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PHARMACEUTICAL PRODUCTS IN THE ENVIRONMENT: SOURCES, EFFECTS AND RISKS

PRODUCTOS FARMACÉUTICOS EN EL AMBIENTE: FUENTES, EFECTOS Y RIESGOS

Jhon F. NARVAEZ V.[1], Claudio JIMENEZ C.[2*]

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ABSTRACT

Pharmaceuticals and personal care products have become an environmental problem in recent years. Their physicochemical properties and persistence in the environment have allowed the distribution of degradates and parent compounds in water, soil, air and food. The widespread use of pharmaceuticals and personal care products in hospitals, domestic residences, agricultural and industrial facilities has increased their discharge into the water bodies, and its toxicity has started to manifest in different biological components of ecosystems. The development of methods for sample treatment and instrumental analysis techniques has enabled the separation, identification and quantification of active ingredients and degradates with higher environmental impact, at concentrations of parts per billion or even parts per trillion. In addition, *in vitro* and *in vivo* assays have demonstrated their ecotoxicity in water, driving them to the classification of emerging organic pollutants, whose waste is indeterminate. Although their adverse effects are still unknown, they could have strong implications for global public health. This review presents the dynamics and the development of research over the past ten years about the presence of non-steroidal anti-inflammatory analgesics, antihypertensives, antibiotics and other drugs in water bodies. Similarly, it described the impact of pharmaceutical activity, hospital services and domestic effluents on water quality.

Keywords: Toxicity tests, environmental pollutants, organic pollutants, analgesics, pharmaceuticals and personal care products.

RESUMEN

Los productos farmacéuticos y los productos para el cuidado personal han representado un problema ambiental en los últimos años. Sus propiedades fisicoquímicas y su persistencia en el ambiente han permitido la distribución de muchos metabolitos parentales en el agua, en el suelo, en el aire y en los alimentos. Su amplio uso hospitalario, doméstico, agrícola e industrial ha aumentado las descargas en los cuerpos de agua y su impacto ambiental y toxicidad han empezado a manifestarse en los diferentes componentes biológicos de los ecosistemas. El desarrollo de metodologías de tratamiento de muestra y las técnicas instrumentales de análisis han permitido la separación, identificación y cuantificación a concentraciones de partes por billón e incluso de partes por trillón de principios activos y productos de degradación de gran impacto ambiental. Adicionalmente, los ensayos *in vitro* e *in vivo* han demostrado su ecotoxicidad acuática, permitiendo clasificar estas sustancias como contaminantes orgánicos emergentes, cuyos vertimientos son indeterminados y su impacto sobre los ecosistemas es silencioso pero de grandes repercusiones para la salud pública mundial. Esta revisión presentó la dinámica y el desarrollo

[1] Grupo de Investigación en Gestión y Modelación Ambiental (GAIA). Facultad de Ingeniería. Universidad de Antioquia. A.A. 1226. Medellín-Colombia.

[2] Grupo de Investigación Aplicada al Medio Ambiente (GAMA). Facultad de Ingeniería. Corporación Universitaria Lasallista. Caldas, Colombia.

[*] Corresponding author: clajimenez@lasallistadocentes.edu.co

de investigaciones durante los últimos diez años de la presencia de analgésicos-antiinflamatorios no esteroideos, antihipertensivos, antibióticos y otros fármacos en cuerpos de agua. De igual forma, describió el impacto de la actividad farmacéutica, los servicios hospitalarios y los efluentes domésticos sobre la calidad del agua.

Palabras clave: productos farmacéuticos y para el cuidado personal, ecotoxicidad, contaminantes orgánicos emergentes, antibióticos, analgésicos.

INTRODUCTION

The Pharmaceuticals and personal care products (PPCPs) are a wide range of organic compounds used for personal health or cosmetics reason, which include therapeutic drugs, phytotherapeutic, biotechnological products, veterinary drugs, fragrances, and cosmetics. These substances have diverse physicochemical properties such as partition coefficient octanol-water (K_{ow}), distribution coefficient-biosolids-water (K_p) and solubility, that describe the environmental dynamic and in most cases, non-specific biological activity (1).

Sometimes, the PPCPs are partially transformed by humans, pets, farm animals among others, triggering the early presence of degraded and parent compounds in water bodies. In some cases, analytical methods don't enable to identify metabolites and the relation between these substances and the adverse effects in the environment are still unknown. Besides, pharmaceutical formulations are complex and incorporate a variety of aids, which increase the unspecific effects on the biota. However, in some previous studies, the greater ecotoxicological potential, recalcitrant properties, and the bioaccumulation have been reported.

Two decades ago, the environmental analysis focused on detection of pollutants, such as pesticides, polychlorinated biphenyls (PCBs), phthalates, dioxins (PCDDs), furanones (PCDFs), polyaromatic hydrocarbons (PAHs) and flame retardants, but in the 90s, wastewater treatment plant (WWTP) began to show concentration of PPCPs. This led to the conclusion that PPCPs enter to water bodies, and that WWTP are not able to remove some active ingredients, thus driving to the appearance of possible epidemiological implications (2).

The first study of drugs in WWTP was reported in 1976 by Keith in Kansas. In this research was evaluated the occurrence of pharmaceuticals in wastewater and their adverse effects on fauna and flora, so US. Food and Drug Administration (FDA) and the European Union (EU) took some actions related with improve the remotion of xenobiotics in WWTP (3). Subsequently, studies focused in the identification and methodologies quantification of active ingredients and degradates, toxicity assay, removal processes and bioremediation (4-7). The principal sources of PPCPs are animal and human excretion, wastewater of pharmaceutical industry, effluents from hospitals, inadequate disposal of expired drugs, and waste dumping from research institutions and drugs development.

PPCPs are part of the so-called emerging organic contaminants, which enter to wastewater indefinitely and, although their impact on ecosystems still remains unknown, they cause widespread effects on biota and global public health. Furthermore, they are the subject of rigorous investigation in the topic of environmental chemical-analysis due to adverse effect on the aquatic organism. Therefore, this paper aims to examine the research development regarding the presence of pharmaceuticals in different water bodies from the last ten years in an easy way to assimilate, in order to generate social responsibility in the institutions of environmental control in Colombia.

Pharmaceutical products in the water bodies

The discharge of active pharmaceutical ingredients in the environment has been found to present catastrophic effects on the biota of aquatic ecosystems (8). Initially, drugs from agricultural and domestic activities were not considered as environmental pollutants, and additionally, some substances are not biodegradable, having high resistance to environmental transformation processes. Due to possible accumulation processes of degradates and parent compounds their concentrations in water bodies have increased. Therefore, instrumental analysis, separation, quantification and identification methodologies have been developed for detecting some active ingredients or metabolites at low levels in water bodies (parts per billion (ppb)–parts per trillion (ppt)) (9).

Moreover, active ingredients from hospital, residential, agricultural and industrial uses have been found to sweep into aquatic influents. These

drugs come from manufacturing, consumption, and inadequate disposal when their use by date has expired and proper disposal methods are unknown. Once in the environment, natural degradation processes act on the drugs, and produce degradates that increase the trouble in the environmental analysis for metabolites and parent compounds.

Recently, 500 tons per year of analgesics have been reported to be entering the environment. Some, such as acetylsalicylic acid (ASA) and diclofenac acid were found at concentrations of 0.22 and 3.02mg/L respectively in different water bodies in Spain, Italy, Germany, Canada, Brazil, Greece and France (10).

Factors like market demand, frequency of administration, self-medication and use of illegal drugs determine the speed of active ingredients entering aquatic ecosystems as well as the quantity present. In addition, the entry of degradates could contribute to nonspecific disorder in aquatic organism, due to greater absorption and distribution of some molecules that nowadays are unknown (11). In countries like Germany, hundreds of tons of high demand active ingredients are let loose into the environment (12).

Currently, hospitals are incorporating antibiotics into the wastewater system which have promoted the formation of resistant organisms such as *Aeromonas, Salmonella, Escherichia, Pseudomonas* and *Staphylococcus,* among others (13, 14). Additionally, the interruption of the enzymatic activity of microbiota present in the water disrupts the metabolism and biodegradation processes of organic matter in water bodies. The direct discharge of drugs into drainage systems allows metabolites and parent compounds to enter the treatment plants. This represents enormous challenges in the process of decontamination since when complete reduction is not achieved, parent compounds and degradates are able to enter water bodies and on occasions drinking water. Currently, chronic effects on the human health and aquatic organism are unknown.

A study by Oaks and colleagues showed that the death of between 34 and 95% of the population of oriental white-backed vultures, was linked to the consumption of water contaminated with diclofenac, a painkiller widely used by the human population that cause kidney failure and visceral gout in birds (15). Furthermore, it was found that in the degradation processes of carbamazepine, atenolol, metoprolol, diclofenac and trimethoprim in WWTP, effective removal processes were not achieved, with initial reductions only corresponding to 10% of the drugs. At the same time a different study reported reductions in water of only 7% for carbamazepine and 96% for propranolol (16). Finally, in countries like Germany, clofibrate concentrations above 70 ng/L have been reported in water (17). Although this concentration is not toxic for humans, the problems associated with chronic exposure to this active ingredient and its metabolites are not fully understood. However, ecotoxicological evaluation in *Ceriodaphnia dubia* presented a toxic concentration of 0.640 mg/mL. Ifosfamide toxicity tests have determined the teratogenic and mutagenic potential in fish species, while other drugs, such as carbamazepine, fluoxetine and gemfibrozil, have demonstrated effective EC50 concentrations of less than 81 μg/mL, 24 μg/mL 1.18 μg/mL respectively, in microtoxicity assay (18). All these drugs have been found in water bodies (19-21).

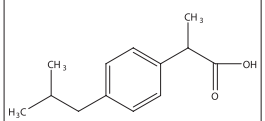
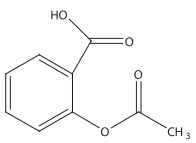
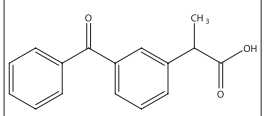
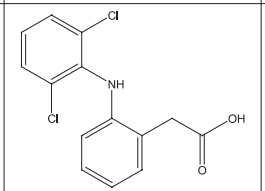
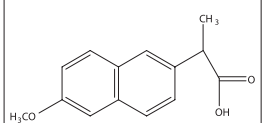
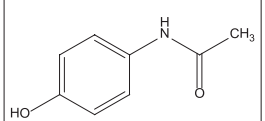
During the course of this review, contamination by drugs widely used around the world such as analgesics, antihypertensives, and antimicrobials will be analyzed. In addition, certain molecules that cause major environmental impact, whose toxic potential classifies them as endocrine disruptors and which are related to the disruption of the development and evolution of cells in aquatic organisms will be addressed.

Analgesics

Analgesics are drugs that are widely consumed all over the world. In Spain, they represent the largest income for the pharmaceutical industry and are the drugs most associated with self-medication, turning them into a public health issue (22-23). In recent years, high sensitive instrumental analysis has detected toxic concentrations of diclofenac and ASA in wastewater (24) (see table 1). Similarly, techniques for processing samples like solid phase extraction (SPE) and those for identification and quantification such as high performance liquid chromatography/electrospray ionization/tandem mass spectrometry (HPLC-ESI-MS/MS) have enabled the analysis of drugs such as naproxen, ibuprofen and acetaminophen in hospital wastewater (25). Farré M. *et al*, 2001 (26), reported concentrations in surface waters of analgesics at different pH and toxic concentrations, assessed with two models *in vivo*. The analysis of surface water indicates the

presence of painkillers such as ASA, naproxen, ibuprofen, diclofenac and ketotifen and some degradates of ibuprofen such as hydroxy-ibuprofen, carboxy-ibuprofen, and carboxihydrotropic acid, which are more toxic than their parent compounds (27). This indicates that the toxicity of some drugs in the environment may be related to metabolic processes, and indicates that the pharmaceutical industry should implement management techniques in WWTP for reducing the discharge of drugs in water bodies and minimize damage on aquatic ecosystems.

Table 1. Analgesics widely used in the pharmaceutical sector.

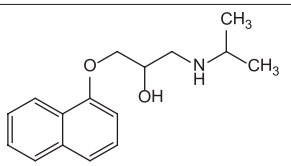
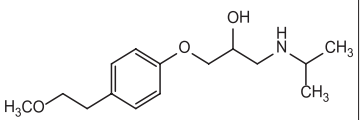
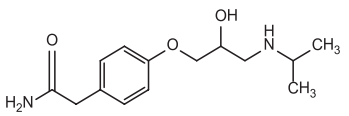
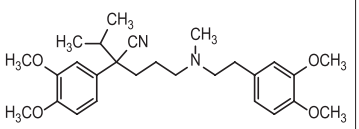
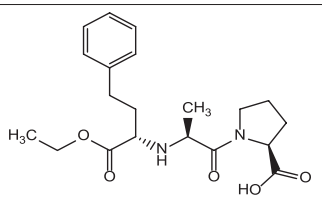
Molecular structure		Concentration in water (μg/L)	Letal concentration (μg/mL)	Identification methods	Reference
Ibuprofen *Log* K_{ow}=3.2		Effluent form hospital wastewater 1.5–151	ToxAlert (12.1) Microtox (19.1)	HPLC-ESI-MS/MS	(25, 28)
Acetylsalicylic acid *Log* K_{ow}=1.43		Surface water 0.34 Effluent form domestic and industrial 1.0	ToxAlert (43.1)	HPLC-ESI-MS/MS	(29)
Ketoprofen *Log* K_{ow}=2.99		Surface water and Sewage sludge pH 2= 28 pH 7= 53	ToxAlert (15.6) Microtox (19.3)	HPLC-ESI-MS/MS	(30-31)
Diclofenac *Log* K_{ow}=4.4		Surface water < 5.1	ToxAlert (13.5) Microtox (13.7)	HPLC –ESI -MS	(32-33)
Naproxen *Log* K_{ow}=2.8		Influent and effluent from WWTP 5.41 – 21.2	*Hydra attenuate* 0.092	HPLC-ESI-MS/MS	(34-35)
Acetaminophen *Log* K_{ow}=1.29		Surface water 3.35 – 15.7. Effluent from hospitals wastewater 186.5	*Daphnia magna* 20.1	HPLC – ESI MS/MS	(36-37)

ToxAlert® Microtox®. Ecotoxicity tests accepted by the EPA. Technique using the marine bacteria *Vibrio fischeri.*

Antihypertensive

Hypertension is the most common cardiovascular disease worldwide. In the U.S.A, 43 million patients have systolic blood pressure values of 140 mmHg or higher, and a diastolic pressure of 90 mmHg or greater (38). This has increased the prescription of antihypertensive drugs such as: Calcium channel blockers, inhibitors of the angiotensin converting enzyme (ACE) and beta blockers, which have been detected in water the recent years. Some antihypertensive Beta blockers such as atenolol, metoprolol and propranolol, have reached levels above 0.017 μg/L in effluents from municipal wastewater and could have adverse effects on aquatic organism (16). Other antihypertensives such as ACE inhibitors and verapamil have also been found in the environment (see table 2).

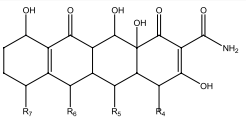
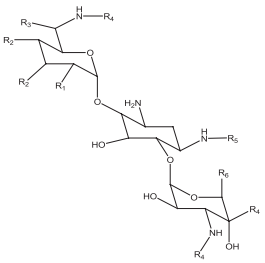
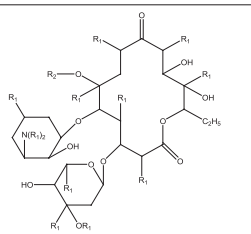
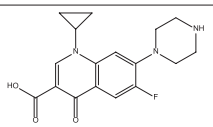
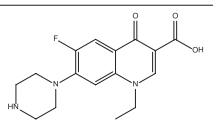
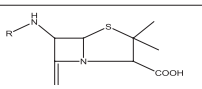
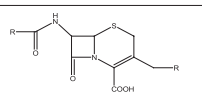
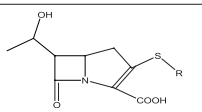
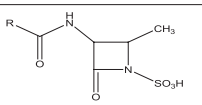
Table 2. Antihypertensive widely used in the pharmaceutical sector.

Molecular structure		Concentration in water (μg/L)	Letal concentration (μg/mL)	Identification methods	Reference
Propranolol *Log* K_{ow}=2.81		Effluent from WWTP 0.1 – 1.09	*Daphnia magna* EC_{50} : 1.6	HPLC–ESI-MS/MS	(39-40)
Metoprolol *Log* K_{ow}=1.8		Influent from WWTP 0.004-2.838	*Daphnia magna* EC_{50} : 0.002	HPLC–ESI-MS/MS	(41-42)
Atenolol *Log* K_{ow}=0.41		Effluent from WWTP 0.66 – 2.432	*Daphnia magna* EC_{50} : 0.002	HPLC–ESI-MS/MS	(42-43)
Verapamil *Log* K_{ow}=5.32		Influent from WWTP 0.51	*Daphnia magna* EC_{50} .11-0.67mM	HPLC–ESI-MS/MS	(44-45)
Enalapril *Log* K_{ow}= 1.52		Influent from WWTP 0.239	*Thamnocephalus-platyurus* 24h-LC_{50} 0.184	HPLC–ESI-MS/MS	(46-47)

Antibiotics

Antibiotics are widely prescribed drugs worldwide. Their success against pathogens in humans and animals and their use in food preservation has increased their demand. However, inappropriate use has facilitated the formation of resistant organisms and ineffective therapies. The resistance of microorganisms is mediated by the expression of genes that encode proteins responsible for the expulsion of antibiotics into the cell exterior (by efflux pumps) (48), synthesis of enzymes that degrade the molecule using inactivators (49) and the modification of their site of action or therapeutic target (50). It has been shown that the presence of antibiotic residues has increased these mechanisms of resistance in some pathogenic microorganisms present in different water bodies. Antibiotics such as tetracyclines (51), aminoglycosides (52), macrolides, beta-lactams and vancomycin (53, 54) have been found in the water (see table 3). Antibiotics sources are hospitals, residential or agricultural origins, from where the parent compounds and degradates are excreted after their ingestion or discarded directly into wastewater. Concentrations of antibiotics found in water have enabled the formation of resistant organisms such as *Aeromonas, Salmonella, Escherichia, Pseudomonas, Staphylococcus* among others (13). When these microorganisms infect humans either through direct contact or by vectors, they can increase mortality in their hosts due to the ineffectiveness of antibiotics used to combat infections. Certainly, the finding of resistant organisms in water bodies is a major issue for hospitals, industries, homes and water treatment plants, as legal precedents, that legislate and monitor the presence of antibiotics in wastewater and their proper disposal, need to be generated in order to avoid global public health problems.

Table 3. Antibiotics widely used in the pharmaceutical sector.

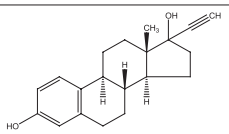
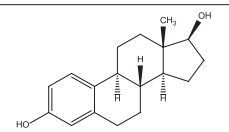
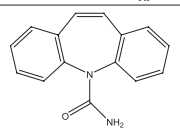
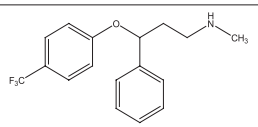
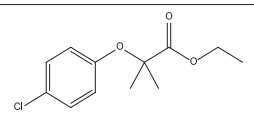
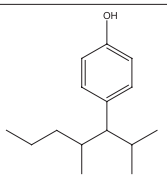
Molecular structure		Concentration in water μg/L	Resistant micro-organisms	Identification methods	Reference
Tetracyclines Chlortetrcycline: *Log* K_{ow}=-1.71		Effluent from WWTP Tetracyclines: 0.278	*Aeromona spp, Acineto-bacter spp.*	UPLC-ESI-MS/MS	(55-57)
Aminoglycosides Gentacine: *Log* K_{ow} =-4.55		Effluent from hospital wastewater Gentamicin 0.4-7.4	*Enterococcus spp.*	HPLC-ESI-MS/MS	(58-59)
Macrolides Clarithromycin: *Log* K_{ow}= 0.41		Influent from WWTP Clarithromycin: 0.267	*Campylobacter spp. Clostridium perfringens*	HPLC-ESI-MS/MS	(60-62)
QUINOLONES					
Ciprofloxacin: *Log* K_{ow}= 0.13		Effluent from WWTP. Norfloxacin: 0.210 Ciprofloxacin: 0.132	*Salmonella typhimurium*	HPLC-ESI-MS/MS	(63-65)
Norfloxacin: *Log* K_{ow}= -0.06					
BETA-LACTAM					
B-Lactam Penicillin G: *Log* K_{ow}= 1.33		Effluent and Influent from WWTP. Penicillin G. 153. Cefalexin. 0.67 – 2.9	*Enterococcus faecium*	HPLC-ESI-MS	(66-68)
Cephalosporins Cefalexin: *Log* K_{ow}= -0.19					
Monobactams Imipenem*Log* K_{ow}= -1.49					
Carbapenems Aztreonam: *Log* K_{ow}= -2.49					

Other potentially endocrine disruptor drugs

The endocrine system is part of more complex biological systems as it is responsible for the synthesis, degradation and release of hormones that regulate biological processes like metabolism and reproduction (69). In recent years, it has been found that some drugs present in water bodies can interrupt or disrupt some endocrine functions. Due to the wide variety of active ingredients and degradates that affect this system, they are addressed as endocrine disruptors (DE) and have caused changes in estrogen and androgen in some fish and amphibian species in aquatic environments (70, 71). Some of them influence the production of hormones such as the gland-stimulating hormone (TSH), the luteinizing hormone (LH) and the follicle stimulating hormone (FSH) in fish species. This has been seen in problems related to the metabolism

and reproduction of the aforementioned species (72, 73). Some drugs such as 17α-etilenestradiol (the oral contraceptive), modulate the production of hormones such as LH and FSH, which decrease the production of testosterone in male frogs and lead to feminization within the species (74, 75). Other drugs such as clofibrate, carbamazepine and fluoxetine also modify the activity of the endocrine system (see table 4). Furthermore, many drugs are not easily removed in WWTP and have been detected by HPLC-ESI-MS/MS in surface water and drinking water. Results show the possible chronic exposure of the human species to the adverse effects of DE (16-76).

Table 4. Endocrine disruptor drugs.

Molecular structure	Concentration in surface water (μg/L)	Toxic concentration (μg/mL)	Identification methods	Reference
 17α-ethinyl estradiol: *Log* K_{ow} = 3.63	Effluent from domestic wastewater 1.2	*Pimephales promelas.* 85 % of Feminization in male. 0.004	HPLC-ESI-MS/MS	(77-78)
 17β-estradiol: *Log*K_{ow} = 3.84	Effluent from sewage treatment plants 0.0032 – 0.055	*Oryziaus latipes* Feminization in male 0.015	HPLC-ESI-MS/MS	(79-81)
 Carbamazepine: *Log* K_{ow} = 3.67	Influent from WWTP 0.07 – 0.8 Surface water: 20	*Potamopyrgus antipodarum.* Change in number of embryos: 0.4-10	UPLC-ESI-MS/MS	(82-83)
 Fluoxetine: *Log* K_{ow} = 3.96	Effluent form WWTP 0.0037	*Daphnia magna* 0.036	HPLC-ESI-MS/MS	(21, 84)
 Clofibrate: *Log* K_{ow} = 3.02	Effluent sewage system 5.1. Lake: 24.7	*Ceriodaphnia dubia* 0.640	HPLC-API-MS/MS	(19, 85)
 Nonylphenol: *Log* K_{ow} = 5.31	Influent from WWTP 1.6-986	*R. esculenta.* Nonylphenol decreases LH plasma levels ($0.0022 \equiv 10^{-8}$ M)	GC–MS m/z =135	(69)

METABOLIC PRODUCTS AS CONTAMINANTS

In metabolic processes, organisms transform xenobiotics into eliminated by-products through hepatic microsomal systems. In this way, drugs are exposed to oxidoreduction and hydrolysis reactions in phase I of metabolism, and subsequently, can be conjugated with glucuronic acid, sulfate groups or amino acids in phase II. It can be noted that functional groups, such as esters and epoxies, are transformable in phase I, while in phase II conjugation reactions are brought about on the hydroxyl groups to increase solubility and guarantee xenobiotic excretion. The enzyme systems with greatest influence on metabolic processes are described as

follows: glycosyltransferases and sulfotransferases act on active ingredients for the phenolic functional groups. Regarding the carboxyl groups, glutathione S-transferases are for electrophilic drugs including halogens or nitro groups, acetyltransferases are for active ingredients like amines and hydrazines and aminoaciltransferases are for carboxylic groups and those with addition of free amino acids (86). Thus, knowledge of the metabolism of xenobiotics can guide the analyst in the search of metabolites and parent compounds in water. A clear example is clofibrate (oral lipid lowering), which is cleaved in phase I of metabolism and whose ionized form cannot be found in water. Equally important, the objective of metabolism is to produce polar products with greater ease of elimination (87). Problems in the identification of metabolites and parent compounds in water bodies occur due to the limited availability of standard reference material in the market, and identification techniques of biotransformation products. This clarifies the need for screening protocols that allow not only structural identification, organic synthesis and toxicological evaluation, but also the structuring of such knowledge within the investigative trends of ecopharmacology (88). The distribution of xenobiotics and transformation products in water depends on the metabolic processes and the biotic or abiotic factors acting on them. In agreement with this, it has been reported that the presence of ASA in water tributaries is accompanied by metabolites such as gentisic acid and hidroxipuric acid (89).

Physicochemical properties of drugs and their distribution in the environment

In contrast to other pollutants in water, drugs are molecules with high biological activity on different organisms. Additionally, their physicochemical properties may limit its persistence in the environment and facilitate their bioaccumulation. Often, the analytical study of drugs in water is performed according to drug groups, which not only assumes a homogeneous group of active ingredients but also identical chemical properties. This is not entirely correct due to differences in molecular weight, structure, crystalline form and polymorphism among other properties.

The molecular complexity of active ingredients lies in their stability, solubility, ionization and polarity that in turn depend largely on environmental properties. Physicochemical parameters of the active ingredients, such as the partition coefficient of octanol/ water (K_{ow}) and the dissociation constant, are values of great importance in environmental models that help to describe their chemical destination in aquatic ecosystems and guide the analyst, regarding their distribution in soil, biomass, sediment and the water column. They also provide information on the ideal matrix for the detection of degradates and parent compounds in the environment. Most drugs behave chemically as weak acids and bases, which means their distribution depends on the pH of the medium and the acidity constant (K_a), or the basicity constant (K_b), factors that determine their ionization in that medium. Generally, acidic active ingredients don't dissociate easily in acidic pH, because of their affinity for lipids, so passing through biological membranes, and the bioaccumulation in the biota increases (90). This indicates that the bile of some fish would be a good matrix for the analysis of the persistence of PPCPs in aquatic ecosystems and biomagnification problems (91). Furthermore, acidic active ingredients don't achieve an easy dissociation in slightly basic media, which increases their solubility in water and their distribution in the aqueous medium. Taking the aforementioned into account, it is clear that the physicochemical properties and structural variety of drugs determine their distribution in the environment, their bioaccumulation and biomagnification problems in the food chain. The study of these chemical properties along with increased sensitivity and limit of detection in spectroscopy, chromatography and separation methods from complex matrices, allow the monitoring of degradates and parent compounds in water bodies.

Toxic effects of active ingredients on the environment

The effects produced by drugs on aquatic biota have not been fully understood yet. Nevertheless, their chemical nature and their mechanisms of action on target organisms may be a way to approach this subject. Many of these drugs are designed to modulate the endocrine system and the immune system, indicating that their presence in water bodies may alter the homeostasis of aquatic organisms (92). Ecotoxicological models use micro-organisms such as fish species and crustaceans among other, to analyze the influence of active ingredients and degradate of the biota. However, they do not fully describe the impact of pollutants on complex and organized aquatic communities. This represents a

challenge for analysts, since the physiological effects of metabolites and parent compounds on target organisms needs to be known in order to correctly choose the species susceptible to the mechanism of action of the toxin and obtain reliable and reproducible results. Ecotoxicity assays approved by the U.S. environmental protection agency (EPA) are reliable assays for the analysis of the acute toxicity of xenobiotics in the environment. Nonetheless, the chronic effects of sub-traces of drugs on aquatic biota are still unknown. Initial toxicity test were implemented by Germany in the European Union guide 92/18 EWG for veterinary pharmaceuticals (93). Nevertheless, some evidence of acute toxicity, behavioral and genetic changes in fish species, amphibians, crustaceans and eukaryotic cells were proposed by the Scandinavian society of cell toxicology in order to extrapolate the results of ecotoxicity to biological systems present in the environment. Similarly, these methods describe potentiation effects as some studies have identified the effects of toxic drug synergy. It has been found that verapamil (an antihypertensive calcium antagonist), may increase the susceptibility of biota to other drugs (94). At present, it is known that aquatic organisms defend themselves using systems that provide resistance to multixenobióticos. These systems are composed of proteins that promote the removal of toxic substances of moderate lipophilicity towards the exterior of the cell. Among such systems stands the glycoprotein P (Pgp), which is one of the first lines of defense for aquatic organisms and whose function is to alter the membrane permeability and dispose of xenobiotics. Some studies have shown that verapamil is attached directly to the active site of Pgp, which increases the toxicity of other active substances in aquatic organisms (95). Drugs such as trifluoperazine, reserpine, quinidine, cyclosporin and progesterone also have a significant effect in inhibiting multixenobiotic resistance.

Ecotoxicological assay are very important forms of analysis in the description of the toxic effects of active ingredients on biota. Usually, the values are expressed as an effective concentration of 50 (EC50) and classify the different substances as either being very toxic to aquatic organisms (<1 mg/ L, evaluated in *Daphnia magna*), toxic (with values around 10 mg/L) or harmful (values ranging from 10-100 mg/L of the active ingredient) (96). Similarly, the multicenter evaluation of *In Vitro* cytotoxicity (MEIC) has become an important reference in toxicology studies due to the fact that the organization has a large catalog of the effects caused by drugs on aquatic organisms. Some of this work, reports the toxicity of 18 drugs on *Daphnia Magna*; drugs like amitriptyline, thioridazine, phenobarbital and ASA, have toxicities of 0.0037 mM, 0.0017 mM, 6 mm and 8.2 mM respectively. Similarly, this study found that the toxicity of some drugs evaluated in *Daphnia Magna*, was higher than the toxicity of some pesticides and other chemicals in common use. The concentration of toxic substances such as phenol, nicotine, cyanide potassium and lindane was 0.078 mM, 0.023 mM, 0.0086 mM and 0.005 mM, respectively (97). Other toxicological guides, such as that generated by the organization for economic cooperation and development (OECD) 202 Part II (*Daphnia magna* Reproduction) reported no observed effect concentration (NOEC) of 10 ug/L and 10 mg/L for clofibrate and ASA respectively. In addition, testing of the luminescent bacteria *Vibrio fischeri* NOEC showed a range of between 5-40 ug/L and 15-60 mg/L for both compounds, respectively (98). Some drugs that come into contact with the environment represent toxic risks that are difficult to identify due to the heterogeneous physiological effects to aquatic biota. Such is the case of selective serotonin reuptake inhibitors (SSRIs), which cause a variety of adverse effects in the target biological systems, and similarly, could disrupt the homeostasis of aquatic biota (99). Considering the above, these drugs are expected to induce subtle but catastrophic changes on aquatic organisms, including: enzyme inhibition, cellular repair damage, cytotoxicity, gradual degeneration, atrophy of organs and tissues, decreased growth, progeria, immune system damage, reproductive abnormalities, decreased environmental adaptation and survival, among others.

Furthermore, studies for ecological risk assessment are needed to identify the dynamics, persistence, transport and processing of drugs in the environment since little is known about their pharmacokinetics and pharmacodynamics. In a retrospective study, the toxicity of drugs such as ASA, Acetaminophen, clofibrate and methotrexate was evaluated and it was found that the parent compounds are not easily detected in ecotoxicity tests, meaning therefore that their impact on aquatic organisms is still unknown (100). The presence of sub-traces of emerging contaminants in drinking water is a big problem for the humans due

to PPCPs are not removed in WWTP. Although it has been found that these concentrations are in sub-therapeutic doses, their chronic exposure could cause catastrophic effects on human beings and different biological systems. Perhaps, the most vulnerable population would be newborn babies, pediatric patients and the elderly. Similarly, chronic exposure to metabolites and parent compounds in water, can lead to synergism or the development of toxic effects. Additionally, it is known about polymedicated patients that suffer a greater extent of unwanted or adverse effects from drugs, due to the interaction between xenobiotics and inhibition of metabolic processes. Moreover, the different toxicological tests still do not assess the risk posed by medication entering the body via drinking water and it should be considered that many active ingredients and by-products are associated with toxic effects such as carcinogenicity, mutagenicity and/or alterations in reproduction.

Qualitative and quantitative analysis of pharmaceutical contaminants in the water bodies

The wide variety of PPCPs, constitute a great analytical complex in the identification and quantification of this substances in different environmental matrices. Treatment and purification methodologies constitute the backbone of eco-pharmacological investigations for the legislation in environmental analysis (101). Analytical methods in ultra traces detection of contaminants in water include sampling process, extraction procedure, cleaning, concentration, and chromatographic detection. Any additional procedure that is included in sampling process prior to quantification becomes a strict control stage for reducing losses by processing and instrumental phases. Some authors consider that the process of sample treatment takes up 80% of the analysis, where the methodologies based on liquid-liquid extraction, solid phase extraction, selective solid phase extraction and biosensor systems are tools of greatest use in environmental analysis (102-103).

Technological developments in environmental monitoring have included passive sampling. These popular methodologies have enabled to analyze PPCPs, metabolites, pesticides and heavy metals at low concentration in water. Some devices such as semi permeable membrane devices (SPMDs) are membranes for sampler xenobiotics with $log\ K_{ow}$ between 4-8 (lipophilic organic compounds), as long as polar organic chemical integrative sampling (POCIS) are membranes for monitoring organic substances with $log\ K_{ow} < 4$ such as metabolites, some antibiotics and other pharmaceutical compounds (104).

Moreover, the developments of different chromatographic techniques related to the identification and quantification has improved the limit of detection and molecular recognition in environmental analysis of degradates and parent compounds. Although gas chromatography with variable detection systems plays an important role in the analysis of many compounds, it is considered that about 90% of total organic compounds can be determined using liquid chromatography –tandem mass spectrometry (LC-MS-MS) (105). Advances in liquid chromatography for PPCPs determination in water are: the use of monolithic columns which allow flows of up to 10 mL/min without significant increases in pressure; high-temperature liquid chromatography (HTLC) (106-109) in which the low viscosity and high diffusivity of the mobile phase at high temperature (> 60°C) decrease significantly the resistance to mass transfer and improve Van Deemter curves. Furthermore, ultrahigh-pressure liquid chromatography (UHPLC) has improved the analysis time and efficiency in environmental monitoring (110-112). UHPLC uses short columns with a smaller particle size of 2.0 microns (1.7 microns), which resist further pressure, has higher resolution peak, better chromatographic separation and reduce the analysis time to around 10 minutes or less. Additionally, the system usually incorporates a static split injection system, pressure regulating valve, lower dead volume of 35 μL, injection volumes of between 0.01 and 500 μL, injection times from 8 seconds, acquisition rates greater than 20 Hz and flows of up to 10 mL/min, among others (113-114).

Perhaps, the most important developments in UHPLC are the use of Fused-core® columns and hydrophilic interaction liquid chromatography (HILIC®). Fused-core columns allow increases in speed of analysis and improve the efficiency of reverse phase separation (115-116). They were initially marketed under the name of HALO and similar technology was developed by Sigma-Aldrich under the name of Ascentis and Phenomenex under the name of Kinetex® (117). For its part HILIC® is a special case of normal phase chromatography, in which the stationary phase is less polar than the mobile phase, facilitating the analysis of polar

molecules that elute with the dead volume in conventional HPLC systems (118). In general, HILIC® mechanism is based on a type of liquid-liquid partition chromatography (LLPC) (119-120).

The wide range of sample treatment techniques, the development of new stationary systems and the design of high-resolution instruments for determination of PPCPs in water, require detection system with high sensitivity. Some columns system associated to ultraviolet-UV, amperometry, fluorescence-FLD, triple quadrupole mass spectrometry-QQQ, the time-of- flight TOF-MS, the quadrupole time of flight-Q-TOF-MS and inductively coupled plasma-mass spectrometry (ICP-MS) enable ultra trace analysis (121-123). Furthermore there are continuous and automated devices known as biosensors for organic pollutant monitoring in water bodies, which allow fast analysis and real time determinations (124-125). Even though their commercialization is still incipient, the numbers of devices that have been developed continue to grow and their projection as a complement to chromatographic techniques is becoming of increasing relevance.

Environmental regulation

Currently, the problems of emerging contaminants represent a widespread challenge for different WWTP, since the active ingredients that are not correctly deposited in wastewater could enter the environment and dramatically affect aquatic organism including humans. Therefore, some authors propose the initial treatment of wastewater with new technologies as advanced oxidation techniques (AOT) for reducing or degrade PPCPs until innocuous products for the environment. For the reduction of PPCPs in the environment, the cooperation and supervision of regulatory and scientific institutions such as the U.S. EPA, FDA and the OECD are necessary (126). The FDA is a scientific agency in charge of legislation of pharmaceuticals entering the market for diagnosis, treatment and the alteration or prevention of disease in humans and animals. Sometimes, the approval and release of active ingredients to the market require the analysis of ecotoxicological effects on biota, where the degradates and parent compounds have contact with the environment, a responsibility in charge of the aforementioned organization. The incursion of new active ingredients in the market, and the discharge of new drugs into water is increasing. In 1998 alone, the FDA approved 30 new molecules (127). Another environmental problem is self-medication, which is a very common activity in the world's population and promotes the introduction of PPCPs and degradates on aquatic ecosystems.

In 1998, finasteride and sildenafil drugs used to treat erectile dysfunction and prostatic hyperplasia respectively were approved, leading to the incorporation of these molecules and degradates in the environment (128). Currently, knowledge about toxic effects of these molecules and their excipients on biota is limited, as well as the synergistic effects that they produce with other substances of anthropogenic origin. The presence of isomers of active ingredients is another challenge for environmental regulatory agencies. However, the FDA requires the development of purification methods that guarantee only the supply of isomers responsible for the desired therapeutic effect. This allows the reduction of the dosage and the presence of molecules with undesired effects, hence leading to the reduction of pollutants in the environment (129). Similarly, the FDA demands regular evaluations to monitor drugs and make sure that they do not exceed 1ppb of the expected introduction concentration (EIC). This value is calculated assuming that the active ingredient is produced over one year, enters the wastewater treatment plant, that this drug is used in proportion to the population and that it is not metabolized (130). However, this value only predicts the concentration of the parent compound in the environment and since most drugs are metabolized, their by-products may have a lesser or greater toxic effect on biota.

Moreover, there are guides with which the risk of some active ingredients on the environment can be assessed. For example, the 92/18/EEC directive proposed a two-stage study to analyze the presence of active substances in the environment. In phase I, predictive environmental concentration (PEC) is evaluated, while in phase II the destination and effects on the biota are predicted (131). Similarly, the guidelines of the european medicines agency (EMEA) were developed in order to observe the impact of veterinary drugs in the environment. These guidelines include algal growth inhibition, studies of bioaccumulation in fish species, toxicity in earthworms, plant growth and respiration inhibition in muds. Currently, the study of degradates and parent compounds in water bodies in Colombia is insufficient, and the amount of pharmaceutical

waste that is dumped into the environment along with the adverse effects on aquatic ecosystems and human health is still unknown. Decrees such as 1575 (2007) and 1594 (1984) for Drinking water and water use respectively, only include the analysis of organic compounds like etanochlorades, chlorobenzene, hexachlorobenzene, halomethanes, haloethers, nitrophenols and some pesticides. It is also necessary to consider regulations for the analysis of PPCPs and by-products in water sources in Colombia (132). Finally, it is of great importance for public health to prevent, remediate, and ensure the absence of PPCPs in water, while bearing in mind that pharmaceutical products are indeed of great need for human beings. Therefore, it is necessary to implement technological solutions that prevent the entry of active ingredients into water and avoid toxic effects on aquatic organism and humans. It is also necessary to the countries that make up the "global village" be aware of this highly significant problem, and formulate policies that will help to the preservation of natural resources, in order to care for the most vital element on our planet: WATER.

CONCLUSIONS

Emerging contaminants have become a serious cause of environmental pollution in the world. Among these are active ingredients of various groups of PPCPs, with some metabolites and parent compounds being found in different water bodies on Earth. Ecotoxicity testing *in vitro* and *in vivo*, have demonstrated the toxic effect of these molecules on the food chain. Furthermore, the identification and quantification of these active ingredients, is a significant step towards making decisions regarding the preservation of water sources. This has been possible thanks to the development of different sample processing techniques and the development of mass filters coupled to gas chromatography, liquid chromatography and complex on line systems that allow detection levels in the order of ppb or even ppt to be reached. Given this situation, it is necessary that the entities responsible for environmental monitoring and care, along with those for the preservation of public health, intervene in the handling and disposal processes of emerging contaminants. Finally, Colombia and other countries need to ensure the quality of water, as we have cited, and since it is possible to find different substances in effluents and influent in treatment plants, the countries should consider applying new technologies for reducing metabolites and parent compounds in water bodies. For example, AOTs and activated sludge techniques have been implemented to reduce pollutants. It is necessary for Colombia to venture into the field of mineralization processes of pollutants in environmental matrices, as it would represent a useful solution for decreasing toxic levels of anthropogenic pollutants in water and would optimize the correct use of this natural resource, which is becoming progressively scarcer on planet earth.

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