**PHARMACEUTICAL PRODUCTS IN THE ENVIRONMENT: SOURCES, EFFECTS AND RISKS**

**PRODUCTOS FARMACÉUTICOS EN EL AMBIENTE: FUENTES, EFECTOS Y RIESGOS**

**ABSTRACT**

Pharmaceuticals and personal care products (PPCPs) have became an environmental problem in recent years. Their physicochemical properties and persistence in the environment allowed the distribution of many metabolites and parent compounds. Their wide hospital, domestic, agricultural and industrial use increased the dumping of related waste into water bodies and toxicity began to be manifested in different biological components of ecosystems. The development of methods for sample treatment and instrumental analysis techniques enabled the separation, identification and quantification at concentrations of ppb or even ppt of active ingredients and degradation products with higher environmental impact. In addition, *in vitro* and *in vivo* tests demonstrated their ecotoxicity in water, allowing them to be classified as emerging organic pollutants whose waste is indeterminate. Although their impact on ecosystems may be silent there are strong implications for global public health.

This review presented the dynamics and development of research over the past ten (10) years of the presence of non-steroidal anti-inflammatory analgesics, antihypertensives, antibiotics and other drugs in water bodies. Similarly, it described the impact of pharmaceutical effluents on water quality. Some databases such as: Science Direct, Pubmed, American Chemical Society (ACS), Informaworld, SpringerLink and SciFinder were consulted.

***Keywords:*** Toxicity Tests, Environmental Pollutants, Organic Pollutants, Analgesics**,**Pharmaceuticals and Personal Care Products.

**RESUMEN**

Los productos farmacéuticos y productos para el cuidado personal (PPCPs), han representado un problema ambiental en los últimos años. Sus propiedades fisicoquímicas y su persistencia en el ambiente permitieron la distribución de muchos metabolitos y compuestos. Su uso hospitalario, doméstico, agrícola e industrial aumentó las descargas en los cuerpos de agua y su impacto ambiental y toxicidad comenzó a manifestarse en los diferentes componentes biológicos de los ecosistemas. El desarrollo de metodologías de tratamiento de muestra y técnicas instrumentales de análisis permitieron la separación, identificación y cuantificación a concentraciones de ppb e incluso de ppt de principios activos y productos de degradación con mayor impacto ambiental; adicionalmente, los ensayos *in vitro* e *in vivo* demostraron su ecotoxicidad acuática, permitiendo clasificarlos como contaminantes orgánicos emergentes, cuyos vertimientos son indeterminados y su impacto sobre los ecosistemas silencioso pero de grandes repercusiones para la salud pública mundial.

Esta revisión presentó la dinámica y el desarrollo de investigaciones durante los últimos diez (10) 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, servicios sobre la calidad del agua. Algunas bases de datos como: Science Direct, Pubmed, American Chemical Society (ACS), Informaworld, SpringerLink y SciFinder, fueron consultadas.

***Palabras clave:*** Pruebas de Toxicidad, Contaminantes Ambientales, Contaminantes Orgánicos, Analgésicos,Productos farmacéuticos y para el cuidado personal

**INTRODUCTION**

The active ingredients of pharmaceuticals, phytotherapeutic and disinfection products, are a wide range of compounds with diverse physicochemical properties (dissociation constant, partition coefficient octanol-water (*Dow*), distribution coefficient-biosolids-water (*Kp*) and ionization), broad stability requirements and, in most cases, non-specific biological activity (1). Most therapeutic active ingredients are obtained by organic synthesis and have molecular weights of between 200 and 1000 Dalton, have ionic character and their absorption and biotransformation processes do not fully take place. Among the pharmacologically active substances, it is important to highlight that many of them are efficiently processed by hepatic microsomal systems of animal cells and those with natural origin are effectively biodegraded in the environment. The biotransformation of biopharmaceutical products obtained by biotechnological methods in most cases is unknown. In addition, first and phytotherapeutic pharmaceutical formulations are complex and incorporate a variety of aids which increase the availability of molecules. Secondly, the transformation products or metabolites of these active ingredients have not yet been studied in depth. However, in some previous studies greater ecotoxicological potential, recalcitrant properties and potential for inclusion in the food chain~~s~~ have been reported.

Analytical development and environmental mitigation after World War II focused on conventional organic pollutants such as agrochemicals, pesticides, Polychlorinated Biphenyls (PCBs), Phthalates, Dioxins (PCDDs), Furanones (PCDFs), Polyaromatic Hydrocarbons (PAHs) and Flame Retardants among others, but in the 90s wastewater treatment systems began to show considerable concentrations of PPCPs. This led to the conclusion that wastewater treatment plants were not able to remove some active ingredients which instead ended up being incorporated into the water cycle, leading to biological systems with unknown and widespread epidemiological implications (2). The first study of drugs in wastewater was reported in 1976 in a wastewater treatment plant in Kansas, The United States, where studies focused on the adverse effects on fauna and flora, allowing the U.S. Food and Drug Administration (FDA) and the European Union (EU) to intervene in wastewater treatment processes (3). Subsequently, studies began to go into more depth regarding active ingredients, metabolites, toxicity, removal processes, bioremediation and analytical methodologies for identification and quantification (4-7). There are many mechanisms that allow pharmaceuticals to enter the environment, such as animal and human excretion, pharmaceutical industry waste, waste generated by the health service, inadequate disposal of out of date or non-consumed products, waste dumping from research institutions and drug development.

Pharmaceutical products are part of the so-called emerging organic contaminants, which enter wastewater indefinitely and although their impact on ecosystems is silent they cause widespread effects to biota and global public health. Furthermore, they are the subject of rigorous investigation in the areas of identification and quantification and constitute a new component to environmental legislation. Therefore, this paper aims to examine the research development regarding the presence of pharmaceuticals in different water bodies from the last ten years in a way that is easy to assimilate, in order to generate social responsibility in the institutions of environmental control in Colombia.

**PHARMACEUTICAL PRODUCTS IN THE WATER CYCLE**

The discharge of active pharmaceutical ingredients in the environment has been found to have catastrophic effects on the biota of aquatic ecosystems (8). Initially, drugs from agricultural and domestic activities were not considered as environmental pollutants because the accumulation processes of metabolites and parent compounds remained unknown. This resulted in an increase in the concentrations of many drugs and their metabolites in water bodies. Additionally, these substances have a low biodegradation and high resistance to environmental transformation processes. Therefore, instrumental analysis techniques and separation, quantification and identification methodologies have been developed since the 90’s in order to detect some of the active ingredients or their metabolites in water and in the environmental in concentrations of parts per billion (ppb) and parts per trillion (ppt) (9).

Moreover, it has been found that active ingredients from hospital, residential, agricultural and industrial uses have been swept into aquatic affluents. 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, which release highly water-soluble metabolites that not only represent great analytical challenges, but also challenges in correct experimental design.

Recently 500 tonnes 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 active ingredients enter aquatic ecosystems as well as the quantity present. In addition, the entry of metabolites and even unchanged active ingredients contribute to the problem due to their low absorption in biological systems (11). In countries like Germany, hundreds of tons of high demand active ingredients are let loose into the environment (12).

Currently hospitals are incorporating large quantities of antibiotics into the wastewater system which have led to 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 presents enormous challenges in the process of decontamination since when complete reduction is not achieved, metabolites and parent compounds are able to enter water bodies and on occasions drinking water. Little is known about the chronic effects that drugs can cause to the environment, but some ecotoxicological tests demonstrate to what extent they may affect biological systems.

A study by Oaks and colleagues showed that the death of between 34-95% 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 wastewater treatment plants, 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 70ng/L have been reported in water (17). Although this concentration is not toxic to humans, the problems associated with chronic exposure to this active ingredient and its metabolites are not fully understood. However, ecotoxicological evaluation in *Ceriodaphnia dubia* did show a toxic concentration of 0.640mg/mL. Ifosfamide toxicity tests have allowed the teratogenic and mutagenic potential in fish species to be elucidated (18) while others such as carbamazepine, fluoxetine and gemfibrozil have demonstrated effective concentrations of 50 EC50 of less than 81μg/mL, 24μg/mL 1.18μg/mL respectively in microtoxicity tests. 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 throughout the world. In Spain they represent the largest income for the pharmaceutical industry (22) and are the drugs most associated with self-medication (23), turning them into a public health problem. In recent years, high sensitivity instrumental analysis techniques have enabled toxic concentrations of diclofenac and ASA in wastewater to be identified and quantified (24), are shown in 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 waste water (25). Farré M *et al*, reported concentrations in surface waters of analgesics at different pH and toxic concentrations, assessed with two (2) models in vivo (26). The analysis of surface water indicates the presence of painkillers such as ASA, naproxen, ibuprofen, diclofenac and ketotifen. Furthermore, metabolites of ibuprofen like hydroxy-ibuprofen, carboxy-ibuprofen, and carboxihydrotropic acid, which are more toxic than their parent compounds, have been detected (27). This indicates that the toxicity of some drugs in the environment may be related to metabolic processes, and indicates that the pharmaceutical industry must implement management techniques and control of waste in its design and production stages to reduce the discharge of drugs in wastewater and minimize damage to aquatic ecosystems.

**Table1.** Analgesics widely used analgesics in the pharmaceutical sector.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Molecular structure** | **Concentration in water (µg/L)** | **Letal concentration ( µg/mL)** | **Identification  methods** | **Reference** |
| Ibuprofen | Wastewater  1.5–151 | ToxAlert (12.1) Microtox (19.1) | HPLC-ESI-MS/MS | (25-28) |
| Acetylsalicylic acid | Surface water 0.34  Wastewater 1.0 | ToxAlert (43.1) | HPLC-ESI-MS/MS | (29) |
| Ketoprofen | Surface water and wastewater pH 2= 28 pH 7= 53 | ToxAlert (15.6) Microtox (19.3) | HPLC-ESI-MS/MS | (30,31) |
| Diclofenac | Surface water  < 5.1 | ToxAlert (13.5) Microtox (13.7) | HPLC –ESI -MS | (32,33) |
| Naproxen | Wastewater 5.41 – 21.2 | *Hydra attenuate* 0.092 | HPLC-ESI-MS/MS | (34,35) |
| Acetaminophen | Surface water 3.35 – 15.7 Hospitals Effluent 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.*

**Antihypertensives**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 mm Hg or greater (38). This has entailed an increase in 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 recent years in water. Some antihypertensive Beta blockers such as atenolol, metoprolol and propranolol, have reached levels above 0.017μg/L in municipal water effluents (16) and have been shown to have toxic effects on aquatic biota. Other antihypertensives such as ACE inhibitors and verapamil have also been found in the environment are shown in Table 2.

**Table 2.** Antihypertensive agents are widely in the pharmaceutical sector

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Molecular structure** | **Concentration in water (µg/L)** | **Letal concentration  ( µg/mL)** | **identification methods** | **Reference** |
| Propranolol | Wastewater in the effluent of treatment plants 0.1 – 1.09 | *Daphnia magna*  EC50 : 1.6 | HPLC–ESI-MS/MS | (39,40) |
| Metoprolol | Wastewater in the affluent of treatment plants  0.004-2.838 | *Daphnia magna*  EC50 : 0.002 | HPLC–ESI-MS/MS | (41,42) |
| Atenolol | Wastewater in the effluent of treatment plants  0.66 – 2.432 | *Daphnia magna*  EC50 : 0.002 | HPLC–ESI-MS/MS | (42,43) |
| Verapamil | Wastewater in the affluent of treatment plants  0.51 | *Daphnia magna*  EC500.11-0.67mM | HPLC–ESI-MS/MS | (44,45) |
| Enalapril | Wastewater in the affluent of treatment plants  0.239 | *Thamnocephalus platyurus* 24h-LC500.184 | 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 therapeutics. 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. Among the most studied antibiotics are tetracyclines (51), aminoglycosides (52), macrolides, beta-lactams and vancomycin (53,54) are shown in Table 3. Antibiotics that enter water bodies may be from hospitals or have residential or agricultural origins, from where the metabolites or parent compounds are excreted after their ingestion, although sometimes they are discarded directly into wastewater. Concentrations of antibiotics found in water have enabled the formation of resistant organisms such as *Aeromonas, Salmonella, Escherichia, Pseudomonas, Staphylococcus* and 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 therapeutics.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Molecular structure** | **Concentration in water µg/L** | **Resistant microorganisms** | **Identification methods** | **Reference** |
| Tetracyclines | Wastewater in the effluent of treatment plants Tetracyclines: 0.278 | *Aeromona spp,*  *Acinetobacter spp.* | UPLC-ESI-MS/MS | (55-57) |
| Aminoglycosides | Hospital wastewater  Gentamicin  0.4-7.4 | *Enterococcus spp.* | HPLC-ESI-MS/MS | (58,59) |
| Macrolides | Wastewater in treatment plants  Clarithromycin: 0.267 | *Campylobacter spp.*  *Clostridium perfringens* | HPLC-ESI-MS/MS | (60-62) |
| QUINOLONES   Ciprofloxacin  Norfloxacin | Wastewater in the effluent of treatment plants  Norfloxacin: 0.210 Ciprofloxacin: 0.132 | *Salmonella typhimurium* | HPLC-ESI-MS/MS | (63-65) |
| BETA-LACTAM  B-Lactam  Cephalosporins    Carbapenems Monobactams | Wastewater in treatment plants  Penicillin G 153  Cefalexin 0.67 – 2.9 | *Enterococcus faecium* | HPLC-ESI-MS | (66-68) |

**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 can interrupt or disrupt some endocrine functions. Due to the wide variety of active ingredients 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, as shown below, are shown in Table 4. Furthermore, many drugs are not easily removed in water treatment plants and have been detected by High-Performance Liquid Chromatography-Electrospray Ionization-tandem Mass Spectrometry (HPLC-ESI-MS/MS) in surface water and water for human consumption. Results show the possible chronic exposure of the human species to the toxic 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 | Wastewater in treatment plants 1.2 | *Pimephales promelas.* 85 % of Feminization in male.  0.004 | HPLC-ESI-MS/MS | (77, 78) |
| 17β-estradiol | Wastewater in treatment plants 0.0032 – 0.055 | *Oryziaus latipes* Feminization in male 0.015 | HPLC-ESI-MS/MS | (79-81) |
| Carbamazepine | Wastewater in the affluent of treatment plant 0.07 – 0.8 Surface water: 20 | *Potamopyrgus antipodarum.* Change in number of embryos: 0.4-10 | UPLC-ESI-MS/MS | (82,83) |
| Fluoxetine | Water treatment plant, in effluent 0.0037 | *Daphnia magna*  0.036 | HPLC-ESI-MS/MS | (21- 84) |
| Clofibrato | Wastewater 5.1 Lake: 24.7 | *Ceriodaphnia dubia*  0.640 | HPLC-API-MS/MS | (19-85) |

**METABOLIC PRODUCTS AS CONTAMINANTS**

In metabolic processes organisms transform xenobiotics into elimination 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 those that 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 aminoaciltransferasas are for carboxylic groups and those with additions 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. Apart from this, 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 in the market of standard reference material 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 and 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 their persistence in the environment and facilitate their bioaccumulation. Often, the analytical study of drugs in water is done according to drug groups, which not only assumes a homogeneous group of active ingredients but also identical chemical properties. This is not entirely correct because differences exist in molecular weight, structure, crystalline form and polymorphism among other properties.

The molecular complexity of active ingredients means their stability, solubility, ionization and polarity depend largely on environmental properties. Physicochemical parameters of the active ingredients such as the partition coefficient of octanol/ water (*Dow*) and the dissociation constant are values of great importance in environmental models that help describe their chemical and dynamic balance 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 metabolites 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 (*Ka*) or the basicity constant (*Kb*), factors that determine their ionization in that medium. Generally, acidic active ingredients do not dissociate easily in acidic pH, because their affinity for lipids, passage through biological membranes and 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 pharmaceuticals in aquatic ecosystems and the problems of biomagnification (91). Furthermore, acidic active ingredients do not 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 detection limits of analytical techniques such as spectroscopy, chromatography and the extraction of analytes from complex matrices, allow trace amounts of metabolites and parent compounds in water bodies, sediments and various aquatic organisms to be isolated, quantified and identified.

**TOXIC EFFECTS OF ACTIVE INGREDIENTS ON THE ENVIRONMENT**

The effects produced by drugs on aquatic biota are not yet fully understood, however, 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 and species of fish and crustaceans among other animals, to analyze the influence of active ingredients on 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 tests approved by the U.S. Environmental Protection Agency (EPA) are reliable tests for the analysis of the acute toxicity of xenobiotics in the environment. However, the chronic effects of sub-traces of drugs on aquatic biota are not fully known. Initial tests of toxicity were implemented by Germany in the European Union guide 92/18 EWG for veterinary pharmaceuticals (93). Nevertheless, some evidence of acute toxicity, behavioral changes 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. 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 is 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 (94-95). Drugs such as trifluoperazine, reserpine, quinidine, cyclosporin and progesterone also have a significant effect in inhibiting multixenobiotic resistance.

Ecotoxicity tests 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 water organisms (<1 mg / L, evaluated in *Daphnia magna*), toxic (with values around 10mg/L) or harmful (values ranging from 10-100mg/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 in 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 0.0037mM, 0.0017mM, 6mm and 8.2mM 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 0.078mM, 0.023mM, 0.0086mM and 0.005mM, 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 10ug/L and 10mg/L for clofibrate and ASA respectively. In addition, testing of the luminescent bacteria *Vibrio fischeri* NOEC showed a range of between 5-40ug/L and 15-60mg/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, can 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 and atrophy of organs and tissues, decreased growth, progeria, immune system damage, reproductive abnormalities and 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 not fully known (100). A big problem for humans is the presence of sub-traces of emerging contaminants in drinking water which are not removed in treatment plants. Although it has been found that these concentrations are in sub-therapeutic doses, their chronic exposure can 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 that polymedicated patients 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 must be considered that 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 CYCLE**

The wide variety of compounds and their chemical heterogeneity constitute an analytical and instrumental challenge of great complexity in the processes of identification and quantification of emerging contaminants in different environmental matrices. Treatment and purification techniques at high resolution constitute the backbone of eco-pharmacological investigations for diagnosis, monitoring and legislation (101). The analytical basis of a method for the determination of ultra traces of contaminants in different matrices is organized in the sampling process, followed by the extraction procedure, cleaning, concentration, chromatographic analysis and detection. Later stages such as identification, confirmation and quantification are severely impacted by the previous procedures. Any additional procedure that is included in stages 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 liquid-liquid extraction (LLE) and SPE have become tools of greatest use (102). Faced with the outlook described above, in recent decades conventional techniques have been fully automated and because of this new and more efficient procedures have developed. New developments are focused within the following categories:

**Based on liquid-liquid extraction**

* Liquid-liquid extraction with Extrelut ®.
* Pressurized liquid extraction (PLE).
* Protein Precipitation (PP).
* Salting-out assisted liquid-liquid extraction (SALLE).
* Liquid-Liquid Extraction (LLE).
* Liquid-Liquid Micro-Extraction (LLME).
* Liquid-Liquid-Liquid Microextraction (LLLME).
* Dispersive Liquid-Liquid Microextraction (DLLM).

**Based on solid phase extraction**

* Solid Phase Micro-Extraction (SPME).
* Turbulent Flow Chromatography (TFC).
* Solid Phase Extraction (SPE).
* Micro-Extraction in Packed Syringe (MEPS).
* Stir Bar Sorptive Extraction (SBSE).
* Monolithic extraction
* Restricted-Access Sorbent (RAM).

**Based on selective Solid Phase Extraction and incorporated into biosensor systems**

* Molecular Imprinted Polymers (MIPs) (103) .
* Aptamers (103).

Among the technological developments achieved in environmental sampling are passive sampling systems such as Semipermeable Membrane Devices (SPMDs) and integrated samplers for Polar Organic Chemical Integrative Sampling (POCIS) used in the monitoring of potentially toxic and imperceptible hydrophilic contaminants by dilution processes in water bodies (104). Undoubtedly, the impact and effectiveness in the process of diagnosis and monitoring of contaminants in different environmental matrices and in particular water, are related to developments of different chromatographic techniques, which contribute to the processes of identification, confirmation and quantification, with detection limits in ppt and even parts per quadrillion (ppq) for some metabolites and parent compounds. Although gas chromatography with variable detection systems plays an important role in the analysis of many compounds (105), it is considered that about 90% of total organic compounds can be determined using liquid chromatography tandem mass spectrometry (LC-MS-MS). Among the advances and trends in liquid chromatography for the determination of pharmaceuticals and personal care products in environmental matrices are: the use of monolithic columns which allow flows of up to 10mL/ 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 notably improve Van Deemter curves, and the development of ultrahigh-pressure liquid chromatography (UHPLC) (110-112). This technique is characterized by allowing the use of short columns with a smaller particle size of 2.0 microns (1.7 microns), which resist further pressure and, ultimately, produce a higher resolution peak and better chromatographic separation, allowing the analysis time to be dramatically shortened to around 10 minutes or less. Additionally, the system usually incorporates a static split injection system, pressure regulating valve, lower dead volume of 35μL, injection volumes of between 0.01 and 500μL, injection times from 8 seconds, acquisition rates greater than 20Hz and flows of up to 10mL/min, among others (113-114).

In the last six years, about 11 references for the UHPLC system by 10 manufacturers have been introduced to the market, while a wide range of columns and stationary phases with applicability to these systems have been developed. The technological parameters overcome in these stages include resistance to broad ranges of pH, mechanical stability and greater selectivity by chemical modifications. Perhaps, the most important developments consist of the 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 terms, how the HILIC ® mechanism works 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 ultra trace determination of emerging organic pollutants in water, require detection systems of high sensitivity and rapid data acquisition to ensure high process efficiency and the correct interpretation of results. Despite their low sensitivity, a good choice of detection systems is represented by on-column systems associated to ultraviolet-UV detection, 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) (121-123). Despite the strengths shown by chromatographic techniques, continuous and automated devices known as biosensors have emerged for monitoring organic pollutants and especially drugs in water bodies (124-125). These devices allow fast, portable and real time determinations. 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 treatment plants, since the active ingredients that are not correctly deposited in waste water can enter the environment and dramatically affect biological systems including humans. Thus, some authors propose the initial treatment of wastewater with new technologies to reduce or degrade these products until they are converted into compounds that are almost innocuous to the environment and direct contact with active ingredients is prevented. For the reduction of pharmaceutical products in the environment, the cooperation and supervision of regulatory and scientific institutions such as the U.SEPA, FDA and the OECD are needed (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 metabolites and parent compounds have contact with the environment, a responsibility in the charge of the aforementioned organization. With the incursion of new active ingredients in the market, 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 metabolites and parent compounds in aquatic ecosystems. This practice, favored by media advertising, induces irrational drug consumption.

In 1998, finisteride and sildenafil were approved, drugs used to treat erectile dysfunction and prostatic hyperplasia respectively, which have led to the incorporation of these molecules and their metabolites in the environment (128). Currently, knowledge of the 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 and therefore leads 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 that 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. On the other hand, there exist 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 Concentrations (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 metabolites and parent compounds in water bodies in Colombia is insufficient and the amount of pharmaceutical waste that is dumped into the environment without the effects on aquatic ecosystems and human health being known is increasing. Decrees such as those of 1575 (2007) and 1594 (1984) for Drinking water and water use respectively, only include the analysis of organic compounds like etanoclorados, chlorobenzene, hexachlorobenzene, halomethanes, haloethers, nitrophenols and some pesticides. It is also necessary to consider regulations for the analysis of active ingredients and by-products in water sources in Colombia (132). Finally, it is of great importance to public health to prevent, remediate, and ensure the absence of pharmaceuticals and personal care products in water, while bearing in mind that pharmaceutical products are indeed of great need for human beings. Thus, it is necessary to implement technological solutions that prevent the entry of active ingredients into water and avoid toxic effects on aquatic ecosystems and humans. It is also necessary that countries that make up the “global village” are aware of this highly significant problem and formulate policies that will help with 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 drugs and personal care products, 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 ppq to be reached. Given this situation it is necessary that the entities responsible for environmental monitoring and care and those for the preservation of public health intervene in the handling and disposal processes of emerging contaminants. Finally, throughout the world advanced oxidation techniques (AOTs) and activated sludge techniques, among others, 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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