**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 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 PPCPs in hospital, domestic, agricultural and industrial has increased their discharge into the water bodies and toxicity has begun to be manifested in different biological components of ecosystems. The development of methods for sample treatment and instrumental analysis techniques has enabled the separation, identification and quantification at concentrations of parts per billion (ppb) or even parts per trillion (ppt) of active ingredients and degradates with higher environmental impact. In addition, *in vitro* and *in vivo* assay have demonstrated their ecotoxicity in water, allowing them to be classified as 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 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 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 (PPCPs), 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 (ppb) e incluso de partes por trillón (ppt) 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 clasificarlos estas sustancias 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, y 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 y analgésicos.

**INTRODUCTION**

The 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 (*Kow*), distribution coefficient-biosolids-water (*Kp*) and solubility, that describe the environmental dynamic and in most cases, non-specific biological activity (1).

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

Two decades ago, the environmental analysis focused on detection of pollutant 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) begun to show concentration of PPCPs. This led to the conclusion that PPCPs enter to water bodies and that WWTP aren’t able to remove some active ingredients which lead 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, then 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 for identification and methodologies quantification of active ingredients and degradatess, toxicity assay, removal processes and bioremediation (4-7). The principal sources of PPCPs are animal and human excretion, wastewater of pharmaceutical industry, effluent form hospitals, inadequate disposal of expired drugs, and waste dumping from research institutions and drug development.

PPCPs are part of the so-called emerging organic contaminants, which enter wastewater indefinitely and although their impact on ecosystems still 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 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 BODIES**

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 weren’t considered as environmental pollutants, additionally, some substances aren’t biodegradable and they have high resistance to environmental transformation processes. Due to possible accumulation processes of degradates and parent compounds have increased their concentrations in water bodies. Therefore, instrumental analysis, separation, quantification and identification methodologies have been developed for detecting some active ingredients or metabolites to low level in water bodies (ppb-ppt) (9).

Moreover, it has been found that active ingredients from hospital, residential, agricultural and industrial uses have been swept 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 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 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 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, 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 34-95% 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 70ng/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 0.640mg/mL. Ifosfamide toxicity tests have determined the teratogenic and mutagenic potential in fish species, while others drugs such as carbamazepine, fluoxetine and gemfibrozil have demonstrated effective concentrations of EC50 of less than 81μg/mL, 24μg/mL 1.18μg/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 in the world. In Spain their represent the largest income for the pharmaceutical industry and are the drugs most associated with self-medication, turning them into a public health problema (22-23). In recent years, high sensitivity instrumental analysis have detedted 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*, reported concentrations in surface waters of analgesics at different pH and toxic concentrations, assessed with two models *in vivo* (26). 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 wáter bodies and minimize damage on aquatic ecosystems.

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Molecular structure** | **Concentration in water (µg/L)** | **Letal concentration ( µg/mL)** | **Identification  methods** | **Reference** |
| Ibuprofen  *Log Kow =* 3.2 | Effluent form hospital wastewater  1.5–151 | ToxAlert (12.1) Microtox (19.1) | HPLC*-*ESI-MS/MS | (25, 28) |
| Acetylsalicylic acid  *Log Kow =* 1.43 | Surface water 0.34  Effluent form domestic and industrial 1.0 | ToxAlert (43.1) | HPLC*-*ESI-MS/MS | (29) |
| Ketoprofen  *Log Kow =* 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 Kow =* 4.4 | Surface water  < 5.1 | ToxAlert (13.5) Microtox (13.7) | HPLC –ESI -MS | (32-33) |
| *Log Kow =* 2.8  Naproxen | Influent and effluent from WWTP  5.41 – 21.2 | *Hydra attenuate* 0.092 | HPLC-ESI-MS/MS | (34-35) |
| Acetaminophen  *Log Kow =* 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.*

**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 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 recent years in water. Some antihypertensive Beta blockers such as atenolol, metoprolol and propranolol, have reached levels above 0.017μg/L in effluents form 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 Kow =* 2.81 | Effluent from WWTP 0.1 – 1.09 | *Daphnia magna*  EC50 : 1.6 | HPLC–ESI-MS/MS | (39-40) |
| Metoprolol  *Log Kow =* 1.8 | Influent from WWTP 0.004-2.838 | *Daphnia magna*  EC50 : 0.002 | HPLC–ESI-MS/MS | (41-42) |
| Atenolol  *Log Kow =* 0.41 | Effluent from WWTP 0.66 – 2.432 | *Daphnia magna*  EC50 : 0.002 | HPLC–ESI-MS/MS | (42-43) |
| Verapamil  *Log Kow =* 5.32 | Influent from WWTP  0.51 | *Daphnia magna*  EC500.11-0.67mM | HPLC–ESI-MS/MS | (44-45) |
| Enalapril  *Log Kow =* 1.52 | Influent from WWTP 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. Antibiotics such as tetracyclines (51), aminoglycosides (52), macrolides, beta-lactams and vancomycin (53, 54) has 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 microorganisms** | **Identification methods** | **Reference** |
| Tetracyclines  Chlortetrcyclinea: *Log Kow =* -1.71 | Effluent from WWTP Tetracyclines: 0.278 | *Aeromona spp,*  *Acinetobacter spp.* | UPLC-ESI-MS/MS | (55-57) |
| Aminoglycosides  Gentacin*: Log Kow =* -4.55 | Effluent from hospital wastewater  Gentamicin  0.4-7.4 | *Enterococcus spp.* | HPLC*-*ESI-MS/MS | (58-59) |
| Macrolides  Clarithromycin: *Log Kow =* 0.41 | Influent from WWTP  Clarithromycin: 0.267 | *Campylobacter spp.*  *Clostridium perfringens* | HPLC-ESI-MS/MS | (60-62) |
| QUINOLONES   Ciprofloxacin: *Log Kow* = 0.13  Norfloxacin: *Log Kow* = -0.06 | Effluent from WWTP  Norfloxacin: 0.210 Ciprofloxacin: 0.132 | *Salmonella typhimurium* | HPLC-ESI-MS/MS | (63-65) |
| BETA-LACTAM  B-Lactam  Penicillin G: *Log Kow=* 1.33    Cephalosporins  Cefalexin: *Log Kow =* -0.19  Monobactams  Imipenem *Log Kow =* -1.49  Carbapenems  Aztreonam: *Log Kow =* -2.49 | Effluent and Influent from WWTP  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 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 Kow =* 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 Kow =* 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 Kow =* 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 Kow =* 3.96 | Effluent form WWTP  0.0037 | *Daphnia magna*  0.036 | HPLC-ESI-MS/MS | (21, 84) |
| Clofibrate  *Log Kow =* 3.02 | Effluent sewage system 5.1 Lake: 24.7 | *Ceriodaphnia dubia*  0.640 | HPLC-API-MS/MS | (19, 85) |
| Ninylphenol  *Log Kow =* 5.31 | Influent from WWTP  1.6-986 | *R. esculenta.* Nonylphenol decreases LH plasma levels (0.0022 ≡ 10-8 M) | GC–MS  m/z =135 | (69, 133) |

**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 isn’t entirely correct due to 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 (*Kow*) and the dissociation constant are values of great importance in environmental models that help describe their chemical fate 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 (*Ka*) or the basicity constant (*Kb*), factors that determine their ionization in that medium. Generally, acidic active ingredients don’t 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 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 moninoring of degradates and parent compounds in water bodies.

**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 and degradate 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 assay approved by the U.S. environmental protection agency (EPA) are reliable assay 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 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 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 (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 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 (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 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 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 0.0037mM, 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, 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 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 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 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 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 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, 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, based on solid phase extraction, based on 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 enabled to analysis PPCPs, metabolites, pesticides and heavy metals to low concentration in water. Some devices such as semi permeable membrane devices (SPMDs) are membranes for sampler xenobiotics with *log Kow* between 4-8 (lipophilic organic compounds), as long as polar organic chemical integrative sampling (POCIS) are membranes for monitoring organic substances with *log Kow* < 4 such as metabolites, some antibiotics and other pharmaceutical compounds (104).

Moreover, the developments of different chromatographic techniques related with 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 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 improve Van Deemter curves. Furthermore, ultrahigh-pressure liquid chromatography (UHPLC) has improved the analysis time and efficiency in environmental monitoring (110-112). UHPLC use 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 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).

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 the charge of the aforementioned organization. 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 PPCPs and degradates on aquatic ecosystems.

In 1998, finisteride 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 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.

Moreover, 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 degradates and parent compounds in water bodies in Colombia is insufficient and the amount of pharmaceutical waste that is dumped into the environment and 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 etanoclorados, 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 to 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 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 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 and 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 water, as we have cited, is possible to find differences substances in effluents and influent in treatment plants, for this reason the countries should think about apply 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.

**REFERENCES**

1. Kümmerer K. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. 3rd rev.ed. Berlin, Germany: Springer; 2008. 28-34 p.

2. Caliman FA, Gavrilescu M. Pharmaceuticals, Personal Care Products and Endocrine Disrupting Agents in the Environment - A Review. CLEAN - Soil, Air, Water. 2009 Apr; 37(4-5): 277-303.

3. Keith CH. Identification and Analysis of Organic Pollutants in Water. Michigan, U.S.A: Ann Arbor Science Publishers; 1976. 517–556 p.

4. Kümmerer K. The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges. J Environ Manage. 2009 Jun; 90(8): 2354-66.

5. Celiz MD, Tso J, Aga DS. Pharmaceutical metabolites in the environment: Analytical challenges and ecological risks. Environ Toxicol Chem. 2009 Dec; 28(12): 2473-2484.

6. Baillie T, Cayen M, Fouda H, Gerson R, Green J, Grossman S, et al. Drug metabolites in safety testing. Toxicol Appl Pharmacol. 2002 Aug; 182(3): 188-196.

7. Onesios K, Yu J, Bouwer E. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation. 2009 Jul; 20(4): 441-466.

8. Mascolo G, Balest L, Cassano D, Laera G, Lopez A, Pollice A, et al. Biodegradability of pharmaceutical industrial wastewater and formation of recalcitrant organic compounds during aerobic biological treatment. Bioresour Technol. 2009 Apr; 101(8): 2585-2591.

9. Mompelat S, Le Bot B, Thomas O. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. Environ Int. 2009 Jul; 35(5): 803-814.

10. Heberer T. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. J Hydrol. 2002 Sept; 266(3-4): 175-189.

11. Halling-Sørensen B, Nors Nielsen S, Lanzky P, Ingerslev F, Holten Lützhøft H, Jørgensen S. Occurrence, fate and effects of pharmaceutical substances in the environment-a review. Chemosphere. 1998 Jan; 36(2): 357-393.

12. Ellis J. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environ Pollut. 2006 Nov; 144(1): 184-189.

13. Baquero F, Martinez J, Cantón R. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol. 2008 Jun; 19(3): 260-265.

14. Khachatourians G. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. CMAJ. 1998 Nov; 159(9): 1129-1136.

15. Oaks J, Gilbert M, Virani M, Watson R, Meteyer C, Rideout B, et al. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature. 2004 Feb; 427(6975):630-633.

16. Ternes T. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 1998 Nov; 32(11): 3245-3260.

17. Stumpf M. Determination of drugs in sewage treatment plants and river water. Vom Wasser. 1996; 86:291-303.

18. Buerge I, Buser H, Poiger T, Müller M. Occurrence and Fate of the Cytostatic Drugs Cyclophosphamide and Ifosfamide in Wastewater and Surface Waters. Environ Sci Technol. 2006 Sept; 40(23): 7242-7250.

19. Sánchez-Argüello P, Fernández C, Tarazona JV. Assessing the effects of fluoxetine on Physa acuta (Gastropoda, Pulmonata) and Chironomus riparius (Insecta, Diptera) using a two-species water-sediment test. Sci Total Environ. 2009 Jan; 407(6): 1937-1946.

20. Quinn B, Gagné F, Blaise C. An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, Hydra attenuata. Sci Total Environ. 2008 Jan; 389(2-3): 306-314.

21. Brooks B, Foran C, Richards S, Weston J, Turner P, Stanley J, et al. Aquatic ecotoxicology of fluoxetine. Toxicol Lett. 2003 May; 142 (3): 169-83.

22. Ministerio de Salud y Consumo. Grupos terapéuticos y Principios activos de mayor consumo en el Sistema Nacional de Salud durante 2003. Inf Ter Sist Nac Salud. 2004 Jun; 28(5): 121-124.

23. Van Gerven J, Schoemaker R, Jacobs L, Reints A, Ouwersloot-Van Der Meij M, Hoedemaker H, et al. Self-medication of a single headache episode with ketoprofen, ibuprofen or placebo, home-monitored with an electronic patient diary. BJCP.1996 Oct; 42(4): 475-81.

24. Richardson S. Water analysis: emerging contaminants and current issues. Anal Chem. 2009 May; 81(12): 4645-4677.

25. Gómez M, Petrovic M, Fernández-Alba A, Barceló D. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. J Chromatogr A. 2006 May; 1114(2): 224-33.

26. Farré M. Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fischeri. J Chromatogr A. 2001 Dec; 938(1-2): 187-197.

27. Buser H, Poiger T, Müller M. Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. Environ Sci Technol. 1999 Jun; 33(15): 2529-2535.

28. Langford K, Thomas K. Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works. Environ Int. 2009 Jul; 35(5): 766-770.

29. Richardson M, Bowron J. The fate of pharmaceutical chemicals in the aquatic environment. Journal of Pharmacy and Pharmacology. 1985 Feb; 37(1): 1-12.

30. Tixier C, Singer H, Oellers S, Müller S. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ Sci Technol. 2003 Feb; 37(6): 1061-1068.

31. Radjenovi J, Jeli A, Petrovi M, Barceló D. Determination of pharmaceuticals in sewage sludge by pressurized liquid extraction (PLE) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Anal Bioanal Chem. 2009 Jan; 393(6): 1685-1695.

32. Pailler J, Krein A, Pfister L, Hoffmann L, Guignard C. Solid phase extraction coupled to liquid chromatography-tandem mass spectrometry analysis of sulfonamides, tetracyclines, analgesics and hormones in surface water and wastewater in Luxembourg. Sci Total Environ. 2009 Aug; 407(16): 4736-4743.

33. Stülten D, Zühlke S, Lamshöft M, Spiteller M. Occurrence of diclofenac and selected metabolites in sewage effluents. Sci Total Environ. 2008 Nov; 405(1-3): 310-316.

34. Quinn B, Gagné F, Blaise C. Evaluation of the acute, chronic and teratogenic effects of a mixture of eleven pharmaceuticals on the cnidarian, Hydra attenuata. Sci Total Environ. 2009 Jan; 407(3): 1072-1079.

35. Santos J, Aparicio I, Callejón M, Alonso E. Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). J Hazard Mater. 2009 May; 164(2-3): 1509-1516.

36. Han G, Hur H, Kim S. Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: Occurrence and toxicity to Daphnia magna. Environ Toxicol Chem. 2006 Jan; 25(1): 265-271.

37. Lin A, Tsai Y. Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. Sci Total Environ. 2009 Jun; 407(12): 3793-3802.

38. He J, Whelton P. Epidemiology and prevention of hypertension. Med Clin North Am. 1997 Sept; 81(5): 1077-1098.

39. Giudice R, Polio A, Garric J. Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment?. Environ Toxicol Chem. 2004 May; 23(5): 1344-1354.

40. Zhou J, Zhang Z, Banks E, Grover D, Jiang J. Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. J Hazard Mater. 2009 Jul; 166(2-3): 655-661.

41. Miège C, Favier M, Brosse C, Canler J, Coquery M. Occurrence of betablockers in effluents of wastewater treatment plants from the Lyon area (France) and risk assessment for the downstream rivers. Talanta. 2006 Nov; 70(4): 739-744.

42. Hernando M, Petrovic M, Fernández-Alba A, Barceló D. Analysis by liquid chromatography-electrospray ionization tandem mass spectrometry and acute toxicity evaluation for [beta]-blockers and lipid-regulating agents in wastewater samples. J Chromatogr A. 2004 Aug; 1046(1-2): 133-140.

43. Rosal R, Rodríguez A, Perdigón-Melón J, Petre A, García-Calvo E, Gómez M, et al. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res. 2010 Jan; 44(2): 578-588.

44. Daughton C, Ternes T. Pharmaceuticals and personal care products in the environment: Agents of subtle change?. Environ Health Perspect. 1999 Dec; 107(Suppl 6): 907-938.

45. Hummel D, Löffler D, Fink G, Ternes T. Simultaneous Determination of Psychoactive Drugs and Their Metabolites in Aqueous Matrices by Liquid Chromatography Mass Spectrometry. Environ Sci Technol. 2006 Nov; 40(23): 7321-7328.

46. Garcia-Ac A, Segura P, Gagnon C, Sauvé S. Determination of bezafibrate, methotrexate, cyclophosphamide, orlistat and enalapril in waste and surface waters using on-line solid-phase extraction liquid chromatography coupled to polarity-switching electrospray tandem mass spectrometry. J Environ Monit. 2009 Jan; 11(4): 830-838.

47. Nalecz-Jawecki G, Persoone G. Toxicity of Selected Pharmaceuticals to the Anostracan Crustacean Thamnocephalus platyurus-Comparison of Sublethal and Lethal Effect Levels with the 1h Rapidtoxkit and the 24h Thamnotoxkit Microbiotests. Environ Sci Pollut Res Int. 2006 Jan; 13(1): 22-27.

48. Kumar A, Schweizer H. Bacterial resistance to antibiotics: active efflux and reduced uptake. Adv. Drug Delivery Rev. 2005 Jul; 57(10): 1486-1513.

49. Wright G. Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv. Drug Delivery Rev. 2005 Jul; 57(10): 1451-1470.

50. Lambert P. Bacterial resistance to antibiotics: modified target sites. Adv Drug Delivery Rev. 2005 Jul; 57(10): 1471-1485.

51. Dang H, Zhang X, Song L, Chang Y, Yang G. Molecular determination of oxytetracycline-resistant bacteria and their resistance genes from mariculture environments of China. J Appl Microbiol. 2007 Jul; 103(6): 2580-2592.

52. Shakil S, Khan R, Zarrilli R, Khan A. Aminoglycosides versus bacteria–a description of the action, resistance mechanism, and nosocomial battleground. J Biomed Sci. 2008 Jan; 15(1): 5-14.

53. Roberts M, Sutcliffe J, Courvalin P, Jensen L, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob Agents Chemother. 1999 Dec; 43(12): 2823-2830.

54. Akinbowale O, Peng H, Barton M. P514 Class 1 integron mediates antibiotic resistance in Aeromonas spp. from rainbow trout farms in Australia. Int J Antimicrob Agents. 2007 Mar; 29: S113.

55. Li B, Zhang T, Xu Z, Fang H. Rapid analysis of 21 antibiotics of multiple classes in municipal wastewater using ultra performance liquid chromatography-tandem mass spectrometry. Anal Chim Acta. 2009 Jul; 645(1-2): 64-72.

56. Guardabassi L, Petersen A, Olsen J, Dalsgaard A. Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. Appl Environ Microbiol. 1998 Sept; 64(9): 3499-3502.

57. Ko W, Yu K, Liu C, Huang C, Leu H, Chuang Y. Increasing antibiotic resistance in clinical isolates of Aeromonas strains in Taiwan. Antimicrob Agents Chemother. 1996 Jun; 40(5): 1260-1262.

58. Zarrilli R, Tripodi M, Di Popolo A, Fortunato R, Bagattini M, Crispino M, et al. Molecular epidemiology of high-level aminoglycoside-resistant enterococci isolated from patients in a university hospital in southern Italy. J Antimicrob Chemother. 2005 Sept; 56(5): 827-835.

59. Löffler D, Ternes T. Analytical method for the determination of the aminoglycoside gentamicin in hospital wastewater via liquid chromatography-electrospray-tandem mass spectrometry. J Chromatogr A. 2003 Jun; 1000(1-2): 583-588.

60. Segura P, García-Ac A, Lajeunesse A, Ghosh D, Gagnon C, Sauvé S. Determination of six anti-infectives in wastewater using tandem solid-phase extraction and liquid chromatography–tandem mass spectrometry. J Environ Monit. 2007 Jan; 9(4): 307-313.

61. Soge O, Tivoli L, Meschke J, Roberts M. A conjugative macrolide resistance gene, mef (A), in environmental Clostridium perfringens carrying multiple macrolide and/or tetracycline resistance genes. J Appl Microbiol. 2009 Dec; 106(1): 34-40.

62. Ding J, Ren N, Chen L, Ding L. On-line coupling of solid-phase extraction to liquid chromatography-tandem mass spectrometry for the determination of macrolide antibiotics in environmental water. Anal Chim Acta. 2009 Feb; 634(2): 215-221.

63. Brown K, Kulis J, Thomson B, Chapman T, Mawhinney D. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Sci Total Environ. 2006 Aug; 366(2-3): 772-783.

64. Costanzo S, Murby J, Bates J. Ecosystem response to antibiotics entering the aquatic environment. Mar Pollut Bull. 2005 Nov; 51(1-4): 218-223.

65. Nakata H, Kannan K, Jones P, Giesy J. Determination of fluoroquinolone antibiotics in wastewater effluents by liquid chromatography-mass spectrometry and fluorescence detection. Chemosphere. 2005 Feb; 58(6): 759-766.

66. Gulkowska A, Leung H, So M, Taniyasu S, Yamashita N, Yeung L, et al. Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. Water Res. 2008 Jan; 42(1-2): 395-403.

67. Granelli K, Elgerud C, Lundström Å, Ohlsson A, Sjöberg P. Rapid multi-residue analysis of antibiotics in muscle by liquid chromatography-tandem mass spectrometry. Anal Chim Acta. 2009 Apr; 637(1-2): 87-91.

68. Li D, Yang M, Hu J, Zhang Y, Chang H, Jin F. Determination of penicillin G and its degradation products in a penicillin production wastewater treatment plant and the receiving river. Water Res. 2008 Jan; 42(1-2): 307-317.

69. Kloas W, Urbatzka R, Opitz R, Würtz S, Behrends T, Hermelink B, et al. Endocrine disruption in aquatic vertebrates. Ann N.Y Acad Sci. 2009 Apr; 1163(suppl 1): 187-200.

70. Björkblom C, Olsson P, Katsiadaki I, Wiklund T. Estrogen-and androgen-sensitive bioassays based on primary cell and tissue slice cultures from three-spined stickleback (Gasterosteus aculeatus). Comp Biochem Physiol C: Toxicol Pharmacol. 2007 Sept; 146(3): 431-442.

71. Hutchinson T, Ankley G, Segner H, Tyler C. Screening and testing for endocrine disruption in fish—biomarkers as “signposts,” not “traffic lights,” in risk assessment. Environ Health Perspect. 2006 Apr; 114(S-1): 106-114.

72. Kloas W, Lutz I, Einspanier R. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. The Sci Total Environ. 1999 Jan; 225(1-2): 59-68.

73. Tsai P, Lunden J, Jones J. Effects of steroid hormones on spermatogenesis and GnRH release in male Leopard frogs, Rana pipiens. Gen Comp Endocrinol. 2003 Dec; 134(3): 330-338.

74. Witschi E, Allison J. Responses of Xenopus and Alytes to the administration of some steroid hormones. Anat Rec. 1950 ; 108: 589-590.

75. Pettersson I, Berg C. Environmentally relevant concentrations of ethynylestradiol cause female-biased sex ratios in Xenopus tropicalis and Rana temporaria. Environ Toxicol Chem. 2007 Dec; 26(5): 1005-1009.

76. Ternes T, Bonerz M, Schmidt T. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr A. 2001 Dec; 938(1-2): 175-185.

77. Länge R, Hutchinson TH, Croudace CP, Siegmund F, Schweinfurth H, Hampe P, et al. Effects of the synthetic estrogen 17alpha-ethinylestradiol on the life-cycle of the fathead minnow. Environ Toxicol Chem. 2001 Oct; 20(6): 1216-1227.

78. PG L, Baatrup E. Male zebrafish (Danio rerio) courtship behaviour resists the feminising effects of 17alpha-ethinyloestradiol-morphological sexual characteristics do not. Aquat Toxicol. 2008 Feb; 87(4): 234-244.

79. Ying G, Kookana R, Ru Y. Occurrence and fate of hormone steroids in the environment. Environ Int. 2002 Dec; 28(6): 545-51.

80. Grung M, Lichtenthaler R, Ahel M, Tollefsen K, Langford K, Thomas K. Effects-directed analysis of organic toxicants in wastewater effluent from Zagreb, Croatia. Chemosphere. 2007 Feb; 67(1): 108-120.

81. Koger C, Teh S, Hinton D. Determining the sensitive developmental stages of intersex induction in medaka (Oryzias latipes) exposed to 17 [beta]-estradiol or testosterone. Mar Environ Res. 2000 Jul; 50(1-5): 201-206.

82. Oetken M, Nentwig G, Löffler D, Ternes T, Oehlmann J. Effects of pharmaceuticals on aquatic invertebrates. Part I. The antiepileptic drug carbamazepine. Arch Environ Contam Toxicol. 2005 Oct; 49(3): 353-361.

83. Batt A, Kostich M, Lazorchak J. Analysis of Ecologically Relevant Pharmaceuticals in Wastewater and Surface Water Using Selective Solid-Phase Extraction and UPLC- MS/MS. Anal Chem. 2008 May; 80(13): 5021-5030.

84. Lajeunesse A, Gagnon C, Sauvé S. Determination of Basic Antidepressants and Their N-Desmethyl Metabolites in Raw Sewage and Wastewater Using Solid-Phase Extraction and Liquid Chromatography- Tandem Mass Spectrometry Anal Chem. 2008 Jun; 80(14): 5325-5333.

85. Zorita S, Boyd B, Jönsson S, Yilmaz E, Svensson C, Mathiasson L, et al. Selective determination of acidic pharmaceuticals in wastewater using molecularly imprinted solid-phase extraction. Anal Chim Acta. 2008 Sept; 626(2): 147-154.

86. James M. Overview of in vitro metabolism of drugs by aquatic species. Vet Hum Toxicol. 1986 Feb; 28(Suppl 1): 2-8.

87. Benet LZ, Cummins CL. The drug efflux-metabolism alliance: biochemical aspects. Adv Drug Delivery Rev. 2001 Oct; 50(Suppl 1): S3-S11.

88. Rahman S, Khan R, Gupta V, Uddin M. Pharmacoenvironmentology – a component of pharmacovigilance. J Environ Health. 2007 Jul; 6(1): 20.

89. Ternes T, Stumpf M, Schuppert B, Haberer K. Simultaneous determination of antiseptics and acidic drugs in sewage and river water. Vom Wasser. 1998; 90: 295-309.

90. Kümmerer K. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. 3rd rev.ed. Berlin, Germany: Springer; 2008. 26 p.

91. Guarino A, Lech J. Metabolism, disposition, and toxicity of drugs and other xenobiotics in aquatic species. Vet Hum Toxicol. 1986 Feb; 28(Suppl 1): 38-44.

92. Fent K, Weston A, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat Toxicol. 2006 Feb; 76(2): 122-159.

93. Boudou A, Ribeyre F. Aquatic ecotoxicology: from the ecosystem to the cellular and molecular levels. Environ Health Perspect. 1997 Feb; 105(Suppl 1): 21.

94. Epel D. Use of multidrug transporters as first lines of defense against toxins in aquatic organisms. Comp Biochem Physiol A: Mol Integr Physiol. 1998 May; 120(1): 23-28.

95. Kurelec B. The multixenobiotic resistance mechanism in aquatic organisms. Crit Rev Toxicol. 1992 Jan; 22(1): 23-43.

96. Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett. 2003 May15; 142(3): 185-194

97. Lilius H, Hästbacka T, Isomaa B. Short Communication: A comparison of the toxicity of 30 reference chemicals to Daphnia Magna and Daphnia Pulex. Environ Toxicol Chem. 1995 Oct; 14(12): 2085-2088.

98. Ferrari B, Paxeus N, Giudice R, Pollio A, Garric J. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol Environ Saf. 2003 Jul; 55(3): 359-370.

99. Kulkarni G. In vivo stimulation of ovarian development in the red swamp crayfish, Procambarus clarkii (Girard), by 5-hydroxytryptamine. Invertebrate reproduction & development. 1992 May; 21(3): 231-239.

100. Henschel K, Wenzel A, Diedrich M, Fliedner A. Environmental Hazard Assessment of Pharmaceuticals 1. Regul Toxicol Pharm. 1997 Jun; 25(3): 220-225.

101. Jjemba PK. Pharma-Ecology: The Occurrence and Fate of Pharmaceuticals and Personal Care Products in the Environment. New Jersey, U.S.A: John Wiley & Sons, Inc; 2008. 81-116 p.

102. Nováková L, Vlcková H. A review of current trends and advances in modern bio-analytical methods: Chromatography and sample preparation. Anal Chim Acta. 2009 Dec; 656(1-2): 8-35.

103. Jiménez C, León D. Biosensores: aplicaciones y perspectivas en el control y calidad de procesos y productos alimenticios. Vitae. 2009 Sept-Nov; 16 (1):144-154.

104.Petrović M, Barceló D. Analysis fate and removal of pharmaceuticals in the water cycle. 1st rev.ed. Amsterdam, Netherlans: Elsevier; 2007. 171-197 p.

105. Pietrogrande MC, Basaglia G. GC-MS analytical methods for the determination of personal-care products in water matrices. TrAC, Trends Anal Chem. 2007 Dec; 26(11): 1086-1094.

106. Motokawa M, Kobayashi H, Ishizuka N, Minakuchi H, Nakanishi K, Jinnai H, et al. Monolithic silica columns with various skeleton sizes and through-pore sizes for capillary liquid chromatography. J Chromatogr A. 2002 Jun; 961(1): 53-63.

107. Ishizuka N, Kobayashi H, Minakuchi H, Nakanishi K, Hirao K, Hosoya K, et al. Monolithic silica columns for high-efficiency separations by high-performance liquid chromatography. J Chromatogr A. 2002 Jun; 960(1-2): 85-96.

108. Roy A, Miller M, Meunier D, Willem deGroot A, Winniford W, Van Damme F, et al. Development of Comprehensive Two-Dimensional High Temperature Liquid Chromatography Gel Permeation Chromatography for Characterization of Polyolefins. Macromolecules. 2010 Mar; 43(8): 3710-3720.

109. Greibrokk T, Andersen T. High-temperature liquid chromatography. J Chromatogr A. 2003 Jun; 1000(1-2): 743-755.

110. Hartonen K, Riekkola M-L. Liquid chromatography at elevated temperatures with pure water as the mobile phase. TrAC, Trends Anal Chem. 2008 Jan; 27(1): 1-14.

111. Portolés T, Ibáñez Ma, Sancho JV, López FJ, Hernández Fl. Combined Use of GC-TOF MS and UHPLC-(Q)TOF MS To Investigate the Presence of Nontarget Pollutants and Their Metabolites in a Case of Honeybee Poisoning. J Agric Food Chem. 2009 Apr; 57(10): 4079-4090.

112. Welch CJ, Wu N, Biba M, Hartman R, Brkovic T, Gong X, et al. Greening analytical chromatography. TrAC Anal Chem. 2010 Jul-Aug; 29(7): 667-680.

113. Liu G, Snapp HM, Ji QC, Arnold ME. Strategy of Accelerated Method Development for High-Throughput Bioanalytical Assays Using Ultra High-Performance Liquid Chromatography Coupled with Mass Spectrometry. Anal Chem. 2009 Oct; 81(22): 9225-9232.

114. Wu N, Collins DC, Lippert JA, Xiang Y, Lee ML. Ultrahigh pressure liquid chromatography/time-of-flight mass spectrometry for fast separations. J Microcolumn Sep. 2000 Nov; 12(8): 462-469.

115. Cunliffe JM, Maloney TD. Fused-core particle technology as an alternative to sub-2-mum particles to achieve high separation efficiency with low backpressure. J Sep Sci. 2007 Nov; 30(18): 3104-3109.

116. Gallart-Ayala H, Moyano E, Galceran MT. On-line solid phase extraction fast liquid chromatography-tandem mass spectrometry for the analysis of bisphenol A and its chlorinated derivatives in water samples. J Chromatogr A. 2010 May; 1217(21): 3511-3518.

117. Gritti F, Leonardis I, Shock D, Stevenson P, Shalliker A, Guiochon G. Performance of columns packed with the new shell particles, Kinetex-C18. J Chromatogr A. 2010 Mar; 1217(10): 1589-1603.

118. Dejaegher B, Pieters S, Vander Heyden Y. Emerging Analytical Separation Techniques with High Throughput Potential for Pharmaceutical Analysis, Part II: Novel Chromatographic Modes. Comb Chem High Throughput Screen. 2010 Jul; 13: 530-547.

119. Dejaegher B, Heyden YV. HILIC methods in pharmaceutical analysis. J Sep Sci. 2010 Feb; 33(6-7): 698-715.

120. Dejaegher B, Mangelings D, Heyden YV. Method development for HILIC assays. J Sep Sci. 2008 May; 31(9): 1438-1448.

121. Richardson SD. Environmental Mass Spectrometry:  Emerging Contaminants and Current Issues. Anal Chem. 2006 May; 78(12): 4021-4046.

122. Richardson SD. Environmental Mass Spectrometry: Emerging Contaminants and Current Issues. Anal Chem. 2008 May; 80(12): 4373-4402.

123. Richardson SD. Environmental Mass Spectrometry: Emerging Contaminants and Current Issues. Anal Chem. 2010 May; 82(12): 4742-4774.

124. Petrović M, Barceló D. Analysis fate and removal of pharmaceuticals in the water cycle. 1st rev. ed. Amsterdam, Netherlans: Elsevier; 2007. 279-334 p.

125. Hansen P-D. Biosensors and Eco-toxicology. Eng Life Sci. 2008 Feb; 8(1):26-31.

126. OECD (Organisation for Economic Co-operation and Development). Guidance document for the development of oecd guidelines for the testing of chemicals. [Internet]. Paris: OECD, Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology; 2009 Aug 05 [Updated 2009 Oct; cited 2010 Feb]. Available from: http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=ENV/JM/MONO(2006)20/REV1&doclanguage=en

127. Food and Drug Administration (FDA). FDA Modernization Act of 1997 - Guidance for the Device Industry on Implementation of Highest Priority Provisions [Internet]. Rockville: Center for Devices and Radiological Health, U.S. Department of Health and Human Services; 1998 Feb 06 [updated 2008 Dec 12; cited 2010 Feb 24]. Available From: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm094527.htm

128. Michels D, Jonnard A, Bretscher C, et al. Review of Global Competitiveness in the Pharmaceutical Industry [Internet]. Washington, DC: U.S. International Trade Commission, Office of Industries; 1999 Apr 1 [update 2008 Dec; cited 2010 Feb] Available from: http://www.usitc.gov/publications/332/working\_papers/pub3172.pdf

129. Rogers R. Sepracor: Skating on thin ‘ice’. Chem Eng News. 1998; 30: 11-13.

130. FDA (Food and Drug Administration). Guidance for Industry Environmental Assessment of Human Drug and Biologics Applications. [Internet]. Rockville, Maryland: FDA, U.S. Department of Health and Human Services; 1998 Jul 1 [Updated 2008 Dec; cited 2010 Feb]. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070561.pdf

131. Kloskowski R, Fischer R, Binner R and Winkler R, editors. Draft guidance on the calculation of predicted environmental concentration values (PEC) of plant protection products for soil, ground water, surface water and sediment, in Human and environmental exposure to xenobiotics, Proceedings of the XI Symposium of Pesticide Chemistry; 1999 Sep 835-850; Pavia, Italy: La Goliardica Pavese; c2000.

132. Ministerio de la protección social. Decreto 1575 de 2007, Control de la Calidad del Agua para Consumo Humano. [Internet]. Colombia : 2007. [Updated 2008 Dic; cited 2010 Feb]. Available from: <http://www.minproteccionsocial.gov.co/Normatividad/DECRETO%201575%20DE%202007.Pdf>.

133. Xia K, Bhandari A, Das K, Pillar G. Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids. J Environ Qual. 2005 Jan; 34(91): 91-104.