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MOLECULAR MODELING AND IN SILICO HUMAN AND ENVIRONMENTAL TOXICOLOGICAL STUDY FOR THE ANTIBIOTICS TRIMETHOPRIM AND SULFADOXINE

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ABSTRACT

Sulfadoxine in combination with trimethoprim are commonly used for broad-spectrum antimicrobial therapy in veterinary medicine. Although widespread resistance limits use in ruminants, the combination is still useful in the treatment of gastrointestinal and respiratory infections in calves, including neonatal colibacillosis. The pharmacokinetic profile of sulfadoxine and trimethoprim differs from each other due to their chemical properties: sulfadoxine acts as a weak acid while trimethoprim is a weak base, thus concentrating at different sites in the body. Sulfadoxine and trimethoprim are two antimicrobial agents that are often used in combination due to their different mechanisms of action, acting synergistically against bacterial infections. The package inserts, both from national and international companies, state that the pharmaceutical product containing the two antibiotics has as its target the treatment of respiratory and gastrointestinal diseases in cattle and horses. One of the major challenges is related to the fact that antibiotics produced by the pharmaceutical industry result in antibiotic residues in the environment, both in water bodies and in the soil. These residues contaminate the food chain, affecting fish, crustaceans and even organisms such as Tetrahymena pyriformis, a protozoan widely distributed in freshwater environments. This contamination mechanism is very worrying because it not only compromises the health of ecosystems, but will also affect the humans

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who consume the contaminated food. The in silico toxicological study revealed that the compounds did not present toxicity in the AMES test, did not present carcinogenicity and did not have Acute Oral Toxicity.

Keywords: Antibiotic. Sulfadoxine. Trimethoprim. Environmental Contamination. Human and Environmental In Silico Toxicology.

INTRODUCTION

Antibiotics are drugs that can be natural or synthetic, and are responsible for inhibiting the growth of bacteria, or causing their death. Thus, they are called bactericidal when they cause the death of the bacterium, or bacteriostatic when they inhibit microbial growth (Guimarães, Momesso & Pupo, 2010). It is known that animal production in Brazil has gradually increased in recent years, consequently the use of antibiotics has also grown.

In Brazil, animal production is one of the most expressive activities. To ensure productivity and maintain the competitiveness of the sector, it is common to use drugs for both treatment and disease prevention. Among the most commonly used drugs, antibiotics are one of the most frequently prescribed classes (Regitano and Leal, 2010). Since humans consume products of animal origin, humans are exposed to the antibiotic residues contained in these foods, so regulatory and research agencies have been paying special attention to this study (Capleton *et al*, 2006). Thus, the subject deserves to be highlighted in the present study, as it will evaluate the pharmacokinetic and toxicological impacts of the antibiotics Sulfadoxine and Trimethoprim on humans and the environment.

Most antibiotics given to animals are not completely metabolized, being excreted in urine and feces, both in the original form and partially metabolized. The application of manure and sewage sludge as fertilizers is one of the main ways to spread these compounds into the environment. In the environment, antibiotic residues can accumulate in the soil, leach or be transported to water bodies by surface runoff. Some waste can be absorbed by plants, accumulating in their tissues and posing a risk to human health through the consumption of contaminated plant foods (Regitano & Leal, 2010). Sulfonamides have a low sorption potential at soil organic and mineral exchange sites, which facilitates their transport to watercourses (Sarmah et al., 2006).

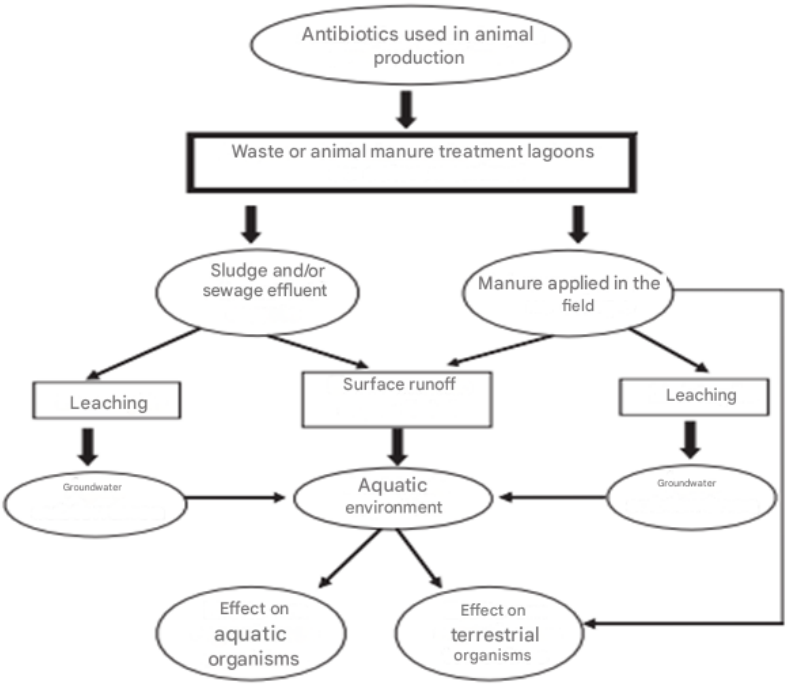
Sulfadoxine is a type of sulfa characterized by its rapid absorption by the body, followed by a very slow excretion. Due to these pharmacokinetic properties, it provides a prolonged action, which makes it effective in treatments that require a sustained release of the drug in the body. Trimethoprim, a diaminopyrimidine, is structurally similar to dihydrofolic acid and works by blocking the enzyme dihydrofolate reductase, which is responsible for converting dihydrofolic acid to tetrahydrofolic acid. Although this enzyme is present in mammals and bacteria, trimethoprim has an affinity 20 to 60 thousand times greater for the bacterial enzyme than for that of mammals. This makes trimethoprim a very safe chemotherapy drug (Spinosa, 2006).

Although trimethoprim can be used alone, its combination with sulfa offers significant advantages, as it broadens the spectrum of action, being effective against Gram-positive

and Gram-negative bacteria. In addition, this combination reduces the incidence of bacterial resistance. Unlike the isolated use of each of these chemotherapy drugs, the association of sulfa and trimethoprim has a bactericidal effect (Cordeiro et al., 2008).

Figure 1 shows the main forms of environmental exposure to drugs used in veterinary medicine.

Figure 1: Main forms of environmental exposure to drugs used in veterinary medicine.



Source: Regitano & Leal, 2010.

When this waste is in the soil, processes such as leaching, surface runoff, and erosion can transport it to water bodies. In addition, it is common to find small amounts of antibiotics, both for human and animal use, in samples of sludge or effluents from urban sewage treatment plants. The disposal of these effluents in water bodies or the use of sludge and effluents as organic fertilizers are significant sources of environmental contamination by a variety of drugs (Regitano & Leal, 2010). Once the soil and animals are contaminated, humans can be exposed to the waste through the consumption of meat and products derived from these animals, resulting in human contamination.

LITERATURE REVIEW

This chapter addresses the theoretical definitions essential to understand the work in question. In addition, the factors that motivated this proposal will be highlighted, especially in the context of technological innovation. The literature review concludes with statistical data provided by regulatory agencies, such as MAPA and ANVISA, emphasizing the

relevance of the sectors involved in our country and the rational use of the antibiotics Trimethoprim and Sulfadoxine.

REGULATORY AGENCIES AND MEAT CONSUMPTION IN BRAZIL

As the two active ingredients act both in antibiotics for veterinary use, in the treatment of respiratory and gastrointestinal diseases, and in antibiotics for human use for the treatment of malaria, it is necessary to mention both regulatory bodies.

The Ministry of Agriculture, Livestock and Supply (MAPA) is the government agency responsible for regulating and inspecting various sectors of agriculture in Brazil, including the control of veterinary drugs. One of MAPA's attributions is to ensure the quality, safety and efficacy of veterinary products marketed in the country, in order to protect animal health and, indirectly, public health. To this end, MAPA conducts inspections, grants registrations for veterinary products, and monitors the production chain, from manufacturing to commercialization (B Rawley, 2024).

The National Health Surveillance Agency (ANVISA), on the other hand, is the regulatory body responsible for inspecting and regulating products intended for human use, including medicines, food, cosmetics, and health products (Brasil, 2024).

According to Conab, in 2023, the production of the three main types of meat in Brazil is estimated at approximately 29.6 million tons, as indicated in the updated product supply table, including poultry, cattle and pork. These data show a significant increase when compared to previous years. As we are dealing with drugs for veterinary use, this brings some concern, because increasing meat production, consequently increasing the use of antibiotics for the treatment of animal diseases.

When it comes to drug misuse, the inappropriate use of antibiotics and other drugs in the production of meat and milk can result in the presence of residues of these products in food consumed by humans. In addition, the improper disposal of medicines and excessive use in agricultural practices can also lead to soil contamination and, consequently, the water table, affecting water quality.

The contamination of water bodies due to improper disposal of pharmaceuticals can also negatively impact aquatic ecosystems and fauna. Finally, environmental and food contamination can affect the entire food chain, including humans, who can also be contaminated by ingesting contaminated food or water.

GASTROINTESTINAL AND RESPIRATORY DISEASES

Digestive system disorders in horses and cattle are a major concern for producers and veterinarians due to their significant economic impact and the complexity involved in controlling, treating, and preventing these conditions. The incidence of these diseases has increased with the intensification of production, as many foods have been incorporated into the animals' diets with the aim of increasing production rates, often without taking into account the welfare of the animals, nor their metabolism and physiology (Brandolt, 2016).

In the case of the respiratory tract of horses, it presents peculiar characteristics in relation to other species of domestic animals, being adapted to meet metabolic needs whose spectrum of variation ranges from low levels of basal requirements in relation to body volume, to high demands for gas exchange during intense long-term efforts. Some of these characteristics, combined with environmental conditions and individual predispositions, facilitate the installation of infectious processes (Brandolt, 2016).

The complex of respiratory diseases in cattle, on the other hand, results from an imbalance between the animal's natural defenses and the external factors that promote the disease. This imbalance is more common in animals that cannot adapt to changes in the environment or overcome these modifications. Stress affects the cleaning and defense mechanisms of the respiratory system, facilitating the proliferation of microorganisms and the production of toxins (Laval; Carraud; Filleton, 1994).

Gastrointestinal diseases affect cattle and horses with great frequency and have a direct impact on the economy, demonstrating the need to modernize veterinary medicine with concepts of population medicine based on epidemiology that allows the establishment of preventive and disease control strategies.

SULFADOXINA

Sulfadoxine is a sulfonamide, which works by inhibiting the enzyme *dihydropteroate* synthetase. This enzyme is essential for the synthesis of folic acid in bacteria, which in turn is necessary for the production of nucleotides and, consequently, for the synthesis of bacterial DNA. By inhibiting *dihydropteroate* synthetase, sulfadoxine interferes with folic acid synthesis, leading to the interruption of bacterial growth and proliferation (Aucamp, 2016).

New antimicrobials, such as sulfadoxine, are available in the Brazilian market and are widely used in the treatment of bacterial infections, especially in combination with other drugs, such as trimethoprim. Sulfadoxine is a long-acting sulfonamide, acting as a competitive inhibitor of *dihydropteroate* synthase, essential in the synthesis of bacterial folic

acid. Its use is prevalent in veterinary medicine, although it also has applications in some human infections, such as malaria, in combination with other medications. Sulfonamides are categorized based on their chemical structure and mechanisms of action, with sulfadoxine being one of the best known for its efficacy and long-term use (Aucamp, 2016).

As for pharmacokinetic and pharmacodynamic properties, sulfadoxine is often administered orally as part of a combination with other drugs, showing relatively slow but effective absorption in the gastrointestinal tract. Sulfonamides are rapidly absorbed in the gastrointestinal tract and reach peak concentrations within 4 to 6 hours. The drugs penetrate the blood-brain barrier and cross the placental barrier in varying concentrations according to the type of sulfa. They have good permeation in inflammatory exudates. They are metabolized mainly in the liver and excreted in the urine (Dereti, 2003).

TRIMETHOPRIM

Trimethoprim is a folic acid antagonist that works by inhibiting the enzyme *dihydrofolate* reductase, which is responsible for converting *dihydrofolate* to *tetrahydrofolate*, an active form of folic acid. By blocking this conversion, trimethoprim disrupts nucleotide synthesis and, consequently, bacterial DNA synthesis. Like sulfadoxine, trimethoprim interferes with the production of bacterial DNA, but acts at a different stage of the folic acid synthesis process (Kaartinent, 2000).

Trimethoprim is widely used in the treatment of bacterial infections, often in combination with sulfonamides such as sulfadoxine. Trimethoprim is an antibiotic that inhibits the enzyme dihydrofolate reductase, which is essential for the synthesis of folic acid in bacteria, and has a spectrum of action that makes it effective against a wide range of pathogens. Its use is common in both human and veterinary medicine, especially in the treatment of urinary, respiratory, and gastrointestinal tract infections (Kaartinent, 2000).

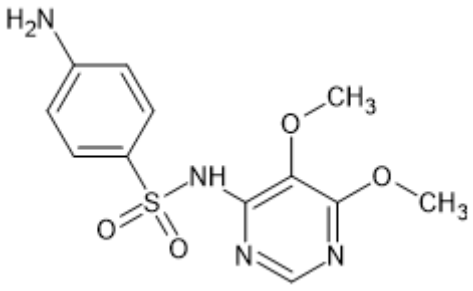
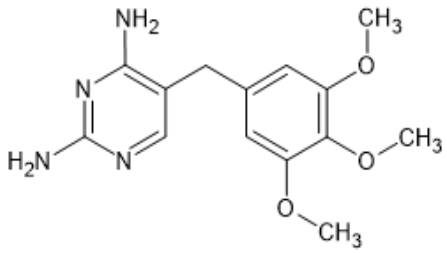
Regarding pharmacokinetic and pharmacodynamic properties, trimethoprim is usually administered orally, with good absorption in the gastrointestinal tract, which allows its rapid availability in the body. The distribution of trimethoprim in tissues is wide, reaching therapeutic concentrations in different organs, including the lungs, kidneys, and prostate. Trimethoprim can be administered both orally and parenterally, being completely absorbed in the gastrointestinal tract and distributed widely to all tissues of the body. It reaches high concentrations in the lungs and kidneys, and reaches high levels in the cerebrospinal fluid. Its excretion occurs mainly by the kidneys and, as it is a weak base, its elimination is intensified with the decrease in urinary pH (Rang, 2001).

SULFADOXINE-TRIMETHOPRIM COMBINATION

Sulfadoxine in combination with trimethoprim are commonly used for broad-spectrum antimicrobial therapy in veterinary medicine. Although widespread resistance limits use in ruminants, the combination is still useful in the treatment of gastrointestinal and respiratory infections in calves, including neonatal colibacillosis. The pharmacokinetic profile of sulfadoxine and trimethoprim differs from each other due to their chemical properties: sulfadoxine acts as a weak acid while trimethoprim is a weak base, thus concentrating at different sites in the body. (Kaartinen, 2000; Golan, 2009).

Sulfadoxine is a sulfonamide consisting of pyrimidine with methoxy substituents at positions 5 and 6 and a 4-aminobenzene sulfonamide group at position 4. Trimethoprim is an aminopyrimidine antibiotic whose structure consists of pyrimidine 2,4-diamine and 1,2,3-trimethoxybenzene linked by a methylene bridge. It functions as an EC 1.5.1.3 (dihydrofolate reductase) inhibitor, a xenobiotic, an environmental contaminant, a drug allergen, an antibacterial drug, and a diuretic. It is a member of methoxybenzenes and an aminopyrimidine. Figure 1 shows the two-dimensional (2D) chemical structure of Sulfadoxine and Trimethoprim.

Figure 2 - Two-dimensional (2D) chemical structure of the active ingredients.

Active ingredient	Two-dimensional (2D) drawing
Sulfadoxine	
Trimethoprim	

Source: ChemSketch® Freeware version 2021.

ENVIRONMENTAL IMPACTS OF ANTIBIOTIC CONTAMINATION

The disposal of expired drugs is a significant public health issue, as these drugs can be considered toxic waste based on their chemical composition. Due to their potential for

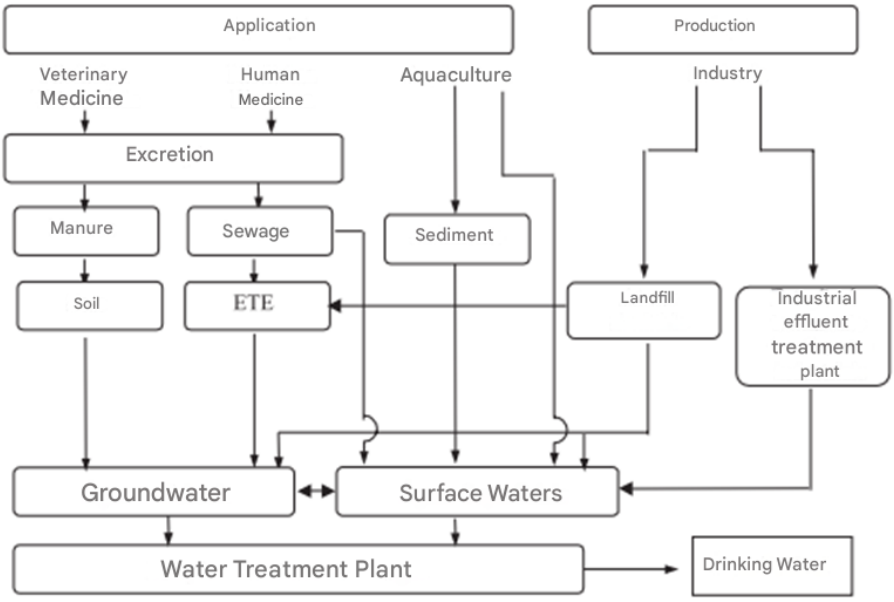
environmental contamination, which varies according to the level of toxicity, they should not be treated in the same way as common waste (Tannus, 2017).

Studies have revealed the presence of medicines, cosmetics and personal hygiene products in surface and groundwater, as well as in drinking water and even in soils that receive the application of sewage sludge. These compounds, when present in soil and water, can cause contamination, which even in a sewage treatment network is not completely removed (Tannus, 2017).

The main forms of environmental contamination by drugs occur through their intentional use, when they are eliminated by the body after oral consumption, injection or infusion; by the removal of topical medications during bathing, and by the improper disposal of expired or no longer unwanted medications, either in the sewer or in the garbage (Carvalho et al., 2009).

Although the high consumption of antibiotics and their presence in surface waters are evident, it is not yet clear whether the contamination originates from effluents or from animal production residues. The current legislation does not include pharmaceutical products in lists of priority compounds. However, as knowledge about the occurrence, toxicity, and efficacy of antibiotic elimination methods increases, it is expected that it will be possible to establish legal limits for these compounds. As for the routes of contamination of antibiotics in the aquatic environment, there are several ways, the main ones being through excreta and effluents resulting from industrial processes. In addition, in rural areas, where there is often no adequate sanitation system and excreta is disposed of in septic tanks, cross-contamination of soils also occurs, which in turn affects surface waters (Homem, 2011).

Figure 3: Routes of contamination of veterinary and human drug residues.



Source: Bila & Dezotti, 2003.

CHEMOINFORMATICS

Chemoinformatics is a multidisciplinary area capable of providing relevant advances, especially in Medicinal Chemistry, through the use of computer science resources to predict various molecular descriptors (Araújo; Dick; Motta, 2022). The term chemoinformatics was coined by Frank Brown in 1998, defining it as "a mixture of information resources to transform data into information and information into knowledge, in order to make better and faster decisions in the area of identification and optimization of leading compounds".

The field of chemoinformatics has developed significantly, moving from practical and technical approaches to the representation, manipulation and processing of individual chemical structures to its central function today: the exploration of chemical databases and the discovery of new compounds with desired activities or properties. Through the exploration of these databases, it is possible to obtain various information that helps to understand the behavior of certain groups of compounds and create computational models. These models are used to predict the activity of molecules that do not yet have experimental data, such as results from *in vitro* and *in vivo assays* (Alves *et al.,* 2018).

It is crucial to note that chemoinformatics is deeply interconnected and often overlaps with other areas of chemistry that use computational technology. This includes computational chemistry, which employs theoretical methods to calculate molecular properties; molecular modeling, which uses three-dimensional graphs and optimization techniques to analyze the nature and action of chemical compounds and proteins; and computer-aided drug planning, which focuses on the application of computational

techniques to assist in the discovery and development of new bioactive molecules (Alves *et al.*, 2018).

Although a variety of high-throughput *in vitro* screening methods are widely used, it is still challenging to obtain data on ADMETOX from the compounds (HOU & WANG, 2008). Deficiencies in pharmacokinetic studies and drug metabolization are the main causes of failures in the clinical phase of drug development. Studies conducted by *in silico methods* show that the prediction of absorption, distribution, metabolization, excretion and toxicity (ADMETOX) profiles plays a crucial role in the discovery of new drugs (Yang et al., 2018).

The purpose of this research is to use chemoinformatics methods in order to investigate the *in silico* human and environmental toxicological profile of the antibiotics Trimethoprim and Sulfadoxine.

MATERIALS AND METHODS

The calculations, simulations, computational modeling and prediction of molecular descriptors will be carried out at the Laboratory of Medicinal Chemistry and Advanced Computational Technologies at the Montes Claros campus of the Federal Institute of Northern Minas Gerais (IFNMG).

COMPUTATIONAL MOLECULAR MODELING

Initially, the chemical structure of the antibiotics Sulfadoxine and Trimethoprim were drawn two-dimensionally (2D) and visualized three-dimensionally (3D) using the ACD/ChemSketch® Freeware computer program version 2021 (Advanced Chemistry Development, Inc., 2021). All chemical structures were saved in a file of the type MDL molfiles (.mol).

IN SILICO ENVIRONMENTAL TOXICOLOGICAL PREDICTION FOR THE ANTIBIOTICS SULFADOXINE AND TRIMETHOPRIM

The present study was carried out with the help of the Chinese international platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/) coordinated by Professor Yun Tang, Leader of the Molecular Modeling and Design Laboratory (LMMD), School of Pharmacy of the East China University of Science and Technology (YANG, et al., 2018).

IN SILICO HUMAN TOXICOLOGICAL PREDICTION FOR THE ANTIBIOTICS SULFADOXINE AND TRIMETHOPRIM

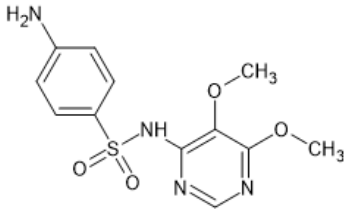
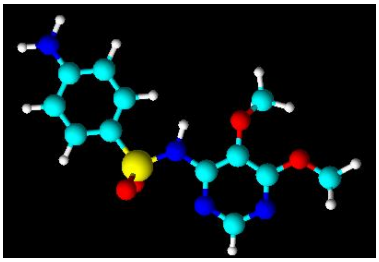
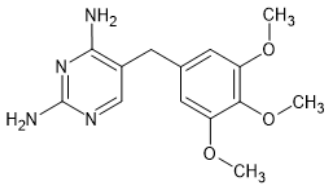
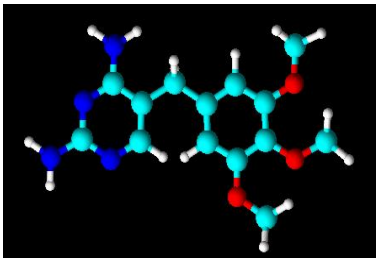
The study was carried out with the help of the Chinese international online platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), coordinated by Professor Yun Tang, Leader of the Molecular Modeling and Design Laboratory (LMMD), School of Pharmacy of the East China University of Science and Technology (YANG, et al., 2018).

RESULTS AND DISCUSSIONS

COMPUTATIONAL MOLECULAR MODELING

In medicinal chemistry, complete knowledge of molecular structure is essential. Therefore, computational modeling allows the visualization of the chemical structures of the molecules under study from a set of tools for the construction, editing and visualization, analysis and storage of complex molecular systems (Barreiro; Rodrigues, 1996). After performing the molecular modeling step (2D Drawing and 3D Drawing) for the chemical structure of Sulfadoxine and Trimethoprim, all structures were saved in a molfiles (.mol) type file. Table 1 shows the 2D and 3D drawings for the antibiotics Sulfadoxine and Trimethoprim.

Table 1: Drawings of the two-dimensional (2D) and three-dimensional (3D) structure of sulfadoxine and trimethoprim.

Compound	Two-Dimensional Design (2D)	Three-dimensional drawing (3D)
Sulfadoxine		
Trimethoprim		

Source: ChemSketch® Freeware version 2021.

IN *SILICO* ENVIRONMENTAL TOXICOLOGICAL STUDY

The evaluation of the environmental toxicity of antibiotics using *in silico* methods is of great importance for the rational planning of new drugs, as it allows predicting the impact

of antibiotics on ecosystems. In this study, parameters such as environmental biodegradation capacity, toxicity in fish, toxicity in *Tetrahymena Pyriformis* and toxicity in bees were analyzed. The preliminary results of these evaluations for the active ingredients Sulfadoxine and Trimethoprim are presented in Table 2.

Table 2: Evaluation of the in *silico* environmental toxicological profile.

Antibiotic	Environmental Biodegradation		Toxicity in Fish		Toxicidade em *Tetrahymena Pyriformis*		Toxicity in Bees	
	Q	P	Q	P	Q	P	Q	P
Sulfadoxine	N	99,6%	N	82,3%	N	64,0%	N	83,31%
Trimethoprim	N	99,49%	N	94,48%	P	89,18%	N	66,16%

Source: admetSAR® version 2.0
Q: Qualitative - N: Negative; P: Positive.
Q: Probability

The results obtained for the evaluation of the *in silico* environmental toxicological profile of sulfadoxine shown in table 2 reveal that Sulfadoxine is not toxic to fish, *Tetrahymena Pyriformis* and bees, but it is not biodegradable in the environment, which indicates that it contaminates the food chain, and also humans, since they consume contaminated food. By analyzing the results obtained for Trimetoprima, it can be analyzed that it has toxicity in *Tetrahymena Pyriformis*, in addition to not being biodegradable in the environment. It has no toxicity for bees or fish.

IN SILICO HUMAN TOXICOLOGICAL STUDY

The in silico *toxicology study* in humans for Sulfadoxine and Trimethoprim was conducted with the objective of predicting the toxicity of the compounds, according to the AMES test (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; CN: non-carcinogenic) and acute oral toxicity, which was classified into categories I, II, III and IV.

To evaluate Acute Oral Toxicity, the derivatives were classified into four categories. Category I (high toxicity) includes compounds with LD50 (Lethal Dose in mg of derivative per kg of body weight) values less than or equal to 50 mg/kg. Category II (moderate toxicity) covers compounds with an LD50 between 50 mg/kg and 500 mg/kg. Category III (low toxicity) refers to compounds with LD50 between 500 mg/kg and 5,000 mg/kg, while category IV (non-toxic) encompasses compounds with LD50 greater than 5,000 mg/kg. Table 3 presents important information on the evaluation of the human toxicological profile *in silico.*

Table 3: Evaluation of the human in *silico* toxicological profile.

Antibiotic	Tested by AMES		Acute Oral Toxicity		Carcinogenic	
	Q	P	C	P	Q	P
Sulfadoxine	NT	64,56%	III	57,64%	NC	54,73%
Trimethoprim	NT	82,27%	IV	62,35%	NC	93,69%

Source: admetSAR®. Version 2.0

The analysis of Table 3 indicates that both Sulfadoxine and Trimethoprim do not demonstrate toxicity in relation to the human toxicity profile, as assessed by the AMES test. In addition, these compounds are not carcinogenic, and as far as Acute Oral Toxicity is concerned, they are classified in categories III and IV, which means they have no toxicity.

CONCLUSION

Sulfadoxine and Trimethoprim were chosen for evaluation because they are antibiotics that are known about their pharmacokinetics and toxicity. In the present study, *in silico* methodologies were used in order to predict molecular properties for the molecules of the antibiotics Sulfadoxine and Trimethoprim.

The in silico environmental *toxicological study* indicates that Sulfadoxine has no toxicity for bees, fish and *Tetrahymena Pyriformis*, but its chemical structure is not biodegradable in the environment, that is, in addition to contaminating the aquatic environment and fish, the chemical structure of the antibiotic remains unchanged in the environment because it does not suffer environmental biodegradation, contaminating the food chain and the human being through food. For Trimethoprima, the environmental in *silico* toxicological study indicates that the antibiotic is not toxic to bees or fish, but it is toxic to *Tetrahymena Pyriformis,* in addition to not being biodegradable in the environment, contaminating the food chain and humans through food.

The human in silico *toxicological study* showed promising data for Sulfadoxine, since the antibiotic is non-toxic (non-mutagenic) as far as the AMES test is concerned, it does not present carcinogenicity and as for acute oral toxicity it falls into category III, that is, it has low toxicity. Promising results were also obtained for Trimethoprim, since the antibiotic is non-toxic as far as the AMES test is concerned, it does not present carcinogenicity and for acute oral toxicity it falls into category IV, that is, it is considered non-toxic.

Finally, it is worth mentioning that the studies carried out so far reveal that the antibiotics Sulfadoxine and Trimethoprim promote environmental and human contamination,

so it is essential to continue the studies for antibiotics, to evaluate the other impacts on humans and the environment.

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