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TOXICOLOGICAL EVALUATION AND IN SILICO PHARMACOKINETICS OF CEFTIOFUR IN HUMAN AND ENVIRONMENTAL HEALTH

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ABSTRACT

Ceftiofur is a broad-spectrum cephalosporin, effective against Gram-positive and Gram-negative pathogens. This antibiotic works by inhibiting the synthesis of the bacterial cell wall. Antibiotics, used in both human and veterinary medicine, are drugs of concern when found in the environment. This is because, even at low concentrations, prolonged exposure to these residues can lead to the emergence of resistant bacteria, compromising human

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health and environmental balance. Direct or indirect contact, whether through the food chain, water resources or animal excreta, represent the main routes of contamination. In the present study, in silico methodologies were employed to predict the molecular properties of the antibiotic Ceftiofur. The in silico environmental toxicology study showed that Ceftiofur is not toxic to bees, but is toxic to fish and crustaceans, and its chemical structure does not degrade in the environment. ADME in silico analysis indicated that the antibiotic does not present a favorable prediction for oral bioavailability, due to violations of the Lipinski criteria and has a moderate intestinal absorption rate and absence of permeability across the blood-brain barrier. In addition, the in silico pharmacokinetic study revealed that Ceftiofur has no inhibitory capacity on any of the five hepatic isoenzymes of cytochrome P450 complex (CYP450). The human in silico toxicology study showed promising results, demonstrating that the antibiotic is non-toxic (non-mutagenic) in the AMES test, has no carcinogenic properties, and is classified in category IV for acute oral toxicity, indicating low toxicity.

Keywords: Antibiotic. Ceftiofur. In Silico Environmental Toxicology. In Silico Human Toxicology and In Silico Human Pharmacokinetics.

INTRODUCTION

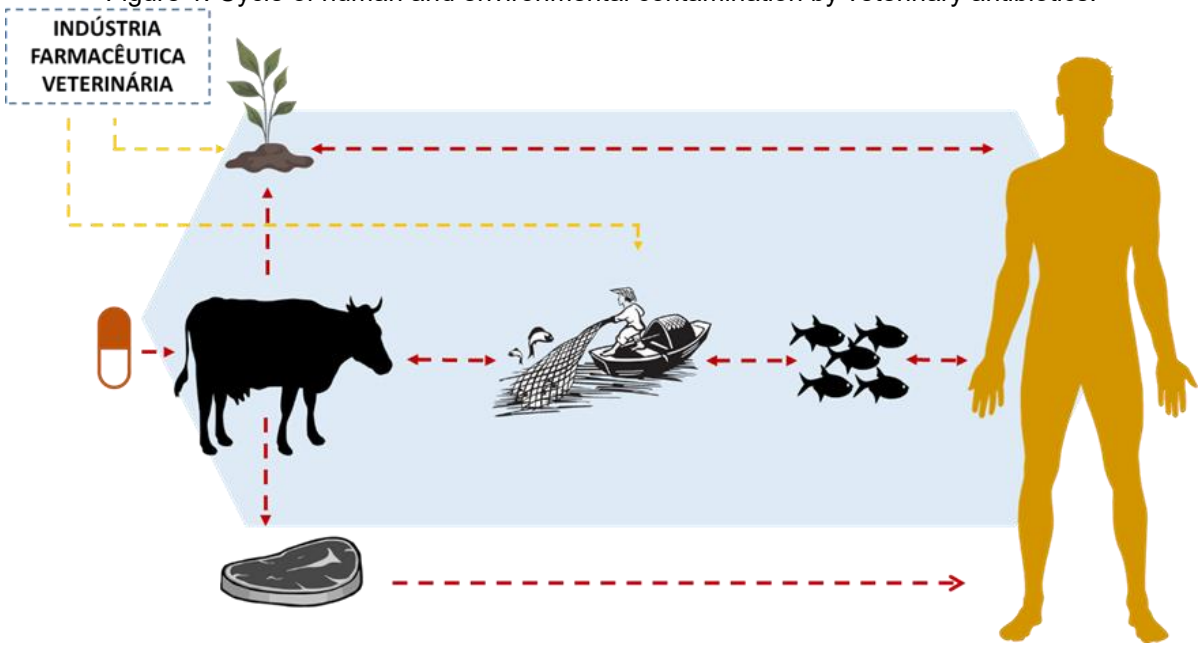
Cattle ranching plays an essential role in the context of Brazilian agribusiness, since Brazil has the second largest commercial herd in the world, estimated at around 200 million animals, which significantly boosts the development of the production chain. However, health challenges still result in losses, and antibiotics occupy the third position in the national ranking of veterinary products, behind only antiparasitics and vaccines. The widespread introduction of antibiotics in agriculture began in the 60s, being recognized as an effective solution to improve intensive agricultural production (OLIVEIRA et al., 2011), (BROWN et al., 2017).

However, antibiotics are considered potentially harmful, since they trigger a process of metabolic resistance and are subject to bioaccumulation and biomagnification phenomena. In addition, they are widely disseminated in the environment, with waste being discarded in significant quantities without prior treatment (MONTAGENERA et al., 2017), (FENT et al., 2006). The inappropriate use of veterinary antibiotics can result in a high concentration of antibiotic residues in products of animal origin, leading to several direct and indirect impacts on consumers, as evidenced in the case of the antibiotic under analysis, ceftiofur (DE SOUZA et al., 2013).

Ceftiofur is a broad-spectrum cephalosporin, effective against Gram-positive and Gram-negative pathogens. This antibiotic works by inhibiting the synthesis of the bacterial cell wall. It is indicated for the treatment of bacterial respiratory infections associated with *Pasteurella multocida*, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*) and *Haemophilus somnus* (AMARANTE, et al., 2018).

Antibiotics, used in both human and veterinary medicine, are drugs of greatest concern when found in the aquatic environment. This is due to the fact that, even at low concentrations, prolonged exposure to these residues can lead to the emergence of resistant bacteria, compromising human health and environmental balance (HERNANDEZ et al., 2007). Drug contamination can occur in different ways, affecting both humans and the environment. Direct or indirect contact, whether through the food chain, water resources or animal excreta, are the main routes of contamination, as illustrated in figure 1.

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



Source: Adapted from Fonseca, 2023.

Contamination of the ecosystem by drugs is manifested due to the incomplete metabolization of antibiotics administered to animal organisms, resulting in the excretion of these compounds by urine and feces, both in the original form and partially metabolized (HALLING-SORENSEN et al., 1998), (SARMAH et al., 2006) and (KEMPER, 2008). Once in the environment, antibiotic residues can accumulate in the soil, leach or be transported to water bodies through surface runoff (DÍAZ-CRUZ et al., 2003). In addition, some of these residues in the soil can be absorbed by plants and accumulate in tissues, posing a risk to human health during harvesting and consumption of foods of plant origin (MIGLIORE et al., 2003) and (BOXALL et al., 2006). This problem is not highlighted in studies that address the environmental and health impacts associated with the presence of antibiotic residues in soil and water.

Despite the crucial relevance of animal production for Brazilian agribusiness, the lack of comprehensive research in this area is remarkable. In this context, the integration of *in silico* methodologies for descitor prediction assumes a strategic position, offering valuable data on human and environmental contamination. Therefore, the present study aims to evaluate, through chemoinformatics, with the aid of *in silico methodologies*, the toxicological impacts on environmental and human health, as well as to predict the pharmacokinetic profile of the antibiotic Ceftiofur on human health.

MATERIALS AND METHODS

To carry out this research, computer programs and online databases of international chemoinformatics platforms were used to obtain the molecular properties (molecular descriptors) of the chemical structure of the antibiotic Ceftiofur.

MOLECULAR MODELING

Initially, the chemical structure of the antibiotic was drawn two-dimensionally (2D) and visualized three-dimensionally (3D) with the aid of the ACD/ChemSketch® Freeware version 2021 program (*Advanced Chemistry Development, Inc*., 2021), then the steric energy of the chemical structure of ceftiofur was tabulated. Subsequently, the steric energy of the three-dimensional chemical structure of the antibiotic was tabulated, the SMILES (*Simplified Molecular Input Line Entry Specification)* code was obtained and exported to international database platforms. Finally, the chemical structure of the antibiotic molecule was saved in a file of the type MDL molfiles (.mol) for further studies.

IN *SILICO* ENVIRONMENTAL TOXICOLOGICAL STUDY

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

IN SILICO PHARMACOKINETIC STUDY FOR ORAL BIOAVAILABILITY

After performing the Molecular Modeling, the SMILES (*Simplified Molecular Input Line Entry Specification) code was obtained* and exported to the international database platform. The human in silico pharmacokinetic study to evaluate the oral bioavailability profile of the antibiotic under study was carried out through the Molinspiration Cheminformatics® (https://www.molinspiration.com) database (GROB, 1986). This database predicts molecular properties in order to evaluate oral bioavailability based on the

Rule *of Five* through interactive calculations of molecular properties, as well as to obtain data tables that can be used in QSAR (Quantitative Structure–Activity Relationship) studies.

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

The ADME study *in silico* (ADME: refers to the *Absorption, distribution, metabolism and excretion*) of the antibiotic ceftiofur was carried out with the objective of predicting the molecular descriptors: Human Intestinal Absorption (HIA: *human intestinal absortion*), Blood-Brain Barrier (BBB) Permeability: *blod-brain barrier*), P-glycoprotein inhibition and antibiotic distribution in the human body. Subsequently, the ADME study was carried out *in silico* in order to predict the inhibition and interaction with the hepatic isoenzymes of the antibiotic cytochrome P450 complex (CYP450). The ADME study *in silico* was carried out with the help of the Chinese platform admetSAR® (http://lmmd.ecust.edu.cn/admetsar2/) (YANG, *et al*., 2018).

IN *SILICO* HUMAN TOXICOLOGICAL STUDY

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.

A human in silico toxicological study was carried out for the antibiotic in order to predict toxicity by the AMES test (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; NC: non-carcinogenic) and Acute Oral Toxicity subdivided into categories also using the admetSAR® platform (http://lmmd.ecust.edu.cn/admetsar2/) (YANG, *et al*., 2018).

RESULTS AND DISCUSSION

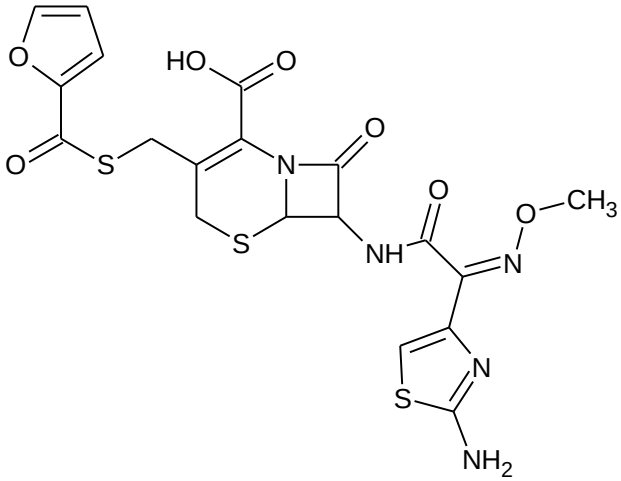
MOLECULAR MODELING

The biological activity of the ligand (antibiotic) is 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).

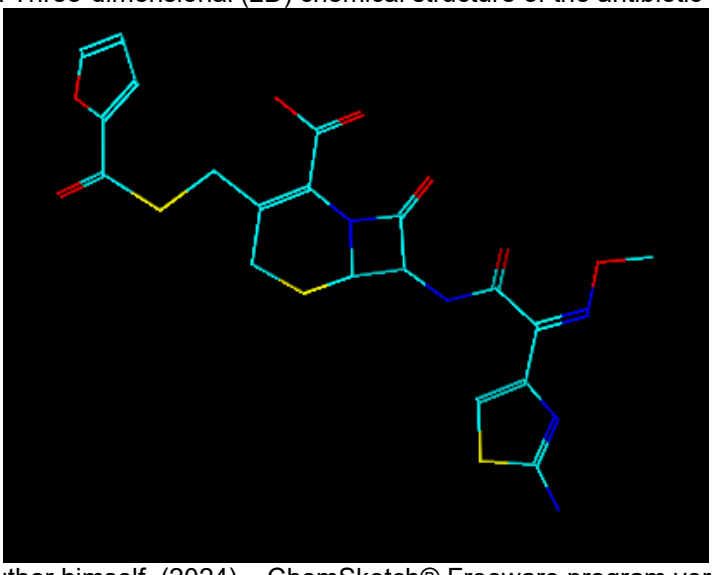
In the Molecular Modeling stage for the chemical structure of the antibiotic Cefquinoma, the minimized chemical structure was saved in a MDL molfiles (.mol) file and the steric energies were tabulated for further studies. Figures 2 and 3 represent, respectively, the chemical structure of the two-dimensional (2D) and three-dimensional (3D) drawings and the molecule of the Ceftiofur antibiotic.

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



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

Figure 3: Three-dimensional (2D) chemical structure of the antibiotic Ceftiofur



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

IN *SILICO* ENVIRONMENTAL TOXICOLOGICAL STUDY

The study of the in *silico* environmental toxicity of antibiotics is of great importance for the rational planning of new drugs, as it allows predicting the impact of these drugs on ecosystems. In this work, parameters such as environmental biodegradation capacity,

toxicity in bees, aquatic toxicity in crustaceans and aquatic toxicity in fish were evaluated. The preliminary results are presented in Table 1, qualitatively [(Q – P: positive or N: negative)] and quantitatively (P = probability).

Table 1: Evaluation of the Environmental Toxicological Profile

Antibiotic	Environmental biodegradation		Testing on bees		Toxicity in crustaceans		Toxicity in fish	
	Q	P	Q	P	Q	P	Q	P
Ceftiofur	N	0,993	N	0,620	P	0,941	P	0,868

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

The environmental toxicological evaluation (Table 1) showed that the antibiotic Ceftiofur presents toxicity in fish and crustaceans, while in relation to bees, it did not present toxicity. The study also revealed that the chemical structure of the antibiotic does not undergo environmental biodegradation, so it remains unchanged in the environment, thus contaminating the aquatic ecosystem and the entire food chain, including humans through contaminated food.

IN SILICO PHARMACOKINETIC STUDY FOR ORAL BIOAVAILABILITY

The analyses to evaluate the oral bioavailability profile are based on the Lipinski rule (Rule of Five). A good drug is expected to have a high rate of human intestinal absorption, solubility in tissue fluids, and permeability across biological membranes. Antibiotics that violate the criteria of Lipinski's Rule do not have good bioavailability when administered orally (VEBER et al., 2002).

Table 2 presents the following molecular descriptors: 1) miLogP: Logarithm of the partition coefficient (measure of molecular hydrophobicity/hydrophilicity); 2) TPSA: Polar surface topological area (Å2); 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 by Veber's Rule: 7) NLR: Number of rotatable bonds; 8) VM: Molecular volume.

Table 2 reveals that ceftiofur violates three Lipinski parameters that refer to molecular weight (PM), Hydrogen Binding Acceptor Sites (ALH) and Polar Topological Surface Area (TPSA), so the antibiotic should not be administered orally by human route, but can still be administered by intravenous or intramuscular administration.

Table 2: Evaluation of the Oral Bioavailability Profile.

Antibiotic	miLogP	PM	DEBT	ALH	TPSA	Violations*	NLR**	VM***
Ceftiofur	0,31	523,57	4	12	177,43	3	9	402,06

Source: Author himself, (2024) - Molinspiration Cheminformatics® Platform.
* Violations: Violations of the Lipinski Rule; ** NLR: Number of Rotatable Links; VM: Molecular Volume.

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

1) Logarithm of the 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 had a value lower than five (5), which means that the compound did not violate 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 property is extremely important for the evaluation of the biological activity of drugs and xenobiotics, since many intermolecular interactions are represented by hydrogen interactions. Therefore, it is ≤ unfavorable for a drug to perform many hydrogen interactions, 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).

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

The first parameter described in table 3 refers to the BBB (*blood-brainbarrier*), a molecular property related to permeability via endothelial cells and which indicates the restriction of the compound regarding the passage of the bloodstream to the Central Nervous System (CNS) (SHARMA et al., 2016), (DOLABELA et al., 2018). It is a descriptor used to evaluate permeability by the blood-brain barrier (BBB). The blood-brain barrier (BBB) has high impermeability and selectivity, regulating the transport of chemical substances between the blood and the Central Nervous System (CNS). BBB is essential for normal brain function, as it protects the CNS from potentially neurotoxic substances present in the blood (BASTOS et al., 2020). The value found in the present study for Ceftiofur was 0.988 (98.80%), indicating that the drug crosses the BBB in a very small way, so it does not act on the central nervous system, corroborating the safety of its use, since it is interesting that a drug does not cross the BBB easily, since this permeability can cause the appearance of adverse effects in the CNS (FELICE et al., 2020).

Evaluation of the molecular descriptor HIA (*human intestinal absorption*) quantitatively demonstrates intestinal absorption of the drug. This parameter is classified according to the intestinal absorption rate: 0 to 20% low absorption rate, 20 to 70% moderate absorption rate, and 70 to 100% high absorption rate (YAKAIAH et al., 2015).

The HIA value for ceftiofur was positive, with a moderate absorption rate, 50.8%, indicating the sum of the intestinal absorption rate with the bioavailability of the unchanged fraction of the analogue that reaches the systemic circulation, i.e., the percentage of the dose that was administered orally and that reaches the hepatic portal system (WANG et al., 2015). The ideal value of IAH will depend on the pharmaceutical purpose of the drug used and the needs of the pathology studied (DOLABELA et al., 2018). However, in the case of drugs administered orally, the ideal is that it has good intestinal absorption.

As Human Intestinal Absorption (IAH), describes the combination of the absorption rate and the absolute bioavailability of the drug that reaches the systemic circulation, when administered orally, the concentration of the drug rarely reaches 100% due to incomplete

absorption and elimination resulting from the first-pass effect in the liver (hepatic biotransformation) (FONSECA, 2023), (HOU, 2008), (ARAUJO et al., 2022), (MOTTA et al., 2023). 3

Glycoprotein P (Gp-P) is a selective descriptor related to the entry of xenobiotics into the tissue, being responsible for the efflux of chemical compounds from the intracellular medium to the extracellular medium, with the function of preventing the entry of drugs into the cell or promoting their elimination, depending on their location. (AMIN, 2013; GOLAN et al., 2017; SANTOS et al. 2022). P-Gp, present in epithelial cells, has the primary function in the excretion and reduction of the bioavailability of several analogues. Gp-P, on the other hand, found in the capillaries of brain vessels, acts as a defense mechanism with the ability to return xenobiotic chemicals to the blood that could cross the BBB (CARREÑO, 2015). Based on the results obtained, it is found that Ceftiofur does not inhibit P-GP with a rate of 86.6%. According to KÖNIG et al., (2013) the induction of P-Gp expression is linked to a decrease in the bioavailability of drugs, while the use of inhibitors of this efflux pump leads to increased plasma levels (ARAUJO et al., 2022).

It is possible to study the transport pathways of a drug using the Caco-2 cell line, human colon adenocarcinoma, because these cells, when differentiated, have morphological and biochemical characteristics that produce functional similarities with the small intestine (spontaneous differentiation into cells that mimic erythrocytes), which makes its applicability viable, mainly because it enables the identification of possible permeability problems of substances, as well as predicting oral absorption and the absorption mechanisms that may be involved (ARTURSSON et al., 2021; CHEN et al., 2012).

Caco-2 epithelial cell permeability, which is an estimating parameter of intestinal permeability as a function of morphophysiological similarity with human enterocytes. This in *vitro method* allows the evaluation of the intestinal absorption capacity of drugs and drugs (FONSECA, 2023) and (MOTTA et al., 2023). In this study, the permeability of Caco-2 was 0.756, which represents low oral absorption. Distribution occurs via the plasma membrane, with a probability of 34.9%.

Table 3: Evaluation of the human in silico Pharmacokinetic Profile (ADME *in silico*).

Antibiotic	BBB		HIA		Distribution		Glycoprotein-P inibiter		Caco-2	
	Q	P	Q	P	Or	P	I	P	Q	P

Ceftiofur	N	0,988	P	0,508	cell membrane	0,349	N	0,866	N	0,756

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

The human ADME *in silico* study on hepatic metabolism aims to predict the inhibitory capacity of cytochrome P450 complex isoenzymes (CYP450) by providing information on whether the antibiotic inhibits any isoenzyme (I: S = yes or N = no) and the likelihood of this inhibition (P). Table 4 presents the predictive evaluation of the *in silico pharmacokinetics* of the antibiotic Ceftiofur, detailing its potential inhibition and interaction with hepatic isoenzymes of the CYP450 complex.

Table 4: Evaluation of the inhibitory Human in silico *Pharmacokinetic Profile* (ADME *in silico*) of cytochrome P450 complex (CYP450) isoenzymes.

	CYP450 1A2		CYP450 2C9		CYP450 2D6		CYP450 2C19		CYP450 3A4	
Antibiotic	I	P	I	P	I	P	I	P	I	P
Ceftiofur	N	0,784	N	0,786	N	0,886	N	0,760	N	0,714

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

Drug metabolism involves a set of chemical reactions that modify molecules and, in general, convert them into a more soluble molecule so that it can be excreted more easily. This process is also known as liver biotransformation. The liver is the organ responsible for metabolizing a drug, due to the presence of a large amount of metabolic enzymes, which transform the drug into active and inactive metabolites and/or facilitate their elimination (GUIMARÃES; SOARES, 2006).

Most human pharmaceuticals are excreted from the body through metabolic reactions catalyzed by the cytochrome P450 (CYP450) and uridine-5'-diphospho-glucuronosyltransferase (UGT) enzyme systems (FROMM et al., 2013). The cytochrome complex (CYP450) belongs to the family of proteins that metabolize endogenous and exogenous substances, making them more polar and water-soluble. Drugs and other substances can induce or inhibit this enzyme, which can result in interactions between drugs, which consequently can decrease the efficacy or increase the toxicity of a substance (AKRAM et al., 2024).

There are isoenzymes of the cytochrome P-450 complex, which act in the metabolism of drugs and other substances, such as CYP1A2, CYP2D6, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, thus contributing more frequently to the metabolism of most drugs. In the study in question, it was observed that Ceftiofur does not have the ability to inhibit any of the five isoenzymes of the cytochrome P450 complex (CYP450) analyzed. Therefore, the antibiotic does not interfere with hepatic metabolism, promoting the excretion of xenobiotic and/or lipophilic compounds from the body (GALLI & FEIJOO, 2002; DIBAEI et al., 2024). Chemical compounds that can inhibit some hepatic isoenzyme can trigger a series of biochemical processes and adverse effects, affecting the hepatic metabolism of other drugs, leading to the formation of toxic metabolites and causing genetic alterations in the formation of several enzymes (DEVLIN, 2002).

IN *SILICO* HUMAN TOXICOLOGICAL STUDY

The human in silico *toxicology study* was conducted with the objective of predicting antibiotic toxicity, considering the three main tests: mutagenicity test (Ames test - T: toxic; NT: non-toxic), carcinogenicity test (C: carcinogenic; CN: non-carcinogenic) and acute oral toxicity testing in categories (I, II, III and IV). The AMES test is a bacterial assay that uses the *Salmonella typhimurium strain* (TA100 and TA1535) to evaluate the mutagenicity of the antibiotic Ceftiofur (MIRANDA et al., 2021).

In the development of a new drug, several tests are carried out to ensure that its composition meets the necessary requirements to be used by the population, in order to provide quality, efficacy and pharmacological safety. Among the analyses, the evaluation of the relationship between mutagenicity and genotoxicity stands out, where it is possible to detect the possible genetic damage associated with human diseases. The AMES test (mutagenicity test) must be applied to any new substance before it reaches the population (OECD, 1997).

The AMES test was developed by Dr. Bruce Ames and collaborators and plays an important role in genetic toxicology by evaluating the mutagenic/carcinogenic potential of the tested substance and for this purpose, strains of *Salmonella typhimurium* auxotrophic for histidine are used, capable of detecting gene mutations in the evaluated substances (GONÇALVES, 2016). Obtaining a positive result by the AMES test represents an obstacle, as it suggests that the analyzed substance has carcinogenic potential. However, negative results provided the basis for the study of genotoxicity in a single dose (BRASIL, 2013; GONÇALVES, 2016).

Toxicity that occurs rapidly after single exposure to the chemical is considered acute oral toxicity, in which contact with the compound occurs within a period of less than 24 hours. This toxicity is classified according to the United States Environmental Protection Agency (EPA *:* https://www.epa.gov) and classifies the antibiotic into four distinct categories, according to the LD50 (median lethal dose): 1) Category I: LD50 ≤ 50 mg/Kg; 2) Category II: 50 < LD50 < 500 mg/Kg; 3) Category III: 500 < LD50 < 5,000 mg/Kg; 4) Category IV: LD50 > 5,000 mg/kg (GONÇALVES, 2011).

Table 5: Evaluation of the Human in *Silico Toxicological* Profile.

Antibiotic	AMES	P	Carcinogenic	P	Acute oral toxicity	
Certiofur	NT	91,32%	NC	87,00%	IV	53,69%

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

The results of the human *in silico* toxicology profile of Ceftiofur shown in table 5 demonstrate that the test antibiotic is non-toxic (non-mutagenic) based on the AMES test and also has no carcinogenic properties. This means that, in toxicity classification systems, category IV has low toxicity, suggesting that, when ingested in a single dose, Ceftiofur has a relatively low risk of causing serious adverse effects (GONÇALVES, 2011).

CONCLUSION

Ceftiofur was selected for study with the purpose of deepening the understanding of its pharmacokinetics and toxicity, due to its wide use and recognition in cattle breeding. In the present study, *in silico* methodologies were employed to predict the molecular properties of the antibiotic Ceftiofur. The *in silico* environmental toxicology study showed that Ceftiofur is not toxic to bees, but is toxic to fish and crustaceans, and its chemical structure does not degrade in the environment. Thus, in addition to polluting aquatic environments, affecting crustaceans and fish, the chemical structure of the antibiotic remains unchanged in the environment, contaminating the food chain and, eventually, humans through food. ADME in *silico* analysis indicated that the antibiotic does not present a favorable prediction for oral bioavailability, due to violations of the Lipinski criteria related to molecular weight (PM), the number of hydrogen bond acceptor sites (ALH) and polar superficial topological area (TPSA), with a moderate intestinal absorption rate and absence of permeability across the blood-brain barrier. In addition, the *in silico* pharmacokinetic study revealed that Ceftiofur has no inhibitory capacity on any of the five hepatic

isoenzymes of cytochrome P450 complex (CYP450). The human *in silico* toxicology study showed promising results, demonstrating that the antibiotic is non-toxic (non-mutagenic) in the AMES test, has no carcinogenic properties, and is classified in category IV for acute oral toxicity, indicating low toxicity. In addition, it is important to highlight that the results indicate that the antibiotic Ceftiofur contributes to environmental and human contamination. Therefore, it is essential to conduct further studies on the drug to assess its additional impacts on humans and ecosystems. The continuity of this research is necessary to deepen the understanding of these effects.

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