CAPÍTULO
1

In silico Pharmacokinetic and Toxicological Evaluation of Cephalonium Antibiotic in Human and Environmental Health

https://doi.org/10.56238/alookdevelopv1-001

Evelyn Gabriely Martins Fonseca
Graduate in Chemical Engineering
Institution: Federal Institute of the Northern Minas Gerais (IFNMG)
Campus: Montes Claros- Minas Gerais – Brazil
Rua Dois, 300 - Village do Lago I - Montes Claros/MG - CEP: 39404-058
Member of the Medicinal Chemistry and Advanced Computational Technologies Group
E-mail: evelyngabriely5@gmail.com

Luiz Frederico Motta
Bachelor's Degree in Pharmacy and Biochemistry from the University of São Francisco: USF – Bragança Paulista (SP)
Degree in Chemistry from Faculty Oswaldo Cruz – São Paulo (SP)
Postgraduate Lato Sensu in Chemistry by Oswaldo Cruz – São Paulo (SP)
Master's Degree in Physical Chemistry with an emphasis in Medicinal Chemistry from the State University of Campinas: UNICAMP (SP)
Ph.D. in Sciences with an emphasis in Medicinal Chemistry from the State University of Campinas - UNICAMP - São Paulo - SP.
Institution: Federal Institute of the Northern Minas Gerais (IFNMG)
Campus: Montes Claros- Minas Gerais – Brazil
Rua Dois, 300 - Village do Lago I - Montes Claros/MG - CEP: 39404-058
Leader of the Medicinal Chemistry and Advanced Computational Technologies Group
E-mail: luiz.motta@ifnmg.edu.br

ABSTRACT

Antibiotics are drugs that have revolutionized the world scenario for the treatment of infectious diseases caused by bacteria and have reduced morbidity and mortality rates worldwide. Due to the increase in population and veterinary treatment, the use of medicines for health and treatment in animals has increased considerably. In this context, the present study aimed to evaluate the potential pharmacokinetic and toxicological impacts of the veterinary antibiotic Cephalonium on human and environmental health. In this work, predictions of molecular properties for the antibiotic were performed with the aid of in silico methodologies. In silico pharmacokinetic studies have shown promising results, as the antibiotic has a low rate of human intestinal absorption, is not permeable by the blood-brain barrier, and does not inhibit hepatic isoenzymes. The antibiotic showed promising data about the Ames test, showing itself to be non-toxic (non-mutagenic), not carcinogenic, with acute oral toxicity classified as category III (low human toxicity). In the in silico evaluations of environmental toxicity, Cephalonium was not toxic for bees and crustaceans, however, it showed a toxicity for fish. Therefore, it is noteworthy that Cephalonium promotes environmental and consequently human contamination by the presence of antibiotic residues present in food.

Keywords: Antibiotic, Cephalonium, Environmental Contamination, Chemoinformatics, in silico ADME-TOX, in silico Environmental Toxicology.

1 INTRODUÇÃO

Antibiotics are drugs used in the treatment of infectious diseases caused by bacteria and have reduced morbidity and mortality rates worldwide. However, due to the growing population and the inappropriate use and disposal of antibiotics, they are considered a global threat to environmental and human health (GUIMARÃES et al., 2010). It is currently estimated that more than half of the world's production of antimicrobials is used in the treatment of animals (MAGALHÃES et al., 2012).

Antibiotics are considered potentially dangerous, since they result in a resistant metabolization process and are susceptible to bioaccumulation and biomagnification phenomena, are environmentally

ubiquitous, and residues are released in considerable quantities into the environment without prior treatment (MONTAGENERA et al., 2017), (FENT et al., 2006).

Several authors have shown in the literature that the increase in animal husbandry promotes an increase in the use of active principles for the health and treatment of animals useful in food production. In this way, the abuse and inappropriate use of veterinary antibiotics can generate a high concentration of residues in products of animal origin, causing several direct and indirect effects on consumers, since chemical exposures generate antibiotic residues in the animal (DE SOUZA et al., 2013). Therefore, this research deserves it, since it will evaluate the pharmacokinetic and toxicological impacts of the Cephalonium antibiotic on humans and the environment (BARRIOS et al., 2015).

Cephalonium is a semi-synthetic antibiotic of the pharmacological category of beta-lactams, with bactericidal biological activity and a broad spectrum of action, indicated in the treatment of dry udder infections and prevention of future infections that may occur during the drying period of cows. (TAVARES, 2014). This cephalosporin has bactericidal biological activity against most of the causative agents of bovine mastitis such as Staphylococcus aureus (including penicillin-resistant strains), Streptococcus agalactiae, Streptococcus dysgalactiae, Actinomyces pyogenes and Corynebacterium ulcerans, Streptococcus uberis, E. coli, Proteus spp, Klebsiella, Citrobacter spp and Enterobacter (LANGONI et al., 2017).

Veterinary antibiotics when used in animals are not always metabolized in the body, and part of the residues are excreted in the urine and feces. Such animal excreta results in fertilization via the transmission of antibiotics through environmental contamination (DÍAS-CRUZ et al., 2007). Antibiotic residues are absorbed in the soil, accumulate in plant tissues, resulting in contamination in the harvest, and are transported to water bodies, resulting in high human risk due to the consumption of animal/plant foods. (DE SOUZA et al., 2013).

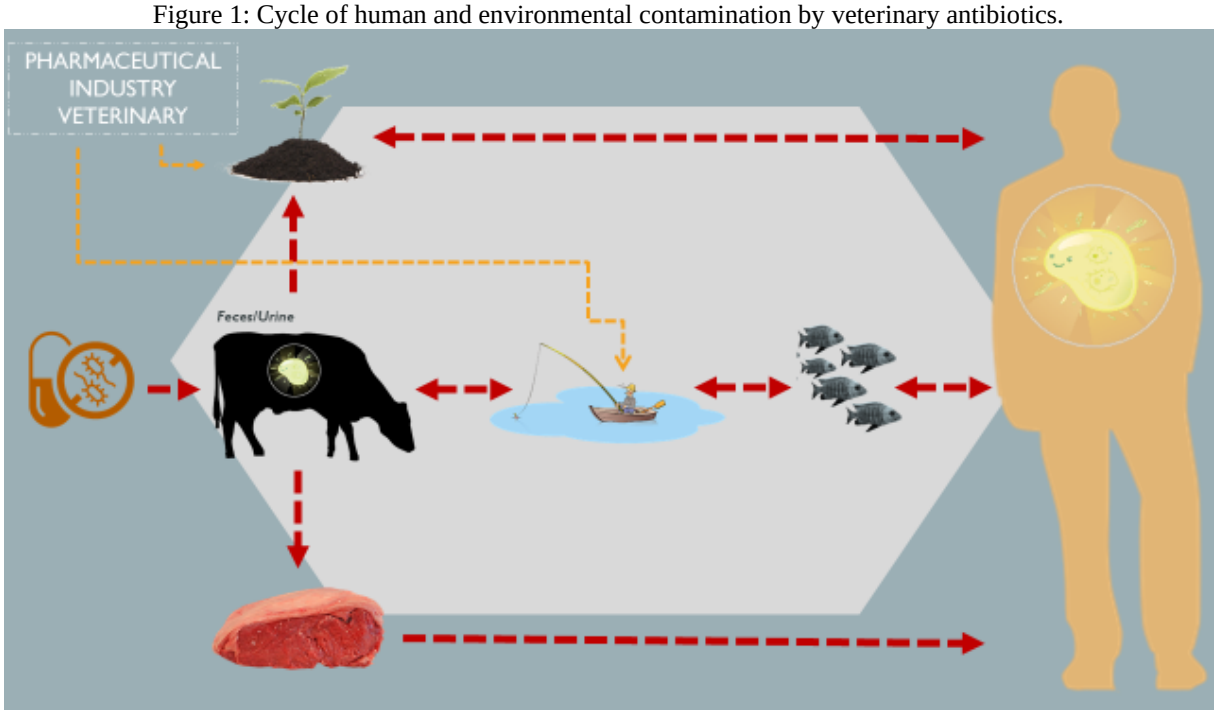
There are reports of several legislations that regulate the use of veterinary drugs in animals that produce food (BOTSOGLOU et al., 2001). However, in Brazil, the ABNT NBR 10004/2004 standard is used to define and classify solid waste, which classifies them as Class I–Hazardous or Class II–Non-Hazardous. All categories of antibiotic waste produced should receive proper treatment and disposal, however, in Brazil, drugs classified as Solid Waste from Health Services (RSS) are the ones that result in the most attention, since the treatment and destination are not effective and may affect human health, as well as the environment and animals (ABRELPE, 2017).

Some studies have revealed that antibiotic residues can cause high imbalance and impact the aquatic environment. It is evidenced that year after year, tons of medicines are produced and remain in the environment (ABRELPE, 2017). Aquatic environmental contamination can be caused in several ways, among which the following stand out: through watercourses, incorrect disposal of medicines,

spraying crops, and by animal excretion. In addition, another contamination pathway observed is correlated with production and disposal by pharmaceutical industries, which even performing the treatment of effluents can result in antibiotic residues in groundwater (BILA, et al., 2003).

Among the drugs commonly found in the aquatic environment, antibiotics, both for human and veterinary use, are the ones that arouse the greatest concern, because even at low residual concentrations, over a prolonged period (environmental half-life rate) they can induce the emergence of resistant bacteria causing an imbalance in human and environmental health (HERNANDEZ et. al, 2007). Therefore, antibiotic residues contaminate humans and the environment through direct and/or indirect contact, via the food chain, water resources, or through animal excreta, and the main routes of contamination are shown in Figure 1.

Figure 1: Cycle of human and environmental contamination by veterinary antibiotics.



Source: Author himself, 2022.

The presence of antibiotic residues in food results in serious risks to human health, as it causes allergic reactions in some individuals, in addition to also causing an imbalance in the human intestinal flora, providing an increase in resistant bacteria, several consequences and adverse reactions (side effects) (WHO, 2022).

2 MATERIAL AND METHODS

In this research, computational programs and databases of international online Chemoinformatics platforms were used to determine the molecular properties (molecular descriptors), taking into account the molecular chemical structure of the Cephalonium antibiotic.

2.1 COMPUTATIONAL MOLECULAR MODELING

Initially, the molecular chemical structure of the antibiotic Cephalonium was drawn two-dimensionally (2D) and later it was visualized three-dimensionally (3D) with the aid of the computer program ACD/ChemSketch® - Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the energy (E1) of the three-dimensional chemical structure of the antibiotic was tabulated. Subsequently, to obtain the local minimum of the chemical structure, Energy Minimization was carried out using the Semi-empirical Quantum Method PM3 (Parametric Method 3) with the aid of the Arguslab® Freeware program version 4.0 (Thompson and Planaria Software LLC, Inc., 1997) and the steric energy (E2) of the chemical structure of the antibiotic was also tabulated for further molecular modeling studies. Finally, the minimized chemical structure of the antibiotic molecule was saved in an MDL molfiles (.mol) file.

2.2 IN *SILICO* PHARMACOKINETIC STUDY FOR HUMAN ORAL BIOAVAILABILITY

With the aid of the computer program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the source code of the file (.mol), the SMILES code (Simplified Molecular Input Line Entry Specification) was obtained and exported. to international online database platforms. Through Chemoinformatics, a human *in silico* Pharmacokinetic study was carried out to predict the descriptor molecular properties of the Cephalonium molecule with the aid of the Molinspiration Cheminformatics® platform database - Molinspiration Property Calculator (mipc) (https://www.molinspiration.com) (GROB, 1986).

The Molinspiration Property Calculator is a desktop program that allows for easy interactive calculations of molecular properties, as well as the generation of data tables that can be used for QSAR studies to correlate biological activity and molecular chemical structure. The program used via the platform is in Java, so it can be used on any platform where Java (version 1.3 or higher) is installed. Java is currently available practically on Windows, LINUX, and Unix. This database predicts molecular descriptors to assess the oral bioavailability of the molecule, taking into account the Lipinski Rule, also known as the Rule of Five.

2.3 *IN SILICO* PHARMACOKINETIC STUDY (*IN SILICO* ADME)

In silico Pharmacokinetic Study (*in silico* ADME) Initially, the *in silico* ADME study (ADME, its acronym in English: refers to the absorption, distribution, metabolism, and excretion) of the antibiotic Cephalonium was carried out, to predict the following parameters Molecular factors: Human Intestinal Absorption Rate (HIA), Blood-Brain Barrier (BBB) Permeability, Caco-2 Epithelial Cell Permeability, P-Glycoprotein Inhibition and Cellular distribution of the antibiotic in the human body.

Subsequently, the *in silico* ADME study was performed to predict the inhibition and interaction with liver enzymes of the cytochrome P450 complex (CYP450) in the process of the Hepatic Metabolization (Hepatic Biotransformation) of the antibiotic. The *in silico* ADME study was carried out with the aid of the Chinese international online platform admetSAR® (http://lmmd.ecust.edu.cn/admetsar2/), coordinated by Professor Doctor Yun Tang, Leader of the Molecular Modeling and Design Laboratory (LMMD), from the School of Pharmacy, East China University of Science and Technology (YANG, et al., 2018).

2.4 *IN SILICO* TOXICOLOGICAL STUDY

In silico toxicological study of the antibiotic was carried out to predict human toxicity by the AMES test (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; NC: non-carcinogenic), and Acute Oral Toxicity in categories. The present study was also carried out with the aid of the Chinese international platform admetSAR® (http://lmmd.ecust.edu.cn/admetsar2) (YANG, et al., 2018).

2.5 *IN SILICO* ENVIRONMENTAL TOXICOLOGICAL STUDY

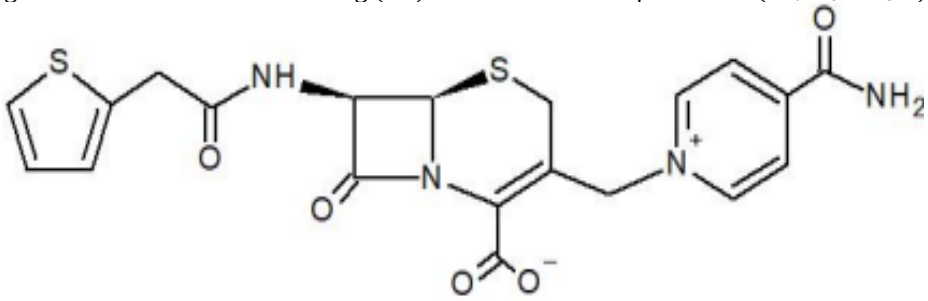
The *in silico* environmental toxicological study of the antibiotic was carried out to predict the environmental biodegradation, and toxicity in fish, bees, and crustaceans. The present study was also carried out with the aid of the Chinese international platform admetSAR® (http://lmmd.ecust.edu.cn/admetsar2) (YANG, et al., 2018).

3 RESULTS AND DISCUSSION

3.1 COMPUTATIONAL MOLECULAR MODELING

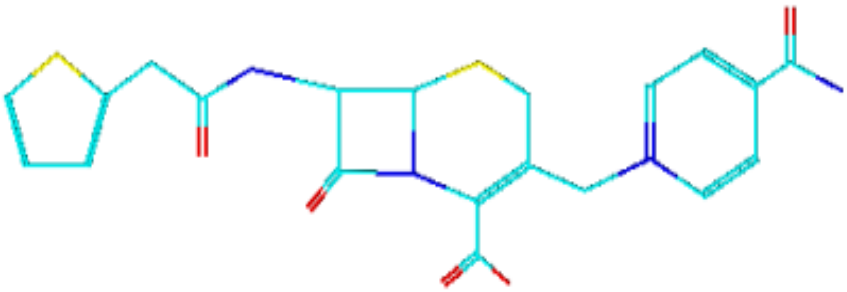
The energetic minimization of the chemical structure of the antibiotic consists of the process in which the atomic coordinates of the molecular structure are altered to reduce its steric energy (greater molecular stability) which corresponds to its local minimum (SANT'ANNA, 2009). After carrying out the Molecular Modeling step (2D Design, 3D Design, and Energy Minimization) for the chemical structure of Cephalonium, the minimized chemical structure was saved in an MDL molfiles file (.mol) and the steric energy (E2) was tabulated. Figures 2 and 3 represent, respectively, the chemical structure of the two-dimensional drawing (2D) and three-dimensional drawing (3D) of the Cephalonium antibiotic molecule.

Figure 3: Two-dimensional drawing (2D) of the antibiotic Cephalonium ($C_{20}H_{18}N_4O_5S_2$).



Source: ChemSketch® Freeware version 2021.

Figure 4: Three-dimensional (3D) drawing of the antibiotic Cephalonium ($C_{20}H_{18}N_4O_5S_2$).



Source: ChemSketch® Freeware version 2021.

3.2 *IN SILICO* PHARMACOKINETIC STUDY FOR HUMAN ORAL BIOAVAILABILITY

The evaluation of the human oral bioavailability profile (Oral Bioavailability) of the antibiotic Cephalonium was performed with the aid of the Chemoinformatics platform Molinspiration Cheminformatics® (https://www.molinspiration.com). The human oral bioavailability profile follows the Lipinski Rule, also known as the Rule of Five. Researcher Christopher Lipinski developed the Rule-of-Five based on the hypothesis that several molecular properties (molecular descriptors) obtained for a range of compounds in the pre-clinical stages and in the phase, I safety evaluation were discarded from the development process of drugs (LIPINSKI et al., 2004), (FARIA et al., 2023).

The rule predicts whether or not a compound has promising properties to be a drug. For a drug to be developed and synthesized, it must have favorable physicochemical properties, that is, it will be based on the United States Adopted Name (USAN), the International Non-proprietary Name (INN) and the huge database Word Drug Index (WDI) to be further evaluated in security phase II (PANDIT, 2008). The rule will indicate whether the antibiotic will obey Lipinski's Rule and will estimate whether Cephalonium molecules have a high rate of human intestinal absorption, plasmatic solubility, in tissue fluids, and permeability by biological membranes when administered/ingested orally.

Lipinski's Rule takes into account the following criteria: Molecular Weight (PM), which should not exceed 500 Da (Dalton); o Log P = miLogP (logarithm of the partition coefficient), whose limit value is 5; Hydrogen Bond Donor Sites = SDLH and Hydrogen Bond Acceptor Sites = SALH, which

should not exceed values 5 and 10 respectively, and the Polar Surface Topological Area (TPSA) which should be less than 140 Å (FARIA et al., 2023), (ARAUJO et al., 2022), (MOTTA et al., 2023).

The described molecular descriptors account for 90% of drugs administered orally that is at the level of phase II clinical development (LIPINSKI et al., 2004). Other descriptors such as the Number of Rotatable Bonds (NLR) and Molecular Volume (MV) were determined as an extension of Lipinski's Rule. The extension of Lipinski's Rule, known as Veber's Rule, indicates molecular flexibility, measured by rotational bonds, which can considerably influence oral bioavailability, that is, the greater the flexibility of the molecule, the less likely it is to be active when administered by orally (VEBER et al., 2002), (FARIA et al., 2023), (CEZÁRIO et al., 2022), (MOTTA et al., 2023).

The analysis of Table 1 reveals promising results for the antibiotic Cephalonium since it does not violate Lipinski's Rule, therefore it presents a good prediction regarding the human oral bioavailability profile. Table 1 shows the following molecular descriptors:

1) miLogP: Partition coefficient logarithm (molecular hydrophobicity measure);

2) TPSA: Polar Surface Topological Area (square angstrom);

3) MW: 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 Lipinski's rule;

7) NLR: Number of Rotatable Links;

8) VM: Molecular Volume in (cubic angstrom).

Table 1 represents the molecular descriptor values obtained after using the online database of the Molinspiration Cheminformatics® platform (https://www.molinspiration.com).

Table 1: Evaluation of the Human Oral Bioavailability Profile.

milogP	TPSA	PM	SALH	SDLH	Violations	NLR	VM
- 5,66	136,51	458,52	6	3	0	7	370,85

Source: Molinspiration Cheminformatics®

3.3 *IN SILICO* PHARMACOKINETIC STUDY (*IN SILICO* ADME)

At this stage of the work, the *in silico* ADME study was carried out to predict the following molecular parameters for Cephalonium: Human Intestinal Absorption Rate (HIA), Permeability through the Blood-Brain Barrier (BBB), Permeability through Caco-2 epithelial cells, Inhibition of P-glycoprotein and cellular distribution of the antibiotic in the human body.

The absolute bioavailability of a drug is understood as the fraction of an orally administered dose that reaches the systemic circulation intact. Absolute bioavailability depends on several factors, including:

1) Drug release system of the presentation form (pharmaceutical form);

2) Permeability of the dissolved drug by the epithelial membrane at the site of absorption in the gastrointestinal tract and;

3) Loss carried out by pre-systemic metabolism (GOLAN et al., 2017).

In pharmacokinetic terms, human intestinal absorption (HIA) represents the sum of the absorption rate and the absolute bioavailability of the drug that reaches the systemic circulation, since when administered orally, the concentration of the drug will always be less than 100% due to the incomplete extension in the process of absorption and elimination of the first-pass effect in the liver (hepatic biotransformation). Thus, the % HIA represents the percentage of dose of the active principle that was administered orally and that reaches the hepatic portal system (HOU, 2008), (ARAUJO et al., 2022), (MOTTA et al., 2023).

Currently, the FDA (Food and Drug Administration) has recommended an in silico study using Caco-2 epithelial cells, as it is an estimative parameter of intestinal permeability due to its morphophysiological similarity with human enterocytes. Due to the complexity regarding the absorption of compounds in the gastrointestinal tract, the prediction of permeability by Caco-2 epithelial cells (from colon adenocarcinoma) was also evaluated (MOTTA et al., 2023).

In the Central Nervous System (brain and spinal cord), endothelial cells lack pores and exhibit little transcytotic activity. For a given analog to cross the blood-brain barrier, it will need to pass through endothelial cells and permeate through the luminal and basement membranes. This permeability through the membranes requires specific physical-chemical properties of the compound and depends on the transport mechanism (THOMAS, 2003), (PATRICK, 2002).

The permeability of chemical compounds through the blood-brain barrier (BBB) consists of passing through endothelial cells that have tight and compacted junctions, which considerably restricts the permeability of the compounds, ensuring that the drug candidate does not cross the BBB, otherwise, it would increase considerably the probability of having side effects (adverse effects) (THOMAS, 2003). In the capillary endothelia of cerebral vessels, the presence of P-glycoprotein is essential, as it acts as a defense mechanism capable of pumping back into the blood the xenobiotic chemical substances that could eventually cross the blood-brain barrier (ARAUJO et al., 2022), (CEZÁRIO et al., 2022), (MOTTA et al., 2023).

The P-glycoprotein molecular property was also evaluated as it is a selective descriptor for the entry of xenobiotics into tissues. P-glycoprotein (present in epithelial cells) has a primordial role in excretion and consequently reduces the absolute bioavailability of several analogs (AMIN, 2013), (ASHORAJ et al., 2003).

Table 2 indicates the evaluation of the in silico pharmacokinetic profile (in silico ADME) of

the following molecular descriptors: Human Intestinal Absorption Rate (HIA), Permeability through the Blood-Brain Barrier (BBB), Permeability through Caco-2 epithelial cells, Inhibition of P-glycoprotein and Cellular distribution of the antibiotic in the human body. Data are presented qualitatively [(Q – P: positive or N: negative)] and quantitatively (P = probability).

Table 2: Evaluation of the human pharmacokinetic *in silico* profile (*in silico* ADME).

BBB		**HIA**		**Distribution**		**P-glycoprotein inhibitor**		**Caco-2**	
Q	**P**	**Q**	**P**	**Q**	**P**	**Q**	**P**	**Q**	**P**
N	95,0%	N	61,4%	Mitochondria	33,2%	N	56,5%	N	90,8%

Source: admetSAR®.

Q: Qualitative; P: Probability.

The analysis of Table 2 reveals that Cephalonium is negative for the HIA parameter (Human Intestinal Absorption) with a probabilistic value equivalent to 61.4%. Therefore, it can be stated that the antibiotic has a moderate intestinal absorption rate, as the intestinal absorption rate is approximately 38.6%. Even if the intestinal absorption rate is moderate, it will still be possible to detect the antibiotic in the blood plasma (YAKAIAH et al., 2015).

The evaluation of the HIA molecular descriptor takes into account the following intestinal absorption rate values as a reference:

1) 0 to 20% low absorption rate;

2) 20 to 70% moderate absorption rate;

3) 70 to 100% high absorption rate.

The results presented in Table 2 also indicated that the parameter BBB (*blood-brain barrier*), was negative in terms of the permeability of the antibiotic through the blood-brain barrier, therefore, Cephalonium molecules do not cross the blood-brain barrier and, therefore, reduces the chances of impacts by xenobiotics on the brain and spinal cord. This molecular descriptor is related to the most important barrier involved in releasing or blocking the passage of chemical substances from the blood to the brain (WANG, 2015).

Hepatic metabolism is the mechanism that promotes changes in the chemical structure of the compound through biochemical reactions resulting in products called metabolites. Hepatic biotransformation, together with excretion, are pharmacokinetic components responsible for drug elimination (ARAUJO et al., 2022).

Hydrophilic (high polarity) drugs are easily excreted in the urine because of their high plasma solubility and poor reabsorption in the renal tubules. Many compounds have high hydrophobicity (lipophilicity), therefore they need to be metabolized into hydrophilic compounds so that they can be excreted via urine. The terms are lipophilic/hydrophobic and lipophobic/hydrophilic refer respectively

to the solubility of the compounds in non-polar and polar media. It is important to note that blood plasma, interstitial fluid, and cellular cytoplasm have high polarity. When hydrophilic drugs reach the bloodstream, they pass slowly through the hepatocyte lipid membrane in the liver without changing their chemical structure and do not have access to liver enzymes (ARAUJO et al., 2022), (MOTTA et al., 2023).

The liver is the main drug-metabolizing organ, and the three major components in the metabolizing process are the reagents (drug or xenobiotic), the product (metabolite), and the catalyst of the biochemical reaction (liver enzymes). Hepatocytes have an apparatus of enzymes responsible for hepatic metabolism, located in the membranes of the smooth endoplasmic reticulum (REL) and rough endoplasmic reticulum (RER) (CEZÁRIO et al., 2022), (MOTTA et al., 2023).

REL enzymes are enzymes of the cytochrome P450 complex, also called microsomal enzymes or mixed-function oxidases. These enzymes are of great importance in the metabolization of drugs and catalyze many reactions of hydroxylation and oxidative hydrolysis of chemical bonds of the -N-C- or -0-C type, metabolizing a variety of lipophilic (hydrophobic) compounds. The cytochrome P450 complex enzyme superfamily is subdivided into families (eg CYP3), subfamilies (eg CYP3A), and finally into isoenzymes (eg CYP3A4) according to the primary structure of the amino acid chain of the cytochrome P450 complex enzyme.

The human *in silico* ADME liver metabolism study for the inhibitory prediction of cytochrome P450 complex enzymes (CYP450) indicates the inhibitory capacity (I: yes/no) and the probability of inhibition (P). Table 3 represents the ability of the *in silico* pharmacokinetic predictive evaluation of the Cephalonium antibiotic in terms of inhibition and interaction with liver enzymes of the cytochrome P450 complex (CYP450).

Table 3: Evaluation of the human pharmacokinetic *in silico* profile (*in silico* ADME) of the inhibitory capacity of the cytochrome P450 complex isoenzymes (CYP450).

CYP4503A4		**CYP4502C9**		**CYP4502C19**		**CYP4502D6**		**CYP4501A2**	
I	**P**	**I**	**P**	**I**	**P**	**I**	**P**	**I**	**P**
No	94,1%	No	90,7%	No	90,2%	No	92,3%	No	90,4%

Fonte: admetSAR®

I: Inhibition; Q: Probability.

Analysis of Table 3 revealed that Cephalonium does not inhibit any of the isoenzymes of the cytochrome P450 complex (CYP450). Therefore, the antibiotic does not affect hepatic metabolism, facilitating the excretion of xenobiotic and/or lipophilic compounds from the body (GALLI & FEIJOO, 2002). Compounds that present the inhibitory capacity of hepatic isoenzymes will result in a cascade of biochemical, and pathophysiological processes and adverse effects, impacting the hepatic metabolism of other drugs, the formation of toxic metabolites, and alterations in the genetics of the

formation of several enzymes. (DEVLIN, 2002).

3.4 *IN SILICO* TOXICOLOGICAL STUDY

The *in silico* toxicological study was carried out to predict the toxicity of the antibiotic taking into account three main tests: mutagenicity test (Ames test - T: toxic; NT: non-toxic), carcinogenicity test (C: in carcinogenic; NC: non-carcinogenic) and Acute Oral Toxicity test in categories (I, II, III, and IV). The Ames test consists of a bacterial assay, in which the Salmonella typhimurium strain (TA100 and TA1535) is used to evaluate the mutagenicity of the Cephalonium antibiotic (MIRANDA et al., 2021).

Another relevant parameter evaluated regarding human toxicity refers to Acute Oral Toxicity, in which the antibiotic is characterized by the administration and exposure of the same in a single dose or varied in 24 hours in which the adverse effects that occur in this time interval are analyzed. The Acute Oral Toxicity test classifies the antibiotic according to the US Environmental Protection Agency (EPA) (https://www.epa.gov) into four distinct categories based on the LD_{50} (median lethal dose) (GONÇALVES, 2011):

1) Category I: DL50 ≤ 50 mg/Kg;
2) Category II: 50 < DL50 < 500 mg/Kg;
3) Category III: 500 < DL50 < 5.000 mg/Kg;
4) Category IV: DL50 > 5.000 mg/Kg

Table 4: Assessment of human *in silico* toxicological profile.

AMES test		**Acute Oral Toxicity**		**Carcinogenic**	
Q	**P**	**C**	**P**	**Q**	**P**
NT	60,0%	III	72,5%	NC	92,0%%

Source: admetSAR®

Q: Qualitative; Q: Probability; C: Category

Table 4 shows qualitative (Q = NT/NC and C) and quantitative (P) information regarding the evaluation of the human *in silico* toxicological profile of the Cephalonium antibiotic. Toxicity assessment revealed promising data for the antibiotic. Cephalonium is non-toxic (non-mutagenic) in terms of the AMES test and also non-carcinogenic. Regarding its Acute Oral Toxicity, it is noted that the antibiotic fits into category III, that is, it has low toxicity. (GONÇALVES, 2011).

3.5 *IN SILICO* ENVIRONMENTAL TOXICOLOGICAL STUDY

The study of the *in silico* environmental toxicity of antibiotics is extremely relevant concerning the rational design of new drugs and such an assessment predicts the impact of the drug (antibiotic) on ecosystems. In the present work, the evaluated parameters were: the capacity of environmental

biodegradation, and toxicity in fish, crustaceans, and bees. The preliminary results obtained are shown in Table 5. The data are presented qualitatively [(Q – P: positive or N: negative)] and quantitatively (P = probability).

Table 5: Evaluation of the environmental *in silico* toxicological profile.

Environmental Biodegradation		**Toxicity in Bees**		**Toxicity in Crustaceans**			**Toxicity in Fish**	
Q	**P**	**Q**	**P**	**Q**	**P**		**Q**	**P**
N	92,5%	N	87,2%	N	60,0%		P	91,4%

Source: admetSAR®

Q: Qualitative; P: Probability.

The evaluation of environmental toxicity (Table 5) revealed that the antibiotic Cephalonium is not toxic to bees and crustaceans, but it presents considerable toxicity to fish and its chemical structure does not undergo environmental biodegradation, that is, the antibiotic, in addition to contaminating the aquatic environment and contaminate the fish, its chemical structure remains intact in the environment because it does not undergo environmental biodegradation, thus contaminating the food chain and, consequently, the human being through the food that will be contaminated.

4 CONCLUSION

Cephalonium was chosen for evaluation in this research because it is an antibiotic that little is known about its human pharmacokinetics and human and environmental toxicity. In the present work, *in silico* methodologies were used to predict molecular properties for the Cephalonium antibiotic molecule. The *in silico* ADME study revealed that the antibiotic has a good prediction for human oral bioavailability, moderate intestinal absorption rate, and does not present permeability through the blood-brain barrier. The *in silico* pharmacokinetic study also revealed that cephalonium cannot inhibit the hepatic isoenzymes of the cytochrome P450 complex (CYP450).

The *in silico* toxicological study showed promising data, since the antibiotic is non-toxic (non-mutagenic) in terms of the AMES test, does not present carcinogenicity and, in terms of Acute Oral Toxicity, falls into category III, that is, it has low toxicity.

The *in silico* environmental toxicological study indicated that Cephalonium is not toxic to bees and crustaceans, but it is highly toxic to fish and that its chemical structure does not undergo 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, contaminating the food chain and humans through food.

In addition, it is worth mentioning that because the results reveal that Cephalonium is a drug that promotes environmental and human contamination, which is why it is essential to continue with

advanced studies of the veterinary drug to evaluate other parameters and other impacts on humans. and in ecosystems.

REFERENCES

Adriana, c. F.; motta, l. F. Estudo qsar clássico de uma série de análogos de pirimidinilsalicilatos (psa) com potencial atividade biológica herbicida. Open science research x, editora científica digital. Curitiba, vol. 10, p. 80800-80817, 2023.

Araujo, l. F.; souza pinto, c. H.; motta, l. F. *In silico* pharmacokinetic and toxicological study of cinnamic acid analogues. Brazilian journal of development, curitiba, vol.8, n.12, p. 80800-80817, dec., 2022.

Acd/advanced chemistry development, inc. Chemsketch® freeware, versão 2021.

Anvisa: agência nacional de vigilância sanitária. Limites máximos de resíduos de medicamentos veterinários em alimentos de origem animal. 2018, 77. Available: <http://antigo.anvisa.gov.br/documents/>. Access date: 20/11/2022.

Ashoraj, y.; dey, c. S.; panchagnula, r.; varma, m. V. S. P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. Pharmacol. Res., 48 (4), p. 347-359, 2003.

Barrios, r. L. A.; sierra, c. A. S.; morales, j. D. C. J. Bacterias resistentes a antibióticos en ecosistemas acuáticos. Producción + limpia, vol. 10, no. 2, p.160-172, 2015.

Botsoglou, n. A.; fletabreouris, d. J. Drug residues in foodpharmacology, food safety, and analysis. 1 ed., nova york, marcel dekker, 2001.

Cezário, s. P. S.; correa, g. V.; motta, l. F. *In silico* pharmacokinetic and toxicological study of flavone analogues. Brazilian journal of development, vol.8, n.12, p. 80782-80799, dec., 2022

De souza, m. I. A.; lage, m. E.; prado, c. S. Resíduos de antibióticos em carne bovina. Centro científico conhecer, vol. 9, no. 16, p. 1917, 2013.

Devlin, thomas. Manual de bioquímica com correlações clínicas. 6a ed. Editora blucher, 2002.

De waterbeemd, h. V.; gifford, e. Admet in silico modelling: towards prediction paradise nat. Rev. drug discov. Vol. 2, p. 192-204, 2003.

Díaz-cruz, m.s. & barceló, d. Recent advances in lcms residue analysis of veterinary medicines in the terrestrial environment. Trac-trends anal. Chem., vol. 26, p. 637-646, 2007.

Fent, k.; weston, a. A.; caminada, d. Ecotoxicology of human pharmaceuticals. Aquatic toxicology. Aquatic toxicology,vol. 76, p. 122–159, 2006.

Grob, s. Molinspiration cheminformatics: cheminformatics on the web, novartis: bratislava university, slovak republic, 1986; available: http://www.molinspiration.com/. Access date: 28 de outubro de 2022.

Golan, d. E.; tashjian jr, a. H.; armstrong, e. J.; armstrong, a, w. Princípios de farmacologia: a base fisiopatológica da farmacologia. Rio de janeiro: guanabara koogan, 2017.

Galli, e.; feijoo, l. Citocromo p-450 y su importancia clínica revisión actualizada. Revista de neuropsiquiatria, 2002.

Gonçalves, n. Z. Avaliação da toxicidade oral aguda e atividade diurética de celtis iguanaea (jacq.) Sargent goiânia, go, 2011. Dissertação de mestrado.

Guimarães, d. O.; momesso, l. S.; pupo, m. T. Antibióticos: importância terapêutica e perspectivas para a descoberta e desenvolvimento de novos agentes. Química nova, vol. 33, no. 3, p. 667-679, 2010.

Hernández, f et al. Antibiotic residue determination in environmental waters by lc-ms. Trends in analytical chemistry, vol. 26, no. 6, p. 466-485, 2007.

Hou, t.; wang, j. Structure-adme relationship: still a long way to go? Expert opin. Drug metab. Toxicol., 4, 759 – 770, 2008.

Langoni, h.; salina, a.; oliveira, g. C.; junqueira, n. B.; menozzi, b. D.; joaquim, s. F. Considerações sobre tratamento das mastites. Pesq. Vet. Bras., vol. 37, no. 11, p. 1261-1269, 2017.

Lipinski, c. A. Lead and drug-like compounds: the rule-of-five revolution. Drug discovery today: technologies, vol. 1, no. 4, 337-341, 2004.

Lpe – associação brasileira de empresas de limpeza pública e resíduos. Panorama dos resíduos sólidos no brasil, 2017. Edição especial de 15 anos. Available in: <https://abrelpe.org.br/pdfs/panorama/>lpe_2017.pdf. Access date: 14/11/2022.

Moda, t. L. Modelagem in silico de propriedades farmacocinéticas para a avaliação de candidatos a novos fármacos. São carlos, sp, 2011. Tese de doutorado.

Magalhães, c.g.; paiva, c.r.; botelho, b.g.; oliveira, a.m.g.; souza, l.f.; nonaka, c.v.; santos, k.v.; farias, l.m. cravalho, m.a.r. in-house validation of premitest, a microbiological screening test with solvent extraction, for the detection of antimicrobial residues in poultry muscles. Food additivies and contaminants. Londres, vol. 29, no.4, p.535-540, abr. 2012.

Miranda, c. C. S., salazar, v. A. C., brito, m. D. R. M. Avaliação in silico da atividade antifúngica de compostos sulfurados presente na petiveria alliacea l. Revista de casos e consultoria, vol. 12, no. 1, 2021.

Montagnera, c. C.; vidala, c.; acayaba, r. Contaminantes emergentes em matrizes aquáticas do brasil: cenário atual e aspectos analíticos, ecotoxicológicos e regulatórios. Química nova, vol. 40, no. 9, p. 1094-1110, 2017.

Motta, l. F.; souza pinto, c. H.; araujo, l. F. Análise farmacocinética e toxicológica *in silico* para derivados do ácido cinâmico. Open science research x, editora científica digital. Curitiba, vol. 10, p. 751-765, 2023.

Motta, l. F.; correa, g. V.; cezário, s. P. S. Análise farmacocinética e toxicológica *in silico* para derivados de flavonas. Open science research x, editora científica digital. Curitiba, vol. 10, p. 177-191, 2023.

Nih: national institutes of health. Available in: <https://pubchem.ncbi.nlm.nih.gov/compound/cefalonium>. Access date: 19/11/2022.
Pandit, n. K. Introdução às ciências farmacêuticas. 1a ed. Artmed: são paulo, 2008.

Patrick, g. L. An introduction to medicinal chemistry. 2a ed. Oxford: new york, 2002.

Sant´anna, c. M. R. Métodos de modelagem molecular para estudo e planejamento de compostos bioativos: uma introdução. Rev. Virtual quim., vol.1, no. 1, p. 49-57. 2009.

Thomas, g. Química medicinal: uma introdução. 1a ed. Guanabara koogan: rio de janeiro, 2003.

Thompson, m. Arguslab® freeware versão 4.0, mark thompson and planaria software, llc., 1997.

Wang, y. In silico adme/t modelling for rational drug design. Quart. Rev. Biophys., vol. 48, p488–515, 2015.

Wise, r. Antimicrobial resistance: priorities for action. Journal of antimicrobial chemotherapy, no. 49, p. 585-586, 2002.

Who - world health organization. Tackling antibiotic resistance from a food safety perspective in europe. Available in: <https://www.euro.who.int/__data/assets/pdf_file/> access date: 14/11/2022.

Yang, h.; sun, l.; li, w.; liu, g.; tang, y. In silico prediction of chemical toxicity for drug design using machine learning methods and structural alerts. Front. Chem., 6, 30, 2018.

Yakaiah, c.; sneha, t.; shalini, t.; srinivas, c.; anand, k. D.; niranjana, k. A.; srinivas. K. V. N. S.; sarfaraz. A.; kotesh. K. J.; feroz. K.; ashok. T. P. G. Synthesis, docking and admet studies of novel chalcone triazoles for anti-cancer and anti-diabetic activity. European journal of medicinal chemistry, 93, 564-573, 2015.