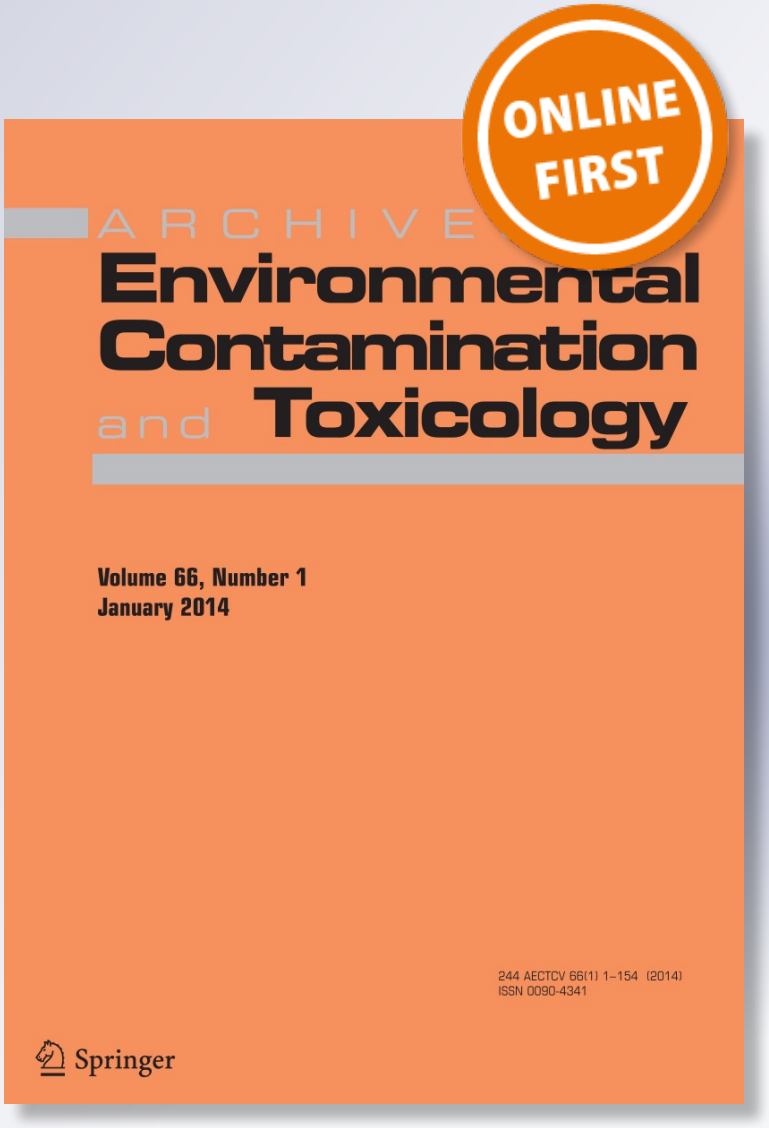
Changes in Hematological Parameters of Cichlasoma dimerus *(Teleostei, Perciformes) Exposed to Sublethal Concentrations of 4-*tert*-Octylphenol*

G. Rey Vázquez & F. Lo Nostro

Archives of Environmental Contamination and Toxicology

ISSN 0090-4341

Arch Environ Contam Toxicol
DOI 10.1007/s00244-014-9997-6



Springer

Arch Environ Contam Toxicol
DOI 10.1007/s00244-014-9997-6

Changes in Hematological Parameters of *Cichlasoma dimerus* (Teleostei, Perciformes) Exposed to Sublethal Concentrations of 4-*tert*-Octylphenol

G. Rey Vázquez · F. Lo Nostro

Received: 30 October 2013 / Accepted: 5 January 2014

Abstract Stress response involves various physiological changes, including alteration in hematological parameters closely related to the response of fish to the environment. 4-*tert*-octylphenol (OP) is one of the worldwide-used surfactants and can pollute the aquatic environment, both marine and freshwater. Previous studies have already shown estrogenic effects of this compound in various wildlife species, e.g., it can disrupt the reproductive system of males organisms including fish. The purpose of this study was to evaluate the effects caused by sublethal concentrations of OP on hematological parameters as biomarkers to assess environmental stress. Adult males and females of *Cichlasoma dimerus* were exposed to waterborne OP during 60 days under semistatic conditions. Experimental groups consisted of control (ethanol 0.005 %), OP 150, and OP 300 μg/L. OP caused hemodynamic stress in *C. dimerus*, which lead to normochromic and normocytic anemia, as well as erythrocytic pathologies such as a significant increase in erythroblasts and amitotic erythrocytes. All of the studied parameters can be used as biomarkers for the presence of xenobiotics in water.

Stress is a general and nonspecific response to any factor disturbing homeostasis. In fish, it may be induced by various abiotic environmental factors (changes in water temperature, pH, O_2 concentration, pollution), biotic interactions (predation pressure, parasitic invasions, or strong competition), and human activities related to fish rearing and harvesting (manipulation, transport, crowding). Under moderate stress conditions, inner balance is usually restored. Under severe or prolonged stress conditions, however, the compensatory abilities of the organism may become exhausted, which results in physiological disturbance or even death (Witeska 2005).

Stress response involves various physiological changes including alteration in blood composition and immune mechanisms. Hematological parameters are closely related to the response of the animal to the environment (Gabriel et al. 2004). These indices have been employed in effectively monitoring the response of fishes to stressors and thus their health status under adverse conditions (Nussey et al. 1995). They can provide substantial diagnostic information once reference values are established under standardized conditions. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* have already been described by Rey Vázquez and Guerrero (2007).

A wide range of chemicals introduced into the environment by human activities may be producing adverse health effects in human and wildlife populations. Among endocrine-disrupting chemicals, various groups of chemicals have estrogen-like effects and are referred to as "xenoestrogens." Alkylphenol polyethoxylates (APEs) are one of the classes of nonionic surfactants whose primary degradation in wastewater treatment plants or in the aquatic environment (rivers, estuaries, coastal marine environments) generates more persistent shorter-chain APEs and alkylphenols (APs) such as 4-*tert*-octylphenol (OP), one of the most biologically active products (Ying et al. 2002). Laboratory and field studies have shown that exposure of male fish to alkylphenolic compounds results in the induction of circulating

G. Rey Vázquez (✉) · F. Lo Nostro
Lab. de Ecotoxicología Acuática, Dpto. de Biodiversidad y Biología Experimental, FCEyN, UBA, Ciudad Universitaria. Pabellón II, 4° piso (C1428EHA), Buenos Aires, Argentina
e-mail: grarey@bg.fcen.uba.ar; grareyva@gmail.com

F. Lo Nostro
bIBBEA, CONICET-UBA, Buenos Aires, Argentina

Published online: 30 January 2014

vitellogenin (Vtg), inhibition of testicular growth, testis abnormalities, and formation of intersex gonads among other signs of reproductive impairment (White et al. 1994; Jobling et al. 1996; Kinnberg et al. 2000; Metcalfe et al. 2001; Knörr and Braunbeck 2002; Rasmussen et al. 2005).

The concentrations of OP used in the present study span the range of concentrations recorded in several countries. In rivers and lakes of developed countries, concentrations of APs rarely exceed 20 μg/L (Blackburn and Waldock 1995; Bennie et al. 1997), although in rivers receiving significant amounts of industrial and/or domestic effluents, point source contaminant levels may reach 600–1,000 μg/L (Warhurst 1995; Ying et al. 2002). APE metabolite concentrations range from <0.1 to 369 μg/L in United States. In the United Kingdom, nonylphenol (NP) levels have been reported to be ≤180 μg/L in the receiving waters for most polluted rivers. In Northeast Spain, NP levels ranged from 6 to 343 μg/L in sewage-treatment plants and ranged from not detected to 644 μg/L in receiving waters (Sole et al. 2000).

Despite cichlids being the most species-rich non-ostariophysan family of freshwater fishes worldwide (Kullander 2003), few studies have dealt with the effect of pollutants on members of this group. The South American cichlid fish *C. dimerus* is a common perciform in quiet shallow waters of the Paraguay River and most of the Paraná River basins (Kullander 1983). This species is representative of teleosts in the La Plata River Basin and results relevant to the Argentinean riverine ecosystems. Considering its successful use in ecotoxicological testing (Moncaut et al. 2003; Rey Vázquez et al. 2009; Genovese et al. 2011, 2012; Piazza et al. 2011; Da Cuña et al. 2011, 2013), this species has been included as one of the suitable native fish species for the determination of the toxicity of xenobiotics. In fact, one of our previous studies showed an estrogen-like action of OP in males of *C. dimerus* based on the induction of Vtg synthesis in the liver. Moreover, the impairment of testis morphology indicated that OP decreases male fertility in this species (Rey Vázquez et al. 2009).

In view of the above-mentioned information and considering the lack of knowledge about side effects of certain xenoestrogens, the aim of this study was to evaluate the effects caused by sublethal concentrations of 4-*tert*-OP on fish hematological parameters.

Materials and Methods

Fish

All adult specimens of *C. dimerus* (4–5 years old) used in this study were captured in Esteros del Riachuelo, a nonpolluted aquatic environment at Corrientes, Argentina (27°25′S, 58°15′W) at the onset of thru reproductive season. A total of 36 sexually mature fish (total length 9–11 cm) were kept in 100-L aquaria at 26.5 ± 1 °C, pH 7.3, under a 12 to 12-h photoperiod and at an average density of 4.6 g/L. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom.

The filtered water used in exposure tests had the following mean values for the physicochemical characteristics: color < 5, turbidity 0.35 NTU, conductivity 219 μS/cm, conductimetric residue 148 mg/L, total alkalinity (CO_3Ca) 36.5 mg/L, chlorides 25.5 mg/L, nitrates (NO^{3-}) < 2.0 mg/L, sulfates 20.7 mg/L, fluorides < 0.5 mg/L, iron < 0.05 mg/L, calcium 17 mg/L, magnesium 3.2 mg/L, sodium 20 g/L, potassium 2.3 mg/L, total hardness (CO_3Ca) 55 mg/L, nitrite (NO^{2-}) 0.13 mg/L, ammonium (NH4+) < 0.05 mg/L, total arsenic < 10 mg/L, dissolved oxygen 8 mg/L, free chlorine mg/L, and manganese < 0.04 mg/L. Analyses were performed by AYSA S. A., Argentina.

Fish were fed once a day with pelleted commercial food (Tetra Holding (US), Inc) and were allowed to acclimate to captivity conditions for 1 month before the start of experimentation.

Experimental Design and OP Concentrations

The test substance, 4-*tert*-OP (>97 % pure) was obtained from Sigma (St. Louis, Missouri, USA). Exposure concentrations of OP were selected from preliminary studies (data not shown), and a 96-h acute toxicity test was performed (LC_{50} at 96 h = 547.21 μg/L).

Before the onset of exposure, each fish ($N = 36$) was transferred to an individual 10-L glass tank under the same physical conditions and alimentary ration except that the layer of gravel on the bottom was removed. Fish were allowed to acclimate to experimental conditions for 1 week before the experiment was started. Fish were then exposed during a 60-day period under semistatic conditions to nominal concentrations of 150 and 300 μg/L OP ($n = 12$ for each treatment). Control group ($n = 12$) tests were performed with ethanol at a final concentration of 0.005 %. Stock solutions were prepared once a week by dissolving OP in 100 % ethanol and storing in the dark at 4 °C. Water was renewed twice a week, during which small aliquots of stock solution were added to filtered tap water to obtain the desired OP concentrations.

To evaluate decreased OP concentration in test water, we measured actual concentrations of OP by reverse-phase high-performance liquid chromatography coupled to fluorescence detection according to Rey Vázquez et al. (2009). Briefly, water samples were taken every 24 h during the last week of the experiment and treated by solid-phase

extraction on C18 with methanol elution before injection in the high-performance liquid chromatograph. Data were acquired and analyzed with Konikrom 5.2 software (Konik Instruments, Spain). For quantifications, calibration curves were constructed for peak areas from injection of standard solutions daily prepared by adding known amounts of OP to control water and processed in the same manner as the samples. For each set of replicate samples, mean and SDs were calculated after interpolation of the OP chromatographic peak area in the calibration curve ($R^2 = 0.99$).

Treatment and Sampling

Initial blood samples were carefully and quickly collected after fish were softly narcotized with fish calmer (Jungle Hypno, USA). Blood was collected by puncture of the caudal vein with a heparin-coated 27G × 1.5″ needle, attached to a 1-mL syringe and processed according to Rey Vázquez and Guerrero (2007). Briefly, blood smears stained with 10 % Giemsa in phosphate-buffered saline and ultrathin sections stained with aqueous uranyl acetate and lead citrate were examined for light and transmission electron microscopy (TEM), respectively. Within the first 2 h after each blood extraction, samples were processed for red blood cell count (RBC), white blood cell count (WBC), and hematocrit or packed cell volume (PCV) as follows. RBC (Kaplow 1955) and WBC+thrombocytes (Natt and Herrick 1952) were determined using a Neubauer hemocytometer. Differential white cell and thrombocyte counts were performed on blood films stained with Giemsa; PCV values were determined by the standard microhematocrit method. Hemoglobin (Hb) in erythrocytes was determined using the cyanmethaemoglobin method (hemogloWiener reactive; Wiener Laboratory, Santa Fe, Argentina). Before measuring the absorbance, Hb test samples were centrifuged to remove dispersed nuclear material. The following indices—mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV)—were calculated according to Seiverd (1964). At the end of each experiment, final blood samples were collected and processed in the same manner as initial blood samples. Duplicate determinations were made for each blood sample. Fish were the, transferred to OP-free water aquaria to recover from exposure (Genovese et al. 2012). All experiments were performed in accordance with international standards on animal welfare (Canadian Council on Animal Care 2005).

Statistical Analysis

Repeated measures analysis of variance, followed by Tukey's test, was used to establish differences between initial and final values of hematological parameters of control and OP-exposed fish (Statistica 7.0; StatSoft, Inc., 2004, Tulsa, USA). Differences were considered significant at $P < 0.05$.

Results

In peripheral blood samples from control animals, all cell types, which have already been described in Rey Vázquez and Guerrero (2007), were observed, such as immature and mature RBCs (erythrocytes) and WBCs (leukocytes: lymphocytes, monocytes, neutrophils, eosinophils) and thrombocytes.

In blood smears of OP-treated fish (150 and 300 μg/L), in addition to the normal cell types, erythrocytes with segmented nuclei, i.e., amitotic erythrocytes, appeared. These atypical erythrocytes are characterized by a constricted or divided nucleus with a slightly basophilic cytoplasm compared with normal erythrocytes (compare between Fig. 1a. b). Under TEM, a homogenous cytoplasm and constricted chromatin in "8 shape" can be seen (Fig. 1c). Erythroblasts, known as reticulocytes, are immature erythrocytes present in peripheral blood. They are slightly more round and have a more basophilic cytoplasm than the mature cell (Fig. 1b).

Initial hematological parameters of control fish did not differ from final control values and were within the normal range previously established for this species by Rey Vázquez and Guerrero (2007). For OP-treated fish, initial values of the hematological indices were not significantly different from those of the control group (Table 1). The initial and final values of the hemogram of OP-exposed fish are also listed in Table 1.

Final values of RBC, PCV, and Hb in OP-treated fish decreased with OP 300 μg/L values being the lowest. Exposure to 150 μg/L OP resulted in a statically significant decrease of PCV compared with initial values. OP at a concentration of 300 μg/L caused a statistically significant decrease in all three aforementioned parameters with PCV showing the greater significance level ($P < 0.01$). The indices MCV, MCH, and MCHC did not vary between experimental conditions (Table 1). Average values for RBC, differential leukocyte count, thrombocytes, amitotic erythrocytes, and erythroblasts are also listed in Table 1. An increase in WBC was observed in OP-treated fish, probably due to the increase in lymphocytes and heterophils. However, these differences were not statistically significant. Similarly, the decrease observed in the number of thrombocytes in OP-exposed fish was statistically nonsignificant.

Amitotic erythrocytes were found in a relatively high proportion in blood of fish exposed to both concentrations of OP; however this parameter only showed significant differences for fish exposed to 300 μg/L OP.

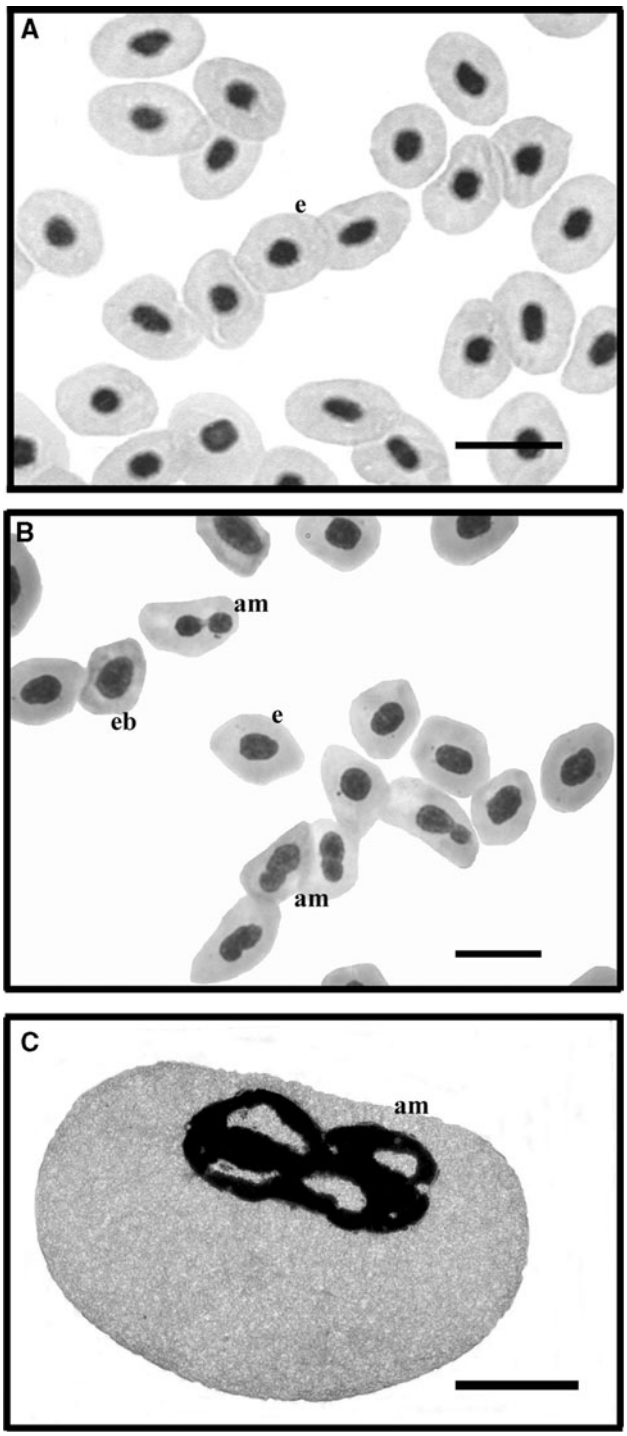


Fig. 1 **a** Blood smears of control *C. dimerus* showing mature erythrocytes. **b** Peripheral blood of OP-treated fish. Note the presence of erythroblasts and atypical erythrocytes such as amitotic erythrocytes. Giemsa stain. *Scale bar* 6 μm. **c** Electron microscopic micrograph of an amitotic erythrocyte showing the typical 8-shaped nucleus. *Scale bar* 2 μm. *Am* amitotic erythrocytes, *e* erythrocyte, *eb* erythroblasts

Peripheral blood of fish normally contains fairly constant quantities of immature elements; however, for all OP-treated individuals a marked increase in the number of circulating erythroblasts was evident ($P < 0.01$ for 150 μg/L; $P < 0.05$ for 300 μg/L) (Table 1).

At the end of the experiment no mortality was observed neither in control nor OP-exposed fish groups.

The decreased concentrations of OP measured in replicate water samples taken every 24 h from the 150 μg/L treatment group are listed in Table 2. The initial nominal and actual OP levels were in good agreement. During the 72-h test period, measured levels of OP decreased to approximately 20 % of the initial concentrations. OP was not detected in samples from the control treatment.

Discussion

The result of this study showed a significant response of the blood of *C. dimerus* exposed to OP consistent with hemodynamic stress symptoms. The concentrations of OP tested produced a strong decrease in RBC values. As a consequence, a decrease of PVC was also observed. Hg concentration in fish exposed to 300 μg/L OP also decreased significantly, whereas on exposure to 150 μg/L OP this effect was less evident. Because MCH and MCV values did not differ significantly, OP exposure would be producing normochromic normocytic anemia.

Mousavi and Yousefian (2012) showed that chloride mercury exposure decreased RBC, hematocrit, and haemoglobin values of *Salmo trutta caspius*, indicating that the subacute mercury concentrations tested may have caused several changes in the hematological parameters of the treated fish. Therefore, estimation of these indices could provide a useful indicator regarding ecosystem pollution.

Martins et al. (2004), and Sabri et al. (2009) also recorded a significant decrease in RBC, PVC, and Hb in parasitized specimens of *Leporinus macrocephalus* and *Clarias garipienus*, respectively; however, anemia subtype differed in these cases because they showed a decrease in the size of RBCs and an increase in the amount of hemoglobin per erythrocyte.

Contaminants introduced by human activities can produce a variety of effects. Studies on *Oreochromis niloticus* with chlorpyrifos (an organophosphorus insecticide) (Girón-Pérez et al. 2006) and endosulfan (an organochlorine insecticide) (Girón-Pérez et al. 2008) reported no change in the hematological indexes analyzed except for alterations in elements of the white-cell series. *Salmo salar* (Petri et al. 2006) and *C. dimerus* (Da Cuña et al. 2011) were also tolerant to endosulfan in terms of the values of the RBC, PVC, and Hb. However, *C. dimerus* exposed to this organochlorine pesticide, unlike the case with OP in our results, showed a decrease of MCV, MCH, and MCHC levels and were therefore thought to have hypochromic microcytic anemia by the investigators of the study. In the hybrid

Table 1 Hematological parameters of *C. dimerus* (both control and exposed) to OP for 60 days

Parameter	Control i	Control f	150 μg/L OPi	150 μg/L OPf	300 μg/L OPi	300 μg/L OPf
RBC ($\times 10^6$ μL^{-1})	3.23 ± 0.97	3.33 ± 1.08	3.00 ± 1.13	2.57 ± 0.08	3.43 ± 0.78	2.07 ± 1.24*
PVC (%)	32.98 ± 2.98	33.40 ± 3.23	35.18 ± 3.6	24.66 ± 6.34**	34.54 ± 2.64	23.08 ± 8.7**
Hb (g/dL)	10.44 ± 2.66	10.26 ± 3.68	10.84 ± 3.30	9.94 ± 3.48	10.33 ± 3.05	6.49 ± 1.73*
MCV (fL)	111.08 ± 27.11	112.53 ± 29.88	103.43 ± 19.44	102.87 ± 23.1	112.48 ± 26.71	122.71 ± 35.84
MCH (pg)	31.54 ± 10.04	34.67 ± 17.16	30.73 ± 8.99	34.38 ± 12.06	27.08 ± 5.05	36.03 ± 13.37
MCHC (g/dL)	30.02 ± 9.67	26.62 ± 11.11	30.21 ± 10.25	45.82 ± 29.00	26.73 ± 7.76	29.01 ± 7.07
WBC ($\times 10^3$ μL^{-1})	11.03 ± 3.85	11.16 ± 3.96	10.89 ± 3.06	17.83 ± 4.57	9.87 ± 2.62	15.25 ± 3.79
Lymphocytes ($\times 10^3$ μL^{-1})	2.97 ± 2.32	3.29 ± 2.91	1.43 ± 1.17	11.79 ± 9.52*	3.84 ± 0.26	5.71 ± 3.89
Monocytes ($\times 10^3$ μL^{-1})	2.13 ± 2.06	2.31 ± 2.71	2.30 ± 1.15	1.40 ± 1.87	0.33 ± 0.86	2.18 ± 2.78
Heterophils ($\times 10^3$ μL^{-1})	3.34 ± 2.15	3.52 ± 2.96	4.48 ± 2.19	3.94 ± 3.90	3.64 ± 3.35	5.62 ± 5.62
Eosinophils ($\times 10^3$ μL^{-1})	1.76 ± 1.93	1.84 ± 2.03	0.90 ± 1.06	0.71 ± 1.22	0.93 ± 0.70	1.74 ± 2.86
Thrombocytes ($\times 10^3$ μL^{-1})	29.86 ± 19.48	32.67 ± 26.28	19.56 ± 12.67	16.44 ± 16.00	22.04 ± 8.64	16.60 ± 22.55
Amitotic erythrocytes ($\times 10^3$ μL^{-1})	0	0	0.48 ± 0.82	12.22 ± 6.65	0	19.46 ± 18.41**
Erythroblasts ($\times 10^3$ μL^{-1})	2.02 ± 1.75	1.59 ± 3.00	1.59 ± 4.69	33.33 ± 16.54**	3.06 ± 0.29	18.66 ± 18.66*

* $P < 0.05$; ** $P < 0.01$

Table 2 Actual concentrations of OP in the aquarium water during the last week of the experiment

Nominal OP concentration 150 μg/L		
Time (h)	Actual concentration (μg/L)	(%)
0	151.95 ± 0.7	101.30 ± 0.5
24	60.30 ± 1.6	40.20 ± 1.1
48	51.45 ± 0.9	34.30 ± 0.6
72	32.25 ± 1.2	21.50 ± 0.8

cichlid *Oreochromis* exposed to sublethal doses of aluminum, Bhagwan and Bhikajee (2000) diagnosed macrocytic hypochromic anemia. Whitmore (1965) found that dietary deficiency of vitamin E induced microcytic anemia in juveniles of *Oncorhynchus tshawytscha*.

Mature erythrocytes of *C. dimerus* exposed to OP did not show variations in its dimensions given the average values of MCV; however, alterations at the nuclear level were observed under light and electron microscopy. Various studies have shown that under certain pathological conditions, several abnormalities can be observed in blood smears with many of them in mature erythrocytes, such as amitosis, nucleus division, and enucleated cells. The medial nucleus division into two lobes is known as "segmentation" and the irregular division into two or three parts is known as "fragmentation" (Yokote 1982). Erythrocytes with bilobed nucleus were observed in the peripheral blood of *C. dimerus*. These amitotic erythrocytes can be considered to be a pathological segmentation because they were only observed in exposed fish. The best known example of amitosis is found in nuclear division or atypical cells of amphibians (Pan 1956; Barni et al. 1995). Erythrocytes with divided nucleus, similar to those of the toad, are commonly distinguished morphologically in triploids of *O. mykiss* (Zou et al. 2006; Han et al. 2007; Wang et al. 2010). Dorafshan *et al.* (2008) found that amitotic divisions of erythrocytes in triploid *Salmo trutta caspius* L. were a mechanism to mitigate stress because triploids could be less tolerant than diploids.

The peripheral blood of fish normally contains a fairly constant number of immature erythrocytes (Hibiya 1982; Genten et al. 2009). Because the number of circulating erythroblasts significantly increased in exposed fish, erythropoiesis has likely occurred in these animals as a compensatory mechanism tending to balance the number of circulating RBCs, although the anemia registered in OP-exposed fish would suggest that this compensation was insufficient. Similar magnitude increases in the number of immature erythrocytes were observed in *O. kisutch* exposed to waste bins (McLeay 1973) or chlorinated residues (Buckley et al. 1976), and in *Colisa fasciatus* exposed to lead (Srivastav and Mishra 1979). Witeska (2005), based on the results of tests performed with lead, copper, cadmium, and zinc, concluded that all metals induce an increase in the frequency of erythroblasts in the common catfish *(Cyprinus carpio).*

The number of white cells can also be affected by physiological and environmental factors. One of the most common responses seen in fish exposed to toxic substances is a decrease in the percentage of lymphocytes and an increase in heterophils (Witeska 2005). However, both activation and suppression of the immune system can be observed with aquatic contaminants due to the great variety in terms of route of exposure, time of exposure, dose, and fish species (Cuesta et al. 2011). Modesto and Martínez (2010) reported an increase in the total number of

leukocytes and lymphocytes and a decrease in neutrophils in juveniles of *Prochilodus lineatus* treated with a formulated herbicide containing glyphosate. In OP-exposed *C. dimerus*, the total leukocyte count showed a slight increase, particularly in individuals treated with 150 μg/L OP, possibly due to the significant increase in the number of lymphocytes.

Under stress conditions, the blood clotting system of fish becomes more active, and therefore thrombocyte count can markedly increase (Casillas and Smith 1977). However, although no significant differences between control and OP-exposed *C. dimerus* were observed for the average number of thrombocytes, a noticeable decrease in this value was observed for both concentrations of OP assayed.

Whenever a static renewal system is employed to perform chemical compound-exposure experiments, the difference between measured and nominal concentrations in exposure chambers tends to be especially noticeable due to glass adsorption, uptake by fish, microbial degradation, and photolysis (Lewis and Lech 1996). Here, we determined that the actual OP concentration decreased to <50 % of the nominal concentration during a 24-h period and resulted only a 20 % decrease at 72 h. Therefore, the effects registered with our experimental design were produced by exposure to concentrations considerably lower than the nominal values. This tendency toward OP decrease was determined from measurements performed only in the 150 μg/L treatment; however, comparable results are expected with the other concentration employed. In a similar experiment, Kinnberg et al. (2000) recorded a comparable decrease of the actual concentration of NP to an average of 15–23 % of nominal concentrations during a 3-day period. Likewise, Metcalfe et al. (2001) found that the measured concentrations of estradiol and ethinyl-estradiol in water during a 48-h period were, on average, 43 and 29 % of the nominal concentrations, respectively, whereas Balch and Metcalfe (2006) registered a decrease of NP level to 29 % of the nominal concentration in the same time period.

Conclusion

Although hematological parameters can vary due to the influence of various intrinsic and extrinsic factors, taken together, the type of anemia and the markedly increased number of circulating immature erythrocytes along with the occurrence of amitotic cells show that all of the evaluated parameters could be used as biomarkers for the presence of xenobiotics in water.

Acknowledgments We are deeply indebted to Graciela Guerrero, friend, colleague, and recognized mentor. We deeply appreciate the revision of the grammar by R. Da Cuña and the valuable comments of F. Meijide. The present work was supported by University of Buenos Aires (X650), and CONICET (PIP 2302).

References

Balch G, Metcalfe C (2006) Developmental effects in Japanese medaka *(Orizias latipes)* exposed to nonylphenol ethoxilates and their degradation products. Chemosphere 62:1214–1223

Barni S, Fraschini A, Prosper E, Vaccarone V, Bernini F (1995) Possible occurrence of amitotic cell division during haemopoiesis in the Urodeles. Comp Haematol Int 45:183–188

Bennie DT, Sullivan CA, Lee HB, Peart TE, Maguire RJ (1997) Occurrence of alkylphenols and alkylphenol mono- and diethoxylates in natural waters of the Laurentian Great Lakes basin and the upper St. Lawrence River. Sci Total Environ 193:263–275

Bhagwan ST, Bhikajee M (2000) Induction of hypochromic macrocytic anaemia in *Oreochromis* hybrid (Cichlidae) exposed to 100 mg/L (sublethal dose) of aluminium. Sci Technol Res J 5:9–20

Blackburn MA, Waldock MJ (1995) Concentrations of alkylphenols in rivers and estuaries in England and Wales. Water Res 29:1623–1629

Buckley JA, Whitmore CM, Matsuda RI (1976) Changes in blood chemistry and blood cell morphology in Coho salmon (*Oncorhynchus kisutch*) following exposure to sublethal levels of total residual chlorine in municipal wastewater. J Fish Res Board Can 33:776–782

Canadian Council on Animal Care (2005) Guidelines on the care and use of fish in research, teaching and testing, CCAC, pp. 94. http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf. Accessed Nov 2010

Casillas E, Smith LS (1977) Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). J Fish Biol 10:481–491

Cuesta A, Meseguer J, Esteban MA (2011) Immunotoxicological effects of environmental contaminants in teleost fish reared for aquaculture. In: Stoytcheva M (ed) Pesticides in the modern world—risks and benefits, 3rd edn, pp. 571

Da Cuña RH, Rey Vázquez G, Piol MN, Verrengia Guerrero N, Maggese MC, Lo Nostro FL (2011) Assessment of the acute toxicity of the organochlorine pesticide endosulfán in *Cichlasoma dimerus* (Teleostei, Perciformes). Ecotoxicol Environ 74:1065–1073

Da Cuña R, Pandolfi M, Genovese G, Piazza Y, Ansaldo M, Lo Nostro F (2013) Endocrine disruptive potential of endosulfan on the reproductive axis of *Cichlasoma dimerus* (Perciformes, Cichlidae). Aquat Toxicol 126:299–305

Dorafshan S, Kalbassi M, Pourkazemi M, Amiri B, Karimi S (2008) Effects of triploidy on the Caspian salmon *Salmo trutta caspius* haematology. Fish Physiol Biochem 34:195–200

Gabriel UU, Ezeri GNO, Opabunmi OO (2004) Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch 1822). Afr J Biotechnol 3:463–467

Genovese G, Da Cuña R, Towle D, Maggese MC, Lo Nostro F (2011) Early expression of zona pellucida proteins under octylphenol exposure in *Cichlasoma dimerus* (Perciformes, Cichlidae). Aquat Toxicol 101(1):175–185

Genovese G, Regueira M, Piazza Y, Towle D, Maggese MC, Lo Nostro F (2012) Time-course recovery of estrogen-responsive genes of a cichlid fish exposed to waterborne octylphenol. Aquat Toxicol 16(114–115):1–13

Genten F, Terwinghe E, Danguy A (2009) Atlas of fish histology. Science, Enfield

Girón-Pérez MI, Barcelós-García R, Vidal-Chavez ZG, Romero-Bañuelos CA, Robledo-Marenco ML (2006) Chlorpyrifos on the hematology and phagocytic activity of Nile tilapia cells (*Oreochromis niloticus)*. Toxicol Mech Methods 16(9):495–499

Girón-Pérez MI, Montes-López M, García-Ramírez LA, Romero-Bañuelos CA, Robledo-Marenco ML (2008) Effect of sub-lethal concentrations of endosulfan on phagocytic and hematological parameters in Nile tilapia (*Oreochromis niloticus*). Bull Environ Contam Toxicol 80:266–269

Han Y, Wang K, Zhang L, Liu M (2007) Comparison on erythrocyte and some haematology indices of diploid and triploid rainbow trout (*Oncorhynchus mykiss*). Freshw Fish 37:52–55

Hibiya T (1982) An atlas of fish histology. Normal and pathological features. Kodansha Ltd., Bunkyō

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP (1996) Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ Toxicol Chem 15:194–202

Kaplow LS (1955) A histochemical procedure for localizing and valuating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood 10:1023–1102

Kinnberg K, Korsgaard B, Bjerregaard P, Jespersen A (2000) Effects of nonylphenol and 17b-estradiol on vitellogenin synthesis and testis morphology in male platyfish, *Xiphophorus maculatus*. J Exp Biol 203:171–181

Knörr S, Braunbeck T (2002) Decline in reproductive success, sex reversal, and developmental alterations in Japanese medaka (*Oryzias latipes*) after continuous exposure to octylphenol. Ecotoxicol Environ Saf 51:187–196

Kullander SO (1983) A revision of the South American Cichlid Genus *Cichlasoma* (Teleostei: Cichlidae). Swedish Museum of Natural History, Stockholm

Kullander SO (2003) Cichlidae. In: Reis RE, Kullander SO, Ferraris CJ Jr (eds) Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre, pp 605–654

Lewis SK, Lech JJ (1996) Uptake, disposition, and persistence of nonylphenol in rainbow trout. Xenobiotica 26:813–819

Martins ML, Tavares-Dias M, Fujimoto RY, Onaka EM, Nomura DT (2004) Haematological alterations of *Leporinus macrocephalus* (Osteichtyes: Anostomidae) naturally infected by *Goezia leporini* (Nematoda: Anisakidae) in fish pond. Arq Bras Med Vet Zoot 56:640–646

McLeay DJ (1973) Effect of a 12-hr and 25-day exposure to Kraft pulpmill effluent on the blood and tissue of juvenile Coho salmon (*Oncorhynchus kisutch*). J Fish Res Board Can 30:395–400

Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ et al (2001) Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 20:297–308

Modesto KA, Martínez CBR (2010) Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere 81:781–787

Moncaut N, Lo Nostro F, Maggese MC (2003) Vitellogenin detection in surface mucus of the South American cichlid fish *Cichlasoma dimerus* (Heckel 1840) induced by estradiol-17β. Effects on liver and gonads. Aquat Toxicol 63:127–137

Mousavi SE, Yousefian M (2012) Effects of exogenous hormones on plasma cortisol, sex steroid hormone and glucose levels in male and female grass carp, *Ctenopharyngodon idellus*, during the spawning induction. Afr J Biotechnol 11(36):8920–8927

Natt MP, Herrick CA (1952) A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poult Sci 31:735–738

Nussey G, Van Vuren JHJ, Preez HH (1995) Effect of copper on the haematology and osmoregulation of the *Mozambique tilapia*, *Oreochromis mossambicus* (Cichlidae). Comp Biochem Physiol C 111:369–380

Pan Y (1956) Study on histology of frog blood. Acta Sci Nat Univ Pekin 1:89–101

Petri D, Glover CN, Ylving S, Kolås K, Fremmersvik G, Waagbø R, Berntssen MH (2006) Sensitivity of Atlantic salmon (*Salmo salar*) to dietary endosulfan as assessed by haematology, blood biochemistry, and growth parameters. Aquat Toxicol 80(3):207–216

Piazza Y, Pandolfi M, Lo Nostro F (2011) Effect of the organochlorine pesticide endosulfan on GnRH and gonadotrops cell populations in fish larvae. Arch Environ Contam Toxicol 61:300–310

Rasmussen TH, Teh SJ, Bjerregaard P, Korsgaard B (2005) Anti-estrogen prevents xenoestrogen-induced testicular pathology of eelpout (*Zoarces viviparus*). Aquat Toxicol 72:177–194

Rey Vázquez G, Guerrero GA (2007) Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue Cell 39:151–160

Rey Vázquez G, Meijide F, DaCuña R, LoNostro F, Piazza Y, Babay P et al (2009) Exposure to waterborne 4-*tert*-octylphenol induces vitellogenin synthesis and disrupts testis morphology in the South American fresh water fish *Cichlasoma dimerus* (Teleostei, Perciformes). Comp Biochem Physiol C 150:298–306

Sabri DM, El-Danasoury MAE-H, Eissa IAE-M, Khouraiba HM (2009) Impact of henneguyosis infestation on hematological parameters of catfish (*Clarias garipienus*). Int J Agric Biol 11:228–230

Seiverd CE (1964) Haematology for medical technologists. Lea and Febiger, Philadelphia, p 946

Sole M, de Alda MJL, Castillo M, Porte C, Ladegaard-Pedersen K, Barcelo D (2000) Estrogenicity determination in sewage treatment plants and surface waters from the Catalonian area (NE Spain). Environ Sci Technol 34:5076–5083

Srivastav AK, Mishra S (1979) Blood dyscrasia in a teleost, *Colisa fasciatus*, after acute exposure to sublethal concentrations of lead. J Fish Biol 14:199–203

Wang B, Liu Y, Chen X, Fan Z (2010) Amitosis-like nuclear division in erythrocytes of triploid rainbow trout *Oncorhynchus mykiss*. J Fish Biol 76:1205–1211

Warhurst AM (1995) An environmental assessment of alkylphenol ethoxylates and alkylphenols. Friends of the Earth, London

White R, Jobling S, Hoare SA, Sumpter JP, Parker MG (1994) Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135:175–182

Whitmore CM (1965) A microcytic anemia of juvenile chinook salmon resulting from diets deficient in vitamin E. fish commission of Oregon Portland, Oregon. Contribution 29:1–31

Witeska M (2005) Stress in fish—hematological and immunological effects of heavy metals. Electron J Ichthyol 1:35–41

Ying GG, Williams B, Kookana R (2002) Environmental fate of alkylphenols and alkylphenol ethoxylates—a review. Environ Int 28:215–226

Yokote M (1982) In: Hibiya T (ed) An atlas of fish histology. Normal and pathological features. Chap. VI. Vascular system. Kodansha Ltd., Bunkyō

Zou S, Li S, Cai W, Yang H (2006) Morphological differences of erythrocyte in autotetraploid, interploid 3n of bluntsnout bream, *Megalobrama amblycephala*. J Fish Sci China 13:891–896