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Environmental elimination estimate and literature review of ecotoxicological aspects of the main widely used antiretrovirals in Brazil

Estimativa de eliminação no meio ambiente e revisão de literatura de aspectos ecotoxicológicos dos principais antirretrovirais mais utilizados no Brasil

Estimación de la eliminación en el medio ambiente y revisión de la literatura sobre aspectos ecotoxicológicos de los principales fármacos antirretrovirales más utilizados en Brasil

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Abstract
*Objective***:** To estimate the consumption and quantity of unaltered antiretrivirals (ARV) eliminated into the environment and to carry out a literature research on aquatic ecotoxicological studies of ARV used in Brazil. *Methodology***:** We requested from one of the ARV dispensing units in the city of Belo-Horizonte (BH) the amount of ARV dispensed in the years 2018-2019-2020. Considering the amount dispensed in 2020, the daily dose, and the elimination rate, the amount of drug in unchanged form in the environment was estimated. For entire municipality of BH and Brazil, we used epidemiological data were used regarding individuals using antiretroviral therapy (ART) in 2020, the proportion of people living with HIV using the main ART regimen, following the same estimation methodology used previously. Structured and individual searches were carried out for each ARV used in Brazil, relating it to ecotoxicology, through Google Scholar, National Center for Biotechnology Information and Scifinder, in addition to the use of the fass.se platform. *Results***:** Four articles presented results of acute or chronic toxicity in ecotoxicological models involving ARV. Dolutegravir and efavirenz were found to be highly toxic in ecotoxicological models. In 2020, approximately 2,167kg of ARVs in unchanged form were released into the environment in BH. In Brazil it was 112,274kg. *Conclusion***:** It is urgent to quantify the main ARV in water bodies. With these data together with ecotoxicological data it will be possible to establish risk criteria for possible measures to control or mitigate these contaminants in the environment, especially actions to improve wastewater/water treatment.
Keywords: Antiretrovirals; Ecotoxicology; Environment.

Resumo

*Objetivo***:** Estimar o consumo e a quantidade de antirretrovirais (ARV) eliminados inalterados no meio ambiente e realizar uma pesquisa bibliográfica sobre estudos de ecotoxicologia aquática de ARV utilizados no Brasil. *Metodologia***:** Solicitamos em uma das unidades dispensadoras de ARV da cidade de Belo Horizonte (BH) a quantidade de ARV dispensada nos anos de 2018-2019-2020. Considerando a quantidade dispensada em 2020, a dose diária e a taxa de eliminação, foram estimadas as quantidades de fármaco na forma inalterada no ambiente. Para todo o município de BH e Brasil, foram utilizados dados epidemiológicos referentes aos indivíduos em uso de terapia antirretroviral (TARV) em 2020, a proporção de pessoas vivendo com HIV em uso do regime principal de TARV, seguindo a mesma metodologia de estimativa utilizada anteriormente. Foram realizadas buscas estruturadas e individuais para cada ARV utilizado no Brasil, relacionando-o à ecotoxicologia, por meio do Google Scholar, National Center for Biotechnology Information e Scifinder, além do uso da plataforma fass.se. *Resultados***:** Quatro artigos apresentaram resultados de toxicidade aguda ou crônica em modelos ecotoxicológicos envolvendo ARV. Dolutegravir e efavirenz foram considerados altamente tóxicos. Em 2020, aproximadamente 2.167kg de ARV na forma inalterada foram liberados no meio ambiente em BH. No Brasil foram 112.274kg. *Conclusão***:** É urgente quantificar os principais ARV em corpos d'água. Com esses dados, somados aos dados ecotoxicológicos será possível estabelecer critérios de risco para possíveis medidas de controle ou mitigação desses contaminantes no meio ambiente, especialmente ações de melhoria nos tratamentos de esgoto/águas.
Palavras-chave: Antirretrovirais; Ecotoxicologia; Meio ambiente.

Resumen

Objetivo: Estimar el consumo y la cantidad de antirretrovirales (ARV) eliminados inalterados en el medio ambiente y realizar una búsqueda bibliográfica sobre estudios de ecotoxicología acuática de los ARV utilizados en Brasil. *Metodología*: Solicitamos en una de las unidades de dispensación de ARV en la ciudad de Belo-Horizonte (BH) la cantidad de ARV dispensada en los años 2018-2019-2020. Considerando la cantidad dispensada en 2020, la dosis diaria y la tasa de eliminación, se estimó la cantidad de fármaco en forma inalterada en el medio ambiente. Para todo el municipio de BH y Brasil, se utilizaron los datos epidemiológicos de las personas que utilizan la terapia antirretroviral (TARV) en 2020, la proporción de personas que viven con el HIV que utilizan el régimen principal de TARV, siguiendo la misma metodología de estimación utilizada anteriormente. Se realizaron búsquedas estructuradas e individuales para cada ARV utilizado en Brasil, relacionándolo con la ecotoxicología, a través de Google Scholar, Centro Nacional de Información Biotecnológica y Scifinder, además del uso de la plataforma fass.se. *Resultados*: Cuatro artículos presentaron resultados de toxicidad aguda o crónica en modelos ecotoxicológicos que involucran ARV. Se encontró que dolutegravir y efavirenz son altamente tóxicos. En 2020, aproximadamente 2167kg de ARV sin cambios se liberaron al medio ambiente en BH. En Brasil fue de 112.274kg. *Conclusión*: Es urgente cuantificar los principales ARV en cuerpos de agua. Con estos datos, sumados a los datos ecotoxicológicos, será posible establecer criterios de riesgo para posibles medidas de control o mitigación de estos contaminantes en el medio ambiente, especialmente actuaciones para mejorar los tratamientos de aguas residuales/aguas.
Palabras clave: Antirretrovirales; ecotoxicología; Ambiente.

1. Introduction

We have observed exponential growth of new drugs and volume produced worldwide with increasing scientific and technological development (Akkari, et al., 2016; Kornis, et al., 2014). The benefits of developing medicines, health products, detergents, disinfectants, surfactants, pesticides, and other organic compounds are evidenced by the improved quality of life of humans, domestic animals, and animals for agricultural activity. However, the cycle of production and consumption generates new types of waste, namely, persistent organic pollutants (POPs) (Tang et al., 2019).

POPs are organic compounds resistant to environmental degradation through biological, chemical, and photolytic processes generating toxicity (RASHED, 2022). An example is the chemical and pharmaceutical industry's production process residues, inappropriately discarded medicines, and their metabolites associated with consumption (Jain, et al., 2013; Ncube, et al., 2018; Rashed, 2022). Due to their persistence, POPs can bioaccumulate and adversely affect human health and the environment (Rashed, 2022).

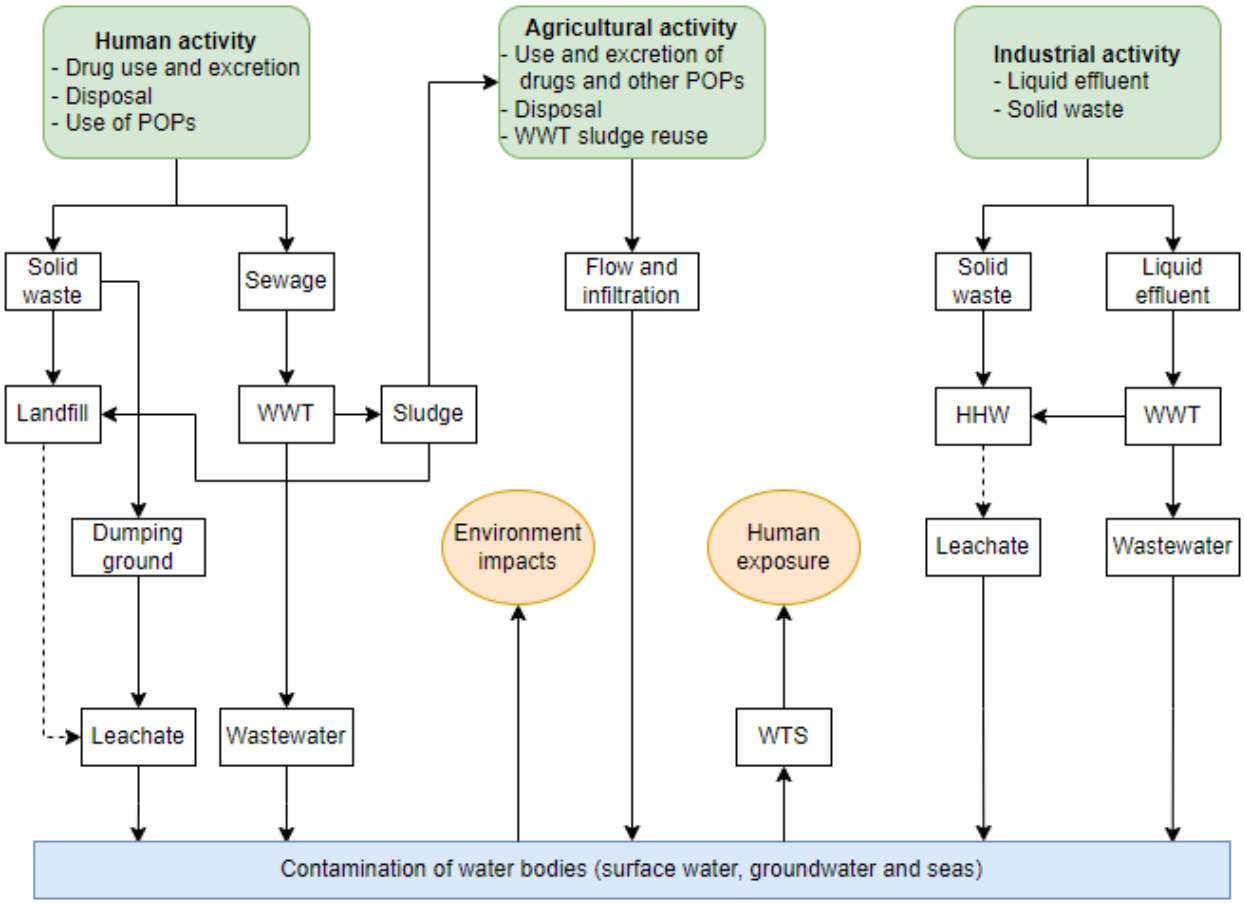
Regarding antiviral (ANV) and antiretroviral (ARV) drugs, we highlight drugs used for the treatment of HIV infection. Approximately 37.6 million people worldwide are currently living with the virus. Of these, 27.4 million are on antiretroviral therapy (ART). Brazil has approximately 920,000 people with the virus, of which 665,000 are on ART (BRASIL, 2020; UNAIDS, 2021). The use of ART is essential for people living with HIV (PLHIV). However, the waste generated should

not be disregarded since it can be associated with generating drug-resistant viral strains. Moreover, they can unbalance the ecosystem, triggering new problems (Kumar et al., 2021; Nannou et al., 2020). Several studies have indicated the presence of ARVs in drinking water and raw water, such as those from rivers, lakes, ponds, and groundwater (Jain et al., 2013; Nannou et al., 2020; Prasse, et al., 2010). A recent literature review performed by Nannou et al. (2020) revealed the presence of ANVs and ARVs in the environment in several studies, and most are concentrated in Europe and South Africa, with 22 and 13 studies, respectively. Central America and South America did not present any relevant study selected by the authors (Nannou et al., 2020).

Estimating pollutants' effects on ecosystems occurs mainly through ecotoxicological tests, using organisms found in the atmosphere, lithosphere, and mainly hydrosphere (Costa, et al., 2008; Gazola, 2020). Most plants for the treatment of effluents containing the new pollutants use conventional sewage treatment methodologies adopted by sanitation companies, which remove organic loads through biological processes. However, they are not consistently effective for the removal of recalcitrant substances. Other processes that include the use of technology, such as advanced oxidative processes, which aim to degrade recalcitrant substances, can be used instead of or associated with conventional processes (Brito & Marinho Silva, 2012; Fioreze, et al., 2014; Von Sperling, 1996).

ARVs reach the environment through waste disposal from the pharmaceutical industry production process (industrial effluent), hospital effluent, and elimination of drugs in unchanged form or through metabolites. Furthermore, the improper disposal of medicines is a source of contamination of the environment (Aquino, et al., 2013; Jain et al., 2013; Ncube et al., 2018). Figure 1 represents the cycle of drugs in the environment after dispensing for consumption and its potential consequences.

Figure 1. Possible routes of drugs and other organics pollutants to the environment.



POPs: Persistent organic pollutants; HHW: Household hazardous waste; WTS: Water Treatment Station; WWT: Wastewater treatment
Source: Adapted from Aquino, Brandt, & Chernicharo (2013)

The impact of these pollutants on the environment can be curbed through the effective treatment of waste and effluents and proper final disposal management. In Brazil, Resolution N° 430 of the National Council for the Environment (CONAMA), of May 13, 2011, requires prior treatment of waste to be disposed of by industries and hospitals (CONAMA, 2011b). Regarding domestic sewage, although universal sanitation is provided for in the Constitution and guaranteed by Law 11,445/2001, in practice, we can observe the lack of universal sewage collection and treatment. According to the *Trata Brasil* Institute, only 54.1% of the Brazilian population has access to adequate sewage collection, and 49.1% of the sewage produced has adequate treatment (Instituto Trata Brasil, 2021). Although some methodologies to mitigate the load of waste released into the environment are in place, an assessment of their efficiency and possible effects on aquatic life is required (CONAMA, 2011b; Gazola, 2020).

1.1 Brazilian legislation and standards for ecotoxicology

The term ecotoxicology was proposed during a meeting in Stockholm at the Committee of the International Council of Scientific Unions (ICSU) in June 1969 by French researcher René Truhaut (Truhaut, 1977 apud Magalhães & Filho, 2008). Truhaut (1977) argues that ecotoxicology is a branch of toxicology, which seeks to study the toxic effects of natural or synthetic pollutants on life in general, whether microbiological, plant or animal. It is a tool to assess the impact of pollutants on the environment and predict possible future damage, thus considering the various interactive possibilities (Magalhães & Ferrão-Filho, 2008; Zagatto & Bertoletti, 2006).

Ecotoxicological tests with aquatic organisms are traditionally used to assess the effect of specific substances or a set of them and contaminated waters. Tests performed with specific substances are used to obtain chemical records, while tests with water are used to obtain data regarding the compliance of the water body or industrial effluent. Toxicity data help evaluate and compare the susceptibility of the aquatic organism to different substances (Costa et al., 2008).

Currently, several environmental protection agencies work on developing and standardizing these tests to organize environmental protection strategies. Some of the international organizations engaged in this work are the United States Environmental Protection Agency (US EPA), the American Society for Testing and Materials (ASTM), the Organization for Economic Cooperation and Development (OECD), the Association of Analytical Communities (AOAC), and the International Organization for Standardization (ISO). In Brazil, the organization responsible for developing and standardizing toxicological testing protocols is the Brazilian Association of Technical Norms (ABNT) and CETESB, which also carries out some standardization work (Costa et al., 2008).

For ecotoxicological tests, the test should be conducted on more than one aquatic biota species, preferably belonging to different trophic levels, such as producers, primary consumers, and secondary consumers. Decomposers such as bacteria and rotifers can also be used besides organisms of different trophic levels (Costa et al., 2008). In Brazilian legislation, the resolutions of the National Council for the Environment (CONAMA) RE 357/2005 and RE 430/2011 establish the possibility of using biological indicators, toxicity tests for interactions of compounds not listed in the resolution, and running ecotoxicological tests in effluents using aquatic organisms of at least two different trophic levels. Box 1 shows the list of the leading Brazilian standards for aquatic ecotoxicological tests (ABNT, 2022; CONAMA, 2005, 2011a)

Box 1. Brazilian standardized aquatic ecotoxicology tests.

Effect	aquatic organism	Species	Trophic level	Rule
Chronic	Fish	*Danio rerio* , *Pimephales promelas*	Secondary consumer	ABNT NBR 15499:2022
Acute	Fish	*Danio rerio, Pimephales promelas*	Secondary consumer	ABNT NBR 15088:2022
Acute	Crustacea	*Daphnia similis, Daphnia magna*	Primary consumer	ABNT NBR 12713:2022
Acute	Crustacea	Anfípodos	Primary consumer	ABNT NBR 16638:2021
Acute	Crustacea	*Artemia sp*	Primary consumer	ABNT NBR 16530:2021
Chronic	Alga	Microalgas marinhas	Primary producer	ABNT NBR 16181:2021
Acute	Crustacea	Copépodos marinhos	Primary consumer	ABNT NBR 16723:2021
Acute	Bacteria	*Aliivibrio fischeri*	Decomposer	ABNT NBR 15411:2021
Chronic	Crustacea	*Hyalella spp*	Primary consumer	ABNT NBR 15470:2021

Box 1. Brazilian standardized aquatic ecotoxicology tests (Cont.)

Effect	aquatic organism	Species	Trophic level	Rule
Chronic	Echinoderm	*Lytechinus variegatus, Arbacia lixula e Echinometra lucunter*	Primary consumer	ABNT NBR 15350:2020
Chronic	Alga	*Chlorella vulgaris, Scenedesmus subspicatus, Pseudokirchneriella subcapitata*	Primary producer	ABNT NBR 12648:2018
Acute	Crustacea	Misideos	Primary consumer	ABNT NBR 15308:2017
Chronic	Crustacea	*Ceriodaphnia dúbia, Ceriodaphnia silvestrii*	Primary consumer	ABNT NBR 13373:2016
Acute	Bivalve mollusc	*Perna perna*	Primary consumer	ABNT NBR 16456:2016
Acute	Crustacea	Anfípodos marinhos	Primary consumer	ABNT NBR 15638:2015

Source: Authors (2022).

Toxicity tests aim to understand the toxic effects of substances on organisms. These tests can assess acute and chronic toxicity. The acute toxicity test evaluates the effects of substances on organisms in a short period as these effects result from single or multiple contacts within 24-96 hours. The parameters evaluated are usually mortality or immobility and metabolic changes or biochemical reactions. The results of these tests can be expressed as LC_{50} (mean lethal concentration), EC_{50} (mean effective concentration), IC_{50} (mean inhibitory concentration), and LD_{50} (mean lethal dose); in other words, the concentration of the substance causing death, immobility, or 50% growth inhibition of test organisms after a specific exposure time (Costa et al., 2008; Gazola, 2020).

Chronic toxicity tests assess the effects of long-term exposure to the substances studied, covering their entire life cycle or part of it. They are used for evaluating sublethal doses, for example, for evaluating treated effluent, since the exposure of organisms to low concentrations does not eliminate the risk of deleterious effects (Costa et al., 2008). The main objective is to understand the effects on the life cycle of organisms, such as, for example, whether there are biological function (reproduction, egg development, and maturation) disorders. Furthermore, they help study the pollutants' bioaccumulation capacity and their carcinogenic and mutagenic potential (Zenker, et al., 2014). Thus, assessing chronic toxicity is crucial to complement the acute toxicity test and obtain adequate information about the studied agents. Chronic toxicity tests are performed for seven to 21 days. Results are usually expressed as NOEC (no observed effect concentration), the highest concentration of toxic agent that does not cause statistically significant deleterious effect on organisms at exposure time and test conditions, and LOEC (lowest observed effect concentration), the lowest concentration of the toxic agent, which causes a statistically significant deleterious effect on organisms at the time of exposure and test conditions, but the results can be expressed as EC_{50} (Costa et al., 2008; Gazola, 2020).

This study aimed to estimate the consumption and amount eliminated in the ARV's unchanged form to the environment in Belo Horizonte and Brazil and search in the scientific literature, in an integrative way, to obtain an overview and more up-to-date ecotoxicological studies on the aquatic environment involving the ARVs used in Brazil.

2. Methodology

2.1 Estimated consumption and elimination of antiretrovirals in Brazil

Due to the difficulty of accurately establishing the amount of ARV consumed, one of the ways to estimate the consumption of medicines is through direct dispensing information from ARV distribution centers. We requested the amount of ARV dispensed in 2018, 2019, and 2020 from one of the ARV dispensing units in Belo Horizonte, Brazil, which provides clinical and outpatient care to PLHIV and those affected by other infectious diseases. Based on the volume dispensed last year, the recommended daily dose of each drug and the elimination rate, we estimated the drug volume in the environment for the municipality studied. The annualized results were obtained by multiplying the recommended daily dose, the percentage eliminated in unchanged form, and the estimated number of pharmacotechnical units dispensed per year, dividing them by one million to express the values in kilograms.

The number of specific ARVs consumed was unavailable for general data related to Belo Horizonte and Brazil, which hampered obtaining estimates of the amount released by these drugs into the environment. Epidemiological data referring to the number of individuals using ART in 2020 in Belo Horizonte and Brazil were employed to estimate the amount of antiretroviral dispensed in the environment, applying the same mathematical equation for quantifying ARVs in the ARV dispensation health unit in Belo Horizonte.

2.2 Ecotoxicity studies

The integrative review was used as method of the research, that consists of building a broad analysis of the literature, contributing to discussions about research methods and results, as well as reflections on carrying out new studies (Cronin & George, 2020; Whittemore & Knafl, 2005).

All the most used ARVs in Brazil were selected and included in the National List of Essential Medicines (RENAME 2020) to conduct the bibliographic search (BRASIL, 2019). After defining which ARVs would be evaluated, we searched for each drug individually on Google Scholar, National Center for Biotechnology Information (NCBI), and SciFinder platforms. The following descriptors were used on Google Scholar for the search strategy: (name of antiretroviral) AND ecotoxicology OR ecotoxicity OR ecotoxicological. In the NCBI, we used: ((Name of the antiretroviral) AND (ecotoxicological) OR

(ecotoxicity) OR (ecotoxicology)). In SciFinder, with each of the terms being searched individually, we used: (antiretroviral name) AND ecotoxicology, (antiretroviral name) AND ecotoxicity, (antiretroviral name) AND ecotoxicological. We included for evaluation publications on any date up to 2021; scientific papers; full-text in English or Portuguese; papers that presented results for ARV toxicity tests included in the RENAME 2020 list, for some test organisms, indicating the concentration of acute or chronic toxicity; papers with information on the initial concentration and after the effluent treatment, of at least one ARV on the RENAME 2020 list. We excluded incomplete texts or texts in other languages, theses, books, literature review papers, and toxicity estimation studies conducted using software if the paper was carried out only for this purpose. Ecotoxicological information on ARVs registered in Sweden was also searched on the Fass website (2016) and inserted in this work as a search engine, as they showed the results of drugs and medicines ecotoxicity tests conducted by drug registration holders.

The ecotoxicity results were discussed from a comparative perspective and analysis of the information obtained. One of the parameters used was the classification of acute ecotoxicity shown in Table 1, proposed by ZUCKER (1985) (Zucker, 1985). Furthermore, chronic toxicity data were also evaluated.

Table 1. Acute ecotoxicity parameters.

Classification	Parameter	Concentration mg/L
Very highly toxic	CL_{50} or CE_{50}	<0.1
Highly toxic	CL_{50} or CE_{50}	0.1 – 1
Moderately toxic	CL_{50} or CE_{50}	1 – 10
Slightly toxic	CL_{50} or CE_{50}	10 – 100
Practically nontoxic	CL_{50} or CE_{50}	>100

Source: Zucker (1985).

3. Results and discussion

Understanding drug elimination aspects is crucial to estimating the percentage not metabolized that is eliminated and knowing the most common metabolites. The physicochemical properties are important parameters to predict some behavior of substances in the environment. Table 2 shows the estimates of drug elimination by the human body in unaltered form and its primary metabolites.

Table 2. Data on the elimination of antiretrovirals found in the 2020 List dispensed in Brazil.

Drug	Recommended daily dose (mg)*	Elimination in unchanged form of drug (%)		Metabolites
		Urine	Faeces	
Abacavir	600	1.2	16	5'-carboxylic (30%); 5'-glucuronide (36%); other metabolites (15%)
Atazanavir	300	7	20	-
Darunavir	1200	7.7	41.2	-
Didanosine**	-	-	-	-
Dolutegravir	50	<1	53	glucuronide ether (18.9%); N-dealkylation metabolite (3.6%); metabolite formed by the oxidation of benzyl carbon (3.0%)
Efavirenz	600	<1	16-61[a]	7-hydroxy-efavirenz; 8-hydroxy-efavirenz; 8,14-dihydroxy-efavirenz
Enfuvirtide	180	-	-	amino acids
Emtricitabine	200	73	14	3'-sulfoxide daystereoisomers; 2'-O-glucuronide
Etravirine	400	-	81.2-86.4	-
Fosamprenavir	1400	-	1%	-
Lamivudine	300	~70%	-	Lamivudine Sulfoxide, Lamivudine 5′ triphosphate
Lopinavir	800	2.2	19.8	12 different metabolites; oxidation metabolites
Maraviroc	600	8	25	Various metabolites
Nevirapine	400	<3	-	2-hydroxynevirapine glucuronide 3-hydroxynevirapine glucuronide 8-hydroxynevirapine glucuronide 12-hydroxynevirapine glucuronide 4-carboxynevirapine
Raltegravir	400	9	-	raltegravir-glucuronide
Ritonavir	400	3.5	33.8	isopropylthiazolyl oxidation metabolite
Saquinavir**	-			
Tenofovir	300	70-80	-	tenofovir alafenamide
Tipranavir	1000	-	52	hydroxylated metabolite and glucuronide
Zidovudine	600	29	-	3′-azido-3′-deoxy-5′-O-beta-Dglucopyranuronosylthymidine, isomeric addition cycle products

* Recommended doses for adults. In cases where doses are variable, the highest recommended doses were used. **Drugs excluded from RENAME 2020. Source: Authors (2022).

According to data from one of the ARV dispensing units in Belo Horizonte (MG), Table 3, we observed an increase of around 20% in the total amount of ARV dispensed from 2018 to 2020, representing approximately 52 kg of ARVs disposed of into the environment. In 2020, more than 2,400,000 ARV pharmacotechnical units were dispensed in this dispensing unit alone to approximately 3,479 people. The highest percentages in the last year were of dolutegravir, lamivudine, and tenofovir, emphasizing the growing number of units of these drugs over the observed triennium, resulting from the guideline that established in 2017 this combination as first-line therapy in the treatment of HIV infection in Brazil. According to data from the Brazilian Ministry of Health, 12,833 PLHIV were on ART (Brazil 665,799) in Belo Horizonte in 2020 (BRASIL, 2022). Thus, based on the proportion of PLHIV using the first-line therapeutic regimen achieved for the Belo Horizonte ARV unit evaluated, at the recommended dosage of one tablet of dolutegravir 50 mg + a tablet of tenofovir 300 mg associated with

Lamivudine 300 mg administered daily, the estimated amount of ARV dispensed to individuals and eliminated unchanged into the environment in Belo Horizonte and Brazil was approximately 559 kg and 28,902 kg, respectively, as shown in Table 4.

Table 3. Data on dispensing and disposal of antiretrovirals from a drug dispensing unit in Belo Horizonte (MG), Brazil.

Drug	Drug Concentration	Pharmaceutical form	Drug elimination rate in unchanged form (%)	Number of pharmaceutical units dispensed			Annual estimate of annual elimination (Kg) in the environment		
				2018	2019	2020	2018	2019	2020
Abacavir	300mg	Tablet	17.2	59,100 (2.9 %)	45,360 (2.1%)	41,880 (1.7%)	3.0	2.3	2.2
Abacavir	20mg/ml	Oral solution	17.2	4 (0%)	0 (0%)	8 (0%)	0.0	0.0	0.0
Atazanavir	300mg	Cápsula	27	207,300 (10.2 %)	178,230 (8.2%)	168,210 (6.9%)	16.8	14.4	13.6
Darunavir	600mg	Tablet	48.9	140,160 (6.9%)	143,400 (6.6%)	155,880 (6.4%)	41.1	42.1	45.7
Dolutegravir	50mg	Tablet	53	194,310 (9.6%)	373,140 (17.2%)	532,380 (21.7%)	5.1	9.9	14.1
Efavirenz	600mg	Tablet	39	37,650 (1.9%)	29,850 (1.4%)	26,610 (1.1%)	8.8	7.0	6.2
Efavirenz	30mg/ml	Oral solution	39	7 (0%)	0 (0%)	8 (0%)	0.0	0.0	0.0
Etravirina	200mg	Tablet	83.8	4,620 (0.2%)	5,100 (0.2%)	4,500 (0.2%)	0.8	0.9	0.8
Lamivudine	150mg	Tablet	70	79,440 (3.9%)	103,080 (4.7%)	134,940 (5.5%)	8.3	10.8	14.2
Lamivudine*	10mg/ml	Oral solution	70	19,920 (1.0%)	8,880 (0.4%)	20,640 (0.8%)	0.4	0.2	0.4
Lopinavir + Ritonavir*	80+20mg/ml	Oral solution	15	4,320 (0.2%)	320 (0%)	0 (0%)	0.1	0.0	0.0
Maraviroc	150mg	Tablet	25	1,320 (0.1%)	1,380 (0.1%)	1,380 (0.1%)	0.0	0.1	0.1
Nevirapine	200mg	Tablet	1	26,760 (1.3%)	18,060 (0.8%)	16,320 (0.7%)	0.1	0.0	0.0
Raltegravir	400mg	Tablet	42	3,960 (0.2%)	3,240 (0.1%)	3,420 (0.1%)	0.7	0.5	0.6
Ritonavir	100mg	Tablet	18.7	328,320 (16.1%)	291,300 (13.4%)	283,650 (11.6%)	6.1	5.4	5.3

Table 3. Data on dispensing and disposal of antiretrovirals from a drug dispensing unit in Belo Horizonte (MG), Brazil (Cont.)

Drug	Drug Concentration	Pharmaceutical form	Drug elimination rate in unchanged form (%)	Number of pharmaceutical units dispensed			Annual estimate of annual elimination (Kg) in the environment		
				2018	2019	2020	2018	2019	2020
Tenofovir	300mg	Tablet	75	5,280 (0.3%)	3,150 (0.1%)	3,180 (0.1%)	1.2	0.7	0.7
Tenofovir+Lamivudine	300mg+300mg	Tablet	75	378,600 (18.6%)	508,530 (23.4%)	637,680 (26.0%)	170.4	228.8	287.0
Tenofovir+Lamivudine+Efavirenz	300mg+300mg+ 600mg	Tablet	61	418,710 (20.6%)	343,170 (15.8%)	322,200 (13.1%)	306.5	251.2	235.9
Zidovudine	100mg	Capsule	29	3,400 (0.2%)	1,900 (0.1%)	400 (0%)	0.1	0.1	0.0
Zidovudine + Lamivudine	300mg+150mg	Tablet	49.5	144,840 (7.1%)	125,520 (5.8%)	121,500 (5.0%)	32.3	28.0	27.1
			Total	2,033,891	2,174,449	2,454,232	601.8	602.4	653.8

Source: Authors (2022).

Table 4. Estimated annual elimination of ARVs in unchanged form in Belo Horizonte and Brazil in 2020.

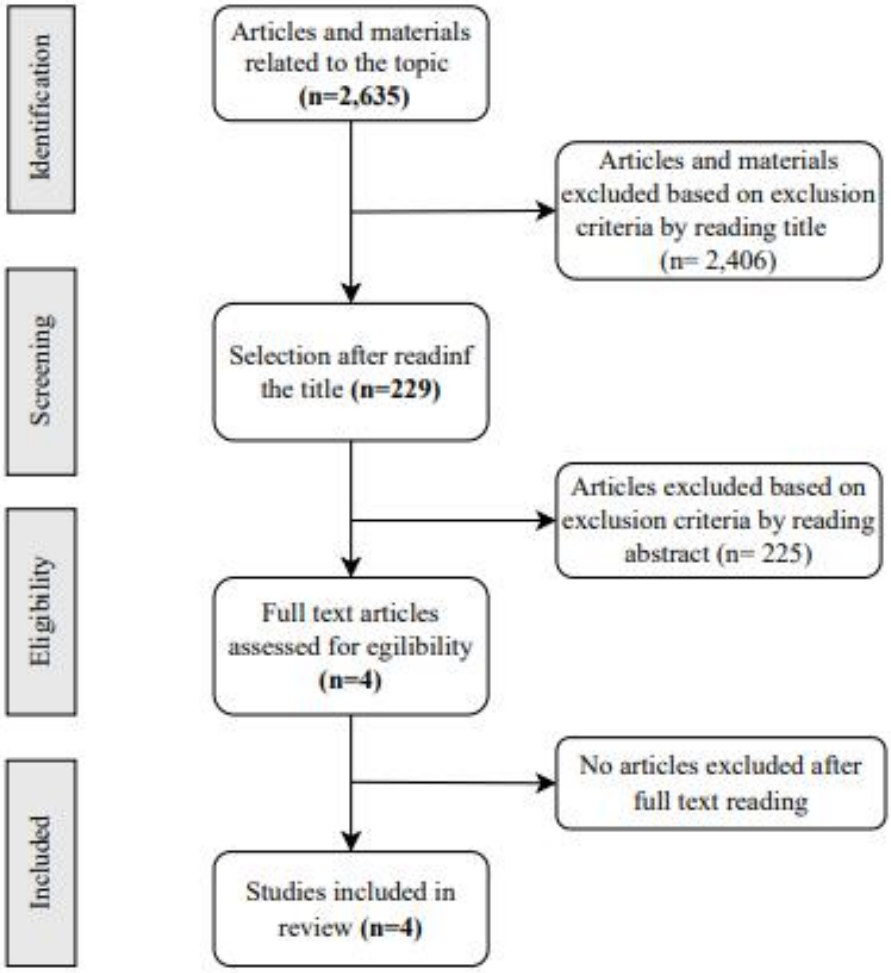
			Belo Horizonte annual estimate		**Brazil annual estimate**	
Drug	**Daily dose (mg)**	**Elimination in unchanged form**	**Pharmacotechnical units**	**Disposal in (Kg) in the environment**	**Pharmacotechnical units**	**Disposal in (Kg) in the environment**
Dolutegravir	50	54%	4,702,295	27.4	243,016,635	1,417
Tenofovir	300	75%	4,702,295	275.1	243,016,635	14,216
Lamivudina	300	70%	4,702,295	256.7	243,016,635	13,269
			Total	559,25	**Total**	28,902

Source: Authors (2022).

These estimates corroborate those described for South Africa, the African country with the most extensive program to combat and prevent HIV infection, which serves more than 5.25 million PLHIV with ART, where it is estimated that 4.32 tons of ARVs are consumed daily, equivalent to approximately 1,576 tons per year (Ncube et al., 2018). We emphasize that these drugs, although released in significant amounts into the environment, are successively diluted in effluents and water bodies and found in ng/mL and pg/mL (Nannou et al., 2020).. Considering also that many of the effluent, sewage, and water treatment systems are not designed to eliminate this type of contaminant, besides the recalcitrant characteristics of some ARVs, there are imminent risks for developing resistant viral strains and possible ecosystem changes in contact with these pollutants (Muriuki et al., 2020; Nannou et al., 2020; Ncube et al., 2018).

Regarding the existing ecotoxicological studies on ARVs dispensed by the Brazilian Unified Health System (SUS) and included in RENAME (2020), after researching databases, we selected four articles with results of acute or chronic toxicity in ecotoxicological models involving ARVs, Figure 2. We did not identify any mention of ecotoxicology involving the ARVs atazanavir, daclatasavir, didanosine, enfurvitide, emtricitrabine, lopinavir, saquinavir and sofosvubir.

Figure 2. Flow of selection of ecotoxicity articles for selected ARVs.



Source: Authors (2022).

Table 5. Complete data on the ecotoxicity of ARVs dispensed by the Brazilian Unified Health System (SUS).

Antiretroviral	Species	Aquatic organism	Kind of study	Period	Parameter	Result (mg/L)	Methodology	Reference
Abacavir	*Daphnia magna*	Crustacea	Acute	48h	EC_{50}	>100		(Minguez et al., 2016)
	Pseudokirchneriella subcapitata	Alga	Acute	72h	EC_{50}	57.32		
	Artemia salina	Crustacea	Acute	48h	EC_{50}	>100		
	Skeletonema marinoi	Bacteria	Acute	72h	CE_{50}	NA		
Atazanavir	*NR*							
Daclatasvir	*NR*							

Table 5. Complete data on the ecotoxicity of ARVs dispensed by the Brazilian Unified Health System (SUS) (Cont.)

Antiretroviral	Species	Aquatic organism	Kind of study	Period	Parameter	Result (mg/L)	Methodology	Reference
Darunavir	*Pseudokirchneriella subcapitata*	Alga	Acute	72h	CE50	>43	OECD 201	(FASS, 2016)
					NOEC	43	OECD 202	
	Daphnia magna	Crustacea	Acute	48h	EC50	>44	OECD 202	
					NOEC	2.6		
	Oncorhynchuss mykiss	Fish	Acute	96h	CL50	>38	OECD 203	
					NOEC	38	OECD 203	
	Daphnia magna	Crustacea	Chronic	21 days	NOEC	19	OECD 211	
	Pimephales promelas	Fish	Chronic	28 days	NOEC	9.4	OECD 210	
Didanosine	*NR*							(FASS, 2016)
Dolutegravir	*Pseudokirchneriella subcapitata*	Alga	Acute	96h	CI50	0.233	OECD 201	(FASS, 2016)
					NOEC	0.095	OECD 201	
	Daphnia magna	Crustacea	Acute	48h	CE50	>6.430	OECD 202	
	Daphnia magna	Crustacea	Chronic	21 days	NOEC	0.834	OECD 211	
	Juvenile Pimephales promelas	Fish	Chronic	28 days	NOEC	0.22	OECD 210	
Efavirenz	*Selenastrum capricornutum*	Alga	Chronic	12 days	CE50	>0.026	FDA 4.01	(FASS, 2016)
					NOEC	0.026	FDA 4.01	
	Daphnia magna	Crustacea	Acute	48h	EC_{50}	1.1	FDA 4.08	
	Lepomis macrochirus	Fish	Acute	96h	CL_{50}	0.85	FDA 4.11	
	Daphnia magna	Crustacea	Chronic	21 days	NOEC	0.16	OECD 211	
	Pimephales promelas	Fish	Chronic	33 days	NOEC	0.07	OCDE 210	
	Oreochromis mossambicus	Fish	Chronic	7 days	LOEC	0.0000103		(Robson, Barnhoorn, & Wagenaar, 2017)
Emtricitabine	*NR*							
Enfuvirtide	*NR*							
Etravirine	*Scenedesmus subspicatus*	Alga	Acute	72h	CE_{50}	>0.0049	OECD 201	(FASS, 2016)
					NOEC	0.0049	OECD 202	
	Daphnia magna	Crustacea	Chronic	21 days	NOEC	0.0091	OECD 211	
	Brachydanio rerio	Fish	Chronic	35 days	NOEC	0.01	OECD 210	

Table 5. Complete data on the ecotoxicity of ARVs dispensed by the Brazilian Unified Health System (SUS) (Cont.)

Antiretroviral	Species	Aquatic organism	Kind of study	Period	Parameter	Result (mg/L)	Methodology	Reference
Fosamprenavir	*Selenastrum caprocornutum*	Alga	Acute	72h	Cl_{50}	>100	OECD 201	(FASS, 2016)
					NOEC	100	OECD 201	
	Daphnia magna	Crustacea	Acute	48h	CE50	>109	OECD 202	
					NOEC	109	OECD 202	
	Juvenilee Ocorhynchus mykiss	Fish	Acute	96h	CL_{50}	>100	OECD 203	
					NOEC	100	OECD 203	
	Ceriodaphnia dubia	Crustacea	Chronic	7 days	NOEC	100	EPA 1002	
Lamivudine	*Selenastrum caprocornutum*	Alga	Acute	72h	CI_{50}	>96.9	OECD 201	(FASS, 2016)
					NOEC	>96.9	OECD 201	
	Daphnia magna	Crustacea	Acute	48h	CE_{50}	>1000	OECD 202	
					NOEC	>1000	OECD202	
	Juvenilee Oncorhyncus mykiss	Crustacea	Acute	96h	CL_{50}	>97.7	OECD 203	
					NOEC	97.7	OECD 203	
	Ceriodaphnia dubia	Crustacea	Chronic	7 days	CE_{50}	>100	EPA 1002	
					NOEC	100	EPA 1002	
	Daphnia magna	Crustacea	Chronic	21 days	CE_{50}	>100	OECD 211	
					NOEC	100	OECD 211	
	Pimephales promelas	Fish	Chronic	96h	CL_{50}	>10	OECD 210	
					NOEC	10	OECD 210	
Lopinavir	*NR*							
Maraviroc	*Pseudokirchneriella subcapitata*	Alga	Acute	72h	CI_{50}	>115	OECD 201	(FASS, 2016)
	Daphnia magna	Crustacea	Acute	48h	CE_{50}	>69	OECD 202	
	Juvenile Ocorhynchus mykiss	Fish	Acute	96h	CL_{50}	>73	OECD 203	
	Ceriodaphnia dubia	Crustacea	Chronic	7 days	NOEC	92	EPA 1002	
Nevirapine	*Selenastrum capricornutum*	Alga	Chronic	10 days	CE_{50}	>43	FDA 4.01	(FASS, 2016)
					NOEC	43	FDA 4.01	
	Daphnia magna	Crustacea	Acute	48h	CE_{50}	76.9	92/69/EEC	
					NOEC	76.9	92/69/EEC	
	Oncorhynchus mykiss	Fish	Acute	96h	CL_{50}	>65	92/69/EEC	
	Pimephales promelas	Fish	Acute	96h	CL_{50}	>99	FDA 4.11	
Nevirapine	*Oreochromis mossambicus*	Fish	Chronic	30 days	NOEC	$1{,}48x10^{-6}$		(NIBAMUREK; BARNHOORN; WAGENAAR, 2019)

Table 5. Complete data on the ecotoxicity of ARVs dispensed by the Brazilian Unified Health System (SUS) (Cont.)

Antiretroviral	Species	Aquatic organism	Kind of study	Period	Parameter	Result (mg/L)	Methodology	Reference
Raltegravir	*Selenastrum capricornutum*	Alga	Acute	96h	CE_{50}	66	OECD 201	(FASS, 2016)
					NOEC	3.8	OECD 201	
	Daphnia magna	Crustacea	Acute	48h	CL_{50}	>100	OECD 202	
	Pimephales promelas	Fish	Acute	96h	CL_{50}	>100	OECD 203	
	Cyprinodon variegatus	Fish	Acute	96h	CL_{50}	>100	OECD 203	
	Daphnia magna	Crustacea	Chronic	21 days	NOEC	9.5	OECD 211	
	Pimephales promelas	Fish	Chronic	33 days	NOEC	9.3	OECD 210	
Ritonavir	*Daphnia magna*	Crustacea	Acute	48h	CE_{50}	>1.5	FDA 4.08	(FASS, 2016)
	Hyalella azteca	Crustacea	Acute	96h	NOEC	1.59	FDA 4.10	
	Lepomis macrochirus	Fish	Acute	24,48,72,96h	CL_{50}	>1.63	FDA 4.11	
Saquinavir	*NR*							
Sofosbuvir	*NR*							
Tenofovir	*seudokirchneriella subcapitata*	Alga	Acute	72h	CE_{50}	69	OECD 201	(FASS, 2016)
					NOEC	18	OECD 201	
	Daphnia magna	Crustacea	Chronic	21days	NOEC	12	OECD 211	
	Pimephales promelas	Fish	Chronic	32days	NOEC	9	OECD 210	
	Aliivibrio fischeri	Bacteria	Acute	15 min	EC_{50}	14.83		(Silva et al., 2019)
	Artemia salina	Crustacea	Acute	36h	EC_{50}	111.82		
	Microcystis novacekii	Bacteria	Chronic	12 days	LOEC	161.01		
Tipranavir	*Desmodesmus subspicatus*	Alga	Acute	96h	EC_{50}	>40.4	OECD 201	(FASS, 2016)
	Daphnia magna	Crustacea	Acute	48h	EC_{50}	5	OECD 202	
					NOEC	3.96	OECD 202	
	Oncorhynchus mykiss	Fish	Acute	96h	EC_{50}	15.4	OECD 203	
					NOEC	1	OECD 203	
Zidovudin3	*Daphnia magna*	Crustacea	Acute	48h	EC_{50}	>1.000	OECD 202	(FASS, 2016)
					NOEC	16	OECD 202	
			Chronic	21 days	EC_{50}	>100	OECD 209	
					NOEC	>16	OECD 209	

NR – No results

Source: Authors (2022).

Considering the parameters presented by Zucker (1985) (Table 1) regarding environmental toxicity, we could say that dolutegravir and efavirenz are highly toxic. Etravirine can be classified as very highly toxic. Tipranavir and ritonavir can be classified as moderately toxic. Abacavir, darunavir, lamivudine, maraviroc, and tenofovir can be classified as mildly toxic. Darunavir, lamivudine, and maraviroc have toxicity values above 38 mg/L, 96.9 mg/L, and 73 mg/L, respectively. Fosamprenavir and zidovudine are practically nontoxic.

We should note that most ARVs do not reach a concentration of 0.1 mg/L in domestic effluent treatment plants or the environment (Nannou et al., 2020). Muriuki et al. (2020) detected a concentration of 1.4635 mg/L for lamivudine in an effluent

treatment plant in Kenya. High concentrations are expected in industrial effluent treatment plants. A study with real effluents from a zidovudine and lamivudine production plant found drug concentrations of 6.29 mg/L and 3.22 mg/L, respectively (Souza, et al., 2010). Therefore, even if a drug is classified as highly toxic and by the studies currently published, there is an indication that the values of environmental concentrations are low. However, higher concentrations can be achieved in industrial effluents.

According to the information shown in Table 5, we can observe that drugs darunavir, fosamprenavir, lamivudine, maraviroc, raltegravir, tenofovir, and zidovudine have high values for NOEC, which in practice are not observed in the environment or a domestic effluent treatment plant (Nannou et al., 2020). Darunavir, lamivudine, and tenofovir have NOEC values below 10 mg/L, which are possible concentrations in a pharmaceutical effluent. Regarding concentration in industrial effluents, the work by Souza et al. (2010) shows that the maximum concentrations of zidovudine and lamivudine, which are 6.29 mg/L and 3.22 mg/L respectively, do not come close to the NOECs for these substances.

Dolutegravir presented an NOEC value of 0.22 mg/L for the fish species *Juvenile Pimephales promelas*. It is challenging to consider the result as data indicating its concentration in the environment or effluent treatment plants were not found in the literature. Etravirine had an NOEC value of 0.0091 mg/L for the crustacean *Daphnia magna*. Although the concentration of NOEC is low, no data on the concentration in the environment and effluent treatment plants is available. We should emphasize that etravirine is not a first-choice drug in Brazil; therefore, its use is reduced.

The chronic toxicity test performed for nevirapine was presented in a paper by Nibamureke et al. (2019). The maximum concentration of the drug found in raw water in South Africa was used in the study, which was $1.48x10^{-6}$ mg/L, determined as NOEC. The study followed OECD guidelines for toxicity testing. It is worth noting that this was the only concentration evaluated (Nibamureke et al., 2019). There are no acute toxicity studies for the species of fish used in the study cited. However, we found some acute toxicity studies with other fish for nevirapine, which promotes toxic effects at concentrations above 65 mg/L. Thus, the NOEC value presented by the paper may be below the actual value. There is no other work or information regarding the chronic toxicity of nevirapine.

Only efavirenz has the result presented as LOEC. The study by Robson et al. (2017) aimed to assess the influence of the maximum concentration of efavirenz in water bodies in the Limpopo province, South Africa (Robson et al., 2017). The maximum concentration was 10.3 ng/l ($1.03x10^{-5}$ mg/L). Tests conducted with *Oreochromis mossambicus* (Tilapia from Mozambique) and histological analysis showed that the concentration induced deleterious effects on the animal's liver. Moreover, a concentration of 20.6 ng/l ($2.06x10^{-5}$ mg/L) was found and induced effects in the liver and other organs. We should emphasize that this study did not follow any international standards for the development of the tests, and only two concentrations were used, the one that appears in the environment and its double. Thus, it is impossible to affirm whether concentrations lower than $1.03x10^{-5}$ mg/L can harm the species. Despite the details, the study obtained important information about the drug.

Although efavirenz was analyzed based on the results of Robson et al. (2017), as the tests did not follow registered protocols, it is interesting to assess chronic toxicity from a second test performed following recognized protocols. As a result, the data obtained through the fass.se website show that the FDA protocols were used. The test was performed with algae for 12 days, and the EC_{50} was determined to be greater than 0.026 mg/L and the NOEC equal to 0.026 mg/L for the species *Selenastrum capricornutum*. Based on other research, we can affirm that the concentrations found in the environment and in domestic effluent treatment plants for this drug do not exceed the NOEC value et al., 2017).

4. Conclusion

Ecotoxicological studies involving antiretrovirals are scarce, and further research with as many of these drugs as possible is required. With significant numbers of medicines used by people living with HIV, it is urgent to quantify the main ARVs in water bodies. With this information and ecotoxicological data, we can establish risk criteria for eventual control or mitigation measures of these contaminants in the environment, mainly to improve the effluent and water treatment of large urban centers. We should also point out that the chronic toxicity of ARVs must be prioritized, especially those with greater consumption and elimination in the unchanged form and considered low-risk in acute toxicity studies or environmental risk assessment. As noted for efavirenz, the concentration at which a chronic effect is observed may be well below the concentration of the not observed effect. The toxicity studies presented were conducted with the drugs in isolation, so further research should be performed to understand the toxic capacity of these substances in complex systems, such as tablets or industrial or domestic effluent.

We strongly encourage the development of new models and ecotoxicological research involving organisms native to the countries, in order to expand the monitoring and knowledge of the environmental impacts caused by organic pollutants, especially drugs.

Conflict of interest

The authors declare no conflict of interest.

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