Environmental Research 182 (2020) 108992

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Survival and malformation rate in oocytes and larvae of *Cyprinus carpio* by exposure to an industrial effluent

Nely SanJuan-Reyes[a,**], Leobardo Manuel Gómez-Oliván[b,*], Ricardo Pérez-Pastén Borja[a], Marlenne Luja-Mondragón[b], José Manuel Orozco-Hernández[b], Gerardo Heredia-García[b], Hariz Islas-Flores[b], Marcela Galar-Martínez[c], Francisco Escobar-Huérfano[b]

[a] *Laboratory of Molecular Toxicology, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu, Esq. Calzada Manuel Stampa s/n, Del. Gustavo A. Madero, Ciudad de México, C.P. 07738, Mexico*

[b] *Laboratory of Environmental Toxicology, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón intersección Paseo Tollocan s/n, Col. Residencial Colón, Toluca, Estado de México, C.P. 50120, Mexico*

[c] *Laboratory of Aquatic Toxicology, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu, Esq. Calzada Manuel Stampa s/n, Del. Gustavo A. Madero, Ciudad de México, C.P. 07738, Mexico*

ARTICLE INFO

Keywords:
Pharmaceutical industry
Pharmaceuticals
NSAIDs
Toxicity
Malformations

ABSTRACT

Pharmaceuticals are used for the prevention or treatment of diseases, and due to their manufacturing process they are continuously released to water bodies. One of the pharmacological groups detected in aquatic environments is non-steroidal anti-inflammatory drugs (NSAIDs) at trace concentrations. This study evaluated the survival and malformation rate in oocytes and larvae of *Cyprinus carpio* (*C. carpio*) after exposure to different proportions of an industrial effluent. Initially, the industrial effluent was sampled from an NSAID manufacturing plant located in the city of Toluca, State of Mexico, subsequently the physicochemical characterization and determination of the concentration of chemical compounds present were carried out. On the other hand, the lethal concentration 50 (LC50) and the effective concentration 50 (EC50) were calculated to determine the teratogenic index (TI), as well as the alterations to the embryonic development and the teratogenic effects on oocytes and larvae of *C. carpio* at the following proportions of the industrial effluent: 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1%, following the Test Guideline 236, which describes a Fish Embryo Acute Toxicity test, the exposure times were 12, 24, 48, 72 and 96 h post-fertilization. The contaminants detected were NaClO (2.6 mg L^{-1}) and NSAIDs such as diclofenac, ibuprofen, naproxen and paracetamol in the range of 1.09–2.68 mg L^{-1}. In this study the LC50 was 0.275%, the EC50 0.133% and the TI 2.068. Several malformations were observed in all proportions of the industrial effluent evaluated, however the most severe such as spina bifida and paravertebral hemorrhage were observed at the highest effluent proportion. The industrial effluent evaluated in this study represents a risk for organisms that are in contact with it, since it contains chemical compounds that induce embryotoxic and teratogenic effects as observed in oocytes and larvae of *C. carpio*.

1. Introduction

Pharmaceuticals are used for the diagnosis, treatment or prevention of diseases and to restore, correct or modify physiological functions (Directive 2004/27/EC; Cherkis, 2013). Every year 4000 pharmaceutical products are marketed for human and animal care (Rehman et al., 2015), and they are continuously released to the environment, mainly as a result of improper disposal, metabolic excretion or manufacturing processes (Hernando et al., 2006). The pharmaceutical industry is one of the main contributors of wastewater as a result of the production process and cleaning of the machinery used, these waters can contain multiple chemical components such as organic solvents, catalysts, additives, reagents, intermediates, raw materials and active pharmaceutical ingredients (APIs) at different concentrations (Sreekanth et al., 2009). It is estimated that approximately half of the pharmaceutical wastewater produced worldwide is discarded without prior treatment (Lange et al., 2006; Enick and Moore, 2007). Although APIs are generally designed to be non-persistent, they can reach the water bodies due to the continuous emissions that are generated, being detected in different aquatic compartments at trace concentrations (ng L^{-1} to low

* Corresponding author.
** Corresponding author.
E-mail addresses: nesajure0905@gmail.com (N. SanJuan-Reyes), lmgomezo@uaemex.mx, lgolivan74@gmail.com (L.M. Gómez-Oliván).

https://doi.org/10.1016/j.envres.2019.108992
Received 19 October 2019; Received in revised form 30 November 2019; Accepted 30 November 2019
Available online 03 December 2019
0013-9351/

Table 1
Physicochemical characteristics of the industrial effluent.

Physicochemical characteristics	Industrial effluent evaluated	NOM-001-SEMARNAT-1996	NOM-073-ECOL-1994
Temperature (°C)	18.4	40	40
Dissolved oxygen (mg L^{-1})	10.1	NI	NI
Conductivity (μS cm^{-1})	138.7	NI	NI
pH	6.9	6.5–8.5	6–9
Chlorides (mg L^{-1})	124	250	NI
Fluorides (mg L^{-1})	4.4	0–15	NI
Hardness (mg L^{-1})	281.2	500	NI
Ammonia (mg L^{-1})	0.94	NI	NI
Total suspended solids (mg L^{-1})	25	60	150
Total phosphorus (mg L^{-1})	5.9	10	10
Total nitrogen (mg L^{-1})	12	25	NI
Biochemical oxygen demand (mg L^{-1})	47	60	100
NaClO (mg L^{-1})	2.6	NI	NI

NI = Not included in the official norm.

Table 2
Mortality and malformation data in oocytes of *Cyprinus carpio* exposed to industrial effluent.

Proportion of industrial effluent (%)	Number of embryos exposed	Mortality (%)	Malformations (%)
0 (control)	60	0	0
0.1	60	23.3	46.6
0.3	60	53.3	66.6
0.5	60	65.0	70.0
0.7	60	71.6	76.6
0.9	60	83.3	81.6
1.1	60	88.3	93.3
		LC50 = 0.275%	EC50 = 0.133%
		CI = [0.212%–0.335%]	CI = [0.067%–0.194%]
		Teratogenic index	
		2.068	

μg L^{-1}) (Hernando et al., 2006; Miller et al., 2018). The main APIs detected in water bodies are non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac (DCF), ibuprofen (IBP), naproxen (NPX) and paracetamol (PCT). These drugs have been consistently detected at higher concentrations compared to other APIs in different effluents from wastewater treatment plants (Metcalfe et al., 2003; Lishman et al., 2006). In Mexico these drugs have been authorized for sale without a prescription and therefore their consumption is increasing (Gómez-Oliván et al., 2009), nevertheless it is essential that they are used rationally. Several studies in Mexico have reported their presence in wastewater, surface water, ground water, and hospital effluents at the following concentrations: DCF (0.001–4.824 μg L^{-1}), IBP (0.015–4.51 μg L^{-1}), NPX (0.052–13.589 μg L^{-1}), PCT (0.0004–14.9 μg L^{-1}) (Siemens et al., 2008; Gibson et al., 2010; Félix-Cañedo et al., 2013; González-González et al., 2014; Neri-Cruz et al., 2015; Pérez-Alvarez et al., 2018; Rivera-Jaimes et al., 2018; Luja-Mondragón et al., 2019). The presence of APIs in the environment is due to their lipophilic and non-biodegradable nature, as well as their biological activity (Velagaleti and Burns, 2006). The global concern for its possible adverse effects on the environment is increasing as it has been shown to affect biota (Andersson and Hughes, 2014). Biomarkers allow to evaluate the effects generated by exposure to a xenobiotic or a mixture of them (Celander, 2011; Hernández et al., 2014, 2019), such is the case of the Fish Embryo Acute Toxicity (FET) test. FET allows to determine the acute or lethal toxicity of chemical products in the embryonic stages of fish (OECD, 2013; Braunbeck et al., 2015). Acute toxicity tests with non-target sensitive species are an integral part of the identification of environmental hazards and the risk assessment of chemical compounds including APIs (Scholz et al., 2013; Bebianno and Gonzalez-Rey, 2015). There are studies that demonstrate the embryotoxic and teratogenic potential of NSAIDs (particularly DCF) in various aquatic species such as *Xenopus laevis*, *Lithobates catesbeianus* (Cardoso-Vera et al., 2017) and common carp *Cyprinus carpio* (Stepanova et al.,

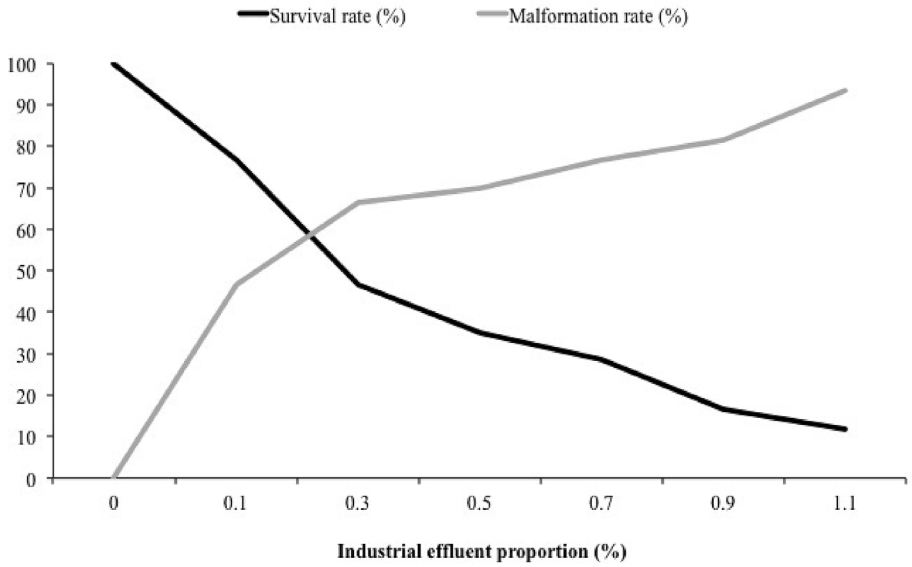

Fig. 1. Survival and malformation rate in oocytes and larvae of *C. carpio* according to industrial effluent exposure.

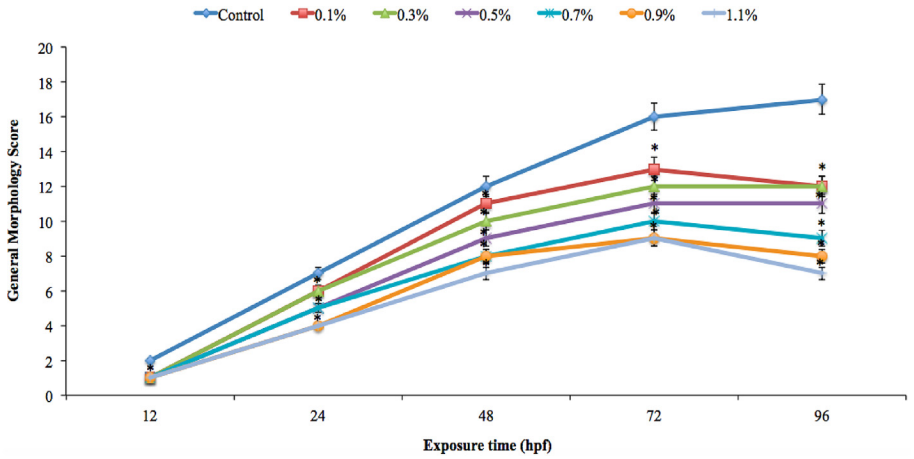

Fig. 2. Exposure time-response curve of the general morphology score according to different proportions of industrial effluent on oocytes and larvae of *C. carpio*. Values are the mean of three replicates ± SEM. *Significantly different from control group.

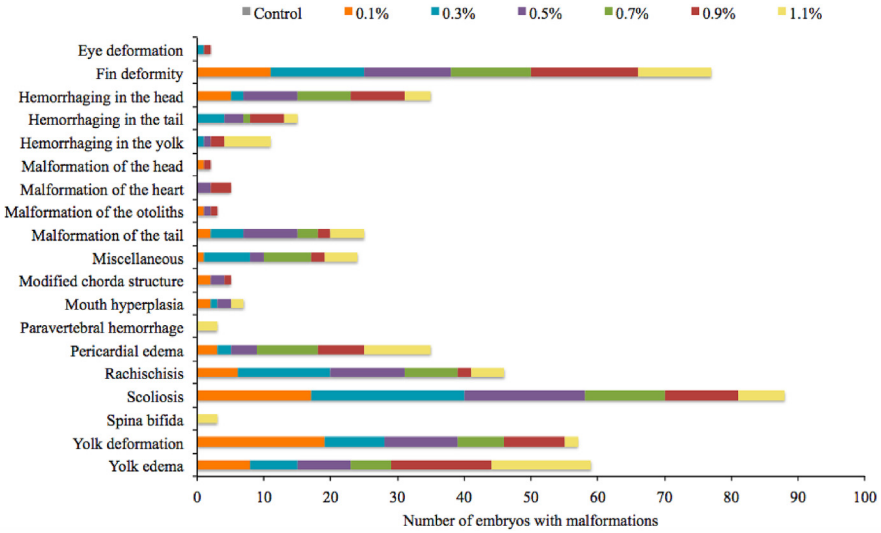

Fig. 3. Teratogenic malformations induced by exposure to industrial effluent for 96 h at the following proportions: 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1%, on *C. carpio*.

2013). In an exposure assessment study conducted by Letzel et al. (2009) determined the specific DCF load that reaches a receiving body of water in Germany, the average per capita per day was 0.28 mg, this study also concludes that at environmentally relevant concentrations (ng L^{-1}), DCF may cause chronic adverse effects in fish populations. In addition, there is evidence that the accumulation of several pollutants (including APIs) in aquatic environments significantly decreases fish populations (Hamilton et al., 2016), such as *Salmo trutta* (Borsuk et al., 2006), salmonids in general (Parrish et al., 1998; Kroglund and Finstad, 2003), in *Anguilla anguilla* (Castonguay et al., 1994a, b; Dekker, 2003), and in European estuarine and coastal fish species (Matthiessen and Law, 2002). FET has been widely used in zebrafish to assess the teratogenic potential of different xenobiotics (Batel et al., 2018; Grünspan et al., 2018; Yao et al., 2018). Nevertheless, there are few studies in other fish species such as common carp (Luja-Mondragón et al., 2019; Pérez-Coyotl et al., 2019). *C. carpio* is grown in more than 100 countries worldwide and represents up to 10% of global annual freshwater aquaculture production (FAO, 2007; Bostock et al., 2010), this organism has a high value as a food source (Xu et al., 2014). Therefore, the present study aimed to evaluate the survival and malformation rate in oocytes and larvae of *C. carpio* after exposure to different proportions of an industrial effluent.

2. Materials and methods

2.1. Sampling of the industrial effluent and physicochemical characterization

The effluent was sampled from an NSAIDs manufacturing plant in Toluca, State of Mexico, which does not have a wastewater treatment plant. It is important to mention that the effluents generated by the pharmaceutical industry of this study reach the Lerma River, this river is part of the Lerma-Chapala-Santiago hydrological system that is one of the most important in Mexico and is particularly distinctive in its endemism of freshwater fish (*Algansea barbata, Chirostoma riojai, Goodea atripinnis, Notropis sallei, Girardinichthys multiradiatus, Poeciliopsis infans*). *C. carpio* is an introduced species and inhabits this aquatic environment (CONAGUA, 2011). The Lerma River is one of the most polluted rivers in Mexico. Its main sources of pollutants are industrial and municipal wastewater, which has seriously affected water quality, causing the extinction of several native species of fauna and flora (Sedeño-Díaz and López-López, 2007).

The methodology described in NMX-AA-003-1980 was followed, which establishes general guidelines and recommendations for sampling wastewater discharges. The samples were taken from the output of the production area, which is directly connected to the drainpipe exiting plant, were collected in 20-L polyethylene containers previously

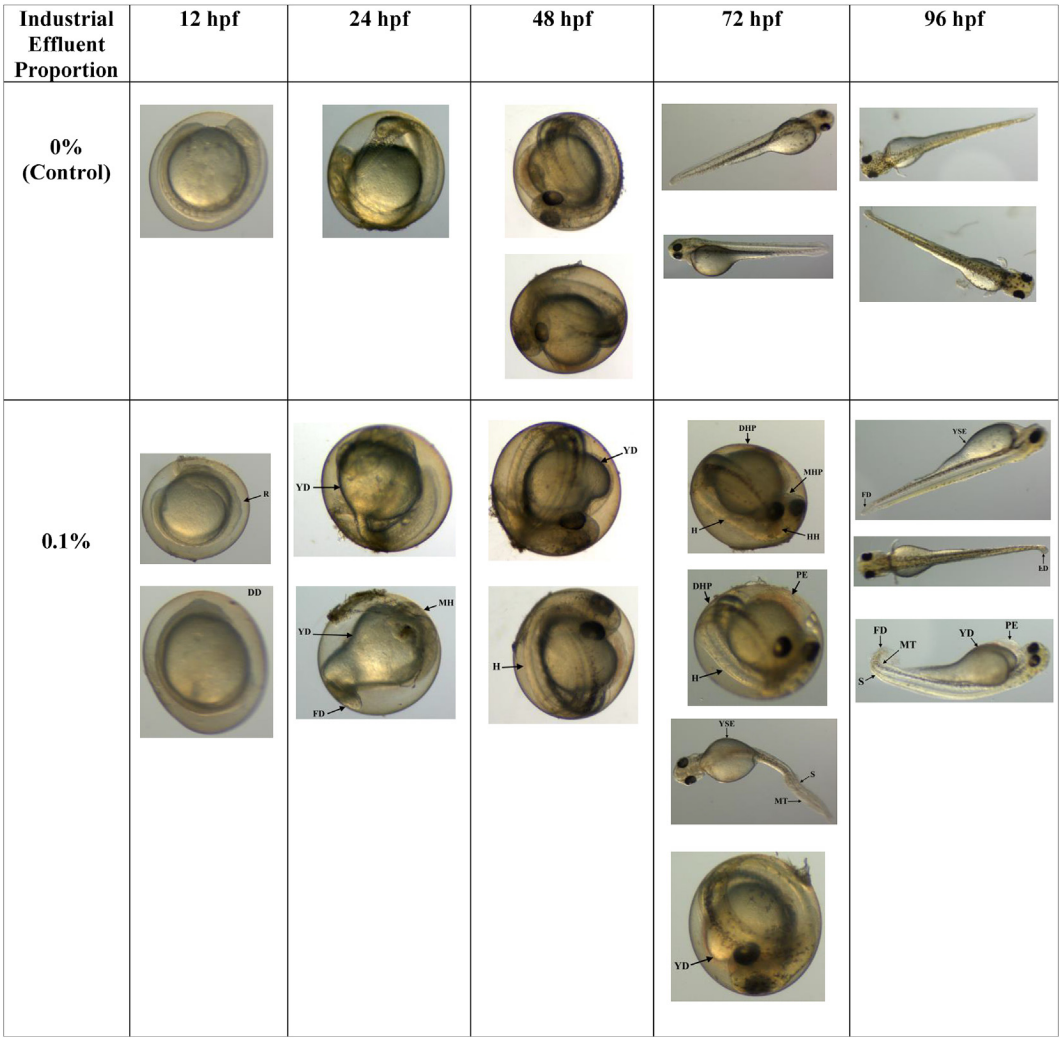

Fig. 4. Representative malformations induced by exposure to industrial effluent at 12, 24, 48, 72 and 96 hpf, on *C. carpio*. Abbreviations: **DD** = Developmental Delay, **DHP** = Delay in the Hatching Process, **ED** = Eye Deformation, **FD** = Fin Deformity, **H** = Hypopigmentation, **HH** = Hemorrhaging in the Head, **HT** = Hemorrhaging in the Tail, **HY** = Hemorrhaging in the Yolk, **M** = Miscellaneous, **MCS** = Modified Chorda Structure, **MH** = Malformation of the Head, **MHE** = Malformation of the Heart, **MHP** = Mouth Hyperplasia, **MO** = Malformation of the Otoliths, **MT** = Malformation of the Tail, **PE** = Pericardial Edema, **PH** = Paravertebral Hemorrhage, **R** = Rachischisis, **S** = Scoliosis, **SB** = Spina Bifida, **YD** = Yolk Deformation, **YSE** = Yolk Edema.

washed with nitric acid (Sigma-Aldrich) 30% and rinsed with water deionized. Samples were labeled, protected from light, and immediately transported to the lab and were stored at 4 °C. The physicochemical characteristics were determined according to official Mexican standards NOM-001-SEMARNAT-1996 and NOM-073-ECOL-1994. The official Mexican norms set the maximum permissible levels of contaminants in wastewater discharges arising in the pharmaceutical and pharmacochemical industries and entering, respectively, domestic waters and resources, and receiving water bodies.

2.2. Extraction of analytes

Water samples (5 mL) were taken directly from the containers with the industrial effluent and collected in glass vials and refrigerated at 4 °C for subsequent determination of concentrations. The samples were vacuum-filtered through 10-μm GF/C glass microfiber filters, followed by 0.45-μm nylon membrane filters (Whatman, Cambridge, UK). A liquid–liquid extraction with 5 mL (1:1, v/v) hexane/ethyl acetate was performed to extract DCF, IBP, NPX and PCT from 1-mL water samples. These samples were centrifuged at 1800 × *g* for 10 min and the upper organic layer was extracted again. This extraction was repeated and organic layers were combined and evaporated to dryness. The procedure was carried out in quintuplicate.

2.3. Quantification of NSAIDs by liquid chromatography–tandem mass spectrometry (LC–MS/MS)

The quantification of NSAIDs from the industrial effluent was carried out following the methodology described by SanJuan-Reyes et al. (2015). Chromatographic analysis was performed on an Agilent 1290 Infinity HPLC unit (Santa Clara, CA), a RRHD Eclipse Plus C18 column (2.1 × 50 mm i.d.; 1.8-μm particle size) was used for the chromatographic separation. Los NSAIDs were quantified on an Agilent 6430 Triple Quadrupole MS equipped with electrospray ionization (ESI) positive mode.

The method DL (MDL) and method QL (MQL) were defined and determined as the minimum detectable amount of DCF, IBP, NPX, and PCT with a signal to noise (S/N) ratio of 3 and 10 for level of detection (LOD) and level of quantification (LOQ), respectively. The data for each API were: 27 and 90 ng L^{-1} for DCF; 30 and 89 ng L^{-1} for IBP; 21 and 76 ng L^{-1} for NPX; 28 and 92 ng L^{-1} for PCT.

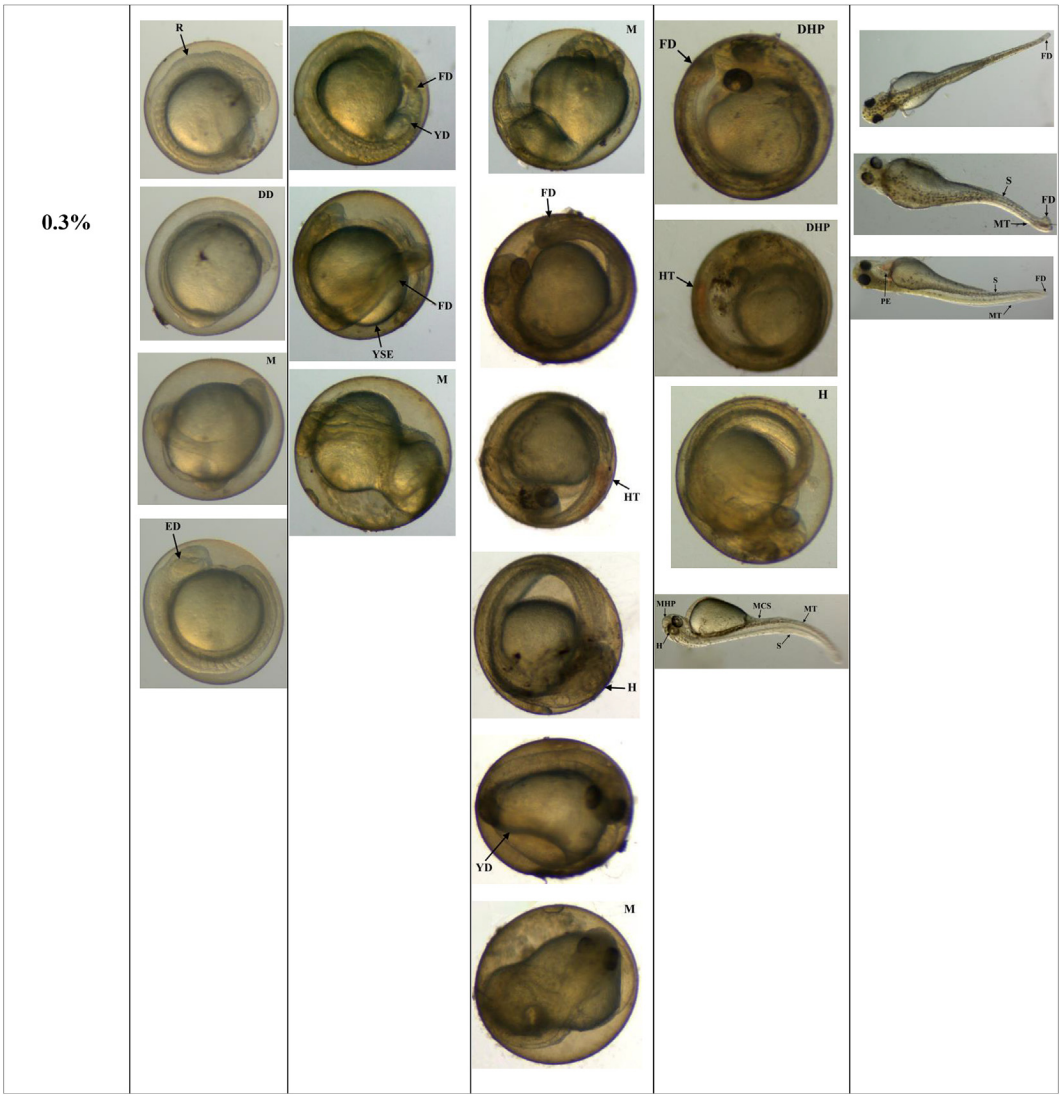

Fig. 4. (*continued*)

2.4. Obtaining and selecting oocytes

Oocytes were obtained by natural fertilization from *C. carpio*-breeding center in Tiacaque, State of Mexico. The fertilization process was carried out *in situ* and two adult females and four adult males were placed in reproductive stages within a fertilization pond with a water level of two-thirds of their capacity. The average size and weight of the breeding fish was 40 cm and 5 kg, respectively. Once spawning and fertilization were done, the parent fish were immediately removed to avoid damage to the eggs that have been laid. Eggs were collected with an adequate size mesh screen, rinsed with Dutch Standard Water [DSW; demineralized water supplemented with $NaHCO_3$ (100 mg L^{-1}), $KHCO_3$ (20 mg L^{-1}), $CaCl_2.2H_2O$ (200 mg L^{-1}), and $MgSO_4.7H_2O$ (180 mg L^{-1}) and then aerated for 24 h] (Hermsen et al., 2011), and were observed under a stereoscopic microscope and only the fertilized eggs were selected [blastula stage; 2 h post-fertilization (hpf)], for subsequent exposure to different proportions of the industrial effluent.

2.5. Tests of embryolethality, embryotoxicity and teratogenesis

These analyzes were carried out in the Toxicology laboratory of the Autonomous University of the State of Mexico. Organisation for Economic Co-operation and Development (OECD) test guideline (236) for fish embryo acute toxicity (FET) was followed. It determines the acute toxicity of chemicals to embryonic fish. Tests were performed as follows: twenty embryos at 2 hpf were randomly selected, for each proportion of the industrial effluent (0.1, 0.3, 0.5, 0.7, 0.9 and 1.1%), a control group was also included. The embryos were placed in sterile flat bottom microplates of 24 wells (1 embryo/well) (Costar, Corning Incorporated, USA), each well contained 2.5 mL of the proportion of the industrial effluent. The microplates were maintained at 24 ± 1 °C with a natural photoperiod of 12:12 h of light/darkness. The developing eggs were observed at 12, 24, 48, 72 and 96 hpf. The tests were carried out in triplicate, in a static environment without renewal. The concentration of dissolved oxygen in the control group and in the different proportions of the industrial effluent was monitored at all exposure times and was greater than 85% of saturation. A Zeiss Stemi 305 stereoscopic microscope coupled with a camera was used for the observations at all exposure times. The embryos and larvae were photographed. The mortality in all negative control groups was less than 10%.

Embryos and larvae were counted after 96 hpf, considering lethality when the oocytes coagulated, or no heartbeat was detected. At the end of these acute toxicity experiments, the LC50 was estimated with its 95% confidence limits ($p < 0.05$).

All morphological transformations occurring during embryonic and larval development were observed. Alterations in embryonic development were recorded according to the scoring system established by Luja-Mondragón et al. (2019), at the specified test times (12, 24, 48, 72

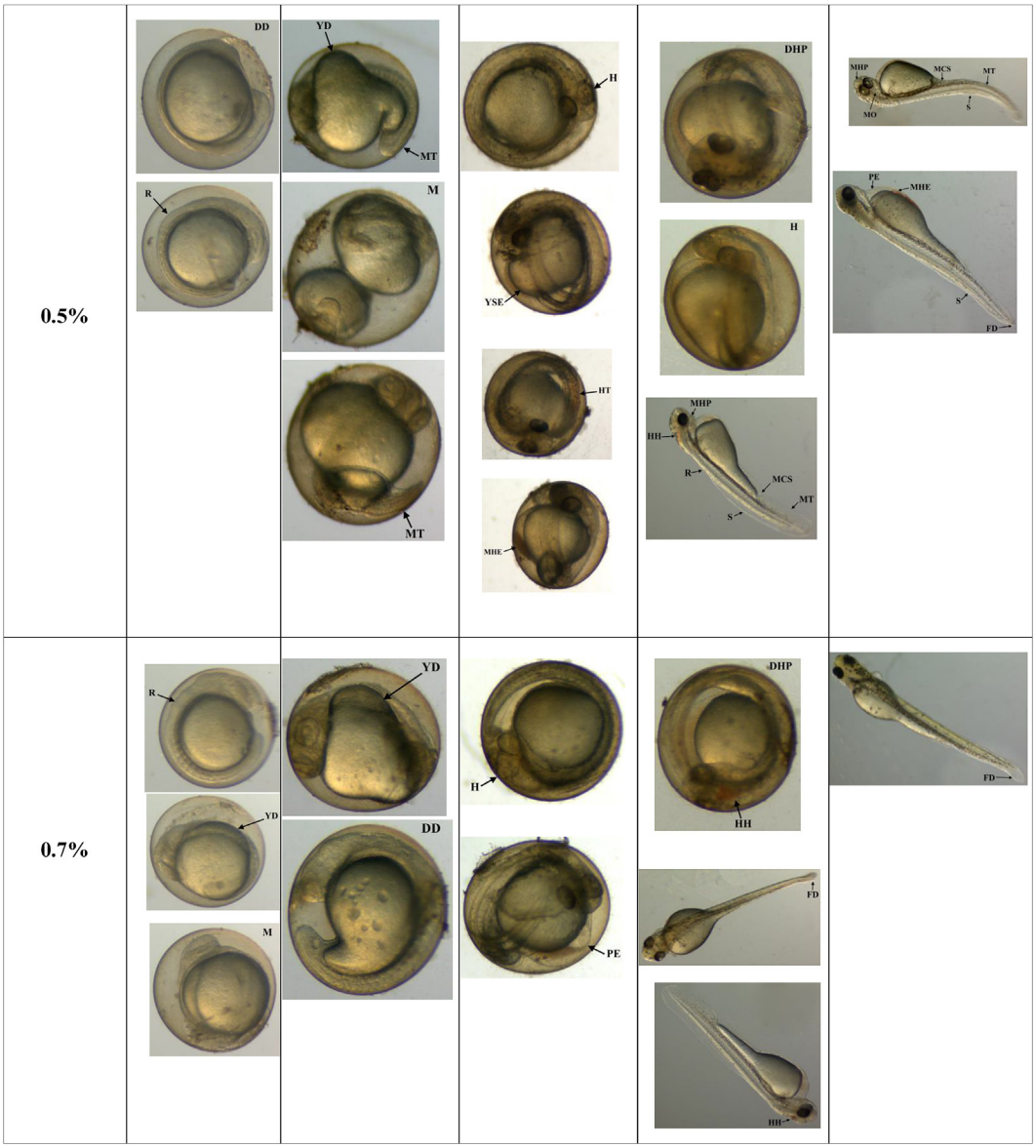

Fig. 4. (*continued*)

and 96 hpf). It is important to mention that dead embryos were not scored. Ultimately, a frequency histogram was created with the teratogenic malformations identified.

The test was considered valid since the control group showed no more than 10% of teratogenic effects at 96 hpf.

2.6. Data analysis

Initially, the mean lethal concentration (LC_{50}) and the effective concentration inducing 50% malformation (EC_{50}) were determined by a probit analysis (EPA Analysis Program v1.5), with the aim of assessing acute toxicity. Teratogenic index (TI) was obtained through the following equation: $TI = (LC_{50})/(EC_{50})$.

Continuous data were analyzed using one-way analysis of variance (ANOVA), with Bonferroni's *post hoc* test for the analysis of homogeneous data. For the analysis of non-homogeneous data, the Kruskal-Wallis test was used. Finally, with the Fisher's exact test, the frequency of abnormal oocytes or embryos was evaluated. Significance was accepted when $p < 0.05$, using the IBM SPSS Statistics 25.

3. Results

3.1. Physicochemical characterization of the industrial effluent

The physicochemical characteristics of the industrial effluent are shown in Table 1. However, with the exception of dissolved oxygen, conductivity, ammonium, and NaClO, since they are not contemplated by official Mexican norms (NOM-001-SEMARNAT-1996 and NOM-073-ECOL-1994), all the remaining parameters do not exceed the limits established by both norms.

3.2. Quantification of NSAIDs

The results of the quantification of NSAIDs are the following: DCF (1.82 ± 0.03 mg L^{-1}), IBP (2.27 ± 0.05 mg L^{-1}), NPX (1.09 ± 0.02 mg L^{-1}) and PCT (2.68 ± 0.05 mg L^{-1}).

3.3. Embryolethality and teratogenicity data of the industrial effluent evaluated

Table 2 shows the mortality percentages obtained for *C. carpio*

Fig. 4. (*continued*)

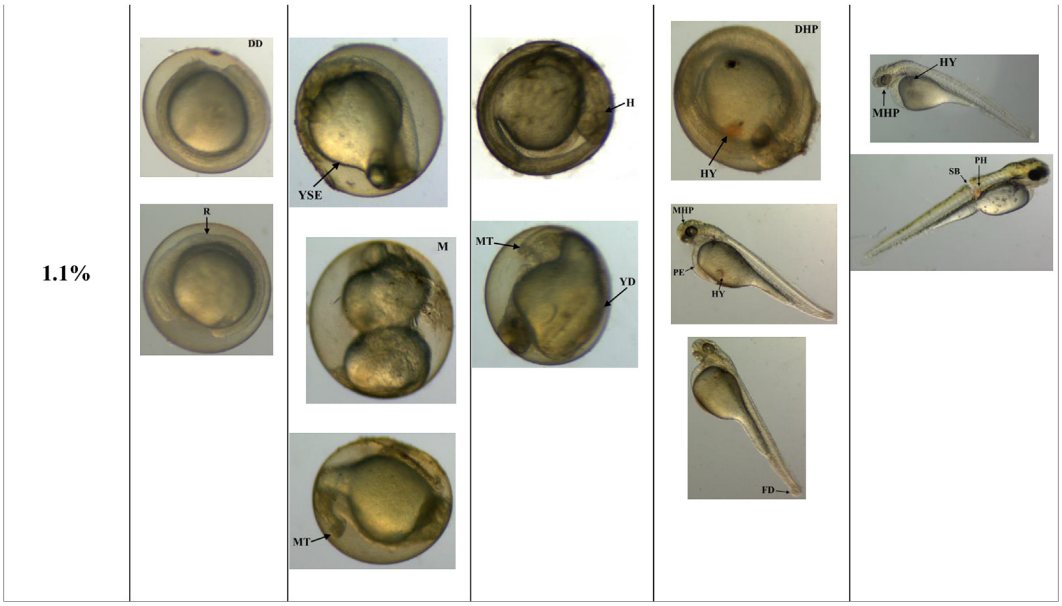

Fig. 4. (*continued*)

exposed to the different proportions of the industrial effluent. In addition, Fig. 1 shows that as the proportion of the industrial effluent increases, the malformation rate also increases while the survival rate decreases. The LC50 value was 0.275% (95% CI: 0.212%–0.335%). On the other hand, the percentages of malformations are also shown, which were used to estimate the EC50, the value was 0.133% (95% CI: 0.067%–0.194%). With the previous data, TI was determined, which was 2.068 in this study. This value indicates that the industrial effluent evaluated in this study is teratogenic.

3.4. Evaluation of embryos

In Fig. 2, the score obtained for each of the proportions of industrial effluent at the different hpf is observed. A significant decrease

($p < 0.05$) can be observed in the score with respect to the control group as the proportions of the industrial effluent increased at all hpf. In the case of 0.1% the decrease was 20%, for the proportion of 0.3% it was 24%, in 0.5% of 31%, for the case of 0.7% it was 39%, for the proportion 0.9% it was 44% and finally for 1.1% a decrease of 48% was observed.

Fig. 3 shows the frequency of the teratogenic alterations observed in the oocytes and larvae of *C. carpio* exposed to the different proportions of the industrial effluent. The most frequent were: fin deformity, rachischisis, scoliosis, yolk deformation and yolk edema. The most severe alterations such as spina bifida and paravertebral hemorrhage were observed at the highest concentration of exposure (1.1%).

Fig. 4 illustrates the teratogenic alterations observed in the oocytes and larvae of *C. carpio*. The control is included where the normal development of the common carp is observed at all times of exposure. However, after 12 hpf developmental alterations begin to be observed, in all the evaluated proportions of the industrial effluent.

4. Discussion

Water is indispensable in pharmaceutical manufacturing operations. The wastewater that leaves the pharmaceutical industries varies in content and concentration of pollutants, it is estimated that approximately half of the pharmaceutical wastewater produced worldwide is discarded without specific treatment (Gadipelly et al., 2014). In this study, the evaluated effluent comes from a pharmaceutical industry that has an NSAIDs manufacturing plant and does not have a wastewater treatment plant, therefore its waste goes directly to the municipal sewer. In Mexico there are two official norms that regulate the discharge of wastewater in the aquatic environment (NOM-001-SEMARNAT-1996 and NOM-073-ECOL-1994), but none of them contemplates the presence of APIs. The physicochemical characterization allows to evaluate the quality of the water and to be able to predict the impact that it will have on the aquatic ecosystem. In this study, the physicochemical characteristics of the industrial effluent were determined, which were within the limits established by both norms, with the exception of dissolved oxygen, conductivity, ammonium, and NaClO, since they are not contemplated by official Mexican norms. Dissolved oxygen represents the concentration of chemical or biological compounds that can oxidize and may have potential for contamination (Popa et al., 2012). The conductivity allows to know the concentration of ionic species present in water, such as ammonium, which is the ionized form of ammonia. The presence of chlorinated compounds in water containing ammonium favors the formation of chloramines (Hahn-Schlam et al., 2006), which are highly oxidizing molecules. Chloramines have been shown to form protein-DNA crosslinks (Kulcharyk and Heinecke, 2001). This could contribute to the damage generated in *C. carpio* embryos due to exposure to industrial effluent. In addition, the teratogenic effect of NaClO has been demonstrated in other aquatic species such as *Daphnia magna* (Ton et al., 2012). The toxic effects of this compound have been related to the generation of reactive oxygen species (ROS), which are produced by the disturbance of different isoforms of cytochrome P450 (Sapone et al., 2007). The effluent analysis showed the presence of NSAIDs such as DCF, IBP, NPX and PCT at concentrations of mg L^{-1}. These pharmaceuticals have also been detected in other industrial effluents at concentrations of ng L^{-1} to μg L^{-1} (Lin et al., 2008; Collado et al., 2014; Scott et al., 2018). The Food and Drug Administration (FDA) requires an environmental evaluation of APIs when the concentration is equal to or greater than 1 μg L^{-1} in the aquatic environment (FDA-CDER, 1998). In addition, according to the European Regulatory Guide established by the European Medicines Agency, the approval procedure for new human pharmaceutical products requires an environmental risk assessment if the concentration of APIs is > 10 ng L^{-1} (EMEA, 2006). LC50 at 96 hpf in this study was 0.275%, Carlsson et al. (2009), reports an LC50 between 2.7 and 8.1% at 144 hpf in zebrafish (*Danio rerio*) due to exposure to an industrial effluent. The occurrence of NSAIDs in the environment has become a topic of great concern due to their potential ecotoxicity in the environment, since they negatively affect aquatic and terrestrial organisms at different trophic levels (Pounds et al., 2008; Gonzalez-Rey and Bebianno, 2014). Further, there are studies that show that the threat of chemical contamination for fish populations is more acute in freshwater environments (Hamilton et al., 2016), which significantly reduces fish populations. The biotransformation of NSAIDs by CYP2C9 generates ROS such as the hydroxyl radical (OH•), and the superoxide anion (O_2•) (Široká and Drastichova, 2004; Uno et al., 2012), as well as hydroxylated metabolites that are even more toxic than the original molecule (Islas-Flores et al., 2014; SanJuan-Reyes et al., 2015). Oxidative stress (OS) occurs when the ROS generation rate exceeds that of their removal, generating deleterious effects such as the oxidation of lipids, proteins, and DNA (Martínez-Álvarez et al., 2005), apoptosis, and embryonic development delays (Pašková et al., 2011). The latter was observed in this study at all proportions of the industrial effluent evaluated. In previous studies, our research group has demonstrated the oxidative damage generated in *C. carpio* after exposure to different NSAIDs as well as by exposure to an industrial effluent (Islas-Flores et al., 2013, 2014, 2017; SanJuan-Reyes et al., 2013, 2015). OS is associated in the pathogenesis of a broad spectrum of defects, including skeletal malformations, and cardiovascular defects (Kovacic and Somanathan, 2014). ROS have an important role in embryonic development, since this may be adversely affected by the reversible reaction of ROS with transduction proteins, thereby altering embryonic signal transduction pathways (Wells et al., 2005; Dennery, 2007). In addition, our results of embryotoxicity and teratogenicity can be explained by the mechanism of action of NSAIDs, through the inhibition of cyclooxygenase (COX-1 or COX-2), which is effected by inhibiting prostaglandin (PG) synthesis (Brausch et al., 2012). It has been shown that PGs are involved in reproduction, as well as in the immune and circulatory systems, and finally in osteo- and chondrogenesis (Peltzer et al., 2019). In this study, alterations at the level of the central nervous system, heart and bone were observed mainly. These alterations may not only affect embryonic survival, but may potentially affect later life-stage fitness parameters (Rangasamy et al., 2018). Inhibition of COX-1 generates a defective formation of the vascular tuve, shortened intersomitic vessels, and causes growth arrest because COX-1 derived prostaglandins are necessary during gastrulation and segmentation periods (Cha et al., 2005, 2006). In this study, hatching delay (72 hpf) was also observed, it has been reported that hatching is mediated by COX-2 derived prostacyclines (Huang et al., 2004). The delay in hatching may be due to the inhibition of the hatching enzyme chorionase, as well as osmotic disorders that interfere with the activity of the hatching enzyme, increased oxygen consumption by embryos/larvae and finally due to behavioral deficits that result in a weakened spontaneous muscle movement (Strmac et al., 2002; Haendel et al., 2004).

Our results are consistent with other authors who have demonstrated the effects of NSAIDs on different aquatic species, such is the case of IBP that generates developmental delay, cardiac anomalies, pectoral fin malformation, decreased hatching rate and growth, spinal curvature, and behavioral alterations in *D. rerio* at concentrations greater than 10 μg L^{-1} (David and Pancharatna, 2009). On the other hand, DCF (1 and 10 μg L^{-1}) affects the early stages of embryonic development of *M. galloprovincialis* (Balbi et al., 2018). In *Xenopus* alterations of the axis, gut, heart, head and eyes were observed, as well as blistering (edema), after exposure to various concentrations of DCF (1 mg L^{-1} to 64 mg L^{-1}) (Chae et al., 2015). NPX (10, 50, 100 and 200 μg L^{-1}) affect early developmental stages including eclosion and growth in *C. carpio* (Sehonova et al., 2017). Finally, it has been shown that PCT (1, 5, 10, 50 and 100 mg L^{-1}) induces alterations in *D. rerio* as developmental delay and hatching, tail and fin malformations, as well as changes in pigmentation, larval behavior and survival (David and Pancharatna, 2009).

5. Conclusions

The industrial effluent evaluated in this study contained as main contaminants NaClO and NSAIDs, these generated embryotoxicity and teratogenicity in *C. carpio*. The main alterations were: fin deformity, rachischisis, scoliosis, yolk deformation and yolk edema. The most severe alterations such as spina bifida and paravertebral hemorrhage were observed at the highest effluent proportion. The use of effect biomarkers based on toxicity endpoints induced by individual chemicals in a mixture could be a useful tool for predicting and preventing the risk of toxicity.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

We give thanks to Biologist Gerardo Ontiveros at the Centro Carpícola Tiacaque, Estado de México.

References

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12, 465–478.
Balbi, T., Montagna, M., Fabbri, R., Carbone, C., Franzellitti, S., Fabbri, E., Canesi, L., 2018. Diclofenac affects early embryo development in the marine bivalve *Mytilus galloprovincialis*. Sci. Total Environ. 642, 601–609.
Batel, A., Borchert, F., Reinwald, H., Erdinger, L., Braunbeck, T., 2018. Microplastic accumulation patterns and transfer of benzo [a] pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. Environ. Pollut. 235, 918–930.
Bebianno, M.J., Gonzalez-Rey, M., 2015. Ecotoxicological risk of personal care products and pharmaceuticals. In: Aquatic Ecotoxicology. Academic Press, pp. 383–416.
Borsuk, M.E., Reichert, P., Peter, A., Schager, E., Burkhardt-Holm, P., 2006. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. Ecol. Model. 192, 224–244.
Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., Corner, R., 2010. Aquaculture: global status and trends. Philos. Trans. R. Soc. B: Biol. Sci. 365, 2897–2912.
Braunbeck, T., Kais, B., Lammer, E., Otte, J., Schneider, K., Stengel, D., Strecker, R., 2015. The fish embryo test (FET): origin, applications, and future. Environ. Sci. Pollut. Res. 22, 16247–16261.
Brausch, J.M., Connors, K.A., Brooks, B.W., Rand, G.M., 2012. Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. In: Reviews of Environmental Contamination and Toxicology, vol. 218. Springer, pp. 1–99.
Cardoso-Vera, J.D., Islas-Flores, H., SanJuan-Reyes, N., Montero-Castro, E.I., Galar-Martínez, M., García-Medina, S., Elizalde-Velázquez, A., Dublán-García, O., Gómez-Oliván, L.M., 2017. Comparative study of diclofenac-induced embryotoxicity and teratogenesis in *Xenopus laevis* and *Lithobates catesbeianus*, using the frog embryo teratogenesis assay: *Xenopus* (FETAX). Sci. Total Environ. 574, 467–475.
Carlsson, G., Örn, S., Larsson, D.J., 2009. Effluent from bulk drug production is toxic to aquatic vertebrates. Environ. Toxicol. Chem. 28, 2656–2662.
Castonguay, M., Hodson, P.V., Couillard, C.M., Eckersley, M.J., Dutil, J.D., Verreault, G., 1994a. Why is recruitment of the American eel, *Anguilla rostrata*, declining in the St. Lawrence River and Gulf? Can. J. Fish. Aquat. Sci. 51, 479–488.
Castonguay, M., Hodson, P.V., Moriarty, C., Drinkwater, K.F., Jessop, B.M., 1994b. Is there a role of ocean environment in American and European eel decline? Fish. Oceanogr. 3, 197–203.
Celander, M.C., 2011. Cocktail effects on biomarker responses in fish. Aquat. Toxicol. 105, 72–77.
Cha, Y.I., Kim, S.H., Solnica-Krezel, L., DuBois, R.N., 2005. Cyclooxygenase-1 signaling is required for vascular tube formation during development. Dev. Biol. 282, 274–283.
Cha, Y.I., Solnica-Krezel, L., DuBois, R.N., 2006. Fishing for prostanoids: deciphering the developmental functions of cyclooxygenase-derived prostaglandins. Dev. Biol. 289, 263–272.
Chae, J.P., Park, M.S., Hwang, Y.S., Min, B.H., Kim, S.H., Lee, H.S., Park, M.J., 2015. Evaluation of developmental toxicity and teratogenicity of diclofenac using *Xenopus embryos*. Chemosphere 120, 52–58.
Cherkis, J., 2013. Andrea Sloan Faces Pharma Firm with History of Indifference. Huffington Post, 26 September.
Collado, N., Rodriguez-Mozaz, S., Gros, M., Rubirola, A., Barceló, D., Comas, J., Rodriguez-Roda, I., Buttiglieri, G., 2014. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environ. Pollut. 185, 202–212.
David, A., Pancharatna, K., 2009. Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, *Danio rerio*. J. Appl. Toxicol. 29, 597–602.
Dekker, W., 2003. Did lack of spawners cause the collapse of the European eel, *Anguilla anguilla*? Fish. Manag. Ecol. 10, 365–376.
Dennery, P.A., 2007. Effects of oxidative stress on embryonic development. Birth Defects Res. Part C Embryo Today - Rev. 81, 155–162.
Directive 2004/27/EC, 2004. Off. J. Eur. Union 30, L136 April.
EMEA, 2006. European Medicines Agency pre-authorization. Evaluation of Medicines for Human Use. Committee for Medicinal Products for Human Use (CHMP), pp. 12 (Doc. Ref. EMEA/CHMP/SWP/4447/00).
Enick, O.V., Moore, M.M., 2007. Assessing the assessments: pharmaceuticals in the environment. Environ. Impact Assess. Rev. 27, 707–729.
FAO Fisheries and Aquaculture Department, 2006. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome.
FDA-CDER, 1998. Guidance for Industry-Environmental Assessment of Human Drugs and Biologics Applications, Revision 1. FDA Center for Drug Evaluation and Research, Rockville.
Félix-Cañedo, T.E., Durán-Álvarez, J.C., Jiménez-Cisneros, B., 2013. The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources. Sci. Total Environ. 454, 109–118.
Gadipelly, C., Pérez-González, A., Yadav, G.D., Ortiz, I., Ibáñez, R., Rathod, V.K., Marathe, K.V., 2014. Pharmaceutical industry wastewater: review of the technologies for water treatment and reuse. Ind. Eng. Chem. Res. 53, 11571–11592.
Gibson, R., Durán-Álvarez, J.C., Estrada, K.L., Chávez, A., Jiménez-Cisneros, B., 2010. Accu- mulation and leaching potential of some pharmaceuticals and potential endocrine disruptors in soils irrigated with wastewater in the Tula Valley, Mexico. Chemosphere 81, 1437–1445.
Gómez-Oliván, L.M., Carmona-Zepeda, F., Galar-Martínez, M., Téllez-López, A., Amaya-Chávez, A., 2009. A study of self-medication in a communitarian pharmacy in the city of Toluca. Rev. Mex. Ciencias Farm. 40, 5–11.
González-González, E.D., Gómez-Oliván, L.M., Galar-Martínez, M., Vieyra-Reyes, P., Islas-Flores, H., García-Medina, S., Jiménez-Vargas, J.M., Razo-Estrada, C., Pérez-Pastén, R., 2014. Metals and nonsteroidal anti-inflammatory pharmaceutical drugs present in water from Madín Reservoir (Mexico) induce oxidative stress in gill, blood, and muscle of common carp (*Cyprinus carpio*). Arch. Environ. Contam. Toxicol. 67, 281–295.
Gonzalez-Rey, M., Bebianno, M.J., 2014. Effects of non-steroidal anti-in ammatory drug (NSAID) diclofenac exposure in mussel *Mytilus galloprovincialis*. Aquat. Toxicol. 148, 221–230.
Grünspan, L.D., Mussulini, B.H.M., Baggio, S., dos Santos, P.R., Dumas, F., Rico, E.P., de Oliveira, D.L., Moura, S., 2018. Teratogenic and anticonvulsant effects of zinc and copper valproate complexes in zebrafish. Epilepsy Res. 139, 171–179.
Haendel, M.A., Tilton, F., Bailey, G.S., Tanguay, R.L., 2004. Developmental toxicity of the dithiocarbamate pesticide sodium metam in Zebrafish. Toxicol. Sci. 81, 390–400.
Hahn-Schlam, F., Miranda-Salgado, G., Perez-Lopez, F., Mayo-Diaz, O., Rojas-Serrano, F., Coras-Merino, P., 2006. Monitoring Texcoco River water quality with ion selective sensors. Agrociencia 40, 277–287.
Hamilton, P.B., Cowx, I.G., Oleksiak, M.F., Griffiths, A.M., Grahn, M., Stevens, J.R., Carvalho, G.R., Nicol, E., Tyler, C.R., 2016. Population-level consequences for wild fish exposed to sublethal concentrations of chemicals–a critical review. Fish Fish. 17, 545–566.
Hermsen, S.A., van den Brandhof, E.J., van der Ven, L.T., Piersma, A.H., 2011. Relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and comparison with their *in vivo* potencies. Toxicol. In Vitro 25, 745–753.
Hernández, A.F., Gil, F., Tsatsakis, A.M., 2014. Biomarkers of chemical mixture toxicity. In: Biomarkers in Toxicology. Academic Press, pp. 655–670.
Hernández, A.F., Gil, F., Tsatsakis, A.M., 2019. Biomarkers of chemical mixture toxicity. In: Biomarkers in Toxicology, second ed. Academic Press, pp. 569–585.
Hernando, M.D., Mezcua, M., Fernández-Alba, A.R., Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69, 334–342.
Huang, J.C., Wun, W.S.A., Goldsby, J.S., Aleksic, N.M., Wu, K.K., 2004. Cyclooxygenase-2-derived endogenous prostacyclin enhances mouse embryo hatching. Human Rep. 19, 2900–2906.
Islas-Flores, H., Gómez-Oliván, L.M., Galar-Martínez, M., Colín-Cruz, A., Neri-Cruz, N., García-Medina, S., 2013. Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (*Cyprinus carpio*). Ecotoxicol. Environ. Saf. 92, 32–38.
Islas-Flores, H., Gómez-Oliván, L.M., Galar-Martínez, M., García-Medina, S., Neri-Cruz, N., Dublán-García, O., 2014. Effect of ibuprofen exposure on blood, gill, liver, and brain on common carp (*Cyprinus carpio*) using oxidative stress biomarkers. Environ. Sci. Pollut. Res. 21, 5157–5166.
Islas-Flores, H., Manuel Gómez-Oliván, L., Galar-Martínez, M., Sánchez-Ocampo, E.M., SanJuan-Reyes, N., Ortíz-Reynoso, M., Dublán-García, O., 2017. Cyto-genotoxicity and oxidative stress in common carp (*Cyprinus carpio*) exposed to a mixture of ibuprofen and diclofenac. Environ. Toxicol. 32, 1637–1650.
Kovacic, P., Somanathan, R., 2014. Recent developments in the mechanism of teratogenesis–electron transfer, reactive oxygen species, and antioxidants. In: Systems Biology of Free Radicals and Antioxidants. Springer, pp. 567–580.
Kroglund, F., Finstad, B., 2003. Low concentrations of inorganic monomeric aluminum impair physiological status and marine survival of Atlantic salmon. Aquaculture 222, 119–133.
Kulcharyk, P.A., Heinecke, J.W., 2001. Hypochlorous acid produced by the myeloperoxidase system of human phagocytes induces covalent cross-links between DNA and protein. Biochemistry 40, 3648–3656.
Lange, F., Cornelissen, S., Kubac, D., Sein, M.M., von Sonntag, J., Hannich, C.B., 2006. Degradation of macrolide antibiotics by ozone: a mechanistic case study with clarithromycin. Chemosphere 65, 17–23.
Letzel, M., Metzner, G., Letzel, T., 2009. Exposure assessment of the pharmaceutical diclofenac based on long-term measurements of the aquatic input. Environ. Int. 35, 363–368.

Lin, A.Y.C., Yu, T.H., Lin, C.F., 2008. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: risk to aqueous environments in Taiwan. Chemosphere 74, 131–141.
Lishman, L., Smyth, S.A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M., Seto, P., 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Sci. Total Environ. 367, 544–558.
Luja-Mondragón, M., Gómez-Oliván, L.M., SanJuan-Reyes, N., Islas-Flores, H., Orozco-Hernández, J.M., Heredia-García, G., Galar-Martínez, M., Dublán-García, O., 2019. Alterations to embryonic development and teratogenic effects induced by a hospital effluent on *Cyprinus carpio* oocytes. Sci. Total Environ. 660, 751–764.
Martínez-Álvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. Rev. Fish Biol. Fish. 15, 75–88.
Matthiessen, P., Law, R.J., 2002. Contaminants and their effects on estuarine and coastal organisms in the United Kingdom in the late twentieth century. Environ. Pollut. 120, 739–757.
Metcalfe, C.D., Koenig, B.G., Bennie, D.T., Servos, M., Ternes, T.A., Hirsch, R., 2003. Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. Environ. Toxicol. Chem. 22 2972-2880.
Miller, T.H., Bury, N.R., Owen, S.F., MacRae, J.I., Barron, L.P., 2018. A review of the pharmaceutical exposome in aquatic fauna. Environ. Pollut. 239, 129–146.
National Water Commission of Mexico (CONAGUA), 2011. Lerma - Chapala Basin Case Study: A Fruitful Sustainable Water Management Experience. http://www.conagua.gob.mx/CONAGUA07/Contenido/Documentos/LermaChapalaBasinCase.pdf.
Neri-Cruz, N., Gómez-Oliván, L.M., Galar-Martínez, M., Romero-Figueroa, M.S., Islas-Flores, H., García-Medina, S., Jiménez-Vargas, J.M., SanJuan-Reyes, N., 2015. Oxidative stress in *Cyprinus carpio* induced by hospital wastewater in Mexico. Ecotoxicology 24, 181–193.
NMX-AA-003-1980, 1980. Wastewater, Sampling. Procuraduría Federal de Protección al Ambiente. Diario Oficial de la Federación (25 March).
NOM-001-SEMARNAT-1996, 1996. Official Mexican Norm Setting the Maximum Permissi- ble Limits of Contaminants in Wastewaster Discharges Entering National Waters and Resources. Procuraduría Federal de Protección al Ambiente. Diario Oficial de la Federación 30 October.
NOM-073-ECOL-1994, 1994. Official Mexican Norm Setting the Maximum Permissible Limits of Contaminants in Wastewater Discharges Arising in the Pharmacochemical and Pharmaceutical Industries and Entering Receiving Water Bodies. Secretaría de Desarrollo Social. Diario Oficial de la Federación (10 June).
OECD, 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECDhttps://doi.org/10.1787/9789264203709-en.
Parrish, D.L., Behnke, R.J., Gephard, S.R., McCormick, S.D., Reeves, G.H., 1998. Why aren't there more Atlantic salmon (*Salmo salar*)? Can. J. Fish. Aquat. Sci. 55, 281–287.
Pašková, V., Hilscherová, K., Bláha, L., 2011. Teratogenicity and embryotoxicity in aquatic organisms after pesticide exposure and the role of oxidative stress. In: Reviews of Environmental Contamination and Toxicology, vol. 211. Springer, pp. 25–61.
Peltzer, P.M., Lajmanovich, R.C., Martinuzzi, C., Attademo, A.M., Curi, L.M., Sandoval, M.T., 2019. Biotoxicity of diclofenac on two larval amphibians: assessment of development, growth, cardiac function and rhythm, behavior and antioxidant system. Sci. Total Environ. 683, 624–637.
Pérez-Alvarez, I., Islas-Flores, H., Gómez-Oliván, L.M., Barceló, D., de Alda, M.L., Solsona, S.P., Sánchez-Aceves, L.M., SanJuan-Reyes, N., Galar-Martínez, M., 2018. Determination of metals and pharmaceutical compounds released in hospital wastewater from Toluca, Mexico, and evaluation of their toxic impact. Environ. Pollut. 240, 330–341.
Pérez-Coyotl, I., Galar-Martínez, M., García-Medina, S., Gómez-Oliván, L.M., Gasca-Pérez, E., Martínez-Galero, E., Islas-Flores, H., Pérez-Pastén, R.B., Barceló, D., de Alda, M.L., Pérez-Solsona, S., Serra-Roig, M.P., Montemurro, N., Peña-Herrera, J.M., Sánchez-Aceves, L.M., 2019. Polluted water from an urban reservoir (Madín dam, México) induces toxicity and oxidative stress in *Cyprinus carpio* embryos. Environ. Pollut. 251, 510–521.
Popa, P., Timofti, M., Voiculescu, M., Dragan, S., Trif, C., Georgescu, L.P., 2012. Study of physico-chemical characteristics of wastewater in an urban agglomeration in Romania. Sci. World J. 1–10 2012.
Pounds, N., MaClean, S., Webley, M., Pascoe, D., Hutchinson, T., 2008. Acute and chronic effects of ibuprofen in the mollusc *Planorbis carinatus* (Gastropoda: *Planorbidae*). Ecotoxicol. Environ. Saf. 70, 47–52.
Rangasamy, B., Hemalatha, D., Shobana, C., Nataraj, B., Ramesh, M., 2018. Developmental toxicity and biological responses of zebrafish (*Danio rerio*) exposed to anti-inflammatory drug ketoprofen. Chemosphere 213, 423–433.
Rehman, M.S.U., Rashid, N., Ashfaq, M., Saif, A., Ahmad, N., Han, J.I., 2015. Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere 138, 1045–1055.
Rivera-Jaimes, J.A., Postigo, C., Melgoza-Alemán, R.M., Aceña, J., Barceló, D., de Alda, M.L., 2018. Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: occurrence and environmental risk assessment. Sci. Total Environ. 613, 1263–1274.
SanJuan-Reyes, N., Gómez-Oliván, L.M., Galar-Martínez, M., Vieyra-Reyes, P., García-Medina, S., Islas-Flores, H., Neri-Cruz, N., 2013. Effluent from an NSAID-manufacturing plant in Mexico induces oxidative stress on *Cyprinus carpio*. Water Air Soil Pollut. 224, 1–14.
SanJuan-Reyes, N., Gómez-Oliván, L.M., Galar-Martínez, M., García-Medina, S., Islas-Flores, H., González-González, E.D., Cardoso-Vera, J.D., Jiménez-Vargas, J.M., 2015. NSAID-manufacturing plant effluent induces geno-and cytotoxicity in common carp (*Cyprinus carpio*). Sci. Total Environ. 530, 1–10.
Sapone, A., Gustavino, B., Monfrinotti, M., Canistro, D., Broccoli, M., Pozzetti, L., Affatato, A., Valgimigli, L., Forti, G.C., Pedulli, G.F., Biagi, G.L., Abdel-Rahman, S.Z., Paolini, M., 2007. Perturbation of cytochrome P450, generation of oxidative stress and induction of DNA damage in *Cyprinus carpio* exposed in situ to potable surface water. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 626, 143–154.
Scholz, S., Sela, E., Blaha, L., Braunbeck, T., Galay-Burgos, M., García-Franco, M., Guinea, J., Klüver, N., Schirmer, K., Tanneberger, K., Tobor-Kapłon, M., Witters, H., Belanger, S., Benfenati, E., Creton, S., Cronin, M.T., Eggen, R.I., Embry, M., Ekman, D., Gourmelon, A., Halder, M., Hardy, B., Hartung, T., Hubesch, B., Jungmann, D., Lampi, M.A., Lee, L., Léonard, M., Küster, E., Lillicrap, A., Luckenbach, T., Murk, A.J., Navas, J.M., Peijnenburg, W., Repetto, G., Salinas, E., Schüürmann, G., Spielmann, H., Tollefsen, K.E., Walter-Rohde, S., Whale, G., Wheeler, J.R., Winter, M.J., 2013. A European perspective on alternatives to animal testing for environmental hazard identification and risk assessment. Regul. Toxicol. Pharmacol. 67, 506–530.
Scott, T.M., Phillips, P.J., Kolpin, D.W., Colella, K.M., Furlong, E.T., Foreman, W.T., Gray, J.L., 2018. Pharmaceutical manufacturing facility discharges can substantially increase the pharmaceutical load to US wastewaters. Sci. Total Environ. 636, 69–79.
Sedeño-Díaz, J.E., López-López, E., 2007. Water quality in the Río Lerma, Mexico: an overview of the last quarter of the twentieth century. Water Resour. Manag. 21, 1797–1812.
Sehonova, P., Plhalova, L., Blahova, J., Doubkova, V., Prokes, M., Tichy, F., Fiorino, E., Faggio, C., Svobodova, Z., 2017. Toxicity of naproxen sodium and its mixture with tramadol hydrochloride on fish early life stages. Chemosphere 188, 414–423.
Siemens, J., Huschek, G., Siebe, C., Kaupenjohann, M., 2008. Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City–Mezquital Valley. Water Res. 42, 2124–2134.
Široká, Z., Drastichova, J., 2004. Biochemical marker of aquatic environment contamination-cytochrome P450 in fish. A review. Acta Vet. Brno 73, 123–132.
Sreekanth, D., Sivaramakrishna, D., Himabindu, V., Anjaneyulu, Y., 2009. Thermophilic treatment of bulk drug pharmaceutical industrial wastewaters by using hybrid up flow anaerobic sludge blanket reactor. Bioresour. Technol. 100, 2534–2539.
Stepanova, S., Praskova, E., Chromcova, L., Plhalova, L., Prokes, M., Blahova, J., Svobodova, Z., 2013. The effects of diclofenac on early life stages of common carp (*Cyprinus carpio*). Environ. Toxicol. Pharmacol. 35, 454–460.
Strmac, M., Oberemm, A., Braunbeck, T., 2002. Effects of sediment eluates and extracts from differently polluted small rivers on zebrafish embryos and larvae. J. Fish Biol. 61, 24–38.
Ton, S.S., Chang, S.H., Hsu, L.Y., Wang, M.H., Wang, K.S., 2012. Evaluation of acute toxicity and teratogenic effects of disinfectants by *Daphnia magna* embryo assay. Environ. Pollut. 168, 54–61.
Uno, T., Ishizuka, M., Itakura, T., 2012. Cytochrome P450 (CYP) in fish. Environ. Toxicol. Pharmacol. 34, 1–13.
Velagaleti, R., Burns, P.K., 2006. The industrial ecology of pharmaceutical raw materials and finished products with emphasis on supply chain management activities. http://www.epa.gov/esd/chemistry/ppcp/images/ecology.pdf.
Wells, P.G., Bhuller, Y., Chen, C.S., Jeng, W., Kasapinovic, S., Kennedy, J.C., Kim, P.M., Laposa, R.R., McCallum, G.P., Nicol, C.J., Parman, T., Wiley, M.J., Wong, A.W., 2005. Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. Toxicol. Appl. Pharmacol. 207, 354–366.
Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., Xu, J., Zheng, X., Ren, L., Wang, G., Zhang, Y., Huo, L., Zhao, Z., Cao, D., Lu, C., Li, C., Zhou, Y., Liu, Z., Fan, Z., Shan, G., Li, X., Wu, S., Song, L., Hou, G., Jiang, Y., Jeney, Z., Yu, D., Wang, L., Shao, C., Song, L., Sun, J., Ji, P., Wang, J., Li, Q., Xu, L., Sun, F., Feng, J., Wang, C., Wang, S., Wang, B., Li, Y., Zhu, Y., Xue, W., Zhao, L., Wang, J., Gu, Y., Lv, W., Wu, K., Xiao, J., Wu, J., Zhang, Z., Yu, J., Sun, X., 2014. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nat. Genet. 46, 1212–1219.
Yao, H., Yu, J., Zhou, Y., Xiang, Q., Xu, C., 2018. The embryonic developmental effect of sedaxane on zebrafish (*Danio rerio*). Chemosphere 197, 299–305.