PHARMACEUTICAL DRUGS IN THE ENVIRONMENT: CHANGE OF THE PARADIGM

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RESUMO

Drogas farmacêuticas foram reportadas num grande número de ecossistemas aquáticos e em efluentes provenientes de estações de tratamento de águas residuais. A presença destas substâncias pode ser responsável pela interferência com um grande número de estruturas biológicas, vias metabólicas e processos regulatórios, possivelmente conducentes a efeitos nocivos e efeitos irreversíveis a vários níveis. Este artigo apresenta as indicações mais recentes que envolvem as drogas farmacêuticas e os efeitos/agressões ambientais, possivelmente indicando que o ciclo do medicamento clássico deve ser reconsiderado.

ABSTRACT

Pharmaceutical drugs have been reported in a large number of aquatic ecosystems, and have also been found in sewage effluents. The presence of these substances may be responsible for the interference with a large number of biological structures, metabolic pathways and regulatory processes leading to deleterious effects and irreversible damage at varied levels. This article presents the most recent indications that implicate pharmaceutical drugs in environmental effects/insults, possibly indicating that the classical cycle of the pharmaceutical drugs in the market must be reconsidered.

1. INTRODUCTION

Pharmaceutical drugs are bioactive compounds, generally developed and synthesized with a strict compliance with several requirements, in order to fully accomplish their role in diagnosis, prophylaxis and treatment of diseases. Pharmaceuticals are thus designed and manufactured in order to allow a good bioavailability, high pharmacological activity, low incidence of side effects, and simple mode of administration. In order to comply with these general needs, pharmaceuticals generally possess common characteristics, such as specific biological activity and relative resistance to biotransformation. However, these common features are also responsible for the main environmental concerns associated to pharmaceutical drugs, since high pharmacological potency may lead to biological activity on non-target species, present in the wild. Concomitantly, resistance to biotransformation implies that pharmaceuticals are somewhat refractory to degradation, and this may lead to a certain degree of environmental persistence. Even if some of the pharmaceutical drugs are not persistent, their continuous input into the aquatic environment is responsible for a residual level been found. Therapeutic agents can thus be considered a major class of chemical compounds, characterized by indiscriminate and continuous use and biological activity (Jones *et al*., 2002; Miao *et al*. 2002; Daughton and Ternes, 1998; Halling-Sörensen *et al*., 1998).

Human populations have been using modern drugs for several decades, in an ever-increasing number, amount, potency and variety of pathologies being treated. Besides its use in modern therapeutics, pharmaceutical drugs must be considered as chemical substances, and the use of therapeutic compounds always implies their later release into the environment (Daughton and Ternes, 1999). They are usually eliminated via urine or faeces, generally as more water-soluble chemicals when compared to the parent compounds. The increased water solubility is of major importance, since it is the cause for the presence of the drugs and/or their metabolites in water streams, rivers, estuaries, and, ultimately, oceans. Furthermore, modern sewage treatment plants are not prepared and even designed to cope with chemically inert and stable compounds such as some pharmaceuticals. This lack of depuration of sewage water from pharmaceuticals leads to an overall low capacity of elimination (Petroviæ *et al*., 2003). The employed treatment methodologies frequently reveal crucial inefficiencies, and elimination rates are not satisfactory, thus leading to a continuous input of these compounds into the environment, as reported by Winkler *et al*. (2001).

The matters of environmental presence of pharmaceutical drugs substantially grew during the nineties, as a consequence of the publication of data showing unexpected levels of contamination by several pharmaceutical drugs, in several ecosystems. These data referred to the contamination of sewage systems, rivers, lakes, subterranean water sources, and oceans. High values of contamination were reported, not surprisingly, in sewage systems (Miao *et al*., 2002; Ternes, 1998); however, high values of contamination were also observed in lakes and even open ocean waters, showing the actual dimension of this environmental question. An example of this situation is the study by Weigel *et al*. (2002), which showed the presence of clofibric acid (concentrations in the range of ng/l), in several sampling sites of the North Sea. Furthermore, an always increasing num-

ber of scientific papers and technical reports evidenced that persistent pharmaceuticals were widely dispersed in the environment, and were not only associated to large human populations. Phenomena of global circulation drove these pharmaceutical compounds far off the vicinity of human populations, and lead to their appearance in earlier considered pristine geographical areas.

When studying the contamination of aquatic environments by pharmaceutical drugs, one must consider several confounding factors. Pharmaceutical residues in the wild are not restricted to prescription drugs, and a large number of pharmaceuticals is also employed in veterinary therapeutics. Furthermore, other drugs may also be used in illegal abuse phenomena, and cannot be followed along normal commercial circuits, thus not being eligible for monitoring since it is impossible to have official statistics of use/consumption. If some of the given examples of contamination were allegedly attributed to parent compounds, some of the examples referred simultaneously to their metabolites and products of environmental degradation. The biological activity of some of these metabolites is similar to the pharmacological activity described for parent compounds, but others are not. Toxicological phenomena assessed in human patients may also be different for the toxicity exerted in animal models, and correlations between human and animal toxicity data are not abundant and well understood (Olson *et al*., 2000). In addition to their biological activity, the lipophylicity of some of the large number of pharmaceutical drugs is well-known, factor that can be held responsible for their ultimate bioaccumulation and/or biomagnification in food webs. Other confounding factor in the interpretation of environmental toxicity data is related to the vast variety of wild organisms and the correspondent variability of pharmacological/metabolic/detoxification pathways. Exposure under natural conditions may also be responsible for the exertion of synergistic effects (Cleuvers, 2003), consequently to the presence of a considerable number of different drug residues. Consequently, effects consequent to pharmaceuticals exposure on wild organisms cannot be easily predicted. However, studies have been performed in order to investigate their effects on non-human species, their metabolism in different classes of organisms and their fate in the environment.

In addition to the difficulties already mentioned, drugs can suffer a wide array of physical and chemical interactions, among each other and with the large number of natural and anthropogenic compounds actually present in the environment. Therefore, toxicological interactions may occur in natural conditions, altering the toxic profiles and activities of pharmaceutical drugs. Furthermore, interactions with sediments, dilution or concentration, photoxidation and other modes of degradation may occur in real scenarios. Consequently, the effects of environmental exposure to human use pharmaceutical drugs are difficult to evaluate and are largely unknown. The relatively low concentrations of these compounds in some areas may not be responsible for acute effects. On the contrary, effects may be subtle and may occur after extremely long periods of time. These long periods of exertion of toxicological activity may be, in the future, erroneously attributed to evolutionary trends (Daughton and Ternes, 1999). In spite of its presence generally in very low concentrations, some studies reported the presence of pharmaceutical residues in unexpected high concentrations (Kümmerer, 2001), which can consequently lead to acute effects over organisms.

Besides human use pharmaceuticals, numerous other compounds have been released into the environment as a consequence of their use. Among these, veterinary/agriculture drugs and personal care products are of high importance, since they represent heavy loads of environmental unfriendly compounds, dumped in large quantities into aquatic ecosystems. The effects of pharmaceuticals after their administration to animals, for veterinary purposes, can also be drastic enough to ask further attention; among veterinary use drugs, antiparasitic (*e.g.* anthelmintic) drugs have been implicated in biological disturbances, as referred by McKellar (1995). Personal care products, in spite of not having a therapeutic purpose, are included in cosmetic preparations, such as shampoos, bubble baths or as adjuvants in dermopharmaceutic products. This is the case of several detergents, whose use is responsible for large amounts being released every year. Furthermore, the biological activity of this type of compounds is also exerted in a large number of organisms after their entrance into the environment. These compounds are often responsible for a decreased efficiency of the sewage treatment plants, since they interfere with the development of microorganisms required to perform aerobic alterations of raw sewage. Again, the issue of degradability is raised, since some of the compounds included in this class are relatively persistent in the environment. Even for substances considered as non-persistent in the environment, their continuous input may be responsible for their continuous presence in some areas.

The present article reviews literature data concerning recent publications related to the assessment of biological effects consequent to exposure to pharmaceutical drugs, under the scope of aquatic environments. It intends to congregate data about the presence of pharmaceutical residues, its known effects, profiles of contamination and directions of future research of this particular area, concerning methodology of toxicology testing.

2. PHARMACEUTICAL RESIDUES IN WASTEWATER

Wastewater is a main responsible for the introduction of chemical contaminants into aquatic ecosystems (Petroviæ *et al*., 2003), since raw sewage is no longer dumped into rivers or oceans in considerable amounts, especially in developed countries. Large efforts have been dedicated during the past decades to the progressive increase in the elimination efficacy of several compounds by sewage treatment plants. As stated by Ternes (1999), the indisputable increase in the effectiveness of sewage treatment plants to deal with nutrient enrichment and microbial contamination, did not contemplate special chemical natural or synthetic compounds, such as pharmaceutical drugs, personal care products or their residues, since these compounds are continuously released in large number and quantities, and pose concerns related to their chemical stability. This type of compounds is generally resistant to degradation, and, in the case of detergents, can even disturb the correct elimination processes, through the impairment of microbial activity, which is held responsible for the complete degradation of organic matter. A large number of studies have been published establishing a connection between pharmaceutical residues in wastewater and their consequent presence in the wild, where they exert deleterious effects on aquatic organisms. Besides the simple environmental question, the presence of drug residues in the water cycle can also be a matter of human

health, since it is of absolute importance the attainment of suitable quality standards of water for human use and consumption (Petroviæ *et al*., 2003). An excellent effort was performed by Daughton and Ternes (1999), in a review article aggregating knowledge about the presence of pharmaceutical drugs residues in aquatic environment. Besides pharmaceutical drugs, the same review also included references of other health-related compounds in the environment, such as diagnostic agents, nutraceuticals, fragrances, sunscreen agents, for instance.

Miao *et al*. (2002) reported the presence of several pharmaceutical residues in the effluents from Canadian sewage treatment plants. This study showed the presence of bezafibrate, diclofenac, fenoprofen, gemfibrozil, ibuprofen, indomethacin and naproxen. Hospital facilities are also a source of pharmaceutical residues that, besides their intrinsic biological activities, can also contain adsorbable organically bound halogens (AOX), which are toxic to humans and other organisms, being also persistent in the environment and possibly bioaccumulated along food webs (Kümmerer *et al*., 1998). Wastewater coming from hospital facilities can be also responsible for the introduction into the environment of mutagenic/carcinogenic substances, such as the antineoplastic agent cyclophosphamide, as shown by Steger-Hartmann (1997). This study showed that this antineoplastic drug is poorly degraded during passage through a sewage treatment plant, but its presence could not be directly correlated with the overall genotoxic potential of the hospital effluent, underlining the deleterious role attributed to complex mixtures of chemical compounds such as pharmaceuticals.

Jones *et al*. (2002) studied the models to assess aquatic environmental fate of the top 25 English prescription pharmaceutical drugs. In this study, the authors were able to predict that many of the considered prescription pharmaceuticals are not likely to degrade to any great extent and not to adsorb to sludge. This means that concentrations of the drugs can be as high as 97–98% of the original influent concentration, indicating that these compounds may be discharged to rivers.

The biodegradibility characteristics of several pharmaceutical compounds, such as sulfonamides, was assessed by Ingerslev and Halling-Sørensen (2000). In this study, the authors observed that this class of chemicals, abundant as a consequence of its widespread use, could not be considered readily degradable in sewage treatment plants, since periods of degradation of 28 days did not cause complete elimination of the tested sulfonamides from screening test system. At the same time, the tested concentrations were not responsible, in most cases, for acute inhibitory effects on growth of bacterial populations. The authors observed that degradation of the majority of sulfonamides by microorganisms present in sewage treatment plants only occurred after an acclimatization period, during which microorganisms suffered adaptative changes in order to be able to cope with the mentioned pharmaceutical residues. However, this conclusion implies that, in spite of not being readily biodegraded by non-adapted bacterial strains, sulfonamides may not be considered as persistent compounds.

The presence of estrogenic compounds in wastewater was also assessed in a large number of studies, namely for the risk assessment regarding endocrine disrupting ef-

fects in the aquatic environment. Ternes *et al*. (1999) reported the discharge of several endocrine active compounds, such as estrone, 17á-estradiol, 17á-ethinylestradiol and 16 á-hydroxyestrone (in concentration in the ng/l-range), by several German and Canadian sewage treatment plants, as a consequence of consumption of oral contraceptives (birth control pills). These discharges are due to the incomplete removal of the mentioned pharmaceutical drugs during passage through the sewage treatment plants; the levels of contamination found in the referred study do not imply that the measured compounds are capable of disrupting endocrine equilibriums in exposed wildlife, but deserve further attention.

The study by Koutsouba *et al*. (2003) showed that both influents and effluents of Greek sewage treatment plants contained the human use drug diclofenac. The presence of this pharmaceutical drug in the influents shows its use in human therapeutics, and is not related to any industrial discharge. The presence of this drug in the effluent from the sewage treatment plants shows that its elimination is far from complete, thus contributing for its ultimate presence in the ecosystems in which it is dispersed.

The study conducted by Ferrari *et al*. (2003) showed the presence of pharmaceutical drugs (clofibric acid, carbamazepine and diclofenac) in effluents of sewage treatment plants in distinct countries, such as France, Greece, Italy, and Sweden.

Besides the sewage related entrance route of pharmaceutical drugs into the environment, direct contamination can also occur through deposition of contaminated manure on soil, as fertilizer. Hamscher *et al*. (2002) determined the presence of persistent tetracyclines residues in soil fertilized with contaminated manure. In general terms, the authors found significant concentrations of tetracycline and chlortetracycline in the soil: highest average concentrations of 86.2 (layer of 0-10 cm of soil depth), 198.7 (layer of 10-20 cm of soil depth), and 171.7 µg/kg (layer of 20-30 cm of soil depth) tetracycline and 4.6-7.3 µg/kg chlortetracycline (all three sublayers) were found. The authors could conclude that tetracyclines enter the environment in significant concentrations via repeated fertilizations with liquid manure. Besides their entrance into the environment, tetracyclines build up persistent residues, and accumulate in soil. These characteristics showed that tetracyclines may have a potential risk to the environment.

3. ANALYTICAL METHODOLOGIES USED IN THE DETERMINATION OF PHARMACEUTICAL RESIDUES IN WATER AND OTHER COMPARTMENTS

The need for accurate, sensitive and routine use methodologies has become a remarkably issue when considering the analysis of pharmaceutical residues in the wild, since the low concentrations in which these compounds are expected make difficult the use of simple technologies. In order to allow the quantification of the majority of pharmaceuticals in waters to the ng/l range, Ternes (2001) summarized the existent knowledge regarding the analytical methodologies applicable to this area of research, taking into account the general characteristics of pharmaceutical drugs. The authors divided resi-

dues according to categories (*e.g.* acidic drugs, betablockers and â$_2$-sympathomimetics, neutral pharmaceuticals, antibiotics, iodinated X-ray contrast media, estrogens) and elaborated analytical protocols devoted to each of the mentioned categories. Techniques such as the combined use of using solid phase extraction (SPE), derivatization, detection and confirmation by gas chromatography /mass spectrometry (GC/MS) and GC/MS/MS or LC-electrospray tandem MS (LC-ES/MS/MS) have proven their efficacy. However, the authors state that the range of applicability of this analytical procedures might be critically reduced in the presence of heavily contaminated samples, such as sludge, and a clean-up step must be included (during sample preparation) or the use of a an appropriate surrogate standard has to be spiked prior to SPE enrichment. A similar methodology was also satisfactorily used for the assessment of the presence of antibiotics (*e.g.* penicillins, tetracyclines, sulfonamides and macrolid antibiotics) in water samples (Hirsch *et al*., 1998). The authors stated that the quantitation limit was of 50 ng/ l for the tetracyclines and 20 ng/ l for all other antibiotics, showing that the developed methodology could be considered sufficiently sensitive to cope with contamination by these specific chemical compounds.

Gas chromatography–mass spectrometry was also the methodology chosen by Koutsouba *et al*. (2003) for the assessment of the levels of contamination of sewage influents and effluents of Greek urban areas by polar pharmaceuticals. A similar methodology was also successfully developed and applied by Weigel *et al*. (2002), for the detection of several anthropogenic compounds (e.g. pharmaceuticals clofibric acid, diclofenac, ibuprofen, ketoprofen, propyphenazone) in seawater samples from the North Sea. Again, the versatility of GC – MS methodology was shown by Ferrari et al. (2003),

River sediment can also be accounted as a sink for large amounts of pharmaceutical drugs delivered into the environment. According to this principle, Löffler and Ternes (2003) developed analytical methodology for the assessment of pharmaceutical residues in river sediments. The developed methodology was similar to the already mentioned procedures, and allowed the detection of a large array of residues, such as antibiotics, acidic pharmaceuticals and parasiticides.

Ahrer *et al*. (2001) developed analytical methodology to be used in the assessment of environmental presence of pharmaceutical drugs, based on the combination of liquid chromatography (LC) or, alternatively, capillary electrophoresis (CE) with mass spectrometry (MS). These methodologies allowed the separation of several drugs, among each one could find paracetamol, clofibric acid, penicillin V, naproxen, bezafibrate, carbamazepine, diclofenac, ibuprofen and mefenamic acid. The applicability of the technique of liquid chromatography, combined with mass spectrometry, could be demonstrated for the analysis of several river water samples, in which concentrations between approximately 2 and 130 ng/l of bezafibrate, carbamazepine, diclofenac and mefenamic acid were found; similar concentrations, according to the authors, have been reported in previous studies assessing the presence of pharmaceutical in European river waters. The combinatory technique of capillary electrophoresis and mass spectrometry showed poorer detection limits than the detection limits obtained by HPLC–MS. This conclusion indicates that the developed methodology can be considered as a valuable

tool for the confirmation of questionable results obtained by HPLC–MS. HPLC was also the methodology selected by Hamscher *et al*. (2002), which determined the presence of persistent tetracyclines residues in soil fertilized with contaminated manure, employing methodology based on high performance liquid chromatography, combined with tandem mass spectrometry.

4. BIOLOGICAL EFFECTS OF DRUG RESIDUES ON TEST ORGANISMS – LABORATORY ASSAYS

4.1. DEATH AND IMMOBILIZATION ASSAYS

Cleuvers (2003) described the toxicity of ten human use pharmaceutical drugs (namely clofibrinic acid, carbamazepine, ibuprofen, diclofenac, naproxen, captopril, metformin, propranolol and metoprolol) to aquatic organisms, including the cladoceran *Daphnia magna*. The effects of the selected residues were quantified through the immobilization of exposed animals, after 24 and 28 hours. Heterogeneity of results among the distinct compounds was a common rule, but toxicity was generally moderate. However, among the tested compounds, the â-blocker propranolol was considered to be the most toxic drug, since it caused immobilization of 50% of the exposed population at a concentration of 7.5 mg/l. On the contrary, lowest toxicity was associated to the analgesic/anti-inflammatory drug naproxen, with an immobilization EC_{50} of 174 mg/l. In spite of the generic low toxicity associated to the selected drugs, the association of clofibric acid (metabolite of several fibrates, blood-lipid regulators) and the anti-epileptic compound carbamazepine showed that combination effects are much more pronounced. According to this conclusion, it was possible for the author to suggest that, if pharmaceutical residues are found in association in aquatic ecosystems, the overall toxicity of water samples must be determined in the complex mixtures, rather than considering isolated pharmaceutical residues. This approach would increase the efficacy of predicting the consequences and effects of pharmaceuticals detected together in the aquatic environment, through the quantification of additive, potentiation and/or synergic effects.

The review by Brooks *et al*. (2003) mentioned the use of several mortality-based assays for the assessment of the environmental toxicity of the pharmaceutical drug fluoxetine. The toxicity tests employed for the determination of the toxicity used several animal species (*e.g.* the crustaceans *Ceriodaphnia dubia* and *Daphnia magna*, and the fish *Pimephales promelas*). The reported LC50s were 234, 820, and 705 mg/l, for *C. dubia*, *D. magna* and *P. promelas*, respectively. In the same review, the authors established a relationship between the presence of fluoxetine and the enhancement of the mortality of sediment-exposed *Chironomus tentans*, in the evaluation of the toxicity of contaminated sediments.

Ferrari *et al*. (2003) tested several pharmaceutical drugs (carbamazepine, clofibric acid, and diclofenac) on the cladoceran species *Daphnia magna* and *Ceriodaphnia dubia*. The authors concluded that, after acute exposure, cladocerans proved to be relatively insensitive to clofibric acid, since the reported EC_{50} (concerning immobilization criterion) were greater than 200 mg/L. A similar trend was observed for carbamazepine, and a

ranking of toxicity was thus established: in decreasing order of toxicity, diclofenac > carbamazepine > clofibric acid. However, chronic exposure was responsible for significant modifications in this ranking of toxicity, since carbamazepine was shown to be the most toxic compound, followed by clofibric acid and diclofenac.

Acute exposure (7 days) of the freshwater cnidarian *Hydra vulgaris* to several environmental relevant concentrations the pharmaceutical drugs ibuprofen, acetylsalicylic acid, paracetamol, amoxicillin, bendroflumethiazide, furosemide, atenolol, diazepam, digoxin and amlodipine was not responsible for lethal effects, as observed by Pascoe *et al*. (2003). These results lead the authors to conclude that, generally, the ecological relevance of the obtained data was low, indicating that acute effects in the wild are not likely to occur.

4.2. ENDOCRINE EFFECTS

Brooks *et al*. published an extensive review article concerning the effects of exposure to the antidepressant fluoxetine on several aquatic organisms, and specially focused on endocrine effects caused by this pharmaceutical drug. Fluoxetine was reported to be implicated in the increase of fecundity of the amphipod *Hyalella azteca* ant the crustacean *Ceriodaphnia dubia*. The induction of fecundity reported for *H. azteca* was considered to be non-significant, and similar responses were previously reported in other studies using distinct test organisms, such as *Daphnia magna* and mussels. In the case of fish, the authors obtained data showing different responses. Japanese medaka (*Oryzias latipes*) exposed during 4 weeks to several concentrations of fluoxetine, did not exhibit significant changes concerning their reproduction features and physiology, in spite of an increase in female circulating estradiol levels. However, embryos resulting from exposed animals showed several abnormalities, such as edema, curved spine, incomplete development (no pectoral fins, reduced eyes), and non-responsiveness.

4.3. INHIBITION OF CULTURE GROWTH

As earlier mentioned in this review, Cleuvers (2003) described the toxicity of ten human use pharmaceutical drugs to aquatic organisms, including the chlorophyte *Desmodesmus subspicatus* and the macrophyte *Lemna minor*, using growth inhibition as end-point. The toxicity of the drug fluoxetine was also quantified using algal species (*e.g. Pseudokirchneriella subcapitata*) as test organism, as referred in the review by Brooks *at al*. (2003). The same algal species (*P. subcapitata*) was also employed by Ferrari *et al*. (2003) for the determination of the effects of the pharmaceutical drugs clofibric acid, carbamazepine and diclofenac, after 96 hours of exposure. The authors observed that this algal species was particularly sensitive to diclofenac.

4.4. NON-SPECIFIC EFFECTS

Anionic detergents, in spite of not being considered pharmaceutical drugs, are also part of the large array of compounds included in human use formulations, such as shampoos,

bubble-baths, shower gels, toothpastes and as adjuvants in pharmaceutical preparations (Sirisattha *et al*., 2004). Due to its continuous use and strong employment under varied forms, heavy loads of these anthropogenic compounds can be responsible for deleterious effects on environmentally exposed wildlife. According to this principle, Csheráti *et al*. (2002) reviewed the literature data in order to collect evidences pointing to the involvement of anionic detergent residues in biological effects on several distinct pathways, biological structures and species. Biological effects consequent to the exposure to detergents are non-specific, such as binding to various bioactive macromolecules, proteins, peptides and DNA. Detergents can also suffer insertion into cell fragments, such as phospholipid membranes, being responsible for general cellular function impairment.

Regeneration of polyps of *Hydra vulgaris* was one of the end-points selected in the study conducted by Pascoe el al. (2003), as an evidence of chemically induced pathological alterations. This end-point showed that organisms exposed for 72 hours to the pharmaceutical drugs diazepam, digoxin or amlodipine most failed to regenerate at concentrations as low at 10 µg/l of the mentioned drugs.

Metabolism of bacterial cells is also an useful end-point when assessing the effects of pharmaceutical contamination of the environment. Ferrari *et al*. (2003) used the microtox test system (containing the bacteria *Vibrio fischeri*) in order to determine the biological effects on microbial metabolism consequent to exposure to clofibric acid, carbamazepine and diclofenac. The selected methodology allowed observing that *V. fishery* was the most sensitive species to the drug diclofenac, among all tested organisms.

4.5. BEHAVIOURAL EFFECTS

The study by Uhler *et al*. (2000) showed that selective serotonin reuptake inhibitors (SSRIs) can exert behavioural alterations in non-target species, such as gastropods. In this study, the authors observed that the embryos of the freshwater gastropod species *Physa elliptica*, showed marked alterations in cilia-driven rotational movement. Among the tested compounds, the authors observed that the antidepressant drugs paroxetine and fluoxetine elicited a dose-dependant response, since these drugs caused an increase in rotation at 10^{-6} and 10^{-5} M but reduced rotation rate below that of baseline at 10^{-4} M.

The assessment of the impact of anthropogenic contamination on the behaviour of freshwater invertebrates is one of the most important tasks when considering pharmaceutical pollutants, since only a small amount of information is available concerning these specific environments. Chronic exposure of the cnidarian *Hydra vulgaris* to increasing concentrations of ibuprofen and acetylsalicylic acid caused a significant decrease in the number of ingested *Artemia* nauplii, as shown by Pascoe *et al*. (2003). However, the same pattern of response was not observed after chronic exposure to the drugs paracetamol, amoxicillin, bendroflumethiazide, furosemide, atenolol, diazepam, digoxin and amlodipine.

5. CONCLUSIONS

It is now possible to conclude that human use pharmaceuticals are not completely innocuous, and can even be responsible for the exertion of the deleterious effects here described. Furthermore, its presence in varied ecosystems has been systematically reported. The number of drugs found in the wild is an ever-increasing subject, and they have been found in previously unsuspected environments. Besides parent compounds, metabolic degradation products are also a matter of debate, since they are also responsible for biological effects. Sewage effluent, as referred, is a strong source of drugs, which cannot be neglected. Improvements in the efficacy of sewage treatment plants are urgently required, in order to cope with the vast amounts of chemical substances being delivered everyday, which are not transformed and not eliminated, during the process of water treatment.

The classical cycle of the human use pharmaceutical drugs involves several steps, such as scientific research in order to develop the drug molecule, the obtainance of a pharmaceutical dosage form in which the pharmaceutical drugs is included, the clinical testing phase, the distribution of the pharmaceutical to the market after approval by regulatory institutions and its ultimate administration to the patient. The cycle here mentioned does not include a thorough evaluation of the environmental toxicity inherent to the pharmaceutical drug *per se*, nor contemplates the definition of the normal expected profile of metabolites formed during biotransformation by the patient's organism. This change of paradigm is crucial to the understanding of the toxic mechanisms underlying not only the parent compound (pharmaceutical drug) but also the biological effects consequent to the metabolites when placed in real environmental conditions. Aquatic ecosystems are evidently more prone to toxic damage since they work as final dumping sites for the majority of human residues, including pharmaceutical drugs. According to the above mentioned toxicity data, it is now possible to delineate a battery of ecotoxicity testing methodologies for the evaluation of the impact consequent to the presence of drugs in the environment. This battery must involve i) the accurate determination of the amount of drug being delivered into the wild by human populations, ii) the profile of metabolites found in wastewater, iii) the amount of drugs being effectively degraded by sewage treatment plants, iv) the overall load of drugs entering the environment, after disposal, v) the biological effects on non-target species, including different level of biological organization of trophic levels, vi) the likely of occurrence of bioaccumulation and/or biomagnification along food chains and vii) the possibility of interference with human biochemical pathways, via food and drinking water.

REFERÊNCIAS BIBLIOGRÁFICAS

AHRER W, SCHERWENK E, BUCHBERGER W (2001). Determination of drug residues in water by the combination of liquid chromatography or capillary electrophoresis with electrospray mass spectrometry. *Journal of Chromatography A* 910: 69 – 78.

BROOKS BW, FORAN CM, RICHARDS SM, WESTON J, TURNER PK, STANLEY JK, SOLOMON KR, SLATTERY M, LA POINT TW (2003). Aquatic ecotoxicology of fluoxetine. *Toxicology Letters* 142: 169-183

CLEUVERS M (2003). Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicology Letters* 142: 185-194.

CSERHÁTI T, FORGÁCS E , OROS G (2002). Biological activity and environmental impact of anionic surfactants. *Environment International* 28 : 337– 348.

DAUGHTON CG, TERNES TA (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* 107: 907-938.

FERRARI B, PAXÉUS N, LO GIUDICE R, POLLIO A, GARRIC J (2003). Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. *Ecotoxicology and Environmental Safety* 55: 359 – 370.

HALLING-SORENSEN B, NORS NIELSEN S, LANZKY PF, INGERSLEV F, HOLTEN LÜTZHOFT HC, JORGENSEN SE (1998). Occurrence, fate and effects of pharmaceutical substances in the environment - A review. *Chemosphere* 36(2): 357-393.

HAMSCHER G, SCZESNY S, HÖPER H, NAU H (2002). Determination of Persistent Tetracycline Residues in Soil Fertilized with Liquid Manure by High-Performance Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry. *Analytical Chemistry* 74: 1509 - 1518.

HIRSCH R, TERNES TA, HABERER K, MEHLICH A, BALLWANZ F, KRATZ K-L (1998). Determination of antibiotics in different water compartments via liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A* 815: 213 – 223.

INGERSLEV F, HALLING-SØRENSEN B (2000). Biodegradability Properties of Sulfonamides in Activated Sludge. *Environmental Toxicology and Chemistry* 19 (10): 2467–2473.

JONES OAH, VOULVOULIS N, LESTER JN (2002). Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. *Water Research* 36: 5013–5022

KOUTSOUBA V, HEBERER T, FUHRMANN B, SCHMIDT-BAUMLER K, TSIPI D, HISKIA A (2003). Determination of polar pharmaceuticals in sewage water of Greece by gas chromatography–mass spectrometry. *Chemosphere* 51: 69 – 75.

KÜMMERER K (2001). Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere* 45: 957-969.

KÜMMERER K, ERBE T, GARTISER S, BRINKER L (1998). AOX-Emissions from Hospitals into Municipal Waste Water. *Chemosphere* 36 (11): 2437-245

LÖFFLER D, TERNES TA (2003). Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* 1021:133 – 144.

MCKELLAR QA (1997). Ecotoxicology and residues of anthelmintic compounds. *Veterinary Parasitology* 72: 413 - 435.

MIAO X-S, KOENIG BG, METCALFE CD (2002). Analysis of acidic drugs in the effluents of sewage treatment plants using liquid chromatography–electrospray ionization tandem mass spectrometry. *Journal of Chromatography A* 952: 139–147

OLSON H, BETTON G, ROBINSON D, THOMAS K, MONRO A, KOLAJA G, LILLY P, SANDERS J, SIPES G, BRACKEN W, DORATO M, VAN DEUN K, SMITH P, BERGER B, HELLER A (2000). Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. *Regulatory Toxicology and Pharmacology* 32: 56 – 67.

PASCOE D, KARNTANUT W, MÜLLER CT (2003). Do pharmaceuticals affect freshwater invertebrates? A study with the cnidarian *Hydra vulgaris*. *Chemosphere* 51: 521 – 528.

PETROVIÆ M, GONZALEZ S, BARCELÓ D (2003). Analysis and removal of emerging contaminants in wastewater and drinking water. *Trends in Analytical Chemistry* 22 (10): 685-696.

SIRISATTHA S, MOMOSE Y, KITAGAWA E, IWAHASHI H (2004). Toxicity of anionic detergents determined by *Saccharomyces cerevisiae* microarray analysis. *Water Research* 38: 61–70.

STEGER-HARTMANN T, KÜMMERER K, HARTMANN A (1997). Biological Degradation of Cyclophosphamide and Its Occurrence in Sewage Water. *Ecotoxicology and Environmental Safety* 36: 174–179.

TERNES TA (2001). Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. *Trends in Analytical Chemistry* 20 (8): 419 – 434.

TERNES TA, STUMPF M, MUELLER J, HABERER K, WILKEN R-D, SERVOS M (1999). Behavior and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. *The Science of the Total Environment* 225: 81 – 90.

TERNES T (1999). Drugs and hormones as pollutants of the aquatic environment: determination and ecotoxicological impacts. *The Science of the Total Environment* 225 1 – 2.

TERNES TA (1998). Occurrence of drugs in German sewage treatment, plants and rivers. *Water Research* 32 (11): 3245 - 3260.

UHLER GC, HUMINSKI PT, LES FT, FONG PP (2000). Cilia-Driven Rotational Behavior in Gastropod (*Physa elliptica*) Embryos Induced by Serotonin and Putative Serotonin Reuptake Inhibitors (SSRIs). *Journal of Experimental Zoology* 286: 414 – 421.

WEIGEL S, KUHLMANN J, HUHNERFUSS H (2002). Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. *The Science of the Total Environment* 295: 131 – 141.

WINKLER M, LAWRENCE JR, NEU TR (2001). Selective degradation of ibuprofen and clofibric acid in tow model river biofilm. *Water Research* 35(13): 3197–3205.