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IN SILICO HUMAN AND ENVIRONMENTAL TOXICOLOGICAL EVALUATION FOR THE ANTIBIOTIC ENROFLOXACIN

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ABSTRACT

Enrofloxacin is an effective veterinary antibiotic in the treatment of bacterial infections in production animals, such as cattle, pigs and poultry, due to its broad spectrum of action against Gram-negative and Gram-positive bacteria. This drug, belonging to the class of fluoroquinolones, is synthesized exclusively for veterinary use and acts by inhibiting DNA-gyrase, an enzyme essential for the replication of bacterial DNA. However, the use of Enrofloxacin presents challenges, such as the generation of waste that contaminates the environment, affecting both soils and waters. In addition, these residues can enter the food chain, contaminating humans. The absence of specific studies on the pharmacokinetics and toxicity of Enrofloxacin, both in humans and in the environment, exacerbates these challenges. For this study, computer modeling of the two-dimensional (2D) chemical structure of Enrofloxacin was performed, followed by three-dimensional (3D) visualization. Subsequently, in silico environmental and human toxicological evaluations were conducted in order to determine the possible toxic impacts of the antibiotic. Enrofloxacin has been observed to contribute to environmental and human contamination, highlighting the importance of conducting additional studies to assess its impacts on humans and the environment.

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INTRODUCTION

The increase in population and the expansion of industrial activity have resulted in high levels of air, soil and water contamination in various parts of the world. In view of this, the growing concern with the environment, which, although it has existed for decades, has never been as evident as it is today (Melo et al., 2009).

In this race for sustainability in line with the Declaration of Ethical Principles, the duty to minimize and predict negative impacts for present and future generations imposes on the engineering class, the science of its responsibility and social visibility, a central role in the development of new technologies and more efficient and integrated processes (Oliveira, 2022).

The engineering challenge with wastewater is a critical issue in environmental preservation and industrial sustainability. Wastewater, which is liquid or gaseous by-products generated by industrial processes, can contain a wide range of contaminants, including the presence of pharmaceuticals. Quantities of antibiotics have been detected in sewage, soil, surface and groundwater in various regions of the world (Frade, 2013).

This has resulted in increasingly stringent standards and legislation, and to this end, they are being implemented to reduce the environmental impact caused by these effluents (Nogueira and Jardim, 1998). In Brazil, public agencies dedicated to the environment have been mobilizing to receive proposals for a sectoral agreement in order to implement a reverse logistics system for drug waste, since 2014 (Brasil, 2024).

These policies are essential in the Brazilian context, considering that it is estimated that the pharmaceutical industry in the country includes about 600 companies, including laboratories, importers, and distributors. According to the IBGE Annual Industrial Survey (PIA-IBGE), in 2010, the pharmaceutical sector was composed of 44 companies in the pharmochemical segment and 500 pharmaceutical laboratories (Brasil, 2024).

More than that, it is worth remembering the importance that human and veterinary drugs play a crucial role in society, being fundamental for improving quality of life and increasing longevity. They are essential for most therapeutic plans, making them an indispensable resource in modern medicine (Bertoldi, 2016).

A wide range of drugs, belonging to various pharmacological categories, are consumed annually around the world. These pharmaceutical compounds include antipyretics, analgesics, lipid regulators, antibiotics, antidepressants, chemotherapeutic agents, contraceptives, in addition to veterinary drugs (Tambosi, 2008).

In this sense, the effluents where drugs are improperly disposed of, come from both consumption, where a significant part of the active ingredient is excreted by the human

body into domestic sewage, and from hospital, industrial and domestic environments, being one of the main causes of the presence of these residues in water bodies (Gil and Mathias, 2005).

For effluents containing such contaminants, more effective treatment would be required. However, this does not always happen, since antibiotics are not removed in Sewage Treatment Plants (ETEs), allowing them to reach the environment (Kümmerer et al., 2010; Bila and Dezotti, 2003; Kümmerer, 2009; Pereira et al., 2012).

When veterinary and human antibiotic residues pollute the environment, the food chain is also affected. Another challenge lies in the fact that, when entering the food chain, these residues also contaminate humans, and there are no *in silico* pharmacokinetic and human and environmental toxicity studies specific to the antibiotic in silico. Research and Development (R&D) is an excellent research "tool" for engineering, as it drives innovation and faces technical and environmental challenges effectively, related to effluent treatment and aligned with Chemoinformatics becomes fundamental to research using *in silico methodologies* (Motta, 2023).

OBJECTIVES

The purpose of this research is to carry out through Chemoinformatics the in *silico* methodologies, in order to obtain the prediction *of in silico* Human Pharmacokinetics , in addition to carrying out the Environmental *and Human in silico* Toxicological study for the antibiotic Enrofloxacin.

LITERATURE REVIEW

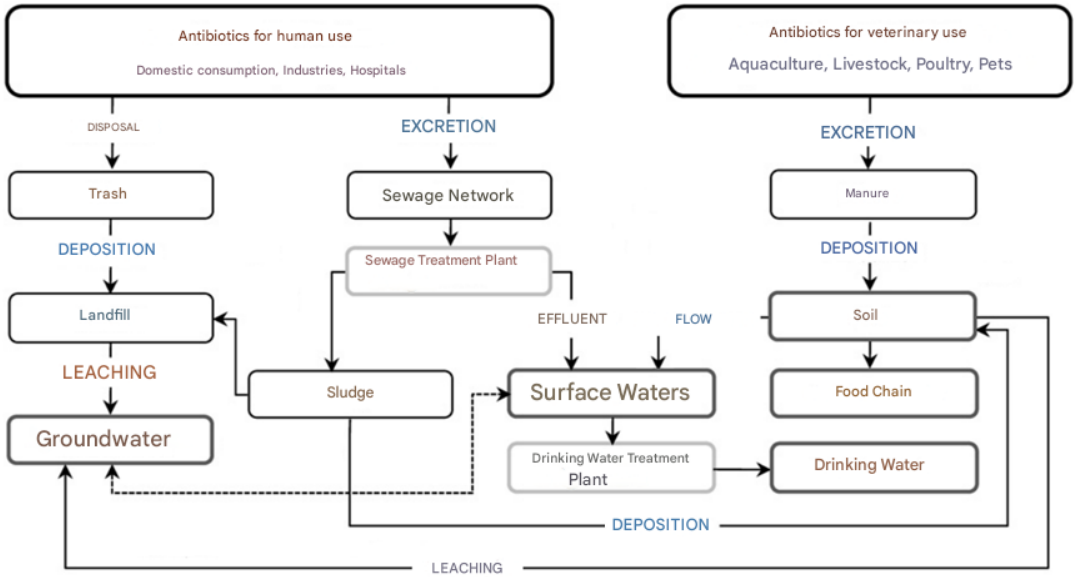
Antibiotics, which are fundamental in combating bacterial infections and reducing morbidity and mortality, represent a global threat to environmental and human health due to their increasing use, driven by population growth (Guimarães et al., 2010).

Considering that they are considered "dangerous" due to their pharmacological activity, which results in resistant metabolization, bioaccumulation, biomagnification and wide environmental presence. In addition, their waste is released into the environment in large quantities without proper treatment (Montagnera et al., 2017; Fent et al., 2006).

Inevitably, small amounts of antibiotics have been detected in sewage, soil, surface and groundwater, in various parts of the world, such as Germany, Brazil, Canada, Holland, England, Italy, Sweden, United States, United Kingdom (Frade, 2013). In this way, it contaminates the ecosystem by some routes through which antibiotics can enter, as illustrated in Figure 1. Antibiotics intended for human and veterinary use can enter the

environment in two main ways: through excretion after being ingested, since the organism has limited capacity to absorb the compounds, or through the incorrect disposal of drugs (Pena et al., 2007; Guinea et al., 2009).

Figure 1: Route of entry of drugs into the environment



Source: Homem e Santos, 2011

In general, effluents are the main sources of contamination of water resources with drugs (Mirzaei et al., 2017). Because the discarded medicines reach the ETE's (Sewage Treatment Station), which do not have treatments capable of decomposing or inactivating the existing drugs in the effluent, or even can be directly released into the beds of streams, rivers and lagoons. The aquatic environment of the watercourses is captured by drinking water treatment plants, which will be distributed for human consumption. The effluent from a WWTP can still contaminate groundwater (Balcioglu and Ötker, 2004; Tambosi, 2008; Homem e Santos, 2011; Bila, 2005).

What is worrying is that antibiotics are formulated to fight bacteria, generally have low biodegradability, and remain unchanged after this type of treatment. Several types of medicines, such as fluoroquinolones, have a low affinity for water and remain preferentially adsorbed in the soil or sludge, coming from the WWTP's generated by biological digestion and being used as fertilizer can be deposited in the soil. When the drug remains in the soil, it can be transferred to the plants, affecting the base of the food chain, and the human being will be ingesting small amounts of drugs without knowledge (Ikehata Et Al., 2006; Kummerer, 2009; Homem e Santos, 2011).

It is known that drugs play an essential role, but when administered in inappropriate doses or prescriptions, they can be harmful to both humans and animals (Kümmerer, 2010; Verlicchi, 2012). According to Barceló (2003), drugs do not need to persist in the

environment resulting in negative effects. Therefore, due to the possibility of contamination of the environment and human beings, drugs fall into a group of substances known as emerging micropollutants, which are found in environmental and biological matrices in very low concentrations, but potentially capable of causing adverse ecological effects and/or on human health (Silva, 2011; Aquino, et al, 2013; Yes, 2010).

What's more, antibiotic resistance is exacerbated by the administration of antibiotics to animals, the residues of which are transferred to humans through the food chain. This process represents a serious public health problem (Costa, 2014).

ECONOMIC ACTIVITIES

The presence of antibiotics in the environment can be attributed to the extensive use of these drugs in agribusiness, which was enhanced with the advent of globalization. In the context of Brazilian agribusiness, especially the livestock sector, it has experienced unprecedented growth driven by technological advances. The intensification of genetic research, improved pest control, and the effectiveness of drugs in the treatment of animal diseases have contributed significantly to this expansion (Castro, 2023).

Data from Embrapa (2022) show that, since the 80s, poultry meat production has increased 22 times, while pork, milk, and beef have also shown significant growth. Annual milk production, for example, has doubled since 2002 due to herd expansion and improved cow productivity.

Simultaneously, the pet market in Brazil, which moved more than 40 billion reais in 2022, has followed this growth trend, along with the technology behind veterinary medicine, providing resources capable of maximizing the prevention, treatment of acute and chronic diseases effectively (Filho, et al., 2024).

Pet owners are increasingly investing in products and services for their pets, which reflects a continuous increase in the demand for quality care and products (Oliveira, 2022). According to ABINPET (Brazilian Association of the Pet Products Industry), the sector recorded an increase in sales and job creation, even in the midst of the economic crisis caused by the COVID-19 pandemic.

However, the growth of the livestock and pet market has significant implications for public and environmental health. The intensive use of antibiotics for treatment as well as growth promoters can result in residues of these drugs in animal products (De Souza, 2013).

As a result, it is necessary to have current regulations, such as the regulations of MAPA (Ministry of Agriculture and Livestock), which aims to control the use of antibiotics in

animal feed, restricting the use of certain drugs to avoid unwanted effects. Careful management of antibiotic use is crucial to ensure animal and human health, maintaining the effectiveness of these drugs and minimizing the risks associated with antibiotic resistance (Gonçalves, 2011).

The inspection is the responsibility of the MAPA agency, however, it is not new that there are debates and questions about the possible impacts on the effectiveness of environmental management and the protection of natural resources. Since many environmental experts, environmentalists and Non-Governmental Organizations (NGOs) have been questioning the fact that the ministry, focused on the promotion of agriculture and livestock, raises concerns about a possible conflict of interest, since the ministry's main mission is to promote agribusiness, which could compromise impartiality in the inspection of environmental issues, presenting a biased inspection (Rodrigues, 2024).

ENROFLOXACINA

The beginning of the use of quinolones occurred in 1962, when Lesher and collaborators described nalidixic acid, which was the precursor of 4-quinolones. However, enthusiasm for these chemotherapeutic agents waned rapidly due to their limited spectrum of antibacterial activity, the rapid emergence of resistance and the associated pharmacokinetic limitations (Bergan, 1988).

Changes in the structure of the chemical groups associated with the fundamental core of 4-quinolone resulted in significant changes in the antimicrobial activity, pharmacokinetic properties, and toxicity of quinolones. Fluoroquinolones, considered as an evolution of the older quinolones, have superior pharmacokinetic characteristics, which makes them an effective choice in the treatment of bacterial infections (Machado, 2009).

Newer fluoroquinolones have a broader spectrum of activity compared to older quinolones. In general terms, they demonstrate high efficacy against aerobic Gram-negative pathogens and have an intermediate action on Gram-positive cocci. However, quinolones, in general, are less effective against staphylococci and streptococci than against Gram-negative bacteria. (Wolfson & Hooper, 1985).

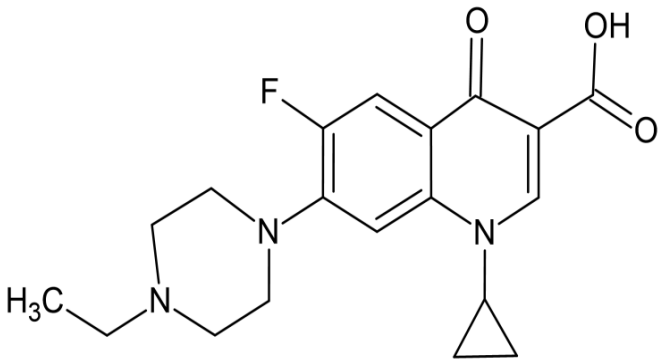
One of the most notable pharmacokinetic characteristics of fluoroquinolones is their wide volume of distribution, which includes tissues such as the central nervous system, bones, prostate, kidneys, liver, female genital tract, and inflammatory fluids. In addition, these drugs have a low plasma protein binding affinity. As a result, the concentration of free fluoroquinolones in serum generally reflects well the concentrations in extracellular fluids, where most infections occur. This wide distribution, combined with their extensive spectrum

of activity, makes fluoroquinolones a preferred choice for the treatment of deep infections of the dermis and pyodermas. (Cavalcante, 2009).

Cyclopropyl acid-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-carboxylic quinoline), enrofloxacin, shown in Figure 2, is a synthetic antimicrobial of the second generation of fluoroquinolones, being used exclusively in veterinary medicine. This drug acts by inhibiting the bacterial DNA gyrase enzyme, has a broad spectrum of action, and is indicated to treat a variety of infections caused by gram-positive and gram-negative bacteria, mycoplasmas, and spirochetes (Frade, 2013).

From the two-dimensional drawing executed in the ChemSketch® Freeware program, version 2021, it can be stated that the molecule of this drug has the following percentage by mass: 63.50% carbon (C), 6.17% hydrogen (H), 5.29% fluorine (F), 11.69% nitrogen (N) and 13.35% oxygen (O). In addition, it has a density of 1.385 ± 0.06 $^{g.cm-3}$ and polarizability of 36.78 ± 0.5 10-24 $^{cm-3}$. Such information is relevant, since it provides additional information in the study of chemical properties, such as its mass per unit volume and its polarization capacity, which can affect the interactions of antibiotic molecules with other substances and their behavior in different environments.

Figure 2: Two-dimensional (2D) drawing of the antibiotic enrofloxacin (C19H22FN3O3).



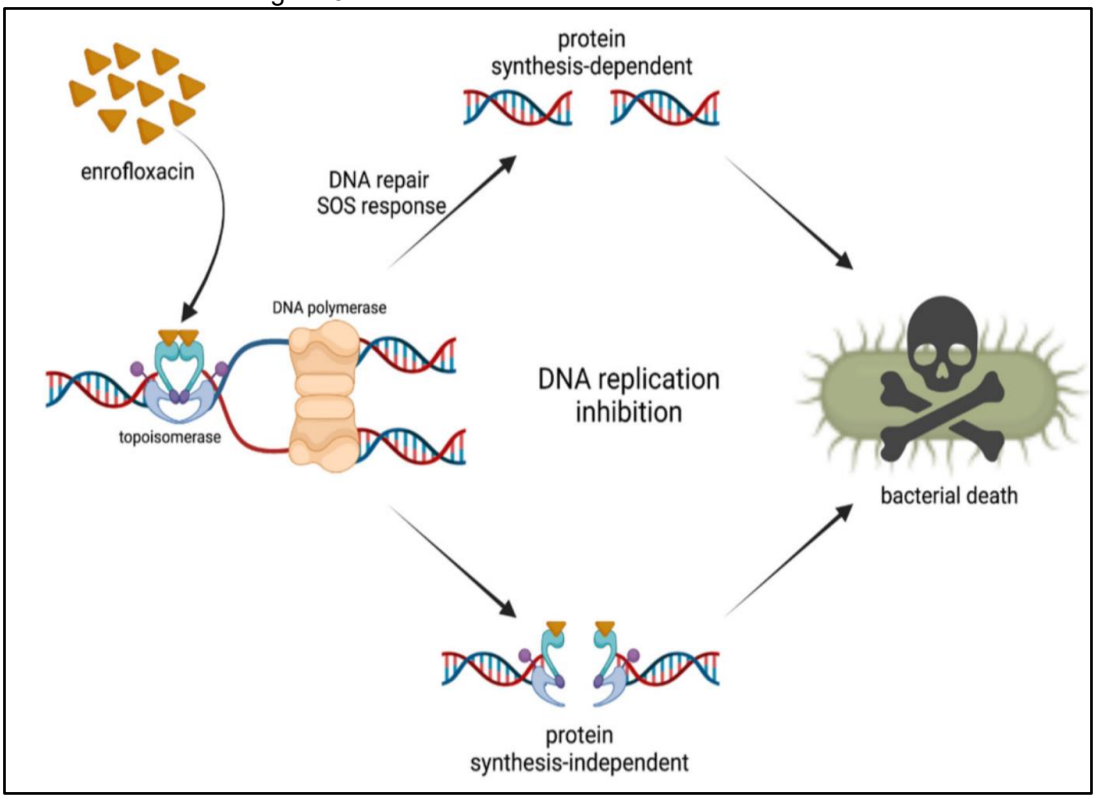
Source: ChemSketch® Freeware version 2021.

Fluoroquinolones are partially biotransformed, being excreted in the urine and bile in high concentrations as an active substance (Górniak, 2006). All fluoroquinolones, including enrofloxacin, are bactericidal and act on the same bacterial enzyme, DNA gyrase (topoisomerase type II) (Vancutsem et al., 1990). Topoisomerases play an essential role in directing and extending the spiraling of DNA strands, facilitating the separation, cutting, and unwinding of DNA (Górniak, 2006).

Enrofloxacin binds to the DNA-topoisomerase complex (figure 3) at the cleavage-binding site, inducing conformational changes in the enzyme. This results in the inhibition of DNA replication, since DNA cleavage is not effectively followed by its binding. The generated DNA fragments are eventually eliminated by bacterial exonucleases, leading to

cell death or activation of DNA repair mechanisms and the S.O.S. response (main paradigm of cellular response to DNA damage in prokaryotes), depending on the drug concentration as indicated in figure 3 (Kohanski et al., 2010).

Figure 3: The mechanism of action of enrofloxacin.



Source: Kohanski et al., 2010.

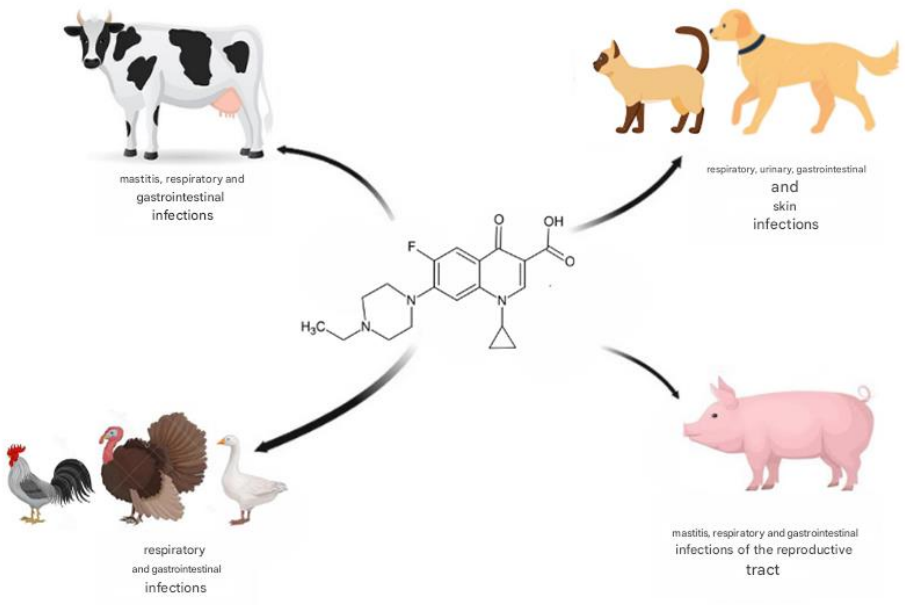
ENROFLOXACIN DISEASES

Enrofloxacin is a broad-spectrum antibiotic used in the treatment of various bacterial infections in animals, such as cattle, poultry, pigs, dogs, and cats. Among the conditions treated with this drug are mastitis, an inflammation of the mammary glands; gastrointestinal infections, which can affect the digestive tract; respiratory infections; and skin diseases.

Mastitis

Mastitis, an inflammation of the mammary gland caused mainly by bacterial infections, is one of the main pathologies in herds in Brazil, especially the pathogens *Staphylococcus aureus* and *Streptococcus agalactiae* due to their high incidence and resistance. In addition to compromising the sustainability of production, mastitis generates significant economic losses, such as reduced milk production, drug costs, and, in extreme cases, the loss of the animal, reinforcing the importance of effective control and proper use of antimicrobials in treatment (Castro, 2023).

Figure 4: Diseases in animals that can be treated with the use of Enrofloxacin.



Source: Author, 2024

Respiratory infections

Respiratory diseases in animals, as well as in humans, represent a significant challenge to veterinary and animal public health. These diseases can be caused by a wide range of pathogens, including bacteria, viruses, and fungi, which affect the respiratory tract of several species. The transmission of these diseases can occur through direct contact, aerosols, or fomites, and symptoms vary according to the causative agent and the species affected, and may include coughing, sneezing, nasal discharge, and, in severe cases, pneumonia and difficulty breathing (Teixeira, 2020).

Gastrointestinal Infections

Gastrointestinal diseases in animals are a significant concern in both production systems and companion animals, affecting several species, such as cattle, pigs, cats, dogs and birds. These diseases can be caused by a variety of etiological agents, including bacteria, viruses, parasites, and eating problems. One of the most common gastrointestinal infections is caused by bacteria of the genus *Campylobacter spp.*, known as campylobacteriosis. These infections are one of the leading causes of foodborne illness (DTA) in humans (Martins, 2023).

CHEMOINFORMATICS

Chemoinformatics integrates computer science and medicinal chemistry to predict and analyze molecular descriptors, facilitating the development of new drugs and

optimization of compounds (Motta, 2023). Through computational models and advanced algorithms, chemoinformatics facilitates the understanding of molecular interactions and the optimization of compounds for the development of new drugs, accelerating the discovery and improvement of therapeutic agents. This approach has been instrumental in advancing research and development in the field of medicinal chemistry (Motta, 2023).

In *silico* methods, widely used in pharmacodynamics, pharmacokinetics, and toxicology, offer advantages such as cost reduction and elimination of the use of animals. In toxicology, *in silico* methods have gained prominence in the pharmaceutical industry due to their proven efficacy in predicting potential toxic effects (Caiana, 2019). Chemoinformatics also contributes to controlling the environmental impact of antibiotics, helping to mitigate bacterial resistance and promoting safer alternatives (Yuan et al., 2011; Fink et al., 2012; Castro, 2023).

MATERIALS AND METHODS

The calculations, simulations, computer modeling and predictions of molecular descriptors will be carried out at the facilities, more specifically at the Laboratory of Medicinal Chemistry and Advanced Technologies on the Montes Claros campus of the Federal Institute of Northern Minas Gerais (IFNMG).

COMPUTATIONAL MOLECULAR MODELING

This phase was initially involved with the molecular modeling of the chemical structure of enrofloxacin, with the execution of its two-dimensional (2D) and three-dimensional (3D) representations, which should be saved in MDL molfiles (.mol). Then, after generating the SMILES code, this algorithm will be used to conduct the toxicological and pharmacokinetic studies *in silico* of the drug. The following computer program will be used: ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021).

IN SILICO ENVIRONMENTAL TOXICOLOGICAL PREDICTION FOR THE ANTIBIOTIC ENROFLOXACIN

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).

HUMAN IN *SILICO* TOXICOLOGICAL PREDICTION FOR THE ANTIBIOTIC ENROFLOXACIN

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).

EXPECTED OUTCOMES

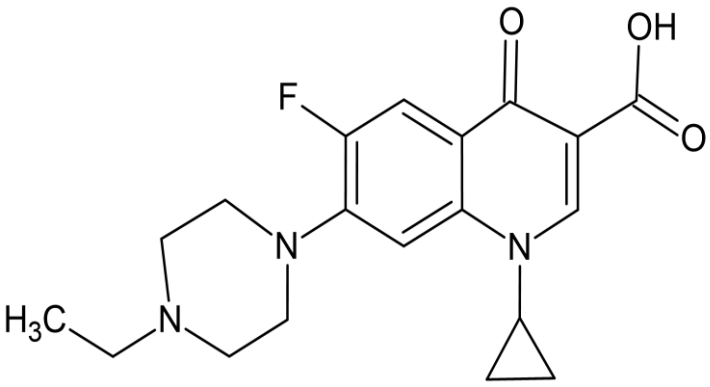
MOLECULAR MODELING

The study of antibiotics requires a deep understanding of medicinal chemistry, where precision in molecular design is key. Justifying the use of the American software program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021), which offers significant benefits in this process, as it allows the visualization of chemical structures in a detailed and intuitive way (Flores and Mól, 2006).

With their tools, researchers can optimize molecular design, predict physicochemical properties, and facilitate the visual communication of complex chemical structures (Batista, 2016). It also allows the two-dimensional and three-dimensional visualization of molecular structures, enabling a better understanding of the models used to represent atomic arrangements and processes related to chemical phenomena (Gilbert, et al., 2000).

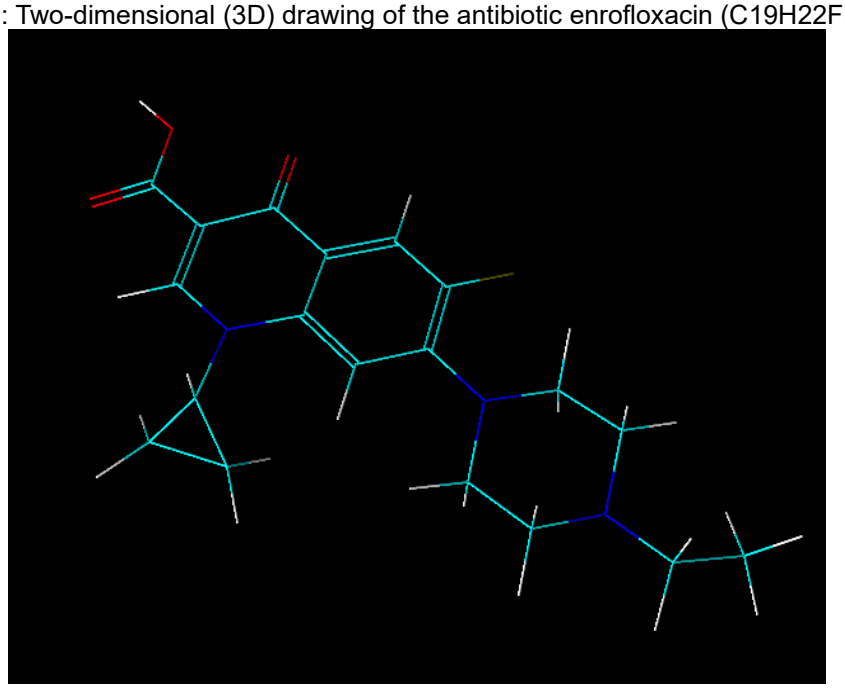
After completing the Molecular Modeling stage, which included the 2D Drawing and 3D Drawing, of the chemical structure of Enrofloxacin, all representations (2D and 3D) were saved in MDL molfiles (.mol). Figures 5 and 6 show, respectively, the two-dimensional (2D) and three-dimensional (3D) chemical structures of the antibiotic Enrofloxacin. By generating the SMILES code: OC(=O)C1=CN(c2cc(N3CCN(CC3)CC)c(F)cc2C1=O)C1CC1, the algorithm allowed the toxicological and pharmacokinetic study of the drug under analysis to be carried out.

Figure 5: Two-dimensional (2D) drawing of the antibiotic Enrofloxacin (C19H22FN3O3).



Source: ChemSketch® Freeware version 2021.

Figure 6: Two-dimensional (3D) drawing of the antibiotic enrofloxacin (C19H22FN3O3).



Source: ChemSketch® Freeware version 2021.

IN *SILICO* ENVIRONMENTAL TOXICOLOGICAL STUDY

The study of environmental in *silico* toxicity is a computational approach used to evaluate the potential toxicity of chemical substances in the environment. From the algorithm obtained, it is possible to predict how a substance can interact with the environment and with the organisms that make up the ecosystem, in addition to collaborating for the rational planning of new drugs (Moschem, 2022), providing existing data on the chemical structure of substances and their behavior under different environmental conditions, predicting the impact of new compounds before their release into the environment, identifying potential risks to the health of aquatic and terrestrial organisms, and to ecosystems in general (Moschem, 2022).

In this study, the following parameters were evaluated: biodegradation capacity in the environment, toxicity in bees, aquatic toxicity in *Tetrahymena pyriformis* and aquatic toxicity in fish. These results were expressed in Table 1, with qualitative data [(Q): positive (P) or negative (N), and quantitative data regarding probability (P).

Table 1: Evaluation of the environmental in *silico* toxicological profile.

Environmental Biodegradation		Toxicity in Fish		Toxicidade em *Tetrahymena Pyriformis*		Toxicity in Bees	
Q	P	Q	P	Q	P	Q	P
N	100,00%	P	99,79%	P	95,94%	N	89,59%

Source: admetSAR®, version 2.0

The evaluation of environmental toxicity, as shown in Table 1, revealed that the antibiotic Enrofloxacin is not toxic to bees, but is toxic to fish and to the single-celled organism *Tetrahymena pyriformis*. In addition, the chemical structure of the antibiotic does not undergo biodegradation in the environment. This means that, in addition to polluting aquatic ecosystems and affecting fish and *Tetrahymena pyriformis*, Enrofloxacin remains unchanged in the environment, contaminating the food chain and potentially humans through contaminated food.

IN SILICO HUMAN TOXICOLOGICAL STUDY

The in silico *toxicological study* focused on the human organism was conducted with the objective of predicting the toxicity of the antibiotic. For this, three fundamental tests were considered. The first was the Mutagenicity Test (AMES test), where the results are classified as Toxic (T) or Non-Toxic (NT). The second test was characterized by Carcinogenicity with the classifications Carcinogenic (C) or Non-Carcinogenic (NC). And, finally, the Acute Oral Toxicity test was carried out, classifying it into four categories (I, II, III, and IV) based on the severity of the effects observed (Fonseca, 2023).

These tests are essential to predict possible risks to human health, offering a detailed analysis of the safety of the antibiotic in question, before its application in clinical contexts. The use of *in silico approaches* is particularly valuable, as it allows for a quick and effective initial assessment, saving time and resources compared to traditional methods (Fonseca, 2023).

The AMES test is highly relevant, as it is able to identify mutations in the genetic material that participates in the synthesis of the amino acid histidine (Kauffmann et al.; 2020). The AMES test is a bacterial assay that investigates the mutagenicity of chemical compounds using the *Salmonella typhimurium* strains TA100 and TA1535 (Miranda et al., 2021).

The evaluation of the Acute Oral Toxicity of analogues, taking into account the classification of the United States Environmental Protection Agency (EPA), categorizes the chemical compounds based on LD50 (median lethal dose) and are described in table 2 below:

Table 2: Antibiotic classification, according to the United States Environmental Protection Agency.

Category	DL50
I	less than or equal to 50 mg/kg
II	greater than 50 mg/kg and less than 500 mg/kg
III	greater than 500 mg/kg and less than 5,000 mg/kg
IV	superior a 5.000 mg/kg

Source: EPA-Environmental Protection Agency: https: (www.epa.gov)

Compounds that fall into categories I and II are considered the most toxic. Category I covers those with high toxicity, posing a significant health risk, even at low doses. Category II compounds, on the other hand, have moderate toxicity, offering a lower risk compared to category I, but still require caution due to their harmful potential (Cheng, et al., 2012). Table 3 provides relevant information regarding the evaluation of the human in *silico* toxicological profile .

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

AMES Test		Acute Oral Toxicity		Carcinogenic	
Q	P	C	P	Q	P
T	91,07%	III	80,00%	NC	86,49%

Source: admetSAR® version 2.0

Table 3 provides qualitative information (NT or T; NC or C) and quantitative (P) on the human in *silico* toxicological profile of enrofloxacin. The analysis of these data reveals results, indicating that the antibiotic is not toxic, mutagenic as to the Ames test, and is also not carcinogenic. Regarding acute oral toxicity, Enrofloxacin is classified in Category III, which denotes low toxicity. These results are confirmed by table 3 (Gonçalves, 2011).

CONCLUSION

Enrofloxacin is an antibiotic widely used in the veterinary market, recognized for its high bioavailability and rapid therapeutic action. Its rational use is essential to maintain efficacy in the treatment of infections, minimizing impacts on public and environmental health.

In silico toxicological studies, enrofloxacin was observed to be non-toxic to bees, but to pose a risk to fish and to the single-celled organism *Tetrahymena pyriformis*. In addition, its chemical structure is not biodegradable, remaining in the environment and contaminating aquatic ecosystems, which can affect the food chain and, eventually, human health through contaminated food.

Although enrofloxacin has been proven to have environmental impacts, the in silico toxicology study concluded that the antibiotic is not carcinogenic and has low acute oral toxicity, being classified in category III by the EPA, which indicates that it is not highly toxic. In addition, the antibiotic showed mutagenic potential in AMES tests, reinforcing its insecurity in this aspect. In view of these results, it is evident that there is a need for further *in silico* studies on human and environmental pharmacokinetics and toxicology, since enrofloxacin contributes to environmental contamination and potentially to human health. The continuity of this research is essential for a deeper understanding of the effects of this drug.

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