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ECOTOXICITY OF THE ANTIRETROVIRAL RALTEGRAVIR ON MICROCYSTIS NOVACEKII, CHLORELLA VULGARIS, AND ARTEMIA SALINA

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Abstract: Raltegravir (RAL), an antiretroviral (ARV) used in HIV treatment, has been detected in aquatic environments, although its ecotoxicological effects are still poorly understood. The aim of this study was to assess the toxicity of the RAL-K (MCR) formulation, both alone and in combination with a dolutegravir-containing medication (MCD), on the aquatic organisms *Microcystis novacekii, Chlorella vulgaris*, and *Artemia salina,* as well as estimate the associated environmental risk. The assays followed ABNT NBR 12648 (2018) and NBR 16530 (2021) standards, with exposures ranging from 6 to 102 mg/L. As a result, the species M. novacekii exhibited the highest sensitivity among the tested organisms, with an EC50 of 44.56 ± 1.77 mg/L, being classified as "slightly toxic" (category 3 – GHS). The combination of MCR + MCD intensified the effects, reducing the EC50 to 20.96 ± 0.95 mg/L. In contrast, the microalga *C. vulgaris* exhibited stimulated cell growth, while *A. salina* did not experience significant acute effects (EC50 > 100 mg/L). The ecological risk assessment indicated high risk in all simulated scenarios: Brazil (RQ = 11.0), Portugal (RQ = 6.06), and South Africa (RQ = 381.51). The results suggest that although RAL is classified as slightly toxic to the tested organisms, its environmental concentration could negatively affect aquatic organisms. Additionally, its combined presence with other ARVs could exacerbate these impacts, highlighting the need for environmental monitoring and improvements in effluent treatment systems.

Keywords: Environmental health; Pharmaceutical contamination; Synergistic effect; Environmental risk; Aquatic organisms.

INTRODUCTION

According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) report, in 2023, approximately 39.9 million people were living with HIV (PLHIV) worldwide, with 30.7 million on antiretroviral therapy (ART), representing 77% of cases. The World Health Organization (WHO) aims to achieve 95% coverage by 2025 (UNAIDS, 2024). Antiretrovirals (ARVs), widely used in HIV infection treatment, are essential for reducing mortality and improving the quality of life for PLHIV (WHO, 2021; Gandhi et al., 2023). However, their intensive and continuous use has led to the increasing detection of these drugs in aquatic environments, where, due to their high stability and bioactivity, they can cause significant ecotoxicological effects and potential risks to human health (Wood et al., 2015; Omotola et al., 2021; Souza et al., 2022; Wallace et al., 2023; Munzhelele et al., 2024).

Among the ARVs used in HIV treatment, raltegravir (RAL), the first integrase inhibitor approved for clinical use, stands out. Although not the first choice for ART, it is used in certain clinical contexts, including treatment for adults, children, and pregnant women living with HIV (FDA, 2011b; Brazil, 2017, 2023). RAL is part of the regimens recommended by the European AIDS Clinical Society (EACS) and is included in the WHO Essential Medicines List, highlighting its importance in managing the infection (WHO, 2017; EACS, 2025). In adults, the medication containing RAL (MCR) is administered in two 400 mg doses, and after hepatic metabolism, a significant portion of the RAL drug is excreted unchanged in feces (51%) and urine (9%) (Nakamura et al., 2023; MSD, 2023).

Different ARVs have been detected in water bodies around the world, with concentrations ranging from ng/L to µg/L in surface waters, groundwater, effluents, and water treatment plants in Africa, America, and Europe (Mlunguza et al., 2020; Cid et al., 2021; Adeola; Forbes, 2021). Although the aquatic toxicity of ARVs such as dolutegravir (DTG) (Souza-Silva et al., 2025), Tenofovir disoproxil fumarate (TDF) (Silva et al., 2019; Souza-Silva et al., 2023), nevirapine (NVP) (Diniz et al., 2022; Cid et al., 2021; Nibamurek et al., 2019), abacavir (ABC) (Minguez et al., 2016), efavirenz (EFZ), and lamivudine (3TC) (Almeida et al., 2021; De Souza et al., 2025) has been investigated, studies on the ecotoxicological effects of RAL are still scarce. Predictive data indicate its presence in different water bodies, with concentrations varying depending on the region and type of aquatic matrix (Almeida et *al., 2023;*

Marzabal et al., 2022; Abafe et al., 2018), underscoring the need for further investigations into the environmental impacts of this drug.

Based on the quantity of MCR distributed by the Brazilian Unified Health System (SUS) between 2022 and 2024, it is estimated that approximately 79,215.38 kg of the medication were made available for consumption in Brazil (Brazil, 2025). Considering pharmacokinetic parameters (MSD, 2023) and adopting a theoretical maximum consumption scenario, about 35,425.43 kg of RAL may have been excreted unchanged into the environment during this period, suggesting a significant environmental load and potential ecotoxicological impacts.

The drug RAL is stable under neutral pH conditions (pH 7.0) and ambient temperature (25°C) (Nakamura et al., 2023). It remains stable when exposed to ultraviolet (UV) light (254 nm), and its significant degradation by hydrolysis occurs only under extreme pH conditions (pH < 1 or pH > 13) (Bhavyasri et al., 2015), suggesting that in natural water bodies, it may not degrade easily, maintaining its chemical integrity and stability.

In addition to RAL, dolutegravir (DTG), another integrase inhibitor, is widely used in first-line HIV therapy in Brazil, and its increasing incorporation into treatment regimens (Chagas dos Santos et al., 2025) may elevate its environmental presence, especially in urban areas (Souza-Silva et al., 2025). In cases of resistance to DTG, this drug can be replaced by RAL in the ART regimen (Brazil, 2022), highlighting the importance of understanding the environmental impact of these compounds, both individually and in combination.

Pharmaceutical residues, although detected in the environment at low concentrations (ng/L to µg/L) (Taheran et al., 2018; Morin-Crini et al., 2022) and considered to pose no direct risk to human health (WHO, 2022), are continuously released into water bodies, and the effects of prolonged exposure remain poorly understood (Adeola et al., 2021). Wallace et al. (2023) demonstrated, in a laboratory assay with RAL concentrations ranging from 10 to 100 mg/L, that this drug can induce cross-resistance to widely used antibiotics, such as clarithromycin and erythromycin, in *Escherichia coli*, suggesting that ARVs in aquatic environments may contribute to the development of multidrug-resistant microbes.

The emergence of resistant strains is particularly concerning, as *E. coli* is a significant agent of human infections (Khan; Gupta, 2020), and the insufficient coverage of basic sanitation, which affects approximately 46% of the global population and 24% of the Brazilian population, may facilitate the spread of these resistant bacteria (UN, 2023; IBGE, 2022).

Although ARVs have the potential to cause biological changes in organisms, cumulative impacts on ecosystems, and risks to the food chain (De Oliveira et al., 2023; Godoy; Kummrow, 2017), the lack of specific guidelines and the inefficiency of wastewater treatments highlight the need for further studies and actions to mitigate the effects of these emerging contaminants (Montagner; Vidal; Acayaba, 2017; Taheran et al., 2018; Patel et al., 2019; Morin-Crini et al., 2022).

Studies on the cyanobacterium *Microcystis novacekii* (Silva et al., 2019) and the microalga *Chlorella vulgaris* (Diniz et al., 2022) indicate that both are effective bioindicators of toxicity due to their tolerance to contaminants and bioremediation capability, showing rapid responses to physicochemical changes and serving as representative organisms in production (Campos et al., 2013; Coronado-Reyes et al., 2022; OECD, 2011; ABNT, 2023; Xiao et al., 2018). Additionally, *Artemia salina*, a primary consumer, is one of the most widely used microcrustaceans in ecotoxicity assessments of substances. Its broad distribution, filtration capacity, and ability to adapt to extreme habitats make it an effective biomarker of environmental quality (Souza et al., 2024).

Given this context, this study aims to evaluate the toxicity of RAL on the aquatic organisms *M. novacekii*, *C. vulgaris*, and *A. salina*, in order to contribute to the understanding of the ecological and environmental impacts of this drug.

MATERIALS AND METHODS

MEDICATIONS

To assess the effects of raltegravir potassium (RAL-K), CAS No. 871038-72-1, the medication containing RAL-K (MCR) Isentress®, manufactured by Merck Sharp & Dohme, was used in this study. The MCR is in the form of pink-coated tablets, oval- shaped, with a water solubility of approximately 71 mg/mL, without buffering, and a final pH of 7.9. The pKa of RAL-K is 6.3, and its solubility increases in slightly alkaline media. The Isentress® formulation contains excipients such as microcrystalline cellulose, monohydrate lactose, dibasic calcium phosphate, hypromello-

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se, poloxamer, sodium stearyl fumarate, magnesium stearate, polyvinyl alcohol, titanium dioxide, macrogol, talc, red and black iron oxides (FDA, 2011).

The medication containing dolutegravir sodium (MCD), CAS No. 1051375-19- 9, used in this study was manufactured by Blanver Farmoquímica e Farmacêutica SA, with a water solubility of approximately 269 mg/L. Each coated tablet contains 50 mg of DTG, combined with excipients such as microcrystalline cellulose, mannitol 25C, povidone K30, sodium starch glycolate, and sodium stearyl fumarate. The coating includes Opadry® II Yellow, composed of partially hydrolyzed polyvinyl alcohol, titanium dioxide, macrogol (PEG), talc, and yellow iron oxide.

Thus, a mixture of MCR and MCD was prepared to evaluate potential synergistic or antagonistic effects on *M. novacekii* and *C. vulgaris*, considering the physicochemical and biological parameters of these drugs. For the preparation of solutions, the active ingredient concentration was taken as declared by the manufacturer, assuming compliance with the quality control standards required for marketed pharmaceutical products.

TEST ORGANISM CULTURES

The cultures of *M. novacekii* and *C. vulgaris* used in this study were isolated from water samples collected from Lake Dom Helvécio (Rio Doce State Park - 42°35′595‖; 19°46′419‖) and the Córrego da Mina stream, located in the São Francisco River basin (19°58′748″; 43°49′259″), both in Minas Gerais, Southeast Brazil. The cultures were maintained in germination chambers at 23 ± 2°C, with a 12/12-hour light/dark photoperiod and a light intensity of 45 ± 5 µmol/m/s. The culture media used were ASM-1 (pH = 8.0 ± 0.2) for the cyanobacterium and BG-11 (pH = 7.5 ± 0.2), with nitrogen (c/N), for the microalga. The cultures are maintained in the Laboratório de Água (LabÁgua) at the Faculty of Pharmacy (FAFAR), Federal University of Minas Gerais (UFMG).

Artemia salina cysts were incubated in a 3.5% saline solution under constant aeration, at a controlled temperature of 25°C, and exposed to light. After 48 hours, the cysts hatched, producing nauplii (the initial stage of the crustaceans). The nauplii were collected and kept under stable conditions until the experiments began. The cyst hatching and nauplii maintenance processes followed the guidelines established by NBR 16530 of 2021 (ABNT, 2021). The experiments with *A. salina* were carried out at the Ecotoxicology Laboratory, Department of Research and Development, Ezequiel Dias Foundation.

PREPARATION OF TEST SOLUTIONS

From MCR, stock solutions were prepared in ASM-1 for the *M. novacekii* assays and in BG-11 (c/N) for the *C. vulgaris* assays. For this, the medications were manually pulverized using a mortar and pestle. The material was then transferred to glass beakers and homogenized using a magnetic stirrer for 10 minutes at room temperature (25°C). After stirring, the material was filtered through qualitative filter paper, and the volume was adjusted to obtain a final concentration of 120 mg/L. From this stock solution, test solutions were prepared at concentrations ranging from 12 to 102 mg/L.

For the stock solution in the *A. salina* test, the MCR was manually pulverized using a mortar and pestle. The material was then transferred to a glass beaker and suspended in a 3.5% saline solution, using a magnetic stirrer for 10 minutes at room temperature (25°C). After stirring, the material was filtered through qualitative filter paper, and the volume was adjusted to obtain a final concentration of 100 mg/L. From the stock solution,

test solutions were prepared at concentrations ranging from 6 to 100 mg/L.

For the preparation of the test solution with the mixture of MCR and MCD, the same procedure described for *M. novacekii* and *C. vulgaris* was followed. The volume was adjusted to the final concentration of 120 mg/L (for both drugs), from which test solutions were prepared at concentrations ranging from 10 to 102 mg/L.

These test concentrations, based on the concentration of the active pharmaceutical ingredient (API) in each medication—RAL--K for MCR and DTG-Na for DTG—were selected according to the toxicity classification of chemical compounds established by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS, 2023). Furthermore, during the preparation, no pH adjustment was necessary for the test solutions, as the pH values remained compatible with the culture media (± 0.2), except for the RAL and DTG mixture, where 0.1M sodium hydroxide (NaOH) was used to adjust the pH of the test solutions.

CELL GROWTH INHIBITION TEST

Acute (4 d) and chronic (14 d) cell growth inhibition assays with *M. novacekii* and *C. vulgaris* were conducted based on the ABNT NBR 12648 standard of 2018, with methodological adaptations. For each assay, suspensions containing approximately 10^6 cells per milliliter of the test organism were transferred to 250 mL glass Erlenmeyer flasks and exposed, in triplicate, to the concentrations of interest of MCR, or to the combination of MCR and MCD, previously prepared.

ASM-1 and BG-11 (c/N) culture media were used as negative controls for the *M. novacekii* and *C. vulgaris* assays, respectively. The average growth rates and growth inhibition curves as a function of the concentrations of the test substances were determined from the absorbance of the samples measured after 4 d and 14 d of exposure. Equations 1 and 2 were used to convert the absorbances of the samples (X), at 680 nm for *M. novacekii* and 695 nm for *C. vulgaris*, to cells per milliliter (y), respectively:

$$y = 10^7 \times X - 13{,}574 \quad (R^2 = 0.9944)$$

(Equation 1)

$$y = 10^5 \times X - 7{,}723 \quad (R^2 = 0.9899)$$

(Equation 2)

ACUTE TOXICITY TEST

The acute toxicity test with *A. salina* was conducted according to the ABNT NBR 16530 standard of 2021 (ABNT, 2021). Forty-eight-hour-old nauplii were distributed in test tubes containing different concentrations of MCR solubilized in a 3.5% saline solution. The concentrations used ranged from 6.25 to 100 mg/L (serial dilution 2x), with test tubes containing only the saline solution used as the negative control. Each test tube received 10 individuals, ensuring proper exposure control. After 48 hours of incubation, the mobility of the nauplii was assessed by counting the mobile and immobile organisms at each tested concentration. The test was performed in quadruplicate (n = 40), ensuring statistical rigor in data analysis.

ENVIRONMENTAL RISK ASSESSMENT

The environmental risk assessment of RAL was conducted using the Risk Quotient (RQ), calculated as the ratio between the measured environmental concentration (MEC) and the Predicted No Effect Concentration (PNEC) for the tested aquatic organisms (Equation 3):

$$RQ = \frac{MEC}{PNEC} \quad \text{(Equation 3)}$$

PNEC values were estimated from the CE50 data obtained in the experiments conducted in this study and from studies available in the literature, applying a division factor of 1,000 (PNEC = CE50 / 1,000) (Cid et al., 2021; Ramos et al., 2025; Souza- Silva et al., 2025). The ecological risk classification was defined as follows:

RQ < 0.01 indicates negligible risk
0.01 ≤ RQ < 0.1 represents low risk
0.1 ≤ RQ < 1 represents medium risk
RQ ≥ 1 indicates high risk

The MEC values were obtained from available literature records, through predictive modeling (QSAR), and chemical analyses in different aquatic matrices (Almeida et al., 2023; Marzabal et al., 2022; Abafe et al., 2018).

STATISTICAL ANALYSIS

The data obtained were analyzed using R software (v4.4.1). The Shapiro-Wilk test was applied to check for normality, and the Wilcoxon test was used for non- parametric data. Logistic regression, log-normal, and Weibull models were fitted using the - drc|| package (Ritz et al., 2015), considering **p < 0.05** as significant.

RESULTS AND DISCUSSION

TOXICITY OF THE RAL-K DRUG (MCR)

This is the first study to experimentally evaluate the ecotoxicological effects of MCR on aquatic organisms. The lack of research using bioassays hinders a precise assessment of the environmental risks associated with this drug. Although studies based on ecological modeling and predictive methods have been conducted (Ramos et al., 2025; Choudhury, Ojha, Ray, 2024), further investigations with organisms from different levels of ecological organization are needed for a more comprehensive understanding of its impacts on aquatic biota.

The study results demonstrated distinct responses between the biological models assessed. The cyanobacterium *M. novacekii* exhibited higher sensitivity to MCR compared to the microalga *C. vulgaris*. Growth inhibition tests indicated that MCR caused initial toxicity to *M. novacekii*, resulting in a significant reduction ($p < 0.05$) in the cell growth rate during the first four days of exposure, with estimated EC10 values of 22.33 ± 1.65 mg/L, EC50 of 44.56 ± 1.77 mg/L, and EC90 of 88.90 ± 9.43 mg/L. These results indicate a dose-dependent response, with an increased inhibition of cell growth as the drug concentration rises.

However, throughout the experiment (14 days of exposure), partial recovery of cyanobacterial growth was observed. In contrast, no detectable toxicity was observed in *C. vulgaris* during the tests, with growth maintained across all tested concentrations (< 100 mg/L), indicating greater tolerance to the drug. Similarly, *A. salina* also exhibited low sensitivity to MCR, with no complete immobilization observed at the highest exposure concentrations tested (< 100 mg/L). Figure 1 shows the dose-response curve (growth inhibition over time) for *M. novacekii*, which experienced a significant reduction in cell density during the first days of exposure, followed by an adaptation and partial recovery phase in subsequent days, possibly associated with the activation of metabolic defense mechanisms against chemical stress.

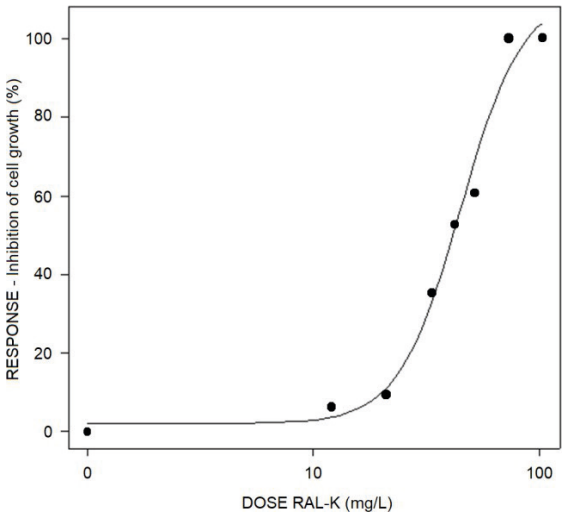

Figure 1 - Dose-response curve of growth inhibition in *M. novacekii* after 4 d of acute exposure to the RAL-K (MCR) - containing drug, at concentrations ranging from 12 to 102 mg/L. The negative control group consisted of ASM-1 culture medium without MCR (0.0 mg/L).

On the other hand, *C. vulgaris* maintained exponential growth at all tested concentrations, indicating that the drug did not affect its cell division rate. The divergence in observed effects may be associated with structural differences between the species, such as variations in cell wall composition and specific detoxification mechanisms. A detailed response to MCR exposure in the test organisms is described in the following sections.

TOXICITY IN M. NOVACEKII

In the growth inhibition assays, *M. novacekii* exhibited acute toxic responses to MCR in a dose-dependent manner. The cell inhibition rate (I%) progressively increased with higher RAL-K concentrations, indicating a significant impact on the cyanobacterium's viability, as shown in Figure 1. Acute exposure (4 d) resulted in an estimated EC50 value of 44.56 ± 1.77 mg/L (Weibull model), classifying RAL-K as Acute Hazard Category 3, according to the criteria established by the Globally Harmonized System of Classification and Labelling of Chemicals – GHS (GHS, 2023).

This category corresponds to substances considered mildly toxic to aquatic organisms in short-duration exposures (10 < EC50 ≤ 100 mg/L). Additionally, the EC10 and EC90 values were determined to be 22.33 ± 1.65 mg/L and 88.90 ± 9.43 mg/L, respectively, further reinforcing the dose-response relationship. Statistical analysis using the Wilcoxon test revealed significant differences between the groups ($p < 0.05$), supporting the reliability of the obtained data.

At concentrations of 72 and 102 mg/L, 100% growth inhibition was observed, suggesting that higher concentrations could have an algicidal effect on the organisms and suppress metabolic activity. As demonstrated by Xian et al. (2025), cyanobacteria may enter a dormant state under chemical stress, maintaining cellular viability without active division. This hypothesis is supported by the partial recovery of growth observed in chronic exposure (14 days).

The cell growth dynamics of *M. novacekii* were monitored over a 14-day exposure to MCR, allowing for the evaluation of potential recovery mechanisms. Figure 2 illustrates the evolution of cell growth at different concentrations over the exposure times (0, 4, and 14 days).

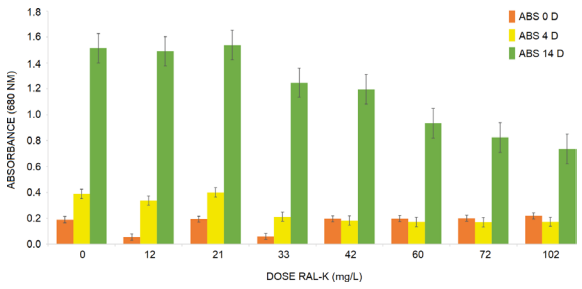

Figure 2 - Cell growth of *M. novacekii* at different concentrations of the RAL-K (MCR)-containing drug, ranging from 12 to 102 mg/L, during acute (4 days) and chronic (14 days) exposures. The negative control group consisted of ASM-1 culture medium without MCR (0.0 mg/L).

It is important to note that the toxicity observed in the acute phase may not only be related to RAL-K in its original form but also to the formation of secondary metabolites with increased toxic potential, as observed by Wroński et al. (2024) in their study, where transformation products of RAL were more toxic than RAL itself for all organisms evaluated.

As illustrated in Figure 2, the exposure time directly influenced the response observed in *M. novacekii*, evidenced by the negative activity on cell growth during acute exposure (4 d), and a partial recovery from toxicity during chronic exposure (14 d), suggesting a possible adaptive mechanism to the chemical stress caused by MCR. This reduction in toxicity may be related to the cyanobacteria's ability to activate detoxification pathways (Wang et al., 2018; Gomes et al., 2022) and the degradation of the drug in the medium, reducing its bioavailability and mitigating impacts on cell growth over time.

Although this study did not isolate the excipients, recent work with ARVs demonstrates that they can significantly modulate toxicity in aquatic organisms. Souza-Silva et al. (2025) observed that excipients such as mannitol and povidone may reduce the toxicity of DTG--Na via complex formation, while surfactants can increase the bioavailability of 3TC. Considering that the MCR evaluated in this studycontains both surfactants (poloxamer) and potentially complexing agents (lactose, hypromellose), it is plausible that similar interactions occurred, emphasizing the need to evaluate complete formulations in ecotoxicological studies (Souza-Silva et al., 2025; Turek et al., 2023; Silva et al., 2014; Jacob et al., 2016).

As a viral integrase inhibitor in humans, RAL-K does not have a direct molecular target in cyanobacteria, but it may have induced oxidative stress, leading to the formation of reactive oxygen species (ROS), damaging cell membranes and photosynthetic pigments such as chlorophyll-A (evidenced by the reduction in absorbance at 680 nm), along with potential damage caused by osmotic imbalance. To clarify the specific mechanisms of toxicity from the RAL-K-containing drug in *M. novacekii*, future tests with biomarkers such as catalase and/or peroxidase are suggested.

The results obtained in this study show similarities to the data reported by Silva et al. (2019) for the ARV TDF, a nucleoside reverse transcriptase inhibitor (NRTI), which exhibited an EC50 of 161.01 mg/L for *M. novacekii*, classifying it as practically non-toxic to the aquatic environment. Similarly, Souza et al. (2025) found that entecavir, an antiviral prescribed for the treatment of hepatitis B, did not exhibit toxicity to *M. novacekii* (EC50 > 100 mg/L).

However, unlike these compounds, MCR demonstrated higher acute toxicity, with an EC50 of 44.56 mg/L, indicating a potentially more relevant environmental impact. This difference may be related not only to the chemical structure of RAL-K (lipophilic characteristics and functional groups) that could interact with components of the cell membrane but also to its pharmaceutical formulation (excipients + active pharmaceutical ingredient).

By using the pharmaceutical formulation (Isentress®) and not just the isolated active pharmaceutical ingredient (API), it is possible to observe the environmental effects caused by the combined presence of API + excipients, since the excipients are also released into the environment and can act in a synergistic or additive manner. Some excipients may modulate toxicity by increasing cellular permeability (Turek et al., 2023) and inducing oxidative stress (Shukla & Trivedi, 2018; Wang et al., 2018) in aquatic organisms. These mechanisms, though documented in other biological models, suggest potential pathways for the acute effects observed in *M. novacekii*.

Souza-Silva et al. (2025) highlighted this aspect when comparing the API and the MCD, another integrase inhibitor like RAL, in different forms. While the formulated drug exhibited an EC50 between 19.5 and 27.6 mg/L (classified as low toxicity), the isolated API was considerably more toxic (EC50 < 2 mg/L). This contrast reinforces the importance of evaluating not only the API but the drug as a whole, including excipients and its commercial presentation.

The combination of DTG, TDF, and 3TC demonstrated high acute toxicity, with an estimated EC50 of 5.6 ± 0.6 mg/L after four days of exposure, categorizing it as toxic. This increased toxicity can be attributed to potential synergistic effects between the APIs and excipients, amplifying the negative impacts on *M. novacekii*, as also observed with the MCR in this study.

In the present study, the reference drug Isentress® 400 mg was used, whose formulation may contain excipients with potential toxicity-modulating effects, and which, as part of the final product, are also released into the environment. These differences between the compounds analyzed emphasize the need to consider the individual physicochemical properties of drugs when assessing their ecotoxicological effects, as toxicity is not only related to the therapeutic class but also to the chemical structure, formulation, environmental behavior, and the response of exposed organisms.

In the cyanobacterium *M. novacekii*, exposure to multiple contaminants can trigger specific biological responses, resulting in varied environmental impacts (Wang et al., 2018; Gomes et al., 2022). In the aquatic environment, drugs coexist with numerous substances whose interactions can lead to synergistic, antagonistic, or additive effects, either amplifying or mitigating ecotoxicological impacts. Therefore, it is essential that future studies evaluate not only the isolated toxicity of each chemical compound but also their combined effects and environmental behavior, considering factors such as exposure time, degradation in the medium, bioaccumulation, and the presence of excipients.

TOXICITY IN C. VULGARIS

In the cell growth inhibition assays with *C. vulgaris*, no acute or chronic toxic effects were observed following exposure to MCR at the tested concentrations. The negative growth inhibition values, or positive values indicating stimulation of cell growth (Table 1), demonstrated a significant effect, with a biomass increase of up to 79% at a concentration of 81 mg/L after 4 d of exposure. However, chronic exposure showed a significant reduction in growth stimulation, although it remained positive after 14 days.

RAL-K (mg/L)	C% - 4 d	C% - 14 d
0.0	0.0 ± 0.2	0.0 ± 0.5
12.0	5.5 ± 2.3	3.9 ± 4.2
21.0	16.1 ± 3.5	3.0 ± 3.9
42.0	33.4 ± 2.3	8.1 ± 1.9
60.0	54.8 ± 1.2	6.4 ± 2.2
81.0	79.0 ± 9.8	9.1 ± 6.2
102.0	58.5 ± 5.3	4.6 ± 5.0

Table 1: Cell growth stimulation rate (C%) of *C. vulgaris* under exposure to the RAL- K - containing drug at different concentrations for 4 and 14 days. The negative control group consisted of BG-11 culture medium with nitrogen and no MCR (0.0 mg/L).

TOXICITY IN *C. VULGARIS*

As established by ISO 8692:2012 (Water quality – Freshwater algal growth inhibition test with unicellular green algae), growth variations up to ± 20% compared to the control are considered within the normal variability of the test system. However, the values observed in this study (up to 79.0%) exceeded this threshold, indicating a biologically relevant effect.

Although MCR did not show apparent toxicity to *C. vulgaris* under isolated conditions, the stimulation of algal growth may have considerable ecological implications. The increase in phytoplankton biomass, for example, could promote the occurrence of algal blooms, which reduce dissolved oxygen in water and compromise the survival of other aquatic organisms. Such imbalances could disrupt trophic chains, favor opportunistic species, and impact biodiversity.

The reduction in growth stimulation from 58.5% at 102.0 mg/L to 79.0% at 81 mg/L over 4 days of exposure may suggest the onset of an inhibitory effect at high concentrations of RAL-K (> 100 mg/L), possibly related to the saturation of cellular tolerance mechanisms, resource depletion, generation of toxic metabolites, or chemical degradation of the compound in the medium. This hypothesis is further supported by the significant reduction in growth stimulation during chronic exposure (14 days) at all tested concentrations. This behavior may represent an inflection point between stimulation and stress, which warrants attention from an ecotoxicological perspective.

The resistance of *C. vulgaris* to MCR may be associated with its thick cell wall, which limits the penetration of xenobiotics, and the presence of detoxification enzyme systems, such as cytochrome P450 and glutathione-S--transferases (Expósito et al., 2021; Rajivgandhi et al., 2022; Herath & Ganehenege, 2023). This microalga also has a recognized capacity for bioremediation, removing heavy metals, surfactants, and various pharmaceuticals from the aquatic environment (Kosarev et al., 2022; Coronado-Reyes et al., 2022).

Another potential factor for the observed growth could be the use of MCR and/or its excipients as supplementary sources of carbon or nitrogen. The RAL-K molecule contains functional groups such as amides and heterocyclic rings that can be metabolized by *C. vulgaris*. Excipients in the formulation, such as lactose and poloxamers, may also have contributed as additional energy sources (De Jesus Oliveira Santos et al., 2023; Zheng et al., 2019; Melo et al., 2018; Abreu et al., 2012).

Previous studies support these findings, showing that pharmaceutical residues can act as metabolic stimuli for microalgae. Procópio et al. (2021) observed a 40% increase in growth rate and a 5% increase in photosynthetic efficiency of *C. vulgaris* in the presence of carbamazepine. Zhang et al. (2019) reported an increase in chlorophyll and lipid production in *C. pyrenoidosa* exposed to low concentrations of diclofenac (2 – 30 mg/L).

Similarly, Ricky, Chiampo, and Shanthakumar (2022) found up to a 46% increase in biomass and a 30% increase in chlorophyll-a in *C. vulgaris* exposed to a concentration of 5 mg/L of the antibiotics ciprofloxacin and amoxicillin. These data reinforce the hypothesis that pharmaceutical contaminants, even at low concentrations, can modulate microalgal metabolism, promoting adaptive responses such as increased biomass, changes in biochemical composition, and activation of antioxidant mechanisms, such as increased superoxide dismutase and catalase enzymes (Zhang et al., 2019).

The results obtained with MCR differ from those reported by Diniz et al. (2022), who observed a significant inhibitory effect of NVP on *C. vulgaris*, with CE50 values of 24.90 mg/L for the active pharmaceutical ingredient (API) and 19.52 mg/L for the pharmaceutical formulation containing NVP. These data suggest that, unlike RAL-K, NVP is classified as toxic to microalgae. These differences may be related to the distinct mechanisms of action: while NVP is a reverse transcriptase inhibitor, RAL acts on HIV integrase, and their physicochemical properties—such as the log Kow of 0.4 for RAL-K and 1.8 for NVP—affect lipophilicity and cellular penetration (NCBI, 2023; Diniz et al., 2022).

Although the data presented here suggest that MCR, analyzed in isolation, does not pose a risk to *C. vulgaris*, it is important to consider that environmental exposure often occurs through mixtures of pharmaceuticals and other chemicals. Studies show that combined toxicity may be greater than the sum of individual effects, with synergistic interactions between ARVs (Omotola et al., 2022; Gomes et al., 2022; Souza-Silva et al., 2025).

For example, the combination of TDF and EFZ resulted in up to a 50% reduction in growth and a 40% reduction in photosynthetic efficiency of *Raphidocelis subcapitata*, with toxicity 30% higher than the sum of the individual effects of the compounds (Gomes et al., 2022). These cumulative effects can cause changes in the structure and functionality of aquatic communities, interfering with competitive dynamics between species and favoring the selection of organisms better adapted to environmental stresses (Godoy et al., 2019; Galus et al., 2013; Richards et al., 2004). Additionally, the bioaccumulation of pharmaceutical residues along the food chain can pose a risk not only to higher aquatic organisms, such as fish, but also potential implications for human health (Zenker et al., 2014; Keerthanan et al., 2021; Khan et al., 2022).

TOXICITY *IN ARTEMIA SALINA*

The results obtained in acute toxicity tests with *A. salina* exposed to MCR demonstrated that the drug did not induce significant immobilization of the organisms, even at the highest tested concentrations (100 mg/L). In none of the three assays conducted was it possible to determine the CE50, as immobilization did not reach 50% of the individuals. The highest observed immobilization response was 27.5 ± 1.26% at the highest tested concentration (100 mg/L). This pattern suggests that MCR exhibits low acute toxicity to *A. salina*, with a CE50 value greater than 100 mg/L, classifying it as ―practically non-toxic‖ according to the Globally Harmonized System (GHS) criteria (CE50 > 100 mg/L) (GHS, 2023).

The experimental data corroborate previous studies on the toxicity of antivirals in *A. salina*. Silva et al. (2019) also did not observe complete immobilization of *A. salina* exposed to TDF, recording a CE50 value of 111.82 mg/L. Minguez et al. (2016) evaluated 48 pharmaceuticals and found that 72% of them had a CE50 greater than 100 mg/L. Antivirals like acyclovir and abacavir, and antibiotics like clarithromycin, clindamycin, and azithromycin were classified as having low toxicity to

A. salina, highlighting that this crustacean has high tolerance to various pharmaceutical compounds. De Souza et al. (2025) further supported this trend, observing a maximum immobilization of approximately 60% at high concentrations of entecavir (250 mg/L), without estimating the CE50 value.

Increasing exposure to MCR resulted in a progressive rise in the immobility of

A. salina, yet without a clear maximum response that would allow for the precise determination of the CE50. Nevertheless, statistical analysis indicated that all tested concentrations showed significant differences compared to the control ($p < 0.05$), suggesting the influence of the drug on the organisms. Figure 3 illustrates this trend, showing the variation in average immobility across the tested concentrations.

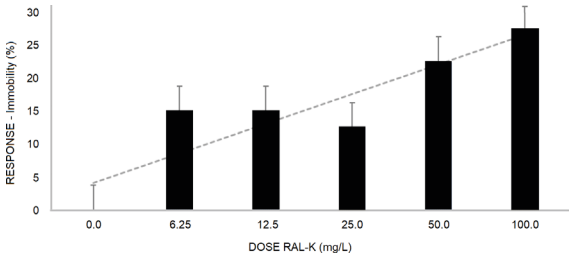

Figure 3: Relationship between the average immobility (%) of *A. salina* and the concentration of the RAL-K containing drug (MCR) after 48 hours of exposure to concentrations ranging from 6.25 to 100 mg/L. The dashed line indicates the trend. The negative control group consisted of saline culture (3.5% sodium chloride) without MCR (0.0 mg/L).

Despite the absence of significant acute toxicity, it is important to consider potential sublethal and ecotoxicological effects, as discussed by Ncube et al. (2018), who emphasize the ability of antiviral drugs to trigger indirect impacts in aquatic ecosystems. The presence of antivirals in the environment could promote the selection of resistant microbial strains, alter trophic dynamics, and interfere with sensitive ecological processes. Furthermore, the interaction of these compounds with other contaminants may lead to combined effects that have not yet been fully investigated. Therefore, although the results of this study suggest that MCR exhibits low acute toxicity to *A. salina*, it is necessary to conduct further analysis on the possible environmental impacts of this drug.

To better understand these effects, it is recommended to conduct complementary studies, including prolonged exposure tests to assess long-term impacts, investigations into metabolic and physiological changes in exposed organisms, and analyses involving other aquatic organisms. These approaches could help elucidate the ecotoxicological risks of ARVs and provide valuable input for more effective monitoring and environmental management policies.

TOXICITY OF THE COMBINATION OF RAL AND DTG MEDICATIONS

Exposure to the mixture of MCR and MCD did not inhibit the growth of *C. vulgaris*, preventing the estimation of CE50 values for both acute and chronic exposure. On the contrary, a significant increase in absorbance was observed at all tested concentrations compared to the control group (0.0 mg/L), as evidenced by ANOVA ($p < 0.05$) and Tukey's test (Table 2). This behavior suggests a stimulatory effect on the growth of the microalga, especially after 14 days of exposure. A similar phenomenon was observed during exposure to RAL alone (Figure 2).

After 4 days of exposure, a reduction in absorbance was observed in the samples exposed to the combination of MCR and MCD, suggesting that, in the early stages, the combination of the drugs may have caused a more pronounced toxic or stressful effect, leading to a temporary inhibition of cell growth compared to RAL alone.

Although the mixture of MCR and MCD caused a possible inhibition in the short term, *C. vulgaris* demonstrated the ability to recover over time. This could be due to adaptive or compensatory processes in the microalga, which, after the initial stress phase, overcame the adverse effects caused by the mixture and restored its biomass. Therefore, the potential reduction in growth observed during the first 4 days seems to be transient, as suggested by the data from 14 days, which show recovery and stimulation in growth.

Additionally, both for the isolated MCR and the mixture of MCR and MCD, the highest concentrations (72 and 102 mg/L) were the ones that stimulated the algal growth the most. This effect may be related to *C. vulgaris*' ability to absorb, metabolize, or even biodegrade complex organic compounds, a mechanism already described for other microalgae in phytoremediation studies (Kosarev et al., 2022; Co-

RAL-K (mg/L)	ABS (4 d) Mean ± SD	p value	ABS (14 d) Mean ± SD	p value
0.0	0.341 ± 0.03	–	0.911 ± 0.06	–
12.0	0.486 ± 0.04	0.037	1.326 ± 0.12	0.018
21.0	0.522 ± 0.05	0.037	1.358 ± 0.11	0.012
42.0	0.440 ± 0.04	0.152	1.299 ± 0.10	0.018
60.0	0.418 ± 0.06	0.245	1.432 ± 0.12	0.008
72.0	0.414 ± 0.05	0.278	1.572 ± 0.13	0.001
102.0	0.593 ± 0.07	0.009	1.626 ± 0.14	0.001

Table 2: Effect of the combination of RAL-containing medication (MCR) and DTG- containing medication (MCD) on the growth of Chlorella vulgaris at different exposure times. The absorbance values at 695 nm (ABS) represent the mean ± standard deviation (SD) of a triplicate. The negative control group consists of BG-11 culture medium with nitrogen and no MCR or MCD (0.0 mg/L).

ronado-Reyes et al., 2022; Zhang et al., 2019).

The difference in response observed between the acute and chronic exposure to the mixture of MCR and MCD, compared to MCR alone, may be related not only to the interaction between the active ingredients but also to the excipients in the pharmaceutical formulation. Previous studies have shown that in some pharmaceutical formulations, the combination of the active pharmaceutical ingredient (API) and excipients may have synergistic effects on toxicity in aquatic organisms.

For example, Turek et al. (2023) observed that the commercial formulation of valsartan (valsartan + excipients) had greater toxicity for *Aliivibrio fischeri* than the pure substance. Certain excipients can increase the toxicity of medications by altering their solubility and bioavailability, making them more accessible to aquatic organisms. Therefore, it is important to consider the excipients in environmental impact assessments, as their interactions may lead to unexpected synergistic effects (Turek et al., 2023). Additionally, the toxicity values of isolated substances for aquatic organisms do not always directly correspond to the toxicity of complete pharmaceutical formulations (Souza-Silva et al., 2025).

It is important to highlight that, although the increase in cell density may initially seem like a positive effect, it does not exclude the ecotoxicological risk associated with the continuous presence of these drugs in the aquatic environment (Richards et al., 2004; Godoy et al., 2019), with potential impacts on more sensitive species (Galus et al., 2013). The accumulation of these substances or their metabolites may cause indirect effects, such as unbalanced algal blooms (Khan et al., 2022) or the transfer of contaminants along the food chain (Zenker et al., 2014).

In contrast to the results obtained for *C. vulgaris*, exposure of *M. novacekii* to the combination of MCR and MCD resulted in significant, dose-dependent growth inhibition ($p < 0.05$), compared to the control group (0.0 mg/L), indicating greater sensitivity of the cyanobacteria to the combination of the drugs. Both acute (4 days) and chronic (14 days) effects were observed. The estimated CE50 for the combination of MCR and MCD was 20.96 ± 0.95 mg/L, indicating toxicity approximately 2.13 times higher than that observed with MCR alone (CE50 44.56 ± 1.77 mg/L) (Table 3).

Parameter	MCR	MCR + MCD
EC50 (4 d)	44.56 ± 1,77 mg/L	20.96 ± 0.95
Maximum inhibition	100% (72 – 102 mg/L)	100% (≥ 35 mg/L)
Recovery (14 d)	Partial	Limited (≤ 20 mg/L)

Table 3. Comparison of the effects of the medication containing RAL-K (MCR) alone and the combination of MCR with the medication containing DTG-Na (MCD) on the cyanobacterium *Microcystis novacekii*, based on acute toxicity (4 days of exposure) and recovery (14 days of exposure) parameters.

In the first 4 days, a marked reduction in absorbance was observed, indicating strong inhibition of cell growth. The combination of MCR and MCD promoted more intense acute toxicity, with significant inhibition ($p < 0.05$) starting from 20 mg/L, without complete recovery after 14 days. While MCR alone allowed partial recovery after 14 days, the combination of MCR and MCD maintained significant inhibition even after chronic exposure (≥ 20 mg/L), suggesting the overcoming of the cyanobacterium's adaptive capacity. This effect may be associated with the generation of more toxic metabolites or the presence of synergistic interactions between the compounds (Godoy et al., 2019; Funke; Prasse; Ternes, 2016).

After 14 days, *M. novacekii* showed slight recovery only at the lowest concentrations (10–15 mg/L), with absorbance values higher than the control. However, at concentrations ≥ 20 mg/L, growth inhibition remained significant ($p < 0.001$), with biomass reduction ranging from 20 to 35% compared to the control.

These results suggest that synergistic or additive effects between the active pharmaceutical ingredients and/or their excipients intensified the toxicity for *M. novacekii*. The increased growth inhibition could reflect the potentiation of oxidative stress, interference with photosynthetic processes, and alterations in cellular metabolism (Souza-Silva et al., 2025; Coronado-Reyes et al., 2022; Cid et al., 2021; Abreu et al., 2012). Additionally, surfactants present in the formulations may have contributed to increased absorption and toxicity in cyanobacteria (Souza-Silva et al., 2025; Turek et al., 2023).

The additive toxicity dynamics observed in this study were similar to those reported by Souza-Silva et al. (2025), where the combination of the active pharmaceutical ingredients DTG and TDF increased acute toxicity in *M. novacekii* by approximately 6 times compared to the isolated DTG.

The differential toxicity between *M. novacekii* and *C. vulgaris* suggests that the combination of MCR and MCD may influence the composition and structure of phytoplankton communities, favoring microalgae over cyanobacteria (Bouzas-Monroy et al., 2022; Coronado-Reyes et al., 2022; Cid et al., 2021). This imbalance may affect the food chain, as cyanobacteria are a food source for zooplankton (Wetzel, 2001). Alterations at this initial trophic level can compromise the ecological stability of the entire aquatic ecosystem. Additional studies are needed to evaluate the bioaccumulation of these drugs and their long-term effects.

In ecotoxicology, it is crucial to consider that substances that appear safe when evaluated alone may exhibit significant adverse effects when combined or released on a large scale into the environment. Thus, the results of this study reinforce the importance of assessing the combined effects of drugs, contributing to future research on the environmental impacts of emerging pollutants and the definition of safe disposal limits, in addition to the development of effective environmental mitigation strategies.

TOXICITY OF PHARMACEUTICAL EXCIPIENTS

Excipients are substances added to pharmaceutical formulations to ensure the stability of the active ingredient, facilitate its administration, absorption, and therapeutic efficacy. Although traditionally considered inert and non-toxic (Bayne et al., 2025), recent studies show that these components can modify the physicochemical properties of drugs, influencing their dissolution, persistence, and interaction with aquatic organisms (Silva et al., 2014; Jacob et al., 2016; Souza-Silva, et al., 2025).

The influence of excipients on the ecotoxicity of drugs has been evidenced in some studies. Silva et al. (2014) observed that the formulated fluoxetine was up to 10 times more toxic to *C. vulgaris* than the isolated active ingredient, highlighting the role of excipients in amplifying toxicity. Similarly, Jacob et al. (2016) analyzed different commercial formulations of nimesulide and found that generic versions showed higher toxicity, attributed to the presence of specific excipients, as the isolated API has low solubility. These findings were reinforced by the same authors when evaluating generic formulations of hydrochlorothiazide, where only the formulation containing sodium lauryl sulfate, sodium amyglycolate, lactose, microcrystalline cellulose, and silicon dioxide showed significant toxicity, suggesting that the excipients, not the active ingredient, were responsible for the adverse effects (Jacob et al., 2016).

Table 4 lists only the water-soluble excipients present in the MCR Isentress® 400 mg formulation, highlighting their potential ecotoxicological impacts on aquatic organisms, as evidenced by the literature. Insoluble excipients were not included in this analysis, as they were retained during the filtration process in the preparation of the test substance.

The scarcity of ecotoxicological data on pharmaceutical excipients hinders a comprehensive evaluation of their environmental impacts. Among the water-soluble excipients present in the tested MCR, poloxamer and polyvinyl alcohol exhibited the highest toxic potential due to their distinct physicochemical properties. Poloxamer, an amphiphilic surfactant widely used in pharmaceutical formulations, can cause adverse effects even with short-term exposures due to its ability to destabilize cell membranes in aquatic organisms and its environmental persistence. These characteristics justify its classification as Category 4 in acute toxicity by the European Chemicals Agency (ECHA, 2025).

Polyvinyl alcohol, in addition to functioning as a film-forming agent, has polymer chains that can interfere with physiological processes in higher organisms and form synergistic complexes with other contaminants. It is considered potentially hazardous to the environment, especially to fish, according to the International Labour Organization (ILO) and the World Health Organization (WHO) (ILO; WHO, 2025). These excipients can alter the physicochemical properties of the drug, increasing its solubility and facilitating absorption by cells, which may have contributed to the acute toxicity observed in *M. novacekii*.

Although many excipients are considered safe, preservatives, surfactants, and plasticizers can induce adverse effects in aquatic organisms when present at environmentally relevant concentrations (10 - 100 mg/L) or in combination with other compounds. This synergistic effect can enhance the toxicity of drugs in the aquatic environment, making it a critical factor to consider in environmental risk assessments (Turek et al., 2023).

The influence of excipients on the toxicity of drugs has also been evidenced in studies with ARVs. Diniz et al. (2022) observed that formulated NVP presented higher toxicity to

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C. vulgaris (CE50 = 19.52 mg/L) compared to the isolated API (CE50 = 24.90 mg/L), suggesting that excipients contributed to the increased toxicity.

Silva-Souza et al. (2025) evaluated the toxicity of the ARVs TDF, 3TC, and DTG, as well as their excipients in *M. novacekii*. The commercial formulation of 3TC was more toxic (CE50 = 60.3 ± 2.7 mg/L in 4 days) than the isolated API (CE50 > 400 mg/L), suggesting that excipients such as microcrystalline cellulose might increase the absorption and toxicity of the drug. On the other hand, the formulation of DTG showed lower toxicity (CE50 = 27.6 ± 5.4 mg/L in 4 days) compared to the isolated API (CE50 = 1.7 ± 0.3 mg/L), indicating that excipients like mannitol and povidone could reduce the bioavailability and consequently the toxicity of DTG. The combination of TDF and 3TC in commercial formulations showed higher toxicity (CE50 = 5.6 ± 0.3 mg/L in 4 days) than the isolated APIs, possibly due to the presence of sodium lauryl sulfate, an excipient with bacteriostatic properties that may enhance toxic effects.

These results demonstrated that excipients present in commercial formulations play an important role in modulating the toxicity of these drugs, which can either increase or reduce their ecotoxicological effects, highlighting the importance of considering the environmental impact of these interactions.

ENVIRONMENTAL RISK ASSESSMENT

The concentration of RAL in water bodies has been reported in the ng/L range, with estimated or detected values in various countries (Table 5). In Brazil, Portugal, and South Africa, concentrations ranged from 40 to 17,000 ng/L, obtained through predictive modeling or chemical analyses.

From the experimental data obtained for *M. novacekii* in this study (CE_{50} = 44.56 µg/L), the PNEC was calculated at 0.04456 µg/L, adopting an assessment factor of 1,000. The RQ values ranged from 0.90 (medium risk) to 381.51 (high risk), depending on the location and environmental concentration. These results indicate significant ecological risk in real exposure scenarios, especially in South Africa and Brazil.

In addition to the observed toxicity, the high excretion of RAL, approximately 83% of the administered dose, with 9% excreted as unchanged drug in urine, contributes to its continuous entry into the environment. In 2024, based on the distribution of medicines by the Brazilian Unified Health System (SUS), it is estimated that up to 313 kg of RAL were excreted unchanged in urine in Brazil, representing a significant source of environmental contamination.

Although less frequently detected compared to other ARVs (Abafe et al., 2018; Rimayi, 2018; K'Oreje et al., 2016), RAL presents concerning characteristics, such as high persistence, low biodegradability, and toxicity to aquatic organisms (Choudhury et al., 2024), which reinforces the need for continuous monitoring and inclusion of this drug in environmental regulatory guidelines. The microalga tolerated the drug more, being the most resistant organism.

CONCLUSION

This study investigated the ecotoxicological effects of the drug RAL-K on different aquatic organisms. The results showed that *M. novacekii* was the most sensitive to exposure to MCR, exhibiting significant inhibition of cell growth, especially when combined with DTG, which intensified the toxicity. On the other hand, *C. vulgaris* and *A. salina* did not exhibit significant toxic effects, with the microalga even showing stimulated growth.

Excipient	Molecular formula	Molecular formula	Aquatic Organism	Assay	Result	Toxicity Category	
Lactose monohydrate	$C_{12}H_{24}O_{12}$	Diluent	Aliivibrio fischeri	Microtox®	CE_{50} = 16,363 mg/L	Virtually non-toxic	
Hypromellose [1]	$C_{56}H_{108}O_{30}$	Coating agent and binder	-	-	-	-	NCBI, 2025.
Poloxamer [1]	$C_5H_{10}O_2$	Surfactant and solubilizer	-	-	-	Acute Toxicity (Acute Tox. 4 (21.3%))[2]	ECHA, 2025.
Polyvinyl alcohol [1]	$(CH_2CHOH)n$	Film forming agent				Potentially hazardous to the environment, especially fish. [3]	ILO; WHO, 2025
Macrogol	$C_4H_6O_2$	Solubilizer and plasticizer	Daphnia magna	Acute toxicity	CL_{50} > 100.0 mg/L	Low toxicity	Schupp et al., 2018

Table 4: Excipients of the commercial drug based on raltegravir potassium (MCR) Isentress® (soluble) and their toxicity in aquatic organisms

Legend: [1] = Ecotoxicological data on aquatic organisms not available. [2] = Toxicity category classified by the European Chemicals Agency (ECHA). [3] = International Chemical Safety Cards (ICSCs) prepared by the International Labour Organization and the World Health Organization.

RAL (ng/L)	Quantification Method	Place	Aquatic Matrix	Reference
40.0	PEC[1]	Alentejo, Portugal	Surface water	Almeida et al., 2023
130.0	PEC[1]	Algarve, Portugal	Surface water	Almeida et al., 2023
190.0	PEC[1]	Centro, Portugal	Surface water	Almeida et al., 2023
270.0	PEC[1]	Lisboa/Vale do Tejo, Portugal	Surface water	Almeida et al., 2023
210.0	PEC[1]	Norte, Portugal	Surface water	Almeida et al., 2023
490.0	PEC[1]	Cubatão, São Paulo, Brazil	STP[3]	Marzabal et al., 2022
61 to 17.000	LC-MS/MS[2]	KwaZulu-Natal, South Africa	STPI [4]	Abafe et al., 2018

Table 5: Raltegravir concentrations reported in water bodies, obtained from various studies using predictive and analytical methods.

Legend: [1] PEC: Predicted Environmental Concentration — modeled values; [2] LC- MS/MS: Liquid Chromatography - Tandem Mass Spectrometry: analytical technique; 3 STP: Sewage Treatment Plant; 4 STPI: Sewage Treatment Plant Influent. (Almeida et al., 2023; Marzabal et al., 2022; Abafe et al., 2018).

Despite the more evident acute effects on *M. novacekii*, the study highlighted the potential risk for other aquatic organisms in chronic exposures, in complex environments, or with multiple contaminants.

The combination of MCR and MCD increased toxicity, suggesting synergistic effects, making it important to evaluate not only isolated drugs but also theircommercial formulations. Environmental risk assessment indicated a high ecological risk in all the studied scenarios (Portugal, Brazil, and South Africa), even with RAL concentrations in the environment at low levels (ng/L).

The chemical stability of RAL and incomplete removal in sewage treatment suggest that the drug may persist and bioaccumulate in aquatic ecosystems. Additionally, the study emphasized the importance of considering the molecular structure of RAL and the excipients in the pharmaceutical formulation, as they can influence the drug's bioavailability and toxicity. The absence of toxicity in some organisms does not mean that RAL is safe for the aquatic environment. The increasing presence of ARVs in the environment, combined with the lack of basic sanitation, may threaten biodiversity and human health.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

ABAFE, A. O. et al. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. **Chemosphere**, v. 200, p. 660-670, 2018.

ABNT Associação Brasileira de Normas Técnicas. **NBR 16530: Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com Artemia sp (Crustacea, Brachiopoda)**. 24 p. Rio de Janeiro, 2021.

ABNT. Associação Brasileira de Normas Técnicas. **NBR 12648: Ecotoxicologia aquática – Toxicidade crônica – Método de ensaio com algas (Chlorophyceae)**. 35p. Rio de Janeiro, 2023.

ABREU, A. P. et al. Mixotrophic cultivation of Chlorella vulgaris using industrial dairy waste as organic carbon source. **Bioresource technology**, v. 118, p. 61–66, 2012.

ADEOLA, A. O.; FORBES, P. B. C. Antiretroviral Drugs in African Surface Waters: prevalence, analysis, and potential remediation. **Environmental Toxicology And Chemistry**, v. 41, n. 2, p. 247-262, 2021.

ALMEIDA, A. et al. Predicted environmental risk assessment of antimicrobials with increased consumption in Portugal during the COVID-19 pandemic; The groundwork for the forthcoming water quality survey. **Antibiotics (Basel, Switzerland)**, v. 12, n. 4, p. 652, 2023.

ALMEIDA, L. C. et al. Chronic toxicity and environmental risk assessment of antivirals in Ceriodaphnia dubia and Raphidocelis subcapitata. **Water Science and Technology**, v. 84, n. 7, p. 1623-1634, 2021.

BABICH, R. et al. Mitochondrial response and resilience to anthropogenic chemicals during embryonic development. **Comparative biochemistry and physiology. Toxicology & pharmacology: CBP**, v. 233, n. 108759, p. 108759, 2020.

BAYNE, A.-C. V. et al. Vitamins as excipients in pharmaceutical products. **European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences**, v. 206, n. 107020, p. 107020, 2025.

BHAVYASRI, K. et al. Development and Validation of Forced Degradation Studies of Raltegravir using RP-HPLC and Characterization of Degradants by LC-MS/MS. **J. Pharm. Sci. & Res**., 2015, Vol. 7(9), p. 685-689.

BOUZAS-MONROY, A. et al. Assessment of the potential ecotoxicological effects of pharmaceuticals in the world's rivers. **Environmental toxicology and chemistry**, v. 41, n. 8, p. 2008–2020, 2022.

BRASIL. Ministério da Saúde. Comissão Nacional de Incorporação de Tecnologias no SUS. **Relatório de Recomendação nº 288: Raltegravir para primeira linha de tratamento de HIV. 2017**. Brasília, DF, 2017.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis. **Protocolo clínico e diretrizes terapêuticas para prevenção da transmissão vertical do HIV, sífilis e hepatites virais**. Brasília, DF: Ministério da Saúde, 2022.

BRASIL. Ministério da Saúde. Secretaria de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde. Portaria SECTICS/MS nº 38, de 3 de julho de 2023. **Diário Oficial da União**: seção 1, Brasília, DF, 5 jul. 2023.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde e Ambiente. Departamento de HIV, Aids, Tuberculose, Hepatites Virais e Infecções Sexualmente Transmissíveis (DATHI/SVSA/MS). **Painel Integrado de Monitoramento do Cuidado do HIV e da Aids**. 2024. Disponível em:<http://mediacenter.aids.gov.br/Transparencia_Ativa/Banco_PIMC_2024.zip>. Acesso em: 19 mar. 2025.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde e Ambiente. Departamento de HIV/AIDS, Tuberculose, Hepatites Virais e Infecções Sexualmente Transmissíveis (DATHI/SVSA/MS). **Solicitação de Acesso à Informação. Plataforma "Fala.BR". Protocolo NUP nº 25072.006646/2025-44. Formulário: resposta ao cidadão**. Recebido em 17/03/2025, 10:52.

BRASIL. Portaria GM/MS nº 888, de 4 de fevereiro de 2021. Estabelece os procedimentos para o controle e a vigilância da qualidade da água para o consumo humano e seu padrão de potabilidade. **Diário Oficial da União**: seção 1, Brasília, DF, 5 fev. 2021.

CAMPOS, M. M. C. et al. Evaluation of the capacity of the cyanobacteriumMicrocystis novacekiito remove atrazine from a culture medium. **Journal of environmental science and health**. Part. B, Pesticides, food contaminants, and agricultural wastes, v. 48, n. 2, p. 101–107, 2013.

CHAGAS DOS SANTOS, I. F. et al. Mutations associated with viral resistance to integrase in individuals initiating dolutegravir-containing antiretroviral therapy: retrospective cohort, Brazil 2017-2019. **AIDS care**, p. 1–11, 2025.

CHMP – COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. **Guideline on the environmental risk assessment of medicinal products for human use**. London: European Medicines Agency, 2006. Disponível em:mhttps://www.ema.europa.eu/en/environmental-risk-assessment-medicinal-products- human-use-scientific-guideline. Acesso em: 30 jan 2024.

CHOUDHURY, A.; OJHA, P. K.; RAY, S. Hazards of antiviral contamination in water: Dissemination, fate, risk and their impact on fish. **Journal of hazardous materials**,v. 476, n. 135087, p. 135087, 2024.

CID, R. S. et al. Toxicity of antiretrovirals on the sea urchin Echinometra lucunter and its predicted environmental concentration in seawater from Santos Bay (Brazilian coastal zone). **Resources**, v. 10, n. 11, p. 114, 2021.

CORONADO-REYES, J. A. et al. Chlorella vulgaris, a microalgae important to be used in Biotechnology: a review. **Food Science and Technology**, v. 42, 2022.

DE JESUS OLIVEIRA SANTOS, M. et al. Blue technology for a sustainable pharmaceutical industry: Microalgae for bioremediation and pharmaceutical production. **Algal research**, v. 69, n. 102931, p. 102931, 2023.

DE OLIVEIRA, Valberto Barbosa et al. Impactos ambientais e toxicológicos pela contaminação de fármacos principalmente antibióticos em ambientes aquáticos: revisão da literatura. **Revista Ibero-Americana de Humanidades, Ciências e Educação**, v. 9, n. 7, p. 838-850, 2023.

DE SOUZA, C. R. et al. Ecotoxicity of Entecavir in *Aliivibrio fischeri*, *Microcystis novacekii*, *Artemia salina*, and *Biomphalaria glabrata*. **Journal of chemical health and safety**, 2025.

DINIZ, J. S. et al. The toxic effects of the antiretroviral nevirapine and a nevirapine- based drug for aquatic organisms. **Research, Society and Development**, v. 11, n. 2, p. 19211225014, 2022.

EACS. European AIDS Clinical Society. **Guidelines Version 12.1**. Bruxelas: EACS, 2025. Disponível em: https://www.eacsociety.org/guidelines/eacs-guidelines/. Acesso em: 29 jan. 2025.

ECHA. EUROPEAN CHEMICALS AGENCY. Poloxamer (EC: 923-642-1), 2025.Disponível em: https://echa.europa.eu/information-on-chemicals/cl-inventory- database/-/discli/details/78630. Acesso em: 12 jan. 2025.

EMEA - European Medicines Agency. Committee for Medicinal Products for Human Use. **Guideline on the Environmental Risk Assessment of Medicinal Products**

for Human Use. London, 21 March 2024. Disponível em: < https://www.ema.europa.eu/en/environmental-risk-assessment--medicinal-products- human-use-scientific-guideline>. Acesso: 20 jun. 24.

EROFEEVA, E. A. Environmental hormesis: From cell to ecosystem. **Current opinion in environmental science & health**, v. 29, n. 100378, p. 100378, 2022.

EXPÓSITO, N. et al. Performance of Chlorella vulgaris exposed to heavy metal mixtures: Linking measured endpoints and mechanisms. **International journal of environmental research and public health**, v. 18, n. 3, p. 1037, 2021.

FDA. Food and Drug Administration. **Clinical Pharmacology and Biopharmaceutics Review: Isentress (Raltegravir Potassium), NDA 203045**. Silver Spring: FDA, 2011. Disponível em: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/203045Orig1s000ClinPh armR.pdf. Acesso em: 8 jan. 2024.

FDA. U.S. Department of Health and Human Services. Center for Drug Evaluation and Research. Center for Biologics Evaluation and Research. **Guidance for Industry**. Washington, DC: U.S. Food and Drug Administration, 2011b.

FUNKE, J.; PRASSE, C.; TERNES, T. A. Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. **Water research**, v. 98, p. 75–83, 2016.

GALUS, M. et al. Chronic effects of exposure to a pharmaceutical mixture and municipal wastewater in zebrafish. **Aquatic toxicology (Amsterdam, Netherlands)**, v. 132–133, p. 212–222, 2013.

GANDHI, R. T. et al. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2022 Recommendations of the international antiviral society-USA panel. JAMA: **The Journal of the American Medical Association**, v. 329, n. 1, p. 63, 2023.

GAO, Q. et al. Investigations on the influence of energy source on time-dependent hormesis: A case study of sulfadoxine to Aliivibrio fischeri in different cultivation systems. **The Science of the total environment**, v. 775, n. 145877, p. 145877, 2021.

GHS. United-Nations. *Globally Harmonized System of Classification and Labelling os Chemicals (GHS)*, 10th ed.; **United Nations Publications**: New York, NY, USA; Geneva, Switzerland, 2023

GODOY, A. A. et al. Single and mixture toxicity of four pharmaceuticals of environmental concern to aquatic organisms, including a behavioral assessment. **Chemosphere**, v. 235, p. 373–382, 2019.

GODOY, A. A. & KUMMROW, F. What do we know about the ecotoxicology of pharmaceutical and personal care product mixtures? A critical review. Critical Reviews in **Environmental Science and Technology**, v. 47, n. 16, p. 1453–1496, 2017.

GOMES, M. P. et al. Do anti-HIV drugs pose a threat to photosynthetic microorganisms? **Chemosphere**, v. 307, n. Pt 2, p. 135796, 2022.

HERATH, C. H.; GANEHENEGE, M. Y. U. **Growth, biomass production, pollutant removal capacity of Chlorella vulgaris cultivated in water polluted with nutrients (PO43- and NO3-) and heavy metal ions (Cd2+ and Pb2+); A strategy for bio-remediation, CO2 utilization and simultaneous biodiesel productivity**. 2023. Disponível em: <http://dx.doi.org/10.21203/rs.3.rs-2898090/v1>.

HSRC. HUMAN SCIENCES RESEARCH COUNCIL. *KwaZulu-Natal's disparate HIV prevalence and pervasive HIV risk behaviour*. 13 dez. 2024. Disponível em: https://www.hsrc.ac.za/. Acesso em: 4 jan. 2025.

IBGE. NSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA**. Censo Demográfico 2022**. Rio de Janeiro: IBGE, 2022.

ILO. INTERNATIONAL LABOUR ORGANIZATION; WORLD HEALTH ORGANIZATION (WHO). International Chemical Safety Cards (ICSC) – Polyvinyl Alcohol (ICSC 1489). Disponível em: https://chemicalsafety.ilo.org/dyn/ icsc/showcard.display?p_version=2&p_card_id=1489

ISO**.** INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. *ISO 8692:2012 – Water quality – Freshwater algal growth inhibition test with unicellular green algae*. Geneva: ISO, 2012.

JACOB, R. S. et al. A toxicity assessment of 30 pharmaceuticals using Aliivibrio fischeri: a comparison of the acute effects of different formulations. **Environmental technology**, v. 37, n. 21, p. 2760–2767, 2016.

KAR, S. et al. Ecotoxicological assessment of pharmaceuticals and personal care products using predictive toxicology approaches. **Green Chemistry**, v. 22, n. 5, p. 1458–1516, 2020.

KEERTHANAN, S. et al. Pharmaceutical and Personal Care Products (PPCPs) in the environment: Plant uptake, translocation, bioaccumulation, and human health risks. **Critical reviews in environmental science and technology**, v. 51, n. 12, p. 1221–1258, 2021.

KHAN, A. H. et al. Impact, disease outbreak and the eco-hazards associated with pharmaceutical residues: a Critical review. **International journal of environmental science and technology: IJEST**, v. 19, n. 1, p. 677–688, 2022.

KHAN, F. M.; GUPTA, R. Escherichia coli (E. coli) as an Indicator of Fecal Contamination in Groundwater: A Review. In: **Sustainable Development of Water and Environment**. Cham: Springer International Publishing, 2020. p. 225–235.

K'OREJE, K. O. et al. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya**. Chemosphere**, v. 149, p. 238-244, 2016.

KOSAREV, A. V. et al. **Possibilities of using Chlorella microalgae to assess the hygienic safety of drinking water sources**. AIP Conference Proceedings. **Anais**...AIP Publishing, 2022.

KUDU, I.; PILLAY, V.; MOODLEY, B. Antiretrovirals (ARVs) in the environment. In: **Emerging Freshwater Pollutants**. p. 227–239.cap 13. 2022. doi: 10.1016/b978-0- 12-822850-0.00006-5

LÓPEZ-DOVAL, J. C. et al. Nutrients, emerging pollutants and pesticides in a tropical urban reservoir: Spatial distributions and risk assessment. **The Science of the total environment**, v. 575, p. 1307–1324, 2017.

MARZABAL, A. L. DE F. et al. Medicamentos antirretrovirais: concentração ambiental estimada e avaliação de risco ecológico em Estações de Tratamento de Esgotos de Cubatão, São Paulo, Brasil. **Research, Society and Development**, v. 11, n. 4, p. e52111427595, 2022.

MELO, R. G. DE et al. Chlorella vulgaris mixotrophic growth enhanced biomass productivity and reduced toxicity from agro-industrial by-products. **Chemosphere**, v. 204, p. 344–350, 2018.

MINGUEZ, L. et al. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. **Environmental Science and Pollution Research**. v. 23(6): p. 4992 - 5001, 2016.

MLUNGUZA, N.Y. et al. Determination of selected antiretroviral drugs in wastewater, surface water and aquatic plants using hollow fibre liquid phase microextraction and liquid chromatography - tandem mass spectrometry. **Journal Of Hazardous Materials**. v. 382: p. 121067, 2020.

MONTAGNER, C. C.; VIDAL, C.; ACAYABA, R. Contaminantes emergentes em matrizes aquáticas do Brasil: cenário atual e aspectos analíticos, ecotoxicológicos e regulatórios. **Quimica nova**, 2017.

MORIN-CRINI, N. et al. Worldwide cases of water pollution by emerging contaminants: a review. **Environmental chemistry letters**, v. 20, n. 4, p. 2311– 2338, 2022.

MSD. MERCK SHARP & DOHME FARMACÊUTICA LTDA. **Isentress: bula do profissional de saúde**. São Paulo: MSD, 2023.

MUNZHELELE, E. P. et al. Pharmaceutical contaminants in wastewater and receiving water bodies of South Africa: A review of sources, pathways, occurrence, effects, and geographical distribution. **Water**, v. 16, n. 6, p. 796, 2024.

Naghavi, Mohsen et al. "Global burden of bacterial antimicrobial resistance 1990– 2021: a systematic analysis with forecasts to 2050." **The Lancet**, vol. 404, no. 10459, 2024, pp. 1199-1226.

NAKAMURA, T. et. al. Conversion of raltegravir carrying a 1,3,4-oxadiazole ring to a hydrolysis product upon pH changes decreases its antiviral activity. **PNAS Nexus**, 2023.

NCBI. NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. Hydroxypropylmethylcellulose. **PubChem**, 2025. Disponível em: https://pubchem.ncbi.nlm.nih.gov/compound/57503849. Acesso em: 14 jan. 2025.

NCBI. NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. Raltegravir. **PubChem**, 2023. Disponível em: <https://pubchem.ncbi.nlm.nih.gov/compound/54671008>. Acesso em: 14 jan 2025.

NCUBE, S. et al. Environmental fate and ecotoxicological effects of antiretrovirals: a current global status and future perspectives. **Water Research**. v. 145: p. 231-247, 2018.

NIBAMUREKE, U.M.C. et al. Nevirapine in African surface waters induces liver histopathology in Oreochromis mossambicus: A laboratory exposure study, **African Journal of Aquatic Science**, v. 44:1, p. 77-88, 2019.

NIELSEN, M. E.; ROSLEV, P. Behavioral responses and starvation survival of Daphnia magna exposed to fluoxetine and propranolol. **Chemosphere**, v. 211, p. 978–985, 2018.

OECD – ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT. Guidelines for testing chemicals freshwater alga and cyanobacteria growth inhibition test. 201. Paris: **OECD Publishing**, 2011.

OMOTOLA, E. O. et al. **Ecotoxicological assays of selected and potentiated antiretroviral and antibiotics**. 2022. Disponível em: <http://dx.doi.org/10.21203/rs.3.rs-1204100/v1>.

OMOTOLA, Elizabeth Oyinkansola et al. Environmental Risk Characterization of an Antiretroviral (ARV) Lamivudine in Ecosystems. **International Journal of Environmental Research and Public Health**, 2021. doi: 10.3390/IJERPH18168358.

PATEL, M. et al. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. **Chemical reviews**, v. 119, n. 6, p. 3510–3673, 2019.

PROCOPIO, Z. S. D. et al. Multifactorial Evaluation of Atenolol, Caffeine, Carbamazepine and Ibuprofen on Raphidocelis subcapitata and Chlorella vulgaris. **Biology**, v. 10, n. 9, p. 926, 2021.

RAJIVGANDHI, G. et al. Green microalgal strain Chlorella vulgaris isolated from industrial wastewater with remediation capacity. **Environmental technology & innovation**, v. 28, n. 102597, p. 102597, 2022.

RAMOS, L. T. et al. Predicted environmental concentration (PEC), environmental risk assessment (ERA) and prioritization of antiretroviral drugs (ARVs) in seawater from Guarujá (Brazilian coastal zone). **Marine environmental research**, v. 204, n. 106964, p. 106964, 2025.

RICHARDS, S. M. et al. Effects of pharmaceutical mixtures in aquatic microcosms. **Environmental toxicology and chemistry**, v. 23, n. 4, p. 1035–1042, 2004.

RICKY, R.; CHIAMPO, F.; SHANTHAKUMAR, S. Efficacy of ciprofloxacin and amoxicillin removal and the effect on the biochemical composition of Chlorella vulgaris. **Bioengineering (Basel, Switzerland)**, v. 9, n. 4, p. 134, 2022.

RIMAYI, Cornelius et al. Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa. **Science of the Total Environment**, v. 627, p. 1008-1017, 2018.

RITZ, C. et al. Dose-response analysis using R. **PloS one**, v. 10, n. 12, p. e0146021, 2015.

SCHUPP, T. et al. A review of the environmental degradation, ecotoxicity, and bioaccumulation potential of the low molecular weight polyether polyol substances. **Reviews of environmental contamination and toxicology**, v. 244, p. 53–111, 2018.

SHUKLA, A.; TRIVEDI, S. P. Anionic surfactant, linear alkyl benzene sulphonate induced oxidative stress and hepatic impairments in fish Channa punctatus. **Proceedings of the Zoological Society**, v. 71, n. 4, p. 382–389, 2018.

SILVA, A. et al. Impact of excipients in the chronic toxicity of fluoxetine on the alga Chlorella vulgaris. **Environmental technology**, v. 35, n. 21–24, p. 3124–3129, 2014.

SILVA, S. R. et al. Toxicity for Aquatic Organisms of Antiretroviral Tenofovir Disoproxil. **Journal of environmental protection**, v. 10, n. 12, p. 1565–1577, 2019.

SOUZA, C. R. DE et al. Ecotoxicological studies of direct and indirect genotoxicity with Artemia: a integrative review. **Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering**, v. 59, n. 6, p. 305–320, 2024.

SOUZA, C. R. et al. Environmental elimination estimate and literature review of ecotoxicological aspects of the main widely used antiretrovirals in Brazil. **Research, Society and Development**, v. 11, n. 10, p. e368111032975, 2022.

SOUZA-SILVA, G. et al. Toxicity of the antiretrovirals tenofovir disoproxil fumarate, lamivudine, and dolutegravir on Cyanobacterium Microcystis novacekii. **Water**, v. 17, n. 6, p. 815, 2025.

SOUZA-SILVA, G. et al. Toxicological evaluation of antiretroviral Tenofovir Disoproxil Fumarate on the mollusk Biomphalaria glabrata and its hemocytes. **Science of the Total Environment**. v. 891: p. 164484, 2023.

TAHERAN, M. et al. Emerging contaminants: Here today, there tomorrow! **Environmental Nanotechnology Monitoring & Management**, v. 10, p. 122–126, 2018.

TUREK, M. et al. Can pharmaceutical excipients threaten the aquatic environment? A risk assessment based on the Microtox® biotest. **Molecules (Basel, Switzerland)**,

v. 28, n. 18, 2023.

UNAIDS**. Fact Sheet 2024**. Geneva: UNAIDS, 2024.

UNITED NATIONS. GHS. **Globally Harmonized System of Classification and Labelling of Chemicals**. 10th Revised Edition, United Nations, New York and Geneva, 2023.

UNITED NATIONS**. Progress on household drinking water, sanitation and hygiene 2000-2023: Special focus on achieving universal access to safely managed services**. Geneva: World Health Organization and United Nations Children's Fund (UNICEF), 2023.

WALLACE, V. J. et al. Bacteria exposed to antiviral drugs develop antibiotic cross- resistance and unique resistance profiles. **Communications biology**, v. 6, n. 1, 2023.

WANG, Y. et al. Effects of yttrium and phosphorus on growth and physiological characteristics of Microcystis aeruginosa. **Journal of Rare Earths**, v. 36, n. 7, p. 781–788, 2018.

WETZEL, R. G. *Limnology: lake and river ecosystems*. 3rd ed. San Diego: Academic Press, 2001.

WHO - WORLD HEALTH ORGANIZATION. **Essential Medicines List. 20th ed**. Geneva, 2017. Disponível em: https://www.who.int/groups/expert-committee-on- selection-and-use-of-essential-medicines/essential-medicines-lists.

WHO - WORLD HEALTH ORGANIZATION. **Global guidance on criteria and processes for validation: elimination of mother-to-child transmission of HIV, syphilis and hepatitis B virus**. 2021. Disponível em: https://www.paho.org/en/documents/global-guidance-criteria-and-processes- validation-elimination-motherchild-transmission.

WHO. WORLD HEALTH ORGANIZATION .**Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda**. Geneva: World Health Organization, 2022.

WOOD, T.P. et al. The Occurrence of Anti-Retroviral Compounds Used for HIV Treatment in South African Surface Water. **Environmental Pollution**. v. 199, p. 235– 243, 2015.

WROŃSKI, M. et al. Electrochemical removal of antiretroviral drug - raltegravir from aquatic media: Multivariate optimization, degradation studies and transformation products characterization. **Journal of environmental management**, v. 370, n. 122584, p. 122584, 2024.

XIAN, X. et al. Cyanobacteria in late lag and exponential phases suit different pre- chlorination and coagulation strategies. **Environmental pollution (Barking, Essex: 1987)**, v. 366, n. 125427, p. 125427, 2025.

XIAO, M.; LI, M.; REYNOLDS, C. S. Colony formation in the cyanobacterium Microcystis. Biological reviews of the **Cambridge Philosophical Society**, v. 93, n. 3, p. 1399–1420, 2018.

ZENKER, A. et al. Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. **Journal of environmental management**, v. 133, p. 378–387, 2014.

ZHANG, Y. et al. The influence of four pharmaceuticals on Chlorellapyrenoidosa culture. **Scientific reports**, v. 9, n. 1, p. 1624, 2019.

ZHENG, H. et al. Cultivation of Chlorella vulgaris in manure-free piggery wastewater with high-strength ammonium for nutrients removal and biomass production: Effect of ammonium concentration, carbon/nitrogen ratio and pH. **Bioresource technology**, v. 273, p. 203–211, 2019.