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IN SILICO EVALUATION OF THE ENVIRONMENTAL, HUMAN AND PHARMACOKINETIC TOXICITY OF THE VETERINARY ANTIBIOTIC CEFQUINOMA

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ABSTRACT

The research developed in this work evaluated, through in silico methodologies, the environmental and human toxicity, the human oral bioavailability and pharmacokinetic parameters for the veterinary antibiotic Cefquinoma. Cefquinoma is a broad-spectrum antibiotic, classified as a fourth-generation cephalosporin. Currently, its use is intended for the treatment of clinical mastitis in lactating cows, caused by bacteria sensitive to it. Currently, the economic losses arising from the pathology result in several additional costs, such as a decrease in production and loss of the animal. Water and soil contamination are topics of high focus nowadays due to the possible impacts that antibiotic residues can cause in the environment. In view of this context, an in silico environmental toxicological study of Cefquinoma was carried out. The study revealed that the antibiotic is not toxic to bees and crustaceans, but it is toxic to fish, its chemical structure does not undergo environmental biodegradation, presenting a total removal rate of only 1.89%, resulting in contamination of the food chain and humans through food. Because the veterinary antibiotic promotes environmental, food and human contamination, it is mandatory to carry out the Toxicological and Pharmacokinetic study in silico human. This study indicated that the antibiotic is not available well via human oral use, as it violated three molecular descriptors of Lipinski's Rule. The ADME in silico study revealed that the antibiotic has a low intestinal absorption rate, does not have permeability through the blood-brain barrier, and does not inhibit P-glycoprotein. The ADME in silico study also indicated that the antibiotic did not inhibit hepatic isoenzymes of the cytochrome P450 complex. The human in silico toxicological study revealed that the antibiotic is not mutagenic in terms of the AMES test, does not present carcinogenicity and in terms of acute oral toxicity it falls into category III (low toxicity), but presents respiratory toxicity, reproductive toxicity, mitochondrial toxicity and human nephrotoxicity.

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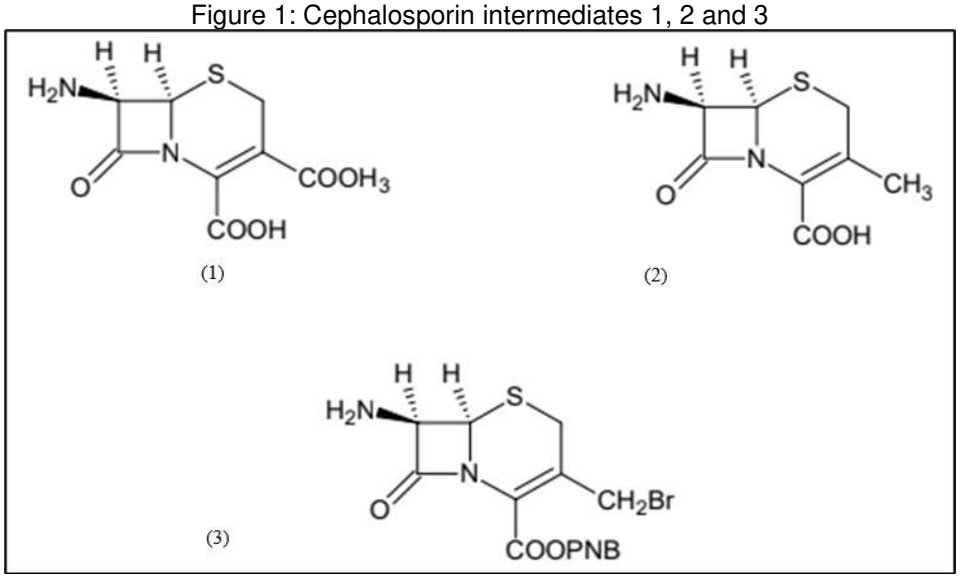
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INTRODUCTION

In recent years, the pharmaceutical industry has undergone a major transformation, which has led the sector to considerably expand its production. Several factors have caused this positive scenario, such as technological innovations, resulting in a greater demand for drugs, especially in the field of antimicrobials. Antimicrobials are chemical substances responsible for inhibiting the growth or eliminating microorganisms. Therefore, they are used in order to prevent or treat a certain infection. In this sense, the cephalosporin class has a prominent position.

Cephalosporins are a class of antibiotics belonging to the beta-lactam group and stand out for their characteristics of broad spectrum of action, low toxicity, favorable pharmacokinetic profile, and ease of use. As for the chemical structure, it has two rings: beta-lactam and dihydrothiazine (SOUZA, 2008). They are derived from three important intermediates, 1) 7-aminopenicillamic acid (7-APA), 2) 7-aminodeacetoxycephalosporanic acid (7-ADCA) and 3) 7-methoxybenzyl ester of phenylacetamide-3-chloro-methyl-4-cephalosporanic acid (GCLE), as shown in Figure 1 (AZEVEDO, 2019). In addition, all of them in clinical use are semi-synthetic derivatives of 7-aminocephalosporanic acid (7-ACA), which was initially obtained from a natural antibiotic (FELICIANO, 2016).

Figure 1: Cephalosporin intermediates 1, 2 and 3



Source: Author Himself, (2023) – ChemSketch Freeware Program Version 2021.

Cephalosporins are also classified according to their spectrum of action, compared to Gram bacteria in the first, second, third, fourth and fifth generation. An example of an antimicrobial derivative belonging to the fourth generation is the antibiotic Cefquinoma.

The antibiotic Cefquinoma stands out for its high efficiency in combating Gram-positive and Gram-negative bacteria, and is for veterinary use only. Its main indication is for

the treatment of clinical mastitis caused by bacteria of the type *Salmonella spp*; *Haemophilus spp*; *Pasteurella spp*; *Klebsiella spp*; *Corynebacterium pyogenes*; *Escherichia coli*; *Staphylococcus aureus*; *Streptococcus dysgalactiae* and *Streptococcus uberis* (MANTILLA, 2020).

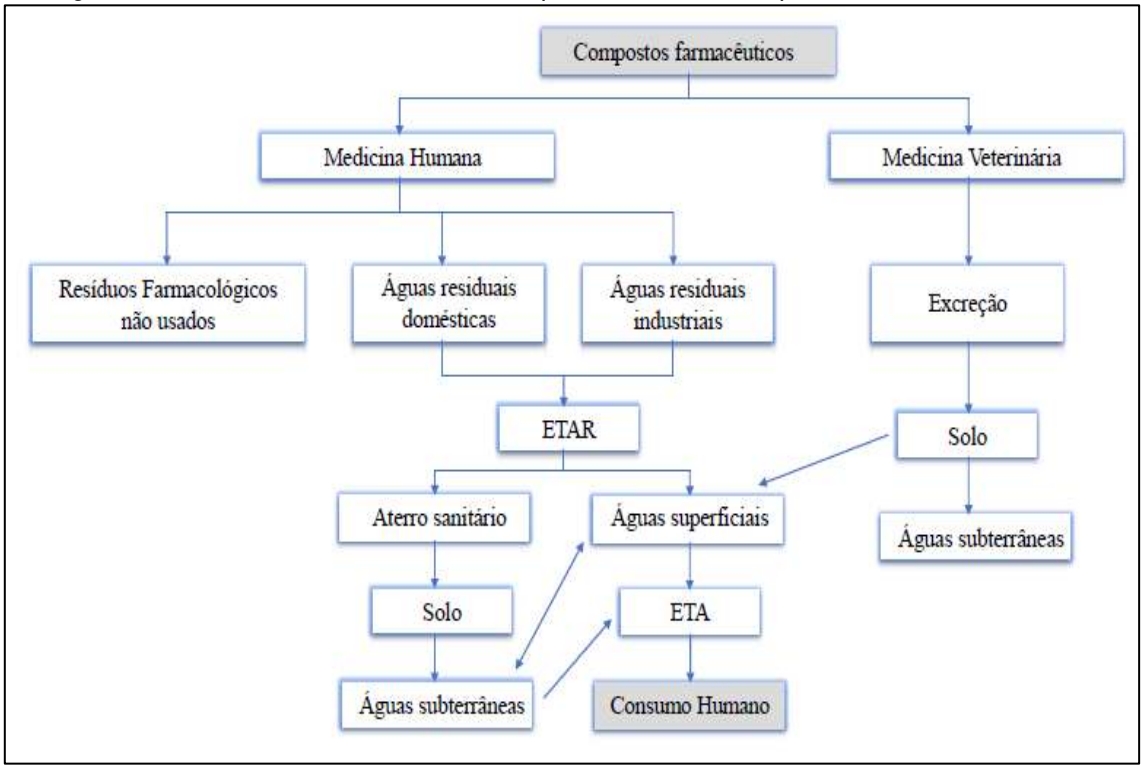
In this context, Brazil occupies a leading place in the large-scale production chain in the various branches of livestock, as well as in the use of widely disseminated veterinary medicines. Thus, the demand for quality products and consequent strict control has required adequate measures in order to ensure process safety, especially with regard to environmental contamination and contamination of the food chain by antimicrobials (LOBATO; DE LOS SANTOS, 2019).

With regard to drugs, there is special concern regarding antibiotics. Studies have shown that such contaminants, when disposed of in the environment inappropriately and without going through any specific treatment process, can result in negative effects, such as biological toxicity, induction of antibiotic resistance in pathogenic bacteria, and genotoxicity (LIMA et al., 2017).

Part of this problem occurs due to the basic sanitation system, which has not kept up with the advance of the pharmaceutical sector, nor the exponential use of it by the population. The presence of these drugs in the aquatic environment results from several parameters, among which refers to their physicochemical characteristics (solubility, polarity, biodegradation, among others) as well as their inadequate management and/or elimination in the environment. There are other forms of contamination as illustrated in Figure 2 (IRIA, 2018).

Through these characteristics and specificities, most of these compounds are not degraded in the conventional treatment systems of the Water Treatment Plants (WTP) and consequently return to the watercourse without adequate treatment. However, in recent years this scenario has been changing, due to more specific analysis and detection techniques, such as liquid chromatography coupled to mass spectrometer (CARVALHO, 2020).

Figure 2: Main routes of contamination of pharmaceutical compounds in the environment



Source: Iria, (2018).

Therefore, the study of contaminants in this category of drug is necessary, in order to evaluate their impact on human health (human toxicity), as well as the implementation of appropriate techniques for water treatment systems. One way to reduce such contaminants is through the use of advanced water treatment techniques (DICK, et al., 2022). However, these techniques are expensive and require specialized professionals with specific knowledge in analytical chemistry, instrumental analysis and organic chemistry. Therefore, an investment is necessary in order to achieve promising results.

The objective of the present work is to perform, through Chemoinformatics, the Environmental and Human in *silico Toxicological study* and Human in silico Pharmacokinetic study in order to predict parameters of Environmental Toxicity, Human Toxicity, Oral Bioavailability, Absorption, Distribution, Metabolization and Excretion (ADME *in silico*) for the antibiotic Cefquinoma with the aid of *in silico methodologies*.

LITERATURE REVIEW

This chapter presents the relevant theoretical definitions of the present work. In addition, the factors that motivated such a proposal in terms of technological innovation will be pointed out. In the literature review, statistical data listed by regulatory agencies, such as MAPA and SINDAN, were presented, emphasizing the importance of the sectors in the country and the consequent rational use of the antibiotic Cefquinoma.

LIVESTOCK IN BRAZIL - GENERAL ASPECTS

In recent years, with the advent of globalization and technological advances, the livestock sector in Brazil has achieved record growth in production and increased productivity. One of the factors responsible for such a feat is due to the expansion of research in genetics, advances in pest control and the effectiveness of drugs in controlling diseases that affect animals.

According to data from Embrapa (2022), from the 80s onwards, poultry meat production increased 22 times, pork, milk and beef production quadrupled, beef cattle production doubled (from 11% to 22%) and annual milk production doubled from 2002 onwards, due to the expansion of the herd and the productivity of the cows.

Also, in the last report of 2022 presented by Brazilian agriculture, livestock activity corresponded to about 369.15 billion and the gross value of production corresponded to 1,207.41 billion. The herds of livestock and livestock products (updated at the end of each year, in reference to the previous year) are shown in Table 1 (MAPA, 2022).

Table 1: Total herds and production of animal origin: Brazil

Herds and production	Total Brazil	Annual Change (%)
Cattle (head)	218.150.298	1,5%
Buffaloes (heads)	1.502.482	4,8%
Horses (head)	5.962.126	1,9%
Pigs - total (head)	41.124.233	1,4%
Swine breeders (head)	4.839.630	1,0%
Goats (heads)	12.101.298	7,1%
Sheep (head)	20.628.699	4,6%
Chickens – total (head)	1.479.363.352	0,9%
Chickens (heads)	252.570.646	1,4%
Quails (heads)	16.512.169	-5,2%
Milk (thousand liters)	35.445.059	1,7%
Chicken eggs (One thousand dozen)	4.767.388	3,3%
Quail eggs (One thousand dozen)	295.904	-6,2%
Bee Honey (Kilograms)	51.507.862	12,0%
Silkworm cocoons	2.742.372	-10,3%
Wool (kg)	7.978.317	-4,4%

Shrimp (Kilograms)	63.169.853	16,3%
Oysters, scallops and mussels	14.297.623	-6,0%
Total peixes (Kg)	551.873.845	4,2%
Fingerlings (Milheiros)	1.369.446	1,6%
Shrimp larvae and powders	12.541.720	4,5%
Mollusk seeds (Milheiros)	26.486	-42,6%

Source: Author – adapted from MAPA data, (2022).

BOVINE MASTITIS AND THE USE OF ANTIMICROBIALS

Brazil is among the largest markets for veterinary products. In 2020, there was a total turnover of 7.586 billion reais in the veterinary products market, highlighting medicines for ruminants, poultry and pigs, which are equivalent to 51%, 14% and 12%, respectively, of the sector's total revenue (PAULA, 2017). In addition, antimicrobials continue to occupy a prominent position in the national market for veterinary products, accounting for the third place in demand (14%), behind only antiparasitic (27%) and biological (22%) (SINDAN, 2020).

Following the numbers mentioned above, it can be seen that the livestock sector represents a significant portion of the country's economy and it is noted that the infectious diseases that affect it generate morbidity and mortality, thus resulting in several losses. It is estimated that more than half of all antimicrobials produced in the world are used in the treatment of animals (MAGALHÃES; SOUZA, 2012).

Antimicrobials correspond to the class of agents responsible for eliminating or inhibiting the growth of microorganisms, whether they are of natural or synthetic origin. Depending on the nature of the active ingredient, it can also be denoted as an antibiotic or chemotherapy (AFONSO, 2008).

Antibiotics are drugs that have the ability to act with selective toxicity, when in low concentrations against other microorganisms. Generally, they are produced by the microorganisms themselves, higher plants, and even by the animal organism itself (MACHADO, et al., 2019).

Although only five classes (penicillins, tetracyclines, macrolides, aminoglycosides, and amphenicols) represent the group of antibiotics, other synthetic drugs (sulfonamides, quinolones, nitrofurans, and nitroimidazoles) and natural products of high molecular weight (polyether antibiotics) are also usually included (PAULA, 2017).

In Brazil, one of the main infections that affect the herd is mastitis, which consists of inflammation of the animal's mammary gland. It is usually caused by infection by several

species of microorganisms, and the bacteria *Staphylococcus aureus* and *Streptococcus agalactiae* are considered to be pathogens of greater relevance, due to their high incidence and resistance rate (ACOSTA, et al., 2016). The economic losses arising from this pathology are due to several additional costs, such as decreased production, loss of the animal, among others. However, the reduction in milk production is considered the main loss.

> Studies conducted in Brazil have shown that mammary quarters with subclinical mastitis produced an average of 25 to 42% less milk than normal mammary quarters. In the United States, it is estimated that the cost per cow/year due to mastitis is approximately US$ 185, which corresponds to an annual cost of US$ 1.8 billion. This amount corresponds to approximately 10% of the total milk sold by producers. Of these, about two-thirds correspond to the reduction in milk production due to subclinical mastitis (Embrapa, 2021, p.1).

This infectious disease still has impacts on public health, since it represents the main cause of the emergence of antibiotic resistance, as well as in the transmission of pathologies in humans as a result of antibiotic residues and resistant bacteria (LOBATO; DE LOS SANTOS, 2019). Due to this scenario, it is essential to better understand the drugs used in the treatment of this pathology, as well as its effective control.

CEFQUINOMA

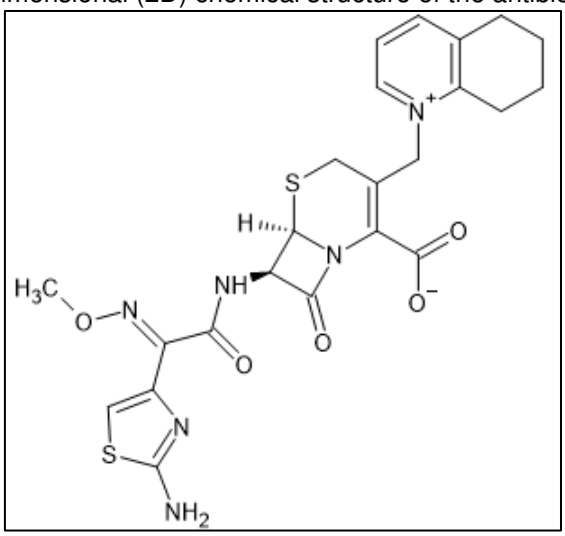
Among the main groups of antibiotics, β-lactams stand out with significant importance in the treatment of several pathologies. This group includes cephalosporins, cefamines, and penicillins, and together they account for more than half of all antibiotics produced and used in the world (HARTWIG et al., 2014).

Cephalosporins are classified as first, second, third, fourth and fifth generation, depending on their biological spectrum of action. Thus, 1st generation cephalosporins have a satisfactory response against Gram-positive organisms, while higher generations (2nd, 3rd, 4th) have a broader spectrum against aerobic Gram-negative bacilli. Finally, the last generation (5th) is active against methicillin-resistant *Staphylococcus aureus* (WERTH, 2022).

New antimicrobials, such as Cefquinoma, are available in the Brazilian market and are widely used in the treatment of mastitis. Cefquinoma is an amino-thiazole-type antibiotic, of the cephalosporin class, with action similar to that of other β-lactam antibiotics. Currently, its use is exclusive in veterinary medicine. In addition, Cefquinoma is a fourth-generation cephalosporin and has different active groups in its chemical structure, with emphasis on the quaternary ammonium group at position C-3 of the aminocephalosporanic

acid nucleus, according to the two-dimensional (2D) structure of the molecule represented in Figure 3 (HARTWIG et al., 2014).

Figure 3: Two-dimensional (2D) chemical structure of the antibiotic Cefquinoma



Source: Author Himself, (2024) - ChemSketch Freeware Program version 2021.

The quaternary ammonium group in the chemical structure of the antibiotic provides a wide range of biological activities against microorganisms and high biological activity against third-generation cephalosporin-sensitive bacteria, mainly Gram-negative bacteria. The explanation for the broad spectrum of action is due to resistance to beta-lactamases produced by chromosomal mutation or induced by bacterial plasmids and high affinity for penicillin-fixing proteins (PBPs) (LITTERIO, 2013).

As for the Pharmacokinetic and Pharmacodynamic properties, it is possible to mention the fact that they are used as bases orally or for parenteral use, as salts in aqueous solution, as well as low intestinal absorption when administered orally and tissue distribution. In addition, they also have ease in crossing the blood-brain barrier during inflammatory processes, time-dependent activity and excretion occurs predominantly via the renal route. However, recent studies show that their use should be avoided in order to prevent super-resistant bacteria and because they are considered antimicrobials listed by the World Health Organization (WHO) as of critical importance for human use (ROSSI, 2017).

ENVIRONMENTAL CONTAMINATION BY ANTIBIOTICS

Currently, the number of pharmaceutical compounds detected in the environment is vast, since the most common drug residues are antibiotics, hormones, anesthetics, anti-inflammatories, among others. Studies corroborate the idea that the rate that is absorbed,

as well as the rate of metabolization and excretion govern the total waste load (SOUZA, 2012).

In general, the main studies obtained in the literature indicate that wastewater is the main source of contamination of other environmental matrices. In addition, antibiotics continue to lead the drugs present in water bodies, such as pharmaceutical contaminants. Much of this situation is justified by the current model of the water treatment process of wastewater treatment plants (WWTPs). The unit operations present in the conventional system (e.g., decantation, flotation, flocculation, coagulation, among others), are insufficient for the removal of more polar drugs (SILVA, et al., 2023).

Another environmental concern refers to the way the drug is found in the environment. When released into the effluent or disposed of inappropriately, it can remain in its original form or be converted into by-products of transformation via photolysis, oxidation, among others. Therefore, these substances can be bioactive and cause toxic effects on humans, aquatic organisms, among others (SOUZA; AQUINO; SILVA, 2020). Despite several studies, it is not known precisely about the fate and behavior of these drugs in the aquatic environment, as well as the degree of impact on organisms.

Antibiotics can have numerous effects on the environment, but the development of resistant bacteria has increased significantly. Generally, they arise due to continued exposure to the drug, even at low concentrations. Additionally, studies show that in aquaculture, there are reports of a population of resistant bacteria in marine sediments. Other authors point to the development of certain human pathologies, such as breast, testicular and prostate cancer, polycystic ovaries and reduced male fertility due to exposure to drugs (BILA; DEZOTTI, 2003).

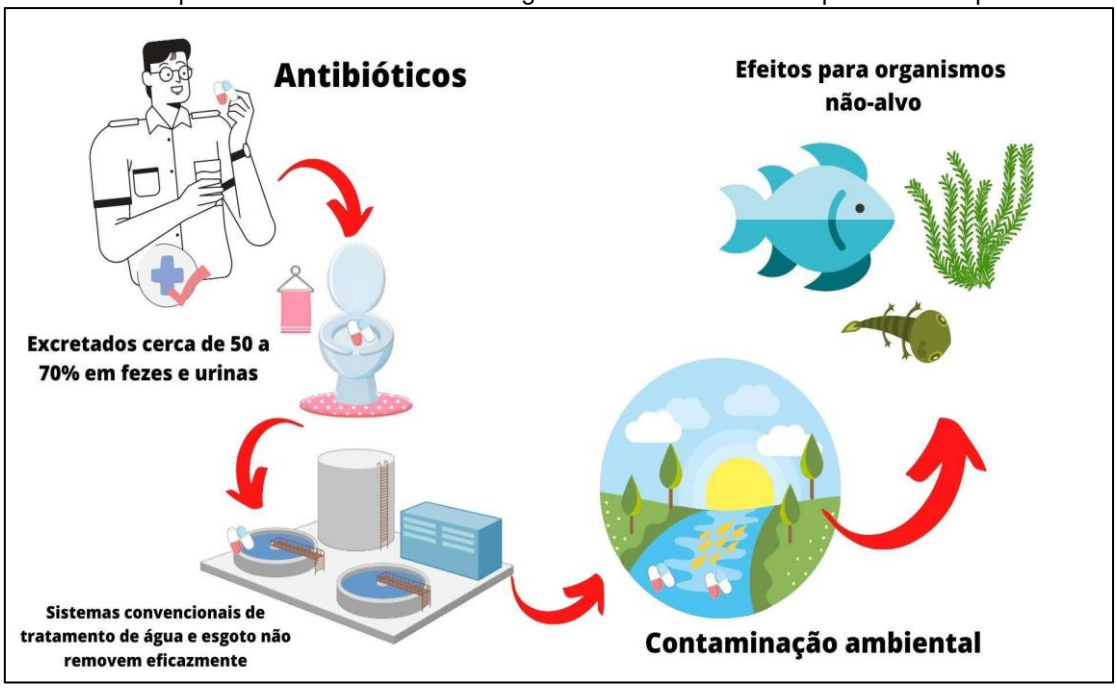
Regarding the idea to define what constitutes toxicity or not, the consequences for human health are taken into account. In Brazil, for example, there are three legislations regarding the presence of contaminants in water. According to Monteiro, (2018), the first legislation refers to the environment and the classification of waste in the aquatic environment, as well as the concentration limits for the safe disposal of treated effluents in water bodies, governed by Conama Resolution No. 357 of 2005. The second establishes the reference standard for effluent discharges into receiving water bodies, described in Conama Resolution No. 430 of 2011 (BRASIL, 2011). Finally, the third presents the standards of potability of water for human consumption, by Ordinance No. 672914 of 2011, of the Ministry of Health. However, these laws do not contain the established limits for antibiotics in the water supply.

Another publication on the disposal of medicines, Silva et al. (2023) explain that Decree No. 10,388, published on June 5, 2020, establishes guidelines on the reverse logistics system for expired or unused household medicines. The flowchart of the process establishes that pharmacies (primary storage) must have collection points for the disposal of medicines. Then, the distributors (secondary storage) forward the waste to manufacturers and importers. The latter, according to the logistics chain, would be responsible for the environmentally appropriate final destination of the waste. However, most of the items that make up the aforementioned guideline are not yet executed.

Despite the high consumption of antibiotics and the fact that they are found in surface waters, it is not yet clear whether the contamination comes from effluents and/or waste from animal production. By law, veterinary pharmaceutical products have not yet been included in the list of priority compounds. However, with the growing knowledge about the occurrence, ecotoxicity and efficiency of antibiotic elimination processes, it is expected that it will be possible to establish legal limits for such compounds (HOMEM, 2011).

Regarding the routes of antibiotic contamination in the aquatic environment, there are several ways, but the main ones occur through excreta and effluents from manufacturing processes. Another detail is that, in rural areas, where there is not always a sanitation system, excreta is dumped into septic tanks and there is also cross-contamination of soils and consequently surface water (HOMEM, 2011). Figure 4 represents the aforementioned process and the respective chain of contamination resulting by antibiotics in the aquatic environment (KITAMURA, 2022).

Figure 4: Schematic representation of the antibiotic-generated contamination process in aquatic environments



Fonte: Kitamura, (2022).

In this context, a key scientific area that evaluates the effects between the environmental and human relationship is environmental toxicology. In antiquity, reports on the subject were already found, as is the case of records of the use of toxic plants around 600 BC by Athenian society. In environmental toxicology, the study of chemical substances and the harmful effects produced by the interaction of environmental contaminants is considered. To determine the adverse effects of a given chemical compound, the phases of intoxication, elucidation, toxicokinetic and toxicodynamics are studied. Thus, it is commonly defined as the science responsible for analyzing and understanding the harmful effects of environmental chemical contaminants on living organisms (SILVA; SILVA; KITAMURA, 2022).

With the implementation of environmental practices and discussions about the relationship between pharmaceutical technological development and environmental management, it became essential that companies carry out the development of their activities, along with sustainable actions about the proper disposal of drugs. Therefore, in view of all the bibliographic surveys, the importance of conducting the present study is reaffirmed, as well as the confirmation or not of the toxicity of the antibiotic Cefquinoma by means of *in silico methods.*

CHEMOINFORMATICS AND *IN SILICO METHODOLOGIES*

Chemoinformatics, according to Abreu et al. (2013), is the area of study in which computational methods are used in order to solve problems related to Chemistry. Another commonly used definition consists of an interdisciplinary science that, through information and computational technology resources, transforms a set of chemical data (data matrix) into knowledge applicable to society (ALVES et al., 2018).

Historically, the term chemoinformatics was coined in 1998 by Frank Brown as a "blend of resources to transform data into information and information into knowledge, in order to make efficient and faster decisions to identify and optimize lead *compounds* or prototypes".

Initially, it dealt only with representations and manipulations of chemical structures. Currently, it is recognized as an interdisciplinary area of knowledge with emphasis on the exploration of chemical and biological databases, the discovery and development of new prototypes (*lead compounds*) with promising biological activity and desirable molecular properties (molecular descriptors), estimation of environmental toxicity of existing chemicals on the market and *virtual* screening of a possible chemical structure of promising chemical compounds (SILVA, 2020).

The term *in silico* refers to the methodologies of computer simulation and obtaining molecular descriptors (molecular parameters = molecular properties) in international database platforms. The importance lies in numerous positive points, such as reduced chemical and biological experimental costs, greater efficiency in processes and speed in results, as well as the unavailability of data to assess (predict) risks and restrictions regarding the use of animals (*in vivo methodology*) (DIAS, 2018).

The rationale of *in silico* methodologies takes into account the assumption that compounds that have similar chemical structures behave in a similar way in terms of human and environmental biological, pharmacokinetic, pharmacodynamic, and toxicological activity, therefore, it should be used as a predictive tool to obtain various information (molecular parameters). Currently, there are several international software and platforms on the market with constant updating of the database (ALVES; STREIT; PIZZOLATO, 2023).

In *silico* approaches have become widely used in preclinical studies for various applications. In addition, there is evidence in the literature for drug modeling and biological performance studies, for application in different pathways (HINES, 2021 apud COSTA, 2022). In addition to these applications, there are also molecular modeling studies, using advanced computational techniques of "molecular docking" and "molecular dynamics", to evaluate the potential inhibitors of proteins associated with different pathologies and analysis of the bioactivity of a chemical compound (ABREU et al., 2013).

In the area of toxicology, *in silico* methods have been widely disseminated in the pharmaceutical industry, since mathematical calculations have proven effectiveness in predicting the possible effects of the pharmaceutical, pharmacokinetic and pharmacodynamic phases. In this context, the U.S. Environmental Protection Agency (EPA) presents the definition of in *silico toxicity* as being the junction of two areas: integration of computing/information technology with cellular, tissue and molecular biology, with the objective of predicting the risk potential of chemical compounds (BRITO, 2010).

The pharmaceutical phase is related to the route of administration of the drugs and the pharmacotechnical way of preparing them, while the Pharmacodynamics phase focuses on the study of the biochemical effects on the molecular target (biological receptors = biological targets), as well as the mechanism of action of the drugs. However, the qualitative and quantitative evaluation of the processes of absorption, distribution, metabolism, excretion and destination of drugs in the body through *in silico* methodologies is evaluated by the study of Pharmacokinetics. In addition, toxic agents and adverse drug reactions are addressed by Toxicology (DELUCIA et al., 2016).

With regard to the route of drug administration, most of these compounds occur orally. Therefore, it seeks to understand how the drug reacts in the body and what its local and systemic effects are. Thus, the route it follows when ingested is to follow the gastrointestinal tract and later to the blood (via intestinal absorption), to the site of action (distribution), to undergo biotransformation in the liver (metabolism) to eliminate xenobiotics and finally, to be eliminated by the body (via renal excretion). This set of steps is known as ADME in *silico assessment* (MODA, 2007).

Among the numerous studies of ADME *in silico* available, the prediction of pharmacokinetic properties stands out, such as CYP450-mediated metabolism (hepatic isoenzymes of the cytochrome P450 complex), intestinal absorption rate, oral bioavailability, passage via the blood-brain barrier to the Central Nervous System (CNS), among others (OLIVEIRA, 2021).

An important application of ADME in *silico* evaluation refers to the prediction of oral drug bioavailability, commonly known as the "Rule of 5" or Lipinski's Rule (LIPINSKI et al., 2004), (FARIA et al., 2023). In this rule, the following relevant molecular descriptors are considered to evaluate the drug molecule:

> molecular mass less than 500 Daltons (molar mass less than 500 g/mol), number of hydrogen bond acceptor sites (SALH) less than or equal to 10, number of hydrogen bond donor sites (SDLH) less than or equal to 5, miLogP (n-octanol/water partition coefficient) less than or equal to 5, and polar topological surface area (TPSA) less than 140 $(A°)^2$. Molecules that violate more than one of the criteria may present oral bioavailability problems (PEREIRA, A. L. D. C, 2019, p.38).

Due to the numerous advantages, the ADME *in silico* evaluation, when compared to traditional approaches (*in vivo and in vitro),* stands out for its greater applicability and speed of results during large-scale virtual screening of molecules.

MATERIALS AND METHODS

In the present work, computer programs and databases of international online chemoinformatics platforms were used in order to predict molecular properties (molecular descriptors) of the molecular chemical structure of the antibiotic Cefquinoma.

COMPUTATIONAL MOLECULAR MODELING

First, the chemical structure of the antibiotic Cefquinoma was drawn two-dimensionally (2D) and visualized three-dimensionally (3D) using the ACD/ChemSketch® Freeware computer program version 2021 (Advanced Chemistry Development, Inc., 2021) and the energy of the three-dimensional chemical structure of the antibiotic was tabulated.

Finally, the chemical structure of the antibiotic molecule was saved in a file of the type MDL molfiles (.mol) for further studies.

TOXICOLOGICAL PREDICTION *IN SILICO* AMBIENTAL

Through the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the use of the file's source code (.mol), the SMILES (*Simplified Molecular Input Line Entry Specification*) code was obtained.

Subsequently, the SMILES code was exported to the online database platform. The environmental in *silico* toxicology study of Cefquinoma was carried out to predict environmental biodegradation, toxicity in fish, bees and crustaceans. 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 PHARMACOKINETIC PREDICTION FOR ORAL BIOAVAILABILITY

Through the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the use of the file's source code (.mol), the SMILES (*Simplified Molecular Input Line Entry Specification) code was obtained*. Subsequently, the SMILES code was exported to the online database platform. The human in silico pharmacokinetic study was carried out with the objective of predicting the molecular descriptors of the antibiotic Cefquinoma with the aid of the Molinspiration Cheminformatics® (https://www.molinspiration.com) database (GROB, 1986). The database predicts the molecular properties in order to evaluate the oral bioavailability of the antibiotic molecule based on Lipinski's Rule, known as the *Rule of Five*.

HUMAN PHARMACOKINETIC IN *SILICO* PREDICTION (ADME *IN SILICO)*

Through the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the use of the file's source code (.mol), the SMILES (*Simplified Molecular Input Line Entry Specification*) code was obtained. Subsequently, the SMILES code was exported to the online database platform.

The first stage of this research was the ADME *in silico* (ADME) *study* for the antibiotic Cefquinoma, to predict the molecular parameters: Human Intestinal Absorption Rate (HIA), Blood-Brain Barrier Permeability (BBB): *blod-brain barrier*), P-glycoprotein inhibition, Caco-2 epithelial cell permeability, and Cellular distribution of the antibiotic in the

human body. In the next step, the ADME in *silico study was performed* to predict the inhibition of hepatic isoenzymes of cytochrome P450 complex (CYP450) in the process of Hepatic Metabolization (Hepatic Biotransformation) of Cefquinoma. This 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).

In order to validate the molecular descriptors obtained from the Chinese online platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), an advanced in silico study was carried out with the aid of the Swiss online database platform SwissADME® The Swiss platform SwissADME® (http://www.swissadme.ch) was developed by Diana, Michielin and Zoete (2017) and also allows the predictive study of the pharmacokinetic descriptors and oral bioavailability of the antibiotic Cefquinoma. The molecular descriptors obtained are: Intestinal Absorption Rate (GI), Blood-Brain Barrier Passage (BBB Permeation), P-Glycoprotein Inhibition (P-gp) and inhibition of hepatic isoenzymes of the cytochrome P450 complex (CYP1A2 Inhibitor, CYP2C19 Inhibitor, CYP2C9 Inhibitor, CYP2D6 Inhibitor and CYP3A4 Inhibitor).

HUMAN IN *SILICO* TOXICOLOGICAL PREDICTION

Through the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the use of the file's source code (.mol), the SMILES (*Simplified Molecular Input Line Entry Specification*) code was obtained. Subsequently, the SMILES code was exported to the online database platform.

The human in silico *toxicological study* of the antibiotic Cefquinoma was carried out with the objective of predicting the toxicity of the drug by the AMES test (T: toxic; NT: non-toxic), carcinogenicity test (C: carcinogenic; NC: non-carcinogenic) and its acute oral toxicity. 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).

In order to complement the study of the human in *silico toxicological profile* , in addition to the three molecular descriptors obtained via the Chinese online platform admetSAR® version

2.0 (http://lmmd.ecust.edu.cn/admetsar2/), other advanced molecular properties were determined using the same online data platform. The descriptors determined are

correlated with: ocular corrosion, eye irritability, hepatotoxicity (liver), dermal sensitivity (skin), respiratory toxicity (airways and lungs), reproductive toxicity (gonads), nephrotoxicity (renal), and mitochondrial toxicity (mitochondria – functional organelle for cellular respiration).

ADVANCED *ENVIRONMENTAL* IN SILICO TOXICOLOGICAL PREDICTION

Through the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the use of the file's source code (.mol) the code SMILES (*Simplified Molecular Input Line Entry Specification*) was obtained. Subsequently, the SMILES code was exported to the online database platform.

The advanced environmental in silico *toxicological evaluation* was performed with the aid of the computer program EPI Suite™ version 4.1. The EPI (Estimation Programs Interface) Suite™ developed by the United States Environmental Protection Agency (EPA) and Syracuse Research Corp (SRC) (EPI Suite™, 2023), robustly calculates environmental and physicochemical properties. Taking into account the calculations obtained in the program, a more advanced environmental toxicological prediction of the antibiotic Cefquinoma will be carried out, the molecular descriptors calculated are: prediction of aquatic biodegradation, total removal, total biodegradation, total sludge adsorption and total air removal.

RESULTS AND DISCUSSION

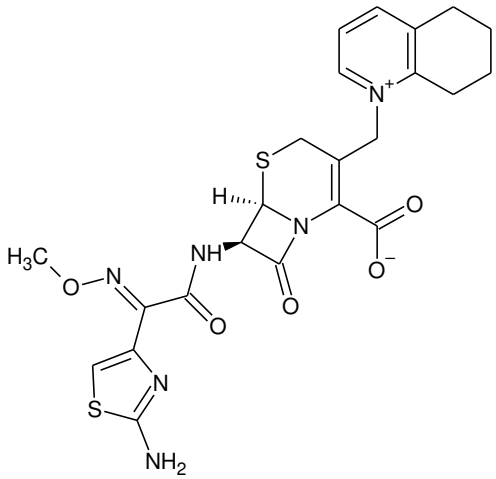
COMPUTATIONAL MOLECULAR MODELING

The molecular stability and biological activity of the ligand (antibiotic) is closely correlated with the intermolecular interactions between the ligand (antibiotic molecule) and the biomacromolecule (biological receptor = biological target). This study is known as SBDD (*Structure Based Drug Design*), in which the free energy of the ligand-receptor intermolecular interactions in the active cleft (active binding groove) of the biological receptor is determined by means of physicochemical parameters (MORRIS and LIM-WILBY, 2008). The energy minimization of the chemical structure consists of the process in which the atomic coordinates of the ligand molecule will be altered in order to reduce the steric energy of the molecular chemical structure that corresponds to its local minimum (more stable chemical structure) (SANT'ANNA, 2009).

In the Molecular Modeling stage for the chemical structure of the antibiotic Cefquinoma, the minimized chemical structure was saved in a MDL molfiles (.mol) file and the steric energies were tabulated for further studies. Figures 5 and 6 represent,

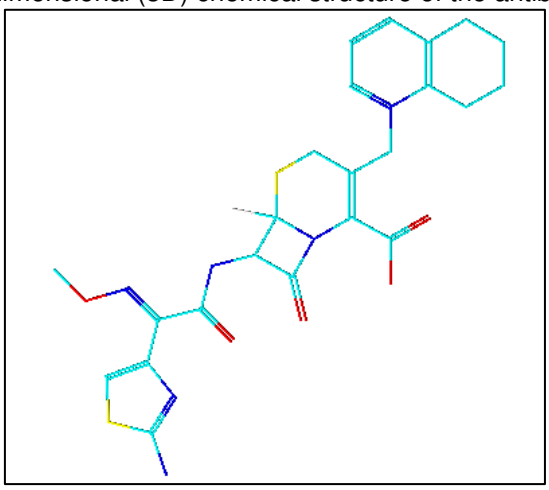
respectively, the chemical structure of the two-dimensional (2D) and three-dimensional (3D) drawings and the molecule of the antibiotic Cefquinoma.

Figure 5: Two-dimensional (2D) chemical structure of the antibiotic Cefquinoma



Source: Author himself, (2024) – ChemSketch® Freeware program version 2021.

Figure 6: Three-dimensional (3D) chemical structure of the antibiotic Cefquinoma



Source: Author himself, (2024) – ChemSketch® Freeware program version 2021.

EVALUATION OF THE *IN SILICO* ENVIRONMENTAL TOXICOLOGICAL PROFILE

After having carried out the molecular modeling with the help of the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and using the source code of the file (.mol), it was possible to generate the SMILES (*Simplified Molecular Input Line Entry Specification*) code and, through it, to carry out the Environmental in *silico* Toxicological study with the help of the Chinese international online platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/).

The information generated by the platform occurs through the database system of each specific compound, in order to predict the profile of *in silico* Environmental Toxicity of the antibiotic Cefquinoma, in which the following molecular descriptors were evaluated: environmental biodegradation, toxicity in fish, bees and crustaceans, as shown in Table 2.

Table 2: Toxicological Profile Assessment *in silico* Environmental

Biodegradation Environmental		Toxicity in bees		Toxicity in Crustaceans		Toxicity in Fish	
What P		Q	P	Q	P	Q	P
N	100,0%	N	80,9%	N	54%	P	99,0%

Source: Author's own, (2024) - admetSAR® Platform Version. 2.0.
Q – Qualitative: (Negative: N), (Positive: P); P: Probability

The analysis of table 2 reveals that the antibiotic Cefquinoma is not toxic to bees or crustaceans, but is toxic to fish and the drug does not undergo environmental biodegradation, that is, its chemical structure remains unchanged in the environment, contaminating the food chain and consequently the human being.

IN *SILICO EVALUATION* OF THE ORAL BIOAVAILABILITY PROFILE

After performing molecular modeling with the aid of the ACD/ChemSketch® Freeware version 2021 computer program (Advanced Chemistry Development, Inc., 2021) and using the file's source code (.mol), it was possible to generate the SMILES (*Simplified Molecular Input Line Entry Specification)* code and, through it, determine the molecular properties of the antibiotic in the international online database platform Molinspiration Cheminformatics® (https://www.molinspiration.com) (GROB, 1986). The molecular descriptors determined in order to analyze the human oral bioavailability of the study drug (Cefquinoma) are shown in Table 3.

The analysis of table 3 reveals that the antibiotic Cefquinoma violates Lipinski's Rule, so it does not have a good prediction regarding the human oral bioavailability profile. Table 3 presents the following molecular descriptors: 1) miLogP: Logarithm of the partition coefficient (measure of molecular hydrophobicity/hydrophilicity); 2) TPSA: Polar surface topological area; 3) PM: Molecular weight (Da), equivalent to molar mass (g/mol); 4) SALH: Hydrogen Bond Acceptor Sites; 5) SDLH: Hydrogen Bond Donor Sites; 6) Violations: Number of violations of the Lipinski rule. It is important to emphasize that the complementation of Lipinski's Rule can also be performed through two other molecular descriptors for human oral bioavailability (Veber's Rule): 7) NLR: Number of rotatable bonds; 8) VM: Molecular volume.

Table 3: Evaluation of the Human Oral Bioavailability Profile

Molecular Descriptions

miLogP	- 5,19
TPSA	153,93
PM	528,62
SALH	11
SDLH	3
Violations	3
NLR	7
VM	436,08

Source: Author himself, (2024) - Molinspiration Cheminformatics® Platform.

For a better understanding of the criteria of Lipinski's Rule for predicting the human oral bioavailability profile, we have:

1) Octanol-water Partition Coefficient Log P (miLogP) 5: molecular descriptor of hydrophilic/hydrophobic nature. This molecular property is related to the intestinal absorption of the antibiotic, bioavailability when administered orally by the human route, hydrophobic/hydrophilic ligand-receptor interactions and also to the process of molecular metabolization. Cefquinoma presented a value lower than five (5), which means that the compound is more hydrophilic and did not violate this criterion of Lipinski's rule. ≤
2) Molecular Weight (PM) 500 Da (Dalton): the antibiotic has a molecular weight greater than 500 Da, so it represents a violation of Lipinski's rule. In addition, this parameter is ≤associated with the absorption and permeation capacity of biological membranes. Therefore, absorption, permeability and bioavailability decrease with the increase in molecular mass.
3) Hydrogen Bond Donor Sites (SDLH)5: the antibiotic has a value lower than five (5), so it did not violate Lipinski's Rule. ≤
4) Hydrogen Bond Acceptor Sites (SALH)10: the antibiotic has a value greater than ten (10), so it represents a violation of Lipinski's rule. This characteristic is extremely important for evaluating the biological activity of drugs and xenobiotics, as many intermolecular interactions involve hydrogen bonding. Therefore, it is ≤ unfavorable for a substance to make too many hydrogen bonds, since this would inversely affect the degree of permeability and also absorption (BARBOSA, 2020).
5) Polar Surface Topological Area (TPSA) 140: a related descriptor as the sum of the polar surfaces of the atoms bonded to the hydrogens in the molecule (usually fluorine, oxygen, and nitrogen). The antibiotic under study has a TPSA value greater than 140 , therefore, as this is a physicochemical descriptor related to

hydrogen bonding, high TPSA values (> 140) are indicative of reduced permeability and bioavailability. In addition, such a result also expresses a violation of Lipinski's rule (MOTTA et al., 2023), $\leq$ Å²Å² Å²(ARAUJO et al., 2022).

Taking into account the results presented in Table 3, it is noted that there were 3 violations of molecular descriptors regarding Lipinski's Rule, i.e., the molecule of the antibiotic Cefquinoma does not have good human oral bioavailability.

It should be noted that in addition to the properties discussed by Lipinski, other parameters were evaluated to predict human oral bioavailability. Veber's Rule is related to molecular flexibility, determined through the number of rotational bonds (NLR) (VEBER et al., 2002), (CEZÁRIO et al., 2022), (MOTTA et al., 2023). Therefore, the greater the flexibility of the molecule, the less likely the chemical structure will have human oral bioavailability (RODRIGUES et al., 2021).

EVALUATION OF THE PHARMACOKINETIC PROFILE *IN SILICO* (ADME *IN SILICO).*

Similarly, from the Chinese online database platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), the ADME in silico study was carried out *to evaluate the human* in silico *pharmacokinetic parameters* , through the variables Human Intestinal Absorption Rate (IAH), Blood-brain Barrier Permeability (BBB), Caco-2 epithelial cell permeability, P-glycoprotein inhibition and Distribution cellular antibiotic in the human body, as shown in Table 4.

Table 4: Evaluation of the Pharmacokinetic profile *in silico* Human (ADME) *in silico*)

	BBB		HIA		Distribution		Glicoproteína P		Caco-2		
	Q	Ꝑ	Q	Ꝑ	Q	Ꝑ	Q	Ꝑ	Q	Ꝑ	
	N	95%	N	85%	Mitochond.	59%	N	74%	N	88,8%	

Source: Author's own, (2024) - admetSAR® Platform Version. 2.0.
Q – Qualitative: (Negative: N), (Positive: P); Ꝑ: Probability

The analysis of Table 4 indicates that the antibiotic has a low intestinal absorption rate (15%), since the evaluation of the molecular descriptor HIA takes into account the following parameters for intestinal absorption as a reference:

1) 0 to 20% low absorption rate;
2) 20 to 70% moderate absorption rate;

3) 70 to 100% high absorption rate.

Even if the intestinal absorption rate is low, it is still possible to detect the antibiotic in blood plasma, as intestinal absorption occurs (YAKAIAH et al., 2015). It is also noted that the antibiotic Cefquinoma is not permeable via the blood-brain barrier (95%), that is, the drug has no impact on the Central Nervous System.

The molecular property of glycoprotein P was also evaluated because it is a selective descriptor regarding the entry of xenobiotics into the tissues. Glycoprotein P (present in epithelial cells) has a primary role in excretion and consequently reduces the absolute bioavailability of the antibiotic. The P-glycoprotein present in the endothelia of the capillaries of brain vessels acts as a "defense mechanism", as it has the ability to return xenobiotic chemical substances to the blood that could eventually cross the blood-brain barrier (GOLAN et al., 2014). With regard to its distribution, it can be stated that the antibiotic has the mitochondria as its cellular target, that is, the organelle responsible for cellular respiration.

From the Chinese online database platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), the ADME in *silico study was performed* to predict the inhibition of hepatic isoenzymes of cytochrome P450 complex (CYP450) in the process of Hepatic Metabolization (Hepatic Biotransformation) of the antibiotic Cefquinoma.

The analysis of table 5 indicates the inhibitory prediction of cytochrome P450 complex isoenzymes by the antibiotic. It is observed that the drug does not inhibit hepatic isoenzymes, that is, the antibiotic does not interfere with hepatic metabolism (biotransformation), facilitating the elimination of xenobiotic and lipophilic molecules from the human body, resulting in no adverse effects in hepatic (liver) terms.

Table 5: Evaluation of Pharmacokinetic Profile *in silico* Human (ADME) *in silico*) inhibitory of cytochrome P450 complex isoenzymes (CYP450)

	CYP4503A4		CYP4502C9		CYP2C19		CYP4502D6		CYP4501A2		
	I	P	I	P	I	P	I	P	I	P	
	N	50%	N	69,1%	N	63,3%	N	85,9%	N	67,2%	

Source: Author's own, (2024) - admetSAR® Platform Version. 2.0.
Q – Qualitative: (Negative: N), (Positive: P); P: Probability
I: Inhibition – (Negative), + (Positive); P – Probability

In addition to the molecular descriptors presented in tables 3 and 4, an advanced in silico pharmacokinetic study was also carried out , with the aid of the Swiss international

online platform SwissADME® (http://www.swissadme.ch), aiming to validate the molecular descriptors obtained by the Chinese online platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/). The molecular descriptors obtained with the aid of the Swiss online platform SwissADME® (http://www.swissadme.ch) were: Intestinal Absorption Rate (GI Absorption), Passage through the Blood-Brain Barrier (BBB Permeation), P-glycoprotein inhibition (P-gp) and inhibition of hepatic isoenzymes of the cytochrome P450 complex (CYP1A2 Inhibitor, CYP2C19 Inhibitor, CYP2C9 Inhibitor, CYP2D6 Inhibitor, CYP3A4 Inhibitor), as shown in Table 6.

From the analysis of the results presented in the table above, it is possible to infer that the antibiotic Cefquinoma does not violate any of the parameters determined with the help of the Swiss online platform SwissADME® (http://www.swissadme.ch). Therefore, it also revealed that the drug under study is not permeable by the blood-brain barrier (BBB), there is low gastrointestinal absorption and does not inhibit P-glycoprotein (P-gp). It should be noted that the largest fraction of glycoprotein P in the human body is found in the columnar epithelial cells of the lower gastrointestinal tract. P-gp is also correlated with drug excretion into the lumen of the gastrointestinal tract. In addition, it is also located in the endothelial cells of the blood-brain barrier (BBB), exerting a protective function and helping in the transport of substances by transepithelial or transendothelial route from epithelial cells and endothelial cells of the capillaries of the blood vessels of the blood-brain barrier (ARAÚJO, 2015).

Table 6: Evaluation of the Pharmacokinetic Profile *in silico* Human (ADME) *in silico*)

GI absorption	Low
BBB Permeation	No
P-gp	No
Inibidor CYP1A2	No
Inibidor CYP2C19	No
Inibidor CYP2C9	No
inibidor CYP2D6	No
Inibidor CYP3A4	No

Source: Author himself, (2024) - SwissADME® platform.

The analysis of Table 6 also indicates that the antibiotic Cefquinoma does not inhibit any of the isoenzymes that make up the cytochrome P450 complex (CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP2E1 and CYP3A4). Hepatic enzymes of the cytochrome P450 complex are the ones that contribute most frequently and significantly to the metabolism of most drugs (BRAZ et al., 2018).

ASSESSMENT OF THE *IN SILICO* HUMAN TOXICOLOGICAL PROFILE

From the Chinese online database platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), a human in silico *toxicological study was carried out* with the objective of evaluating the toxicity parameters of the antibiotic Cefquinoma taking into account the three primary tests: mutagenicity test (Ames test - T: toxic; NT: non-toxic), carcinogenicity test (C: in carcinogenic; CN: non-carcinogenic) and acute oral toxicity test for classification into categories (I, II, III and IV).

When evaluating the human toxicity profile, Table 7 indicates that the antibiotic has no toxicity taking into account the AMES test (mutagenicity), is not carcinogenic and as for acute oral toxicity it falls into category III (low toxicity).

The AMES test is an assay that identifies whether a certain chemical substance is capable of causing nitrogenous base pair substitution, that is, it evaluates the mutagenic capacity of the antibiotic. Therefore, it is an extremely important parameter to be investigated, in order to assess the safety of its use (GONÇALVES et al., 2016).

Acute oral toxicity testing classifies chemical compounds against the four categories of the U.S. Environmental Protection Agency (EPA), classifying them according to their LD50 value (known as the median lethal dose). Therefore, category I compounds have LD50 values less than or equal to 50 mg/kg. In category II, compounds have LD50 values above 50 mg/kg and below 500 mg/kg. Category III includes compounds with LD50 values above 500 mg/kg and below 5000 mg/kg. In category IV, compounds have LD50 values higher than 5000 mg/kg (PALMEIRA et al., 2018). Finally, for carcinogens (carcinogenicity), the test indicated that the antibiotic molecule does not have carcinogenic effects in a systemic way.

Table 7: Toxicological Profile Assessment *in silico* Human

Tested by AMES		Acute Oral Toxicity		Carcinogenic	
What P		C P		What P	
NT	59.0%	III	51.0%	CN	83.0%

Source: Author's own, (2024) – admetSAR® Version. 2.0.
Q: Qualitative; P: Probability; C: Category

To complement the study of the human *in silico toxicological profile* , in addition to the three molecular descriptors obtained from the Chinese online platform admetSAR® version 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), other advanced molecular parameters were also determined using the same international online database platform. The parameters determined for a more robust evaluation of the human toxicity profile were: ocular corrosion, eye irritability, hepatotoxicity (liver toxicity), dermal sensitivity (skin

toxicity), respiratory toxicity (airway and pulmonary toxicity), reproductive toxicity (gonad toxicity), nephrotoxicity (renal toxicity), and mitochondrial toxicity (mitochondrial toxicity: functional organelle of cellular respiration).

The molecular parameters used to evaluate the advanced human toxicity profile are presented in Table 8. The analysis of Table 8 shows that the antibiotic Cefquinoma has a positive profile for four toxicological parameters: Respiratory Toxicity, Reproductive Toxicity, Mitochondrial Toxicity and Nephrotoxicity. The parameters evaluated are extremely relevant in pathophysiological terms for the proper functioning of the human organism.

Table 8: Toxicological Profile Assessment *in silico* Advanced Human

Toxicological parameters Q probability (%)		
Ocular Corrosion N		0,9847
Eye irritability N		0,9599
Hepatotoxicidade	N	0,6066
Skin sensitivity N		0,8345
Respiratory Toxicity	P	0,9778
Reproductive toxicity	P	0,8667
Mitochondrial Toxicity	P	0,9750
Nephrotoxicity	P	0,5491

Source: Author's own, (2024) – admetSAR® Version. 2.0.
Q: Qualitative; P: Positive; N: Negative

The toxicity of a chemical substance refers to its ability to cause some negative damage to a particular organ, as well as alter biochemical processes and enzyme systems. Thus, respiratory toxicity refers to the presence of substances that affect the respiratory tract. While reproductive toxicology evaluates the damage caused to sexual performance and fertility as a result of exposure to certain substances (FRIEDRICH et al., 2015).

Another important parameter refers to mitochondrial toxicity. The mitochondria correspond to an intracellular structure (organelle) responsible for converting energy from food into another energy with added value and transportable to cells and tissues, through the molecule adenosine-triphosphate (ATP). As a result, mitochondria, which are considered the primary source of ATP, are essential for the maintenance and life of eukaryotic cells. Because it has such functionalities, mitochondrial toxicity induced by xenobiotics is being addressed/researched in the pharmaceutical industry (PEREIRA et al., 2012).

Finally, with regard to nephrotoxicity, its incidence varies according to the functionalities employed and the kinetic characteristics of the drugs. In this sense, the most commonly related drugs Renal tissue is vulnerable due to some factors, as it is responsible

for receiving 25% of cardiac output, exposing the different renal cells to plasma flow. In addition, the rate of circulation of drugs in the kidney is much higher than in other tissues and protein-bound drugs are also released in the kidneys (MARTINS; IBRAHIM, 2022).

ADVANCED *IN SILICO* ENVIRONMENTAL PROFILE ASSESSMENT

The most robust environmental in silico *toxicological evaluation* was performed with the aid of the computer program EPI Suite™ version 4.1. The Estimation *Programs Interface (PPE*) Suite™ is a Windows® program that predicts physicochemical and environmental properties developed by the Environmental Protection Agency (EPA) and Syracuse Research Corp (SRC) (EPI Suite™, 2023). Based on the data determined in the program, it was possible to predict the most advanced environmental toxicology of the antibiotic Cefquinoma, as shown in Table 9.

Table 9: Toxicological Profile Assessment *in silico* Advanced Environmental

Molecular Descriptions	Percentage (%)
Aquatic Biodegradation	No
Total Removal	1.89 %
Total Biodegradation	0.09 %
Total Sludge Adsorption	1.80 %
Total Air Removal	0.00 %

Source: Author, (2023) – EPI Suite™ Program version 4.1.

The molecular descriptors presented in table 9, prediction of aquatic biodegradability, total removal, total biodegradation, total sludge adsorption, and total air removal are essential parameters in the evaluation of the environmental behavior of chemical substances. According to the data obtained computationally, it is possible to assess that the antibiotic Cefquinoma does not suffer environmental biodegradation, since its total removal is only 1.89%, corroborating the *in silico* environmental toxicological evaluation carried out with the help of the Chinese online platform admetSAR® version 2.0.

The biodegradability prediction characterizes whether the antibiotic will undergo natural decomposition and its total environmental removal, which reflects on the efficiency of the treatment processes in order to eliminate the antibiotic residues from the environment and the total biodegradation indicates the complete transformation of the antibiotic through biological processes into other harmless products.

The computer program EPI Suite™ provides for the removal of a certain chemical in a typical sewage treatment plant based on activated sludge. To this end, total removal

values and three processes that can contribute to removal are provided: total biodegradation, sludge adsorption and air removal.

It is noted that in the treatment, the antibiotic Cefquinoma is removed from the sludge at a rate of only 1.80%. Total sludge adsorption highlights a compound's affinity with sludge particles, influencing its mobility and potential impact on aquatic ecosystems. Finally, the total air removal assessment is null (0%), addresses the dispersion and fate of chemicals in the air, and is crucial in understanding the potential atmospheric effects. Through the results presented, it is inferred that it is quite inert and cumulative and the joint consideration of these properties, provides a comprehensive view of the interaction of the antibiotic under study with the environment, helping in the process of sustainable management.

CONCLUSION

The antibiotic Cefquinoma was chosen as the object of research because it is a veterinary antimicrobial in which little is known about its environmental, human and human pharmacokinetic toxicity. In this research, *in silico* methodologies were used in order to predict molecular descriptors for the antibiotic Cefquinoma.

The in silico environmental *toxicological study* revealed that the veterinary antibiotic does not have toxicity for bees and crustaceans, but it is toxic for fish and its chemical structure does not suffer environmental biodegradation. Therefore, in addition to contaminating the aquatic environment and fish, the chemical structure of the antibiotic remains unchanged in the environment because it does not undergo environmental biodegradation, presenting a total removal rate of only 1.89%, resulting in contamination of the food chain and humans through food.

The ADME *in silico study* indicated that the antibiotic does not have a good prediction regarding human oral bioavailability, has a low intestinal absorption rate, does not have permeability by the blood-brain barrier, and does not inhibit glycoprotein P. The in silico pharmacokinetic study also revealed that the antibiotic does not inhibit hepatic isoenzymes of the cytochrome P450 complex, that is, there will be no biochemical alteration in the metabolization process in the liver, which facilitates the excretion of xenobiotic and/or lipophilic compounds from the human body.

Initially, the human in *silico* toxicological study indicated promising data, since the antibiotic does not have mutagenic toxicity with regard to the AMES test, does not present carcinogenicity and as for acute oral toxicity it falls into category III, that is, it has low toxicity. However, a more robust human in *silico toxicology study* revealed that the

veterinary antibiotic has respiratory toxicity, reproductive toxicity, mitochondrial toxicity and nephrotoxicity. As a result of the present research, it was possible to evaluate the environmental and human toxicity, the human oral bioavailability and the evaluation of pharmacokinetic descriptors in humans of the antibiotic Cefquinoma.

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