies are needed to validate the data presented and assess the toxicity endpoints of the fungicides.
Keywords: *Botrytis cinerea*; *In silico*; Rats; Narcosis.

Resumo

O fungo *Botrytis cinerea* é um agente patogênico que causa mofo cinzento em culturas de estufa, afetando a qualidade dos produtos e provocando a morte das plantas. O controle com fungicidas tem sido limitado devido à elevada resistência do fungo e como consequência da contínua aplicação dessas substâncias, ocorrem os impactos ambientais. Dessa forma, são necessários novos fungicidas que eliminem o *B. cinerea*, mas que sejam menos tóxicos ao ecossistema. Nesse sentido, novos compostos antifúngicos foram derivados da luotonina A, os quais apresentaram maior eficácia contra o *B. cinerea*. O presente estudo objetiva avaliar em modelo *in silico* as consequências dos fungicidas derivados da luotonina A (10a, 10m, 10l, 10r e 10s) no ecossistema, analisando suas propriedades ambientais, ecotoxicidade e segurança toxicológica. As moléculas foram analisadas pelos softwares ECOSAR® e JANUS®, que permitiram analisar a toxicidade em organismos aquáticos e a persistência em água, solo e sedimento. Também foram utilizados as ferramentas GUSAR© e GraphPAD Prism® para aferir a toxicidade em diferentes vias de exposição nos ratos e comparar as doses entre as vias, utilizando o teste estatístico *one way anova*. Os resultados indicaram que o composto 10s apresenta baixa toxicidade para peixes e Daphnia magna tanto no teste agudo, como no crônico. Em algas verdes, na avaliação aguda o 10s exibiu baixa inibição no crescimento da biomassa, enquanto no ensaio crônico, os fungicidas 10l, 10r e 10s apresentaram baixo efeito inibitório. Na avaliação de toxicidade em ratos, todos os compostos demonstraram ser nocivos à saúde ao serem ingeridos. Diante desses resultados, são necessários novos estudos *in silico* e *in vivo* para validar os dados apresentados e avaliar os pontos finais de toxicidade dos fungicidas.
Palavras-chave: *Botrytis cinerea*; *In silico*; Ratos; Narcose.

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1. Introduction

Botrytis cinerea is a multi-host necrotrophic fungus that affects greenhouse crops such as fruit, vegetables, and ornamentals, damaging product quality and killing plants. *B. cinerea* causes gray mold, infecting stems, flowers, leaves, and fruit (ZUPAROV *et al.*, 2020).

Fungicides are constantly applied to control this pathogen, but *B. cinerea* is highly resistant due to its short life cycle and rapid reproduction (HARPER *et al.*, 2023). In addition, the continuous use of chemical products can have consequences for farmers health and environmental impacts. (GAVA *et al.*, 2021).

In this context, it is necessary to create new fungicides that are less aggressive to the ecosystem. As an alternative, new compounds can be synthesized from structures with proven antifungal activity.

Among them, luotonin A, which can be extracted from the therapeutic plant *Peganum nigellastrum*, belongs to the quinoline alkaloid group and has activity against the fungus *B. cinerea*. Based on this molecule, new compounds were synthesized which showed greater antifungal activity when compared to luotonin A. The synthesis process began with anthranilamide being treated with diethyl oxalate at reflux, obtaining intermediate compounds that reacted with other reagents, resulting in derivatives 10a, 10m, and 10l. Derivative 10a was then modified using boron tribromide with a demethylation reaction and then reacted with paraformaldehyde and various amines, giving rise to molecules 10r and 10s (YANG, Guan Zhou *et al.*, 2020).

Given this, the study aims to predictively evaluate the environmental properties, ecotoxicity, and toxicological safety of the luotonin A derivatives (10a, 10m, 10l, 10r, and 10s) to understand their effects as fungicides in the ecosystem.

2. Literature review

2.1 The importance of computational analysis in pesticide evaluation

Pesticides are used in agriculture to kill pests, contain diseases, prevent and reduce damage caused by fungi, reduce damage to crops, and increase quality.

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However, the high application of these chemicals has led to their accumulation in the environment and especially in water bodies (YANG; WANG; HAO; *et al.*, 2020). As a result, they generate toxicity and adverse effects on non-target organisms due to their complex and varied structures.

In this scenario, there is a need to develop new pesticides that do not cause so many consequences for the ecosystem. However, the regulation of these substances is based on *in vitro* and *in vivo* tests, which are generally expensive, and extensive and may not strictly follow the ethical rules of animal studies. Therefore, chemical regulatory agencies are encouraging the use of computational tools in the development and risk assessment of new pesticides, reducing the cost of experiments and optimizing the process of analyzing these compounds (YANG; WANG; CHANG; *et al.*, 2020).

Quantitative structure-activity relationships (QSAR) models use mathematical methods to predict the activities and properties of molecules, based on their conformations. These models are used as alternatives to experiments with animals, microorganisms, invertebrates, and cell and tissue cultures (GALIMBERTI; MORETTO; PAPA, 2020).

3. Methodology

3.1 (ECOSAR®) Ecological Structure Activity Relationships

The ECOSAR® tool uses the QSAR method to evaluate the ecotoxicity of compounds in aquatic organisms. The model is based on the octanol-water partition coefficient (log Kow) to predict acute toxicity, analyzed by LC50 (lethal concentration 50%) in fish and *Daphnia magna*, and EC50 (effective concentration 50%) in green algae. ECOSAR® also assesses chronic toxicity, in which the ChV (chronic value) is applied to analyze the aforementioned organisms (BU *et al.*, 2021).

Toxicity can also be classified as high (acute < 1.0; chronic < 0.1 mg/L), moderate (acute between 1.0 and 100 mg/L; chronic between 0.1 and 10 mg/L) and low (acute > 100; chronic > 10 mg/L) (WRIGHT *et al.*, 2022b).

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3.2 JANUS®

JANUS® is a software program that integrates various *in silico* models to evaluate toxicity, ecotoxicity, and other environmental properties. From these models, molecules are classified as persistent, bioaccumulative, toxic (PBT), carcinogenic, mutagenic, reprotoxic (CMR), and endocrine disruptors (ROGIERS *et al.*, 2020).

The results are provided based on experimental and predicted data and are then analyzed according to the score acquired, in which 0 indicates a low risk, 0.5 a moderate risk, and 1 a high risk (RONCAGLIONI; LOMBARDO; BENFENATI, 2022).

3.3 (GUSAR©) General Unrestricted Structure-Activity Relationships

GUSAR© is an online platform that predicts the LD50 (median lethal dose) of chemical substances for rats in the following exposure routes: oral, intravenous (IV), intraperitoneal (IP), and subcutaneous (SC). Predictions are made based on experimental data on the acute toxic effect in rats (AHMED *et al.*, 2021).

Toxicity is assessed according to the standards of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which classifies molecules into six classes when ingested. These are class I (LD50 ≤ 5 mg/kg) - deadly; class II (5 < LD50 ≤ 50 mg/kg) - fatal; class III (50 < LD50 ≤ 300 mg/kg) - toxic; class IV (300 < LD50 ≤ 2000 mg/kg) - harmful; class V (2000 < LD50 ≤ 5000 mg/kg) - dangerous; class VI (LD50 > 5000 mg/kg) non-toxic (MUSLIKH *et al.*, 2022).

3.4 Statistical assessment

The statistics were carried out using GraphPad Prism® software which, using the one-way ANOVA analysis, verified significant differences in the normalized means of LD50 of the routes of exposure to the fungicides. The test applied was the Turkey test for multiple comparisons, in which the differences had to have a P-value of less than 0.05 to be identified.

4. Results and Discussion

4.1 Environmental biomonitoring

The intensive application of fungicides to protect crops can have harmful consequences for the ecosystem. Through spraying, these substances can enter the soil and, after a period, be carried by leaching or runoff into bodies of water and groundwater resources, leading to adverse effects on aquatic organisms (SIKANDAR; MOHSIN; MALIK, 2023).

In order to analyze the dynamics and toxicity of Luotonin A derivatives, Table 1 was produced, containing data on the properties, persistence and toxicity of the fungicides analyzed.

Table 1 shows that the log kow varies between 0.98 and 3.565. Therefore, the compounds can be classified as hydrophilic (10s), i.e. substances with a greater affinity for water and with a log kow < 2, and hydrophilic-lipophilic (the other molecules), with an affinity for both water and soils and sediments. In addition, these chemicals are characterized by having a log kow between 2 and 4 (IANCU *et al.*, 2024).

Given the influence of log kow on the persistence of the compounds, it can be seen that the fungicides were not persistent in water, lasting between 7 and 26 days. In soil, they were also not persistent, remaining in the compartment for between 23 and 34 days. In the sediment, derivatives 10a, 10l and 10m showed a persistence of 157 days, while 10r and 10s are very persistent products that can remain in the sediment for 227 days.

As they remain in the sediment for a long time, the organisms that interact most with these molecules are green algae. These algae are used as bioindicators to assess water quality, as they can use the nutrients present in the environment to grow their biomass. Green algae have been evaluated in the acute test by the EC50 and the inhibition of the increase in their biomass (SALO; SALOVIUS-LAURÉN, 2022).

The evaluation of the acute toxic effect indicated that most of the fungicides (10a, 10l, 10m, and 10r) showed moderate inhibition of algae growth, with the

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EC50 varying between 9.273 and 43.395 mg/L. Compound 10s was the only one to show low inhibition with a concentration of 706.876 mg/L.

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Table 1. Environmental dynamics and ecotoxicity of fungicides

							Neutral organic					
				Persistence (Days)			Acute (mg/L)			Chronic (mg/L)		
Compounds	**MW**	**log kow**	**Solubility (mg/L)**	**Water**	**Soil**	**Sediment**	**Fish 96h**	***D. magna* 48h**	**G. algae 96h**	**Fish**	***D. magna***	**G. alage**
10a	317.35	3.245	16.44	nP-7	nP-34	**P-157**	**19.857***	12.649	15.156	2.223	1.699	5.127
10l	347.38	2.809	25.64	nP-7	nP-34	**P-157**	**53.537***	**32.757***	**33.233***	5.715	3.933	10.279
10m	323.30	3.565	8.081	nP-26	nP-34	**P-157**	**10.440***	6.849	**9.273***	1.210	0.999	3.350
10r	398.47	2.728	55.71	nP-23	nP-23	**vP-227**	**72.655***	44.122	43.395	7.688	5.188	13.199
10s	400.44	0.98	1684	nP-23	nP-34	**vP-227**	**2710.3***	1400.6	706.876	237	105.1	150.09

Source: Authors

Notes: MW - Molecular weight; log kow - Octanol-water partition coefficient; nP - Not persistent; P – Persistent; vP - Very persistent; * - Concentration greater than solubility.

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The next organisms to be evaluated by LC50 are fish and *D. magna*. Fish are used as environmental bioindicators because they are easy to determine their behavioral state, are sensitive to water pollution, and are long-lived, making it possible to assess water quality in the short and long term. *D. magna* are evaluated in toxicity tests given their importance in the food chain, as they consume primary producers (phytoplankton) and are consumed by secondary consumers (fish). Consequently, damage to this species can harm the food chain (AL-KHAZRAJI; THAKIR; EL-HADEETI, 2020).

Table 1 shows that most of the molecules (10a, 10l, 10m, and 10r) were moderately toxic to fish, with LC50s ranging from 10.440 to 72.655 mg/L. The chemical 10s, in contrast to the others, had a low toxic effect with a concentration equivalent to 2710.3 mg/L. In *D. magna*, the derivatives generally showed moderate toxicity with the LC50 ranging from 6.849 to 44.122 mg/L. The exception among these compounds was 10s, which showed a low toxic effect and a concentration of 1400.6 mg/L.

Table 1 also shows that the fungicides had concentrations higher than their solubility in fish (all), *D. magna* (10l), and green algae (10l and 10m). When this occurs in fish and *D. magna*, narcosis toxicity is generated, in which the organisms suffer a general anesthetic effect that impacts their locomotion. In algae, basal toxicity occurs, which impairs the growth of the biomass of these living beings (WRIGHT *et al.*, 2022a).

To assess the chronic toxic effect, the organisms were analyzed by ChV. In fish, the ChV values (1.210 to 7.688 mg/L) indicated that derivatives 10a, 10l, 10m, and 10r were moderately toxic, while 10s showed low toxicity and a concentration equivalent to 237 mg/L. In *D. magna*, the compounds showed the same toxicity behavior, differing only in the ChV values, in which the moderate toxic effect ranged from 0.999 to 5.188 mg/L, and the low toxic effect was 105.1 mg/L. About green algae, molecules 10a and 10m showed a moderate inhibitory effect with concentrations of 5.127 and 3.350 mg/L, while chemicals 10l, 10r, and 10s showed low inhibition with ChV values ranging from 10.279 to 150.09 mg/L.

Since the fungicides showed persistence in the sediment, chronic exposure to green algae can lead to bioaccumulation of these compounds, increasing concentration in consumer organisms and, consequently, biomagnification in the

food chain. Other consequences of chronic exposure are changes in the development of aquatic organisms, reproduction, behavior, and sublethal effects (KHOSHNOOD, 2023).

4.2 Toxicological safety

Rats are constantly used in the toxicity assessment of agricultural chemicals to understand their toxic effects on humans. This is due to the metabolic similarity between these rodents and humans, as well as the fact that they are small animals with short life spans and gestation periods (GAD, 2019). In this context, Table 2 was structured to assess toxicity in rats, using the LD50 in the IP, IV, oral, and SC exposure routes.

Table 2. Toxicity in rats by routes of exposure

Compounds	LD_{50} (mg/kg)				
	IP	IV	Oral	Toxicity class	SC
10a	296.8	100.8	857	4	296.6
10l	207.8	141.8	1061	4	396.5
10m	141.4	116	875.2	4	238
10r	205.8	119	424.5	4	210.5
10s	498	162.9	365.1	4	611.4

Source: Authors

Notes: DL50 - Lethal dose 50%; IP – Intraperitoneal; IV – Intravenous; SC – Subcutaneous.

Table 2 shows that the doses of fungicides in the IP route ranged from 141.4 to 498 mg/kg. Next, in the IV route, the LD50 values were the lowest of all the routes of administration, ranging from 100.8 to 162.9 mg/kg. In the SC route, the lethal doses ranged from 210.5 to 611.4 mg/kg. Finally, the oral route showed the highest LD50 results, ranging from 365.1 to 1061 mg/kg, indicating that the compounds, when ingested through contaminated food or water, will be harmful to the body's health. The possible effects of this intoxication are endocrine disruption, infertility, immune suppression, and carcinogenicity (XIAO *et al.*, 2022).

In addition to the toxicity assessment carried out based on Table 2, Figure 1

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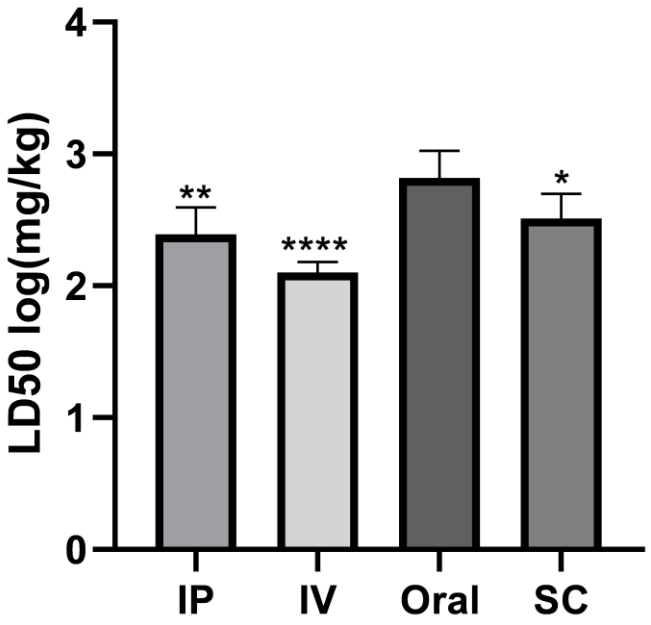
was developed, illustrating the average of the log LD50 values in the exposure routes, to identify significant differences between the routes.

Figure 1. Graph of the average LD50 in the routes of exposure



Source: Authors

Note: * - Significant difference between routes.

Looking at Figure 1, it can be seen that the lowest average LD50 is exhibited by the IV route, which shows a significant difference with the SC and oral routes. This last route has the highest average, since among the routes analyzed it is the main route of exposure to pesticide residues. The oral route, as well as showing a significant difference with the IV route, also shows a difference with the IP route. These differences are reinforced by the P-value, which was 0.0001.

5. Conclusion

The results of the ecotoxicity assessment on aquatic organisms indicated that the luotonin A derivatives do not show persistence in water and soil, but are persistent in sediment. In the acute effect test, 10s showed low toxicity/inhibition, while the other compounds showed moderate toxicity/inhibition. In the chronic toxicity evaluation, most of the molecules showed moderate toxicity, except for

10s which showed a low toxic effect, but all the fungicides showed a narcosis effect on fish and *D. magna*. In green algae, moderate inhibition was caused by substances 10a and 10m, while the other compounds showed low inhibitory effects.

The predicted toxicological safety in rats indicated that all the derivatives are harmful to health and can have adverse effects on the body when ingested. Thus, even though fungicides do not show high toxicity, studies are needed to evaluate the possible adverse effects of applying chemical products that are less aggressive to the ecosystem. Among these studies, new in silico analyses can be carried out with QSAR models to evaluate toxicity endpoints and in vivo tests to corroborate the data acquired.

References

AHMED, Sium et al. A comprehensive in silico exploration of pharmacological properties, bioactivities and COX-2 inhibitory potential of eleutheroside B from Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. **Journal of Biomolecular Structure and Dynamics**, v. 39, n. 17, p. 6553–6566, 2021. Available at: https://doi.org/10.1080/07391102.2020.1803135. Accessed May 22, 2024.

AL-KHAZRAJI, Hind I.; THAKIR, Baraa M.; EL-HADEETI, Sawsan A.K. Bioindicators of pesticides pollution in the aquatic environment: A review. **Plant Archives**, v. 20, p. 1607–1618, 2020. Available at: https://l1nq.com/Z3ACZ. Accessed May 22, 2024.

BU, Qingwei et al. Performance Comparison between the Specific and Baseline Prediction Models of Ecotoxicity for Pharmaceuticals: Is a Specific QSAR Model Inevitable?. **Journal of Chemistry**, v. 2021, 2021. Available at: https://doi.org/10.1155/2021/5563066. Accessed May 22, 2024.

GAD, Shayne C. Rodent Models for Toxicity Testing and Biomarkers. **Biomarkers in Toxicology**. [*S. l.*]: Elsevier Inc., 2019. p. 7–73. Available at: http://dx.doi.org/10.1016/B978-0-12-814655-2.00002-5. Accessed May 22, 2024.

GALIMBERTI, Francesco; MORETTO, Angelo; PAPA, Ester. Application of chemometric methods and QSAR models to support pesticide risk assessment starting from ecotoxicological datasets. **Water Research**, v. 174, p. 1–14, 2020. Available at: https://doi.org/10.1016/j.watres.2020.115583. Accessed May 22, 2024.

GAVA, Angelo et al. Occurrence and impact of fungicides residues on fermentation during wine

production– A review. **Food Additives & Contaminants - Part A**, v. 38, n. 6, p. 943–961, 2021. Available at: https://doi.org/10.1080/19440049.2021.1894357. Accessed May 22, 2024.

HARPER, Lincoln A. et al. Fungicide resistance characterized across seven modes of action in Botrytis cinerea isolated from Australian vineyards. **Pest Manag Sci.**, v. 80, n. 4, p. 1326–1340, 2023. Available at: https://doi.org/10.1002/ps.6749. Accessed May 24, 2024.

IANCU, Vasile-Ion et al. Occurrence and distribution of azole antifungal agents in eight urban Romanian waste water treatment plants. **Science of the Total Environment**, v. 920, p. 1–9, 2024. Available at: https://doi.org/10.1016/j.scitotenv.2024.170898. Accessed May 24, 2024.

KHOSHNOOD, Zahra. Acute and Chronic Effects of Pesticides on Non-Target Aquatic Organisms. **Transylv. Rev. Syst. Ecol. Res.**, v. 25, n. 3, p. 71–78, 2023. Available at: https://doi.org/10.2478/trser-2023-0022. Accessed May 24, 2024.

MUSLIKH, Faisal Akhmal et al. In Silico Molecular Docking and ADMET Analysis for Drug Development of Phytoestrogens Compound with Its Evaluation of Neurodegenerative Diseases. **Borneo Journal of Pharmacy**, v. 5, n. 4, p. 357–366, 2022. Available at: https://doi.org/10.33084/bjop.v5i4.3801. Accessed May 24, 2024.

ROGIERS, Vera et al. The way forward for assessing the human health safety of cosmetics in the EU - Workshop proceedings. **Toxicology**, v. 436, p. 1–14, 2020. Available at: https://doi.org/10.1016/j.tox.2020.152421. Accessed May 23, 2024.

RONCAGLIONI, Alessandra; LOMBARDO, Anna; BENFENATI, Emilio. The VEGAHUB Platform: The Philosophy and the Tools. **Alternatives to Laboratory Animals**, v. 50, n. 2, p. 121–135, 2022. Available at: https://doi.org/10.1177/02611929221090530. Accessed May 23, 2024.

SALO, Tiina; SALOVIUS-LAURÉN, Sonja. Green algae as bioindicators for long-term nutrient pollution along a coastal eutrophication gradient. **Ecological Indicators**, v. 140, p. 1–10, 2022. Available at: https://doi.org/10.1016/j.ecolind.2022.109034. Accessed May 23, 2024.

SIKANDAR, Minahil; MOHSIN, Ayesha; MALIK, Alina. Ecological Risk Assessment of Inorganic Arsenic and Mercuric Fungicides Through Biological Tools. **Indonesian Journal of Innovation and Applied Sciences**, v. 3, n. 2, p. 133–145, 2023. Available at: https://doi.org/10.47540/ijias.v3i2.742. Accessed May 23, 2024.

WRIGHT, R. Tracy et al. Methodology document for the ecological structure-activity relationship model (ECOSAR) class program. **U.S. Environmental Protection Agency**, p. 1–40, 2022a.

Available at: https://l1nq.com/bIiFC. Accessed May 23, 2024.

WRIGHT, R. Tracy et al. Operation manual for the ecolological Structure-Activity Relationship Model (ECOSAR) Class Program. **U.S. Environmental Protection Agency**, p. 1–42, 2022b. Available at: https://l1nq.com/Wn3YP. Accessed May 23, 2024.

XIAO, Jinjing et al. Incorporating Tenax into the in vitro method to improve the predictive capability of bioaccessibility of triazole fungicides in grape. **Food Chemistry**, v. 396, p. 1–8, 2022. Available at: https://doi.org/10.1016/j.foodchem.2022.133740. Accessed May 27, 2024.

YANG, Guan Zhou et al. Discovery of luotonin A analogues as potent fungicides and insecticides: Design, synthesis and biological evaluation inspired by natural alkaloid. **European Journal of Medicinal Chemistry**, v. 194, p. 1–12, 2020. Available at: https://doi.org/10.1016/j.ejmech.2020.112253. Accessed May 27, 2024.

YANG, Lu; WANG, Yinghuan; CHANG, Jing et al. QSAR modeling the toxicity of pesticides against Americamysis bahia. **Chemosphere**, v. 258, p. 1–8, 2020. Available at: https://doi.org/10.1016/j.chemosphere.2020.127217. Accessed May 27, 2024.

YANG, Lu; WANG, Yinghuan; HAO, Weiyu et al. Modeling pesticides toxicity to Sheepshead minnow using QSAR. **Ecotoxicology and Environmental Safety**, v. 193, p. 1–7, 2020. Available at: https://doi.org/10.1016/j.ecoenv.2020.110352. Accessed May 27, 2024.

ZUPAROV, Mirakbar A. et al. In Vitro Efficacy Testing of Fungicides on Botrytis cinerea causing Gray Mold of Tomato. **International Journal on Emerging Technologies**, v. 11, n. 5, p. 50–55, 2020. Available at: https://l1nq.com/3RaRv. Accessed May 27, 2024.