Toxicology and Environmental Health Sciences
https://doi.org/10.1007/s13530-021-00116-3

ORIGINAL ARTICLE

Risk of chlorine dioxide as emerging contaminant during SARS-CoV-2 pandemic: enzyme, cardiac, and behavior effects on amphibian tadpoles

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Accepted: 21 October 2021

Abstract

Objective The use of chlorine dioxide (ClO_2) increased in the last year to prevent SARS-CoV-2 infection due to its use as disinfectant and therapeutic human treatments against viral infections. The absence of toxicological studies and sanitary regulation of this contaminant represents a serious threat to human and environmental health worldwide. The aim of this study was to evaluate the acute toxicity and sublethal effects of ClO_2 on tadpoles of *Trachycephalus typhonius*, which is a common bioindicator species of contamination from aquatic ecosystems.

Materials and methods Median lethal concentration (LC50), the lowest-observed effect concentration (LOEC), and the no-observed effect concentration (NOEC) were performed. Acetylcholinesterase (AChE) and glutathione-S-transferase (GST) activities, swimming behavior parameters, and cardiac rhythm were estimated on tadpoles of concentrations ≤ LOEC exposed at 24 and 96 h. ANOVA and Dunnett's post-hoc comparisons were performed to define treatments significance ($p \leq 0.05$).

Results The LC50 of ClO_2 was 4.17 mg L^{-1} (confidence limits: 3.73–4.66). In addition, NOEC and LOEC values were 1.56 and 3.12 mg L^{-1} ClO_2, respectively, at 48 h. AChE and GST activities, swimming parameters, and heart rates increased in sublethal exposure of ClO_2 (0.78–1.56 mg L^{-1}) at 24 h. However, both enzyme activities and swimming parameters decreased, whereas heart rates increased at 96 h.

Conclusion Overall, this study determined that sublethal concentrations of ClO_2 produced alterations on antioxidant systems, neurotoxicity reflected on swimming performances, and variations in cardiac rhythm on treated tadpoles. Thus, our findings highlighted the need for urgent monitoring of this chemical in the aquatic ecosystems.

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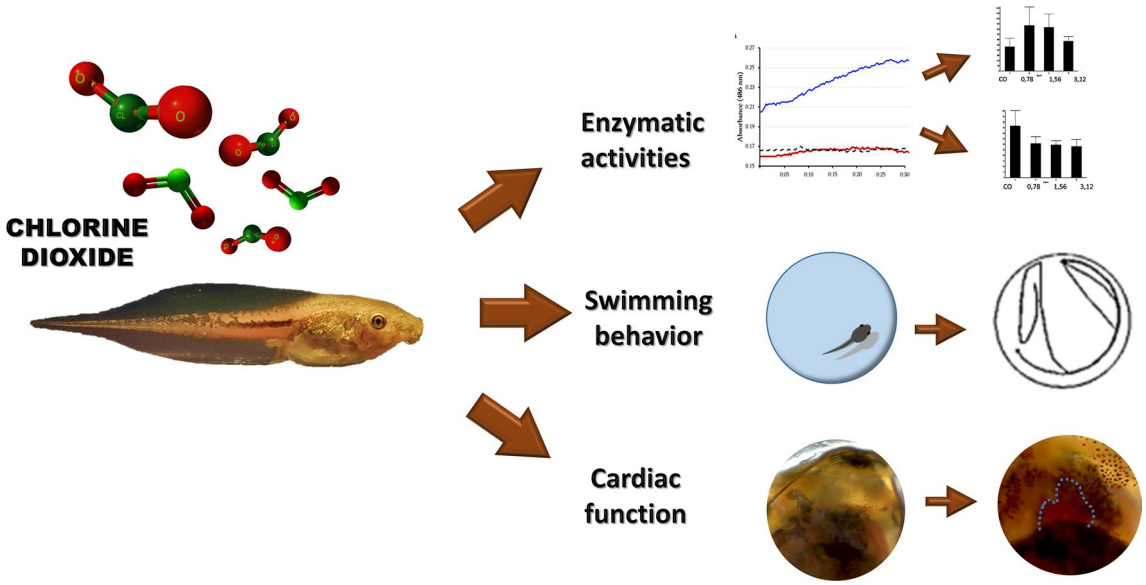
Published online: 03 November 2021

Graphical abstract



Keywords Chlorine disinfectant · COVID-19 treatment · Anurans · Sublethal toxicity

Introduction

Chlorine dioxide (ClO_2) is a disinfection agent that is massively used due to its high oxidizing capacity that deactivates chlorine-resistant pathogens and prevents biofilm formation [1]. In the last decades, drinking water treatments and industrial processes increase the use of ClO_2, which replaces other chlorine disinfectants [2, 3]. Several studies determined that the consumption of ClO_2 solutions is a preventive and therapy tool against different viral human infections, such as HIV/AIDS [4, 5]. The occurrence of ClO_2 had an exponential increase in surface water during the last two years due to its use as treatment and preventive therapy against SARS-CoV-2 [6, 7]. Although ClO_2 lacks of scientific and sanitary approval as medical treatment against SARS-CoV-2 for human consumption, the ClO_2 began to be commonly commercialized specially in Latin America [8–10]. Moreover, the increased use of ClO_2, together with other pharmaceutical products and disinfection agents poses a serious threat to aquatic systems, animal and human health [11, 12].

In addition, treatment protocols to prevent waterborne pathogens in public water systems and drinking water was determined by the World Health Organization, including also those treatments with ClO_2 water. It has been reported that a concentration higher than 0.5 mg L^{-1} of free chlorine and 2.19 mg L^{-1} ClO_2 inactivate SARS viruses. In general, residual chlorine and ClO_2 concentrations in wastewater treatments do not exceed 6.5 mg L^{-1} and 10 mg L^{-1}, respectively [6, 13, 14]. However, solutions of 20 mg L^{-1} ClO_2 are recommended for SARS-CoV-2 inactivation for domestic use as disinfectant, whereas solutions over 500 mg L^{-1} ClO_2 are suggested for hospital and health unit care disinfections. Thus, maximum concentrations of 0.8 mg L^{-1} ClO_2 and 1.0 mg L^{-1} chlorite ion for drinking waters were recommended by U.S.EPA recommends [15].

During COVID-19 pandemic, sanitation with chlorinated products increased in domestic wastewater, drinking water, and surface water, which pose a healthy human and environmental risk [16]. For example, concentrations of chlorine ranged between 1.5 and 4−5 mg L^{-1} in wastewater treatment plants were used for preventive measure in China [17]. The same study recorded residual chlorine concentration up to 0.4 mg L^{-1} in some Chinese lakes, where had not been detected before the actual COVID pandemic. This concentration produced acute toxicity on freshwater organism [17].

The effect of ClO_2 on virus, bacteria, algae, and plankton has been analyzed during last decades [18–20]. However, few studies described ClO_2 effect on wild organisms, such as aquatic vertebrates. The ClO_2 lethal toxicity has been reported for the early stages of several fishes [21, 22], chironomid larvae [23], and nematodes [24]. It is important to note that ClO_2 toxicity data obtained for common sentinel organisms of model organisms (e.g., Cyprinidae, Daphnidae, Brachionidae, and Hyalelladae) is generally used by U.S.EPA water quality criteria method [25]. The monitoring of ClO_2 on aquatic environments and ecotoxicological studies that include lethally and sublethal parameters of other

sentinel species is needed. To the best of our knowledge, no information exists on ClO_2 toxicity on amphibian tadpoles.

Amphibians present the highest decrease of population than other vertebrates worldwide, due to their complex life cycle that involves physiological, morphological, and behavioral changes during metamorphosis. These biological traits increase amphibian vulnerability to man-made environmental modifications [26–28]. Contaminants (e.g., pharmaceuticals, agrochemicals, health care products) affect survival and produce several sublethal effects on amphibians [29, 30]. Death and sublethal effects at different biological levels (e.g., cell, endocrinology, metabolic and behavior alterations, metamorphosis rates, malformations, among others) were determined for amphibian tadpoles [31–33]. In addition, the cholinesterase (ChEs) enzyme activity is useful to indicate the presence of neurotoxic substances in amphibian tadpoles [34]. Moreover, acetylcholinesterase (AChE) activity is inhibited in presence of neurotoxic substances, leading to acetylcholine accumulation and producing alterations in nerve function that can be lethal on exposed organisms [35]. The alteration in normal AChE activity has been linked with neurotoxic effects on locomotor activity in different invertebrates and vertebrates [36, 37]. Thus, glutathione-S-transferase (GST) activity is used to assess the detoxification process of contaminants [38]. The GST activity is frequently quantified in amphibian tadpoles exposed to several contaminants, such as pharmaceutical residues [39, 40]. However, other sublethal effects of tadpole regarding to physiology, such as heart function and rhythm are poorly explored [41]. Tadpoles' heart rate is considered novel biomarkers in amphibian ecotoxicology [32, 42] and could reflect of what happens at biochemical, physiological, and ecological levels in aquatic organisms exposed to different contaminants [43].

The aim of this study was to evaluate the acute and sublethal effects of ClO_2 on *Trachycephalus typhonius* tadpoles. It has been hypothesized that the ClO_2 produces different biological changes on AChE and GST activities and physiological (cardiac rhythm and swimming behavior) endpoints.

Results and discussion

Mortality

LC_{50} of ClO_2 was 4.17 mg L^{-1} (CL: 3.73–4.66) at 48 h. NOEC and LOEC values were 1.56 and 3.12 mg L^{-1} ClO_2, respectively, at 48 h. The TU for ClO_2 was 23.98, meaning a high acute toxicity substance (Class IV) in the hazard classification system.

The toxicity of ClO_2 has been studied in alkaline solutions when it becomes chlorate [44]. In fishes (feather minnows *Pimephales promelas*), the LC_{50} was determined to be 0.19 mg L^{-1} of ClO_2, whereas LC_{50} was recorded to 63.38 mg L^{-1} of chlorite within 24 h of exposure [45, 46]. In amphibians, the LC_{50} values are only reported for chlorite concentration that varied between 65.69 mg L^{-1} (*Lithobates pipiens*) and 149.60 mg L^{-1} (*Anaxyrus americanus*). Chlorite is much less toxic than its precursor chlorine [25]. No estimation could be made regarding to the toxicity of ClO_2 in terms of values of LC_{50} obtained in this study for *T. thyphonius* with respect to other amphibian species because mortality values of ClO_2 are unknown for other anurans. Time of exposure, acclimation temperature, and chlorine species can be considered as factors that influence the ClO_2 toxicity [46]. For example, the LC_{50} values of ClO_2 varied between 0.41 and 0.23 mg L^{-1} for different instar larvae (1st to 4th) of chironomid species at 24 h [23], whereas these values were 0.022 mg L^{-1} for planktonic crustacean (*Daphnia magna*) and 47 mg L^{-1} for mosquitoes larvae [25].

Enzyme activities

AChE activity significantly increased (51%, $F = 7.18$, $p < 0.01$) in 0.78 mg L^{-1} ClO_2 treated tadpoles with respect to CO (Dunnett's test $p < 0.01$) at 24 h (Fig. 1A, Table S1 in supplementary material). In contrast, AChE activity significantly decreased ($F = 5.16$, $p < 0.01$) between 32.15 and 36.51% in ClO_2 treated tadpoles with respect to CO (Dunnett's test $p < 0.01$) after 96 h of exposure (Fig. 1B, Table S1). In addition, exposure to ClO_2 also altered the AChE activity of larvae of indianmeal moth (**Plodia interpunctella**) [47]. Matsushita et al. [48] who recognized ClO_2 as insecticide, reported that ClO_2 produced a fast increase of AChE activities in *P. interpunctella* than those insecticide-based-malathion or -methidathion formulations. Moreover, the AChE activities decreased with increasing chlorination time in ClO_2 treated *T. thyphonius* tadpoles at 24 and after 96 h, which may be explained in terms of enzyme synthesis as a response to initial inhibition [49]. It is important to note that AChE is involved in the cleavage of the acetylcholine neurotransmitter within the synaptic cleft. An inhibition of AChE activity leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission, consequently affecting the animal behavior [50].

The GST activity of ClO_2 treated tadpoles significantly increased ($F = 4.47$, $p < 0.05$) in 0.78 mg L^{-1} and 1.56 mg L^{-1} concentrations with respect to CO (Dunnett's test $p < 0.05$) at 24 h (87.84% and 79.41%, respectively; Fig. 1B, Table S1). There was a positive correlation between the responses of both AChE and GST enzyme activities (r Spearman = 1; $p < 0.01$) at 24 h. The mean GST activity significantly decreased (30%, $F = 8.19$, $p < 0.01$) in 0.78 mg L^{-1} ClO_2 concentration with respect to CO (Dunnett's test $p < 0.01$) after 96 h (Fig. 1B, Table S1).

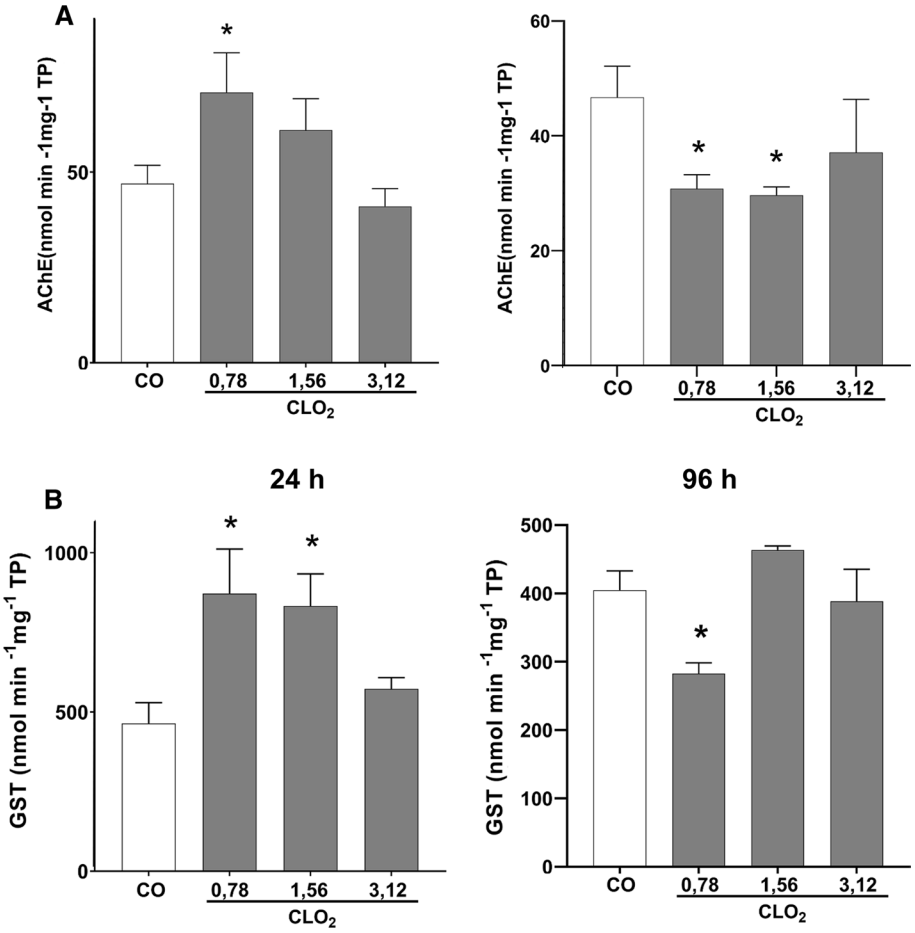


Fig. 1 Acetylcholinesterase (AChE) and glutathione-S-transferase (GST) activities (nmol min^{-1} mg^{-1} protein) in *Trachycephalus typhonius* tadpoles from control (CO) and chlorine dioxide (ClO_2 mg L^{-1}) treatments, at 24 h (left) and 96 h (right) of exposure. $p < 0.05$ (*) and $p < 0.01$ (**) indicates statistical differences with respect to CO

The ClO_2 is recognized as a powerful oxidant that affects the first line of antioxidant defense, which is mediated by GST in animals [51]. Besides, the GST plays a pivotal role in cellular detoxification via conjugation of their electrophilic group with the GSH nucleophilic group of harmful contaminants. In contrast to our results, Elia et al. [52] reported an increase in liver GST activity in carp fishes (*Cyprinus carpio*) exposed to 1.6 mg L^{-1} ClO_2 after 20 days, suggesting that GST acts as a useful biomarker of disinfectants-mediated oxidative stress. The significant decrease in the GST activity in treated tadpoles exposed to 0.76 mg L^{-1} of ClO_2 after 96 h, could be related to the availability of substrate of this enzyme. It is well known that ClO_2 oxidizes the glutathione and produces several products that block substrates for normal reaction [53].

Swimming activity

The mean total distance of ClO_2 treated tadpoles significantly increased ($F = 39.73$ $p < 0.01$) in 0.78 and 3.12 mg L^{-1} ClO_2 concentrations (103.19% and 139.36%, respectively) with respect to CO (Dunnett's test $p < 0.01$) at 24 h (Fig. 2A, Table S1). The mean speed of ClO_2 treated tadpoles increased ($F = 77.18$, $p < 0.01$) in all concentrations (103.16; 78.29 and 139.36% for 0.78; 1.56 and 3.12 mg L^{-1}, respectively; Fig. 2B, Table S1) with respect to CO (Dunnett's test $p < 0.01$). The global activity significantly increased ($F = 53{,}02$, $p < 0.05$) at 1.56 and 3.12 mg L^{-1} ClO_2 concentrations (456.57 and 994.48%, respectively; Fig. 2C, Table S1) with respect to CO (Dunnett's test $p < 0.05$). The effects of ClO_2 concentrations on global activity were negatively correlated with both enzyme activities (r Spearman = -1; $p < 0.01$) at 24 h.

The mean total distance significantly decreased ($F = 44.03$, $p < 0.05$) in 1.56 and 3.12 mg L^{-1} ClO_2 treated tadpoles (43.67 and 66.51%, respectively) with respect to CO (Dunnett's test $p < 0.05$) after 96 h (Fig. 2A, Table S1). Also, the mean speed significantly decreased ($F = 18.04$, $p < 0.05$) in the same concentrations (44.4 and 58.34%, respectively) with respect to CO (Dunnett's test $p < 0.05$; Fig. 2B, Table S1)). Similarly, the global activity significantly decreased ($F = 25.8$, $p < 0.05$) in 0.78 mg L^{-1} ClO_2 treated tadpoles (64.76%) with respect to CO (Dunnett's test $p < 0.05$) after 96 h (Fig. 2C, Table S1).

The observed responses of swimming activity parameters indicated that ClO_2 affects the mechanisms involved

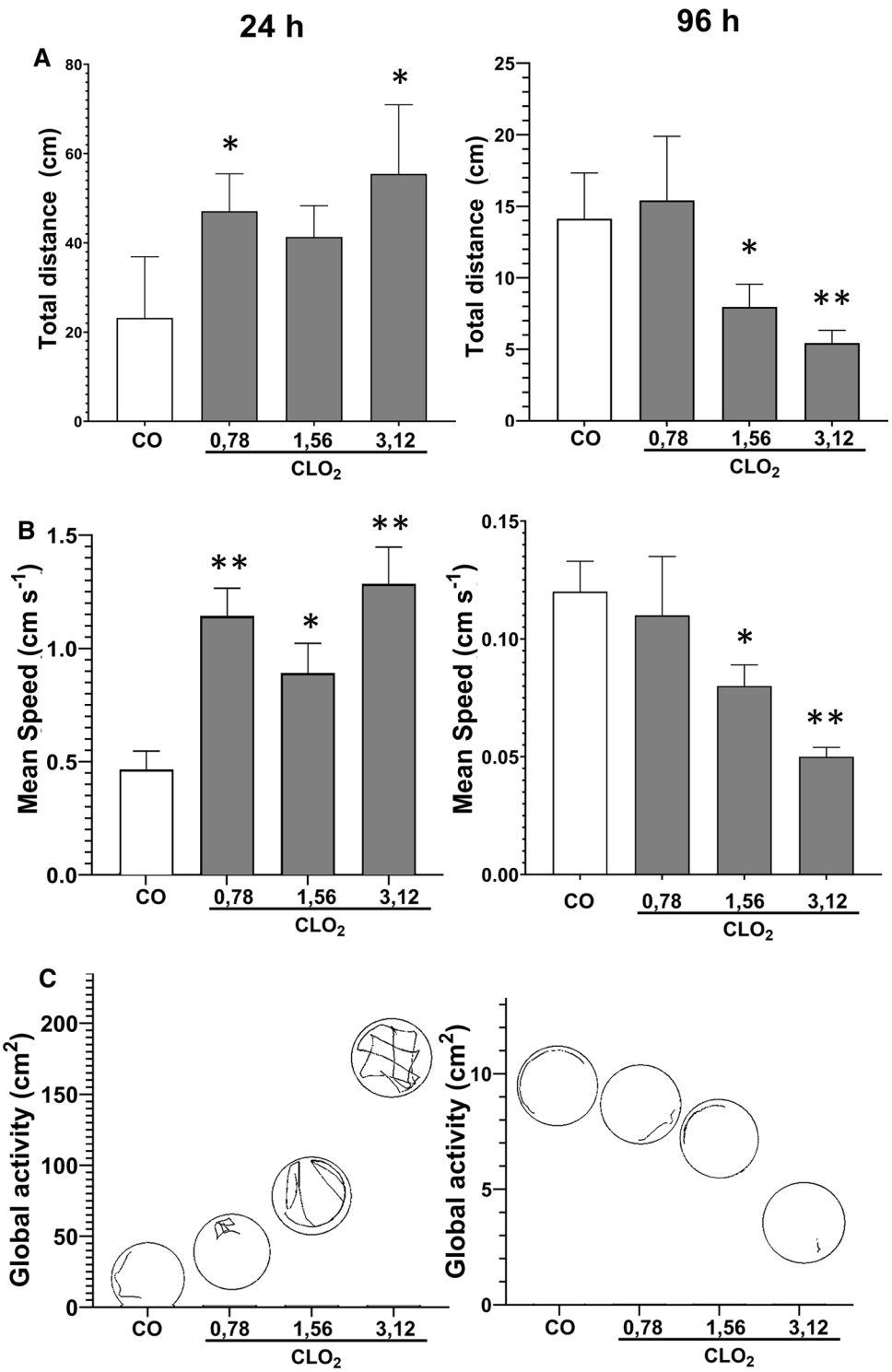


Fig. 2 Swimming activity of *Trachycephalus typhonius* tadpoles of control (CO) and chlorine dioxide (ClO_2 mg L^{-1}) treatments at 24 h (left) and after 96 h (right) of exposure: **A** Total distance moved (cm); **B** Mean speed (cm s^{-1}); **C** Global activity (cm^2), showing the trajectory of individual in each concentration. $p < 0.05$ (*) and $p < 0.01$ (**) indicates statistical differences with respect to CO

in swimming behavior at different times (24 and after 96 h). For example, swimming behavior has been related to AChE activity in invertebrates, fish, and amphibian larvae exposed to different contaminants [32, 34, 39, 54, 55]. In this sense, swimming behavior observed in ClO_2 treated tadpoles at 24 h may be related to neurotoxicity through AChE over-activation [50]. Conversely, decrease in swimming behavior observed in ClO_2 treated tadpoles after 96 h could be related to the disruption of the normal nervous system function by inhibition of the AChE activity and the resulting transduction signal [56, 57]. Regarding to these behavior disparities, a higher expression of AChE genes may occur as a response to acetylcholine neurotransmitters accumulation [58].

Yonkos et al. [45] described gill pathology with epithelial lifting, hypertrophy, hyperplasia, lamellar fusion, and necrosis on feather minnow *P. promelas* exposed to ClO_2. Histological alterations in gills may produce alteration in swimming activity due to its demands of oxygen [59]. In this sense, the decrease of swimming parameters after 96 h of exposure may be related to structural changes in the body of tadpoles such as gills that are related to physiological changes [60]. Moreover, Orme et al. [61] reported a reduced level of thyroxine in Sprague Dawley rats exposed to ClO_2 during development and demonstrated anti-thyroid and neuro-behavioral effects during their development. Thyroid hormones play important roles in neuro-behavioral functioning during anuran development [32], however, studies regarding to thyrotoxicosis and neuro-behavioral performance are scarce.

Cardiac rhythm

The mean heart rate (heartbeats per minute) in 0.78 and 3.12 mg L^{-1} ClO_2 treated tadpoles significantly increased (84.61% and 70.19%; $F = 5.323$, $p < 0.01$) with respect to CO (Dunnett's test $p < 0.01$) at 24 h (Fig. 3, Table S1). Heart rates were positively related (r Spearman = 0.8; $p < 0.01$) to some swimming parameters (total distance, mean speed) and GST activities.

Heart rate showed a significant ($F = 7.25$, $p < 0.01$) increase in treated tadpoles exposed to 0.78, 1.56 and 3.12 mg L^{-1} ClO_2 concentrations after 96 h (75.88%, 84.93% and 69.64%, respectively) respect to CO (Dunnett's test $p < 0.01$; Fig. 3, Table S1). Heart rate showed a negative correlation with AChE (r Spearman = − 1; $p < 0.01$). Results of Spearman correlation between biological endpoints were summarized in Table S2 (supplementary material). The cardiac rhythm is recognized as effective biomarkers for amphibian's health in several ecotoxicological investigations [32, 39, 41, 62]. In the present study, heart rate increased in ClO_2 treated tadpoles at 24 and after 96 h. In contrast, reduction of heartbeats was reported in ClO_2 treated larvae of *P. interpunctella* [47]. The relation among heart rhythm, oxidative stress, and GST activities were determined in tadpoles exposed to other chemicals such as pyriproxyfen, however, mechanisms involved in these relations remain unclear [32]. A study on pigeons (*Columba livia*) suggested that ClO_2 may increase the risk of cardiac rhythm alteration due to an increase in plasma cholesterol levels and size of thrombocytes in blood vessels [63].

Materials and methods

Chemical

Chlorine dioxide was synthesized as described by Hey et al. [64] and Chhetri et al. [65]. Demineralized water (400 mL) was mixed with 25 mL of 4% HCl and 25% $NaClO_2$. This mixture was diluted to 1000 mL in demineralized water after an overnight reaction. This procedure produced an approximately 1 g L^{-1} chlorine dioxide solution, which was used for three replicates of stock solutions of 100 mg L^{-1} ClO_2. The stability of concentration of stock solutions was verified by chlorine dioxide Sensing Strip (Insta-TEST, LaMotte®-USA) [66, 67] in lab conditions immediately after preparation and at every 24 h for three consecutive days. These observations were used for defining the renewal time of treatment solutions. The initial concentration (0 h) was reduced by 30% at 24 h, and more than half at 48 h, so it was decided to renew the solutions at every 48 h.

Organisms and experimental design

Tadpoles (Gosner Stage 28–32) of the veined tree frog *Trachycephalus typhonius* were sampled from temporary small ponds from the Paraná River floodplain, Argentina (31° 11′

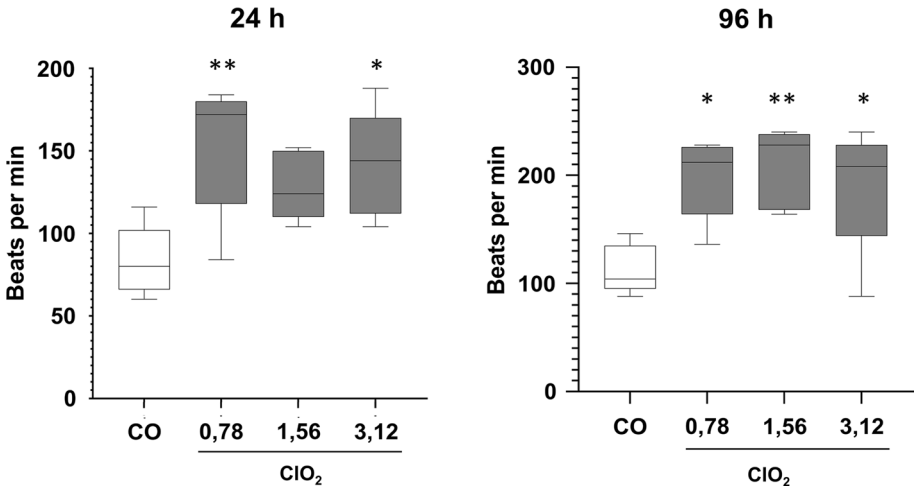


Fig. 3 Heart rates (beats min^{-1}) of *Trachycephalus typhonius* tadpoles of control (CO) and chlorine dioxide (ClO_2 mg L^{-1}) treatments at 24 h (left) and after 96 h (right) of exposure. $p < 0.05$ (*) and $p < 0.01$ (**) indicates statistical differences with respect to CO

31″ S, 60° 9′ 29″ W). Authorization for tadpole sampling was given by the Ministerio de Ambiente de la Provincia de Santa Fe (No 02101–0018518-1). Site of tadpole sampling was contamination-free, as determined in a previous research of our lab [68]. Tadpoles were transported immediately to laboratory in dechlorinated tap water (DTW) and acclimatized. Tadpoles' acclimation period was performed under controlled lab conditions of 12/12 h (light N100 Lx/dark photoperiod cycles) at 24 ± 2 °C for two days. Tadpole behavior such as eat and swim was checked daily.

The experiments followed regulations of ASIH [69]. Tadpoles were euthanized following the protocol of the Animal Euthanasia Guide proposed by the Institutional Animal Care and Use Committee and the Advisory Committee on Ethics and Safety in research of the Facultad de Bioquímica y Ciencias Biológicas of the Universidad Nacional del Litoral (No 388/06).

There is lack of environmental concentration data on aquatic ecosystems and doubts related to the fate of ClO_2 on surface freshwater. For this reason, a first approximation to the toxicity of ClO_2 on tadpoles was performed to determine the median lethal concentration (LC_{50}), similarly as previously performed for different contaminants [39]. A first bioassay was carried out by exposing three replicate solutions each containing 12 tadpoles. The nominal concentrations of ClO_2 assayed were: 12.5, 6.25, 3.125, 1.56, and 0.78 mg L^{-1}. Mortality was recorded at 24 and 48 h to estimate LC_{50}, lowest-observed effect concentration (LOEC), and no-observed effect concentration (NOEC) values.

The sublethal effects (enzyme activities, swimming behavior, and heart rhythm as biomarkers) were evaluated in tadpoles of treatments with ≤ LOEC at 24 and after 96 h of exposure. These effects were analyzed in subsequent bioassays with the same lab conditions. The solutions were renewed at 48 h in the 96 h experiment.

Biomarkers

Enzyme activities

Tadpoles ($n = 7$ per treatment) were weighed (g), and homogenized (1:10, w/v) in ice-cold 25 mM sucrose, 20 mMTris-HCl buffer (pH ¼ 7.4) containing 1 mM EDTA, using a polytron tissue grinder. The homogenates were centrifuged at 10,000 g for 15 min at 4 ± 1 °C, and stored at − 80 °C until measurements. Enzyme activities were quantified after considering total protein concentration by the Biuret method according to Kingsley [70]. AChE activity was determined colorimetrically following Ellman et al. [71] and expressed as nmol min^{-1} mg^{-1}protein (MEC = 13.6×10^3 M^{-1} cm^{-1}). The reaction mixture consisted on 25 mM Tris–HCl and contained 1 mM CaCl2 (pH = 7.6), 10 mL 20 µM acetylthiocholineiodide, and 50 µL 5,5-dithio-bis-(2-nitrobenzoic acid) (3 × 104 M, final concentration). The variation in optical density was measured in duplicate at 410 nm at 25 °C for 1 min, using a Jenway 6405 UV–vis spectrophotometer. GST activity was measured spectrophotometrically according to Habig et al. [72] with modifications of Habdous et al. [73] for mammal serum GST activity. The measurement was performed with 340 nm in 100 mM Na–phosphate buffer (pH 6.5), 20 µL of 0.2 mM 1-chloro-2,4-dinitrobenzene, 50 µL of 5 mM reduced glutathione, and the sample homogenates. GST activity was expressed as nmol min^{-1} mg^{-1}protein (molar extinction coefficient, MEC = 9.61×10^3 M^{-1} cm^{-1}).

Swimming activity

The swimming activity of individual tadpoles was recorded for one minute using a digital video camera (Motic®, 10.0 M pixel) that was mounted in a tripod and placed just above the petri dish. The petri dish was filled with 200 ml of different treatment solutions, following procedures previously established in our lab [39, 74]. For each treatment, three tadpoles were evaluated independently.

Total distance moved (cm), mean speed (cm seg^{-1}), and global activity (cm^2) were evaluated on each tadpole. These behavior parameters were quantified and analyzed using video-tracking software Smart (3.0.02 PanLab Harvard Apparatus ®).

Cardiac rhythm

Cardiac rhythm was evaluated following the methodology described for other native amphibian species [32, 39]. The body of tadpoles ($n = 5$ per treatment) was located in a thin concave plate in ventral side up position. Continual transillumination on heart area (front quarter body) was applied bottom-up with a spot-led light (Luxeon Rebel 3 wat Led). Videos were recorded in completely immobile tadpoles with a remote-triggered portable USB Digital Microscope (Video capture resolution: 640 × 480, 30 fps) at lab conditions (constant 24 °C) during 15 s.

The heart rate (HR; beats. min^{-1}) was quantified from slow-speed digital videos by direct visual examination of maximum systole ventricle beating [39, 75]. The number of beats measured in the recorded 15 s was multiplied by four to obtain the value of a complete one min [76, 77].

Data analyzes

The LC_{50} value and its respective 95% confidence limits (95% CL) were calculated by the trimmed Spearman–Karber method [78]. Post-hoc comparison Dunnett's test was used to analyze means of mortality of LOEC and NOEC values [79]. The toxicity of this compound was estimated according

to the hazard classification system for wastewater discharged into the aquatic environment because the effects of ClO_2 were not previously studied for tadpoles [80]. The obtained LC_{50} value was transformed into Toxic Units (TU) following the criterion of Lajmanovich et al. [81]: $TU = 100 / LC_{50}$. The values were classified into five classes: I (No acute toxicity) = $TU < 0.4$; II (Slight acute toxicity) = $0.4 < TU < 1$; III (Acute toxicity): $1 < TU < 10$; IV (High acute toxicity): $10 < TU < 100$; V (very high acute toxicity): $TU > 100$.

The data of biomarkers were expressed as means ± standard deviation (SD). Kolmogorov–Smirnov test and Levene test were used to confirm normality and homogeneity of variances, respectively. Differences in enzymatic activity of CO and ClO_2 treated tadpoles were analyzed by one-way ANOVA and Dunnett's test for post-hoc comparisons. GraphPad InStad® software was used for statistical analyzes. In addition, Spearman correlations were used to evaluate correlation between all pairs of variables (biochemical, behavioral, and cardiac endpoints, shown in table S2 from Supplementary material) for each treatment at 24 and after 96 h exposure. Values were considered significant at $p < 0.05$. No significant differences were found among replicates ($p > 0.05$), so, replicates from each treatment were pooled.

Conclusion

The present study determined the toxicity of ClO_2 in amphibian tadpoles for first time worldwide. These data would be included in the maximum concentration criterion of chlorine compound toxicity assessment that has only been determined for freshwater fish and invertebrates. Our findings indicate a high risk of ClO_2 exposure to anuran tadpoles that present alterations of AChE and GST enzymes, swimming behavior, and cardiac rhythm. The relevance of our study resides in the fact that this compound that extensively and continuously input to water become a threat for aquatic organisms and aquatic ecosystems functions and services, many of which are essential to human well—being.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13530-021-00116-3.

Acknowledgements We acknowledge Boccioni L.S. for supplying the chemical compounds for test solutions preparation and Lajmanovich R.F. for providing information regarding to the erroneous human ClO_2 use as "miracle mineral solution". We also thanks to ANPCyT FONCyT and UNL for providying subsidies (PICT 2017 N 1069-RCL; CAI+D 2020-PMP). Finally, we acknowledge reviewers for their comments and suggestions.

Author contributions Conceptualization: RL and PP; Methodology: PP, ACB, CM, AA and CC; Formal analysis and investigation: PP, ACB; Writing—original draft preparation and Writing—review and editing: PP, ACB and RL.

Funding This study was supported in part by National Agency for Promotion of Science and Technology, Argentine (ANPCyT FONCyT PICT, No. 1069), and Course of Action for Research and Science Promotion, Argentine (CAI D-UNL, PIC No. 100004 LI).

Availability of data and material Data presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest Paola M. Peltzer, Ana P. Cuzziol Boccioni, Andrés M. Attademo, Candela S. Martinuzzi, Carlina L. Colussi, and Rafael C. Lajmanovich declare that we have no conflict of interest.

Ethics approval Animals were treated according the Institutional Committee for the Care and Use of Animals (IACUC), and approval was obtained from the bioethics committee of the FBCB-UNL (Res. No. 388/06).

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