Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 18(2): 355-364, April 2024

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Acute toxicity assessment of chloroxylenol on the whirligig beetle, *Orectogyrus alluaudi* (Coleoptera: Gyrinidae)

Babatunde O. AMUSAN*, Ayorinde F. KOLEOSHO and Godswill E. RICHARD

Obafemi Awolowo University Ile-Ife, Zoology, Osun State, Nigeria.
**Corresponding author; E-mail: tundeamusan5@yahoo.com*

Received: 23-01-2024 Accepted: 02-04-2024 Published: 30-04-2024

ABSTRACT

This study assessed the acute toxicological effects of different concentrations of chloroxylenol (PCMX), an active ingredient in most liquid cleaning products, on the whirligig beetle *Orectogyrus alluaudi* Regimbart, 1889. This was done with the aim of determining the potential lethal effects of the chemical on this aquatic beetles. Whirligig beetles sourced from an unpolluted stream were subjected to varying concentrations of PCMX in controlled laboratory conditions. Five concentrations ranging from 2 to 32 mg/l were utilized alongside control groups, with exposure durations spanning 24 to 96 hours. Observations revealed alterations in the beetles' behaviour and swimming patterns preceding mortality. Calculated median lethal concentration (LC50) values at 24 to 96 hours demonstrated a concentration-dependent increase in mortality rates, with LC50 values ranging from 21.587 to 7.819 mg/L. This study underscores the detrimental impact of PCMX on aquatic beetle populations, providing valuable insights into the environmental risks associated with common cleaning agents.

Keywords: Chloroxylenol, Whirligig beetle, Mortality rate, Concentration-dependent, Toxicity, Environmental risk.

INTRODUCTION

Aquatic pollution is regularly reported all over the world, but developing countries account for a disproportionate number of these reports (Connor, 2015; Akeredolu et al., 2022). A significant fraction of this pollution is caused by xenobiotics or anthropogenic pollutants which get introduced into the environment as a result of human activity. Xenobiotics interfere with the correct functioning of the environment regardless of the concentration at which they are present (Biel-Maeso et al., 2019). Certain xenobiotics derived from municipal or industrial wastewater have equally been discovered in aquatic environments, particularly in freshwater ecosystems, in fact, the aquatic environment has been shown to be a critical sink for pollutants (Ugwu et al., 2005; Gavrilescu et al., 2015).

Chloroxylenol (4-chloro-3, 5-dimethylphenol or para-chloro-meta-xylenol, PCMX) is a xenobiotic commonly used as an antiseptic and disinfectant for skin disinfection and for cleaning surgical instruments in hospitals (Seibert et al.,2019) due to their antimicrobial properties such as killing of bacteria, fungi, algae, and viruses through disruption of their cell membranes (Wilson and

DOI : https://dx.doi.org/10.4314/ijbcs.v18i2.3

Mowad, 2007). As a result of the recent global pandemic caused by a new type of coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and Ebola, PCMX is now being added to many cosmetics and household chemicals just to improve the antimicrobial properties of such products (Neerukonda and Katneni, 2020; Wu et al*.,* 2020; Tan et al., 2021). The increased usage of PCMX through these ways eventually makes its way into aquatic environments via municipal wastewater treatment facilities, industrial discharges, agricultural runoff, landfill leachate, and atmospheric deposition (Baby et al., 2010; Au et al., 2023)**.** For instance, recently measured PCMX concentrations in surface waters in Jakarta (Indonesia) ranged from 20 to 1200 ng/L, which was clearly higher than previously recorded concentrations (Dsikowitzky et al., 2016, 2017). Furthermore, PCMX has also been found in greater abundance in European rivers (Kasprzyk-Hordern, 2008a, 2008b; 2009). This is an indication that the widespread and indiscriminate use of this chemical portends a serious environmental threat.

In spite of the universal and extensive application of PCMX and its perceived potential as a pollution causative agent, it is disheartening that only a few studies have assessed its potential toxicity to the environment. Available studies like Sreevidya et al. (2018) and Won et al. (2022) have reported that PCMX has the potential to endanger aquatic environmental health when released into the environment. It is also a known fact that when PCMX is released into the environment, it does not dissipate completely but remains in bioavailable forms in the atmosphere, water, and soils due to its physical and chemical properties (El-Badawy et al., 2018). The low volatility and solubility of PCMX in water also mean that it readily binds to soil, sediments and living organisms (Capkin et al., 2017; Sreevidya et al., 2018). As a result, PCMX residue in the aquatic environment poses toxicological risks to aquatic organisms at all trophic levels. Also, recent studies on PCMX toxicity to aquatic organisms have revealed genotoxicity, histopathology, embryonic mortality, neurotoxicity, morphology changes, and endocrine function modulation (Capkin et al., 2017; Sreevidya et al., 2018; Won et al., 2022).

Whirligig beetles, such as *Orectogyrus alluaudi* Regimbart, 1889, are among the aquatic insects that significantly contribute to the macroinvertebrate diversity of Tropical temporary ponds or slow-moving water bodies (Umar et al., 2018). The aquatic beetles are ecologically adapted to removing dead invertebrates from the water's surface, as one of the important roles they play in freshwater ecosystems. Environmental factors, such as stream chemistry and substrate characteristics, have been proposed to threaten their distribution in freshwater ecosystems and this has made them important bio-indicator of water quality (Polhemus, 2011). Given the critical ecological roles of whirligig beetles in freshwater ecosystems and the increasing availability of chloroxylenol residue around the aquatic environment, it is important that the toxicity of this chemical to aquatic life is determined. Hence, this study sought to provide information on the acute response of aquatic animals to Chloroxylenol using *O. alluaudi* as the test organism. The choice of *O. alluaudi* as a test organism was due to its abundance, distribution, and the important role the insect plays in freshwater ecosystems.

MATERIALS AND METHODS

Test chemical

Chloroxylenol is a commercial household antiseptic produced by Reckitt Benckiser Nigeria Limited and sold under the brand name Dettol. For this study, a 165 mL bottle of Dettol containing 4.8 percent w/v chloroxylenol was used. It was properly stored in order for its activity to be preserved before use.

Collection and acclimation of test organisms

Specimens of *Orectogyrus alluaudi* were collected from Opa reservoir, Obafemi Awolowo University, Ile-Ife, Nigeria. The coordinates of the collection location are

07°32.561'N and 04°32.550'E. A D-frame net was used to scoop the beetles from the water's surface. The collected beetles were transported to the Laboratory in two plastic bowls. They were then allowed to acclimatize to the Laboratory conditions and light: dark photoperiod of 12:12 h. The beetle samples were identified using the identification keys provided by (Brinck 1956). Prior to and during PCMX exposure, the test organisms were fed *Drosophila melanogaster* Meigen, 1930 (fruit flies) cultured in the Laboratory, using ripe bananas as culture media.

Toxicity test

The acute toxicity test was carried out in the Laboratory using the standard methods recommended by the American Society for Testing and Materials (ASTM, 2000). A range-finding test was conducted prior to the definitive test to ascertain the tolerable concentrations for the specimens. The following concentrations were chosen based on the range-finding test: 2, 4, 8, 16, 32 mg/L, and control. These concentrations were prepared by dilution of the PCMX stock solution using de-chlorinated tap water of known pH, conductivity, and total dissolved solids (TDS). Each exposure was performed in triplicates to ensure the accuracy and reliability of the results. A 1-litre test container containing ten beetles was utilized for each replicate across all exposure conditions. Exposure of the test organisms to PCMX was carried out for 96-hours, using a static exposure system which meant that there was no test solution renewal. The test was run under a 12:12hour light: dark photoperiod same as was for acclimation. The survival and mortality rates were tracked and recorded twice in each 24 hour period, and dead test organisms were removed to avoid ammonia build-up and dilution effects in the test solutions. A test organism was deemed dead when movement ceased and there was no response to gentle probing. Additionally, this study identified behavioural indicators in the beetles, such as avoidance or agitated behaviour, by visually watching how frequently the beetles tend to avoid the test solution at the exposure doses. Furthermore, as compared to control groups, the beetles' mobility was shown to be disoriented at times. At the start and end of the exposure period, certain physicochemical parameters of the test solutions were determined, including temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), and conductivity, using the American Public Health Association's (APHA, 1995) standard methods for chemical analysis of water.

Statistical analysis

The test chemical's median lethal concentration (LC_{50}) for *O. alluaudi* specimens were calculated using probit analysis recommended by Finney (1971) on IBM SPSS version 26. The physicochemical parameters of the test solutions were compared using two-way ANOVA on Graphpad prism 8.0 to test for significant differences in their values. A Turkeys multiple comparisons test was used to compare the percentage mortality for each concentration and the control at 24 hr, 48 hr, 72 hr, and 96 hr, from which the low observable effect concentration (LOEC) and no observable effect concentration (NOEC) was estimated. The level of significance was set at 5% ($P \leq 0.05$).

RESULTS

Physico-chemical tests

The water temperature remained relatively constant throughout the duration of the test, ranging between 20 and 23 degrees Celsius (°C). The conductivity of the test solutions differed significantly from control at the start of the tests but did not differ much at the end of exposure period (Figure 1). TDS measured at 0 h and at the end of the exposure, i.e., 96 h, were found to differ significantly ($p \leq 0.05$) from control (Figure 2). The DO (mg/l) and pH levels were not significantly different ($p \leq 0.05$) across the treatment compared with the control before and after the exposure (Figure 3 and 4).

Effect of chloroxylenol on beetle behaviour

Observing the beetles' behaviour indicated substantial reactions to different levels of exposure. At the lowest concentration of PCMX (2 mg/l), beetles did not exhibit restlessness or a preference to avoid the treated water. At larger concentrations, however, avoidance/agitation behaviour became more prominent, with beetles moving rapidly as if attempting to flee the test medium. This avoidance response grew stronger over time, especially at the highest levels of exposure, resulting in confusion in beetle movement when compared to the control group's whirligig pattern. Disorientation became more common after 24 hours of exposure, along with a significant drop-in general activity and movement. Overall, avoidance behaviour increased with concentration, indicating a dose-dependent response to sodium hypochlorite exposure.

Effect of chloroxylenol on beetle survival

There was no mortality recorded during the first 24 hours of tests at concentrations less than 16 mg/l; nevertheless, 16.67% and 100% mortality were found during the same test period at 16 mg/l and 32 mg/l test concentrations, respectively. After 24 hours of exposure, a statistically significant increase in mortality ($p \leq 0.05$) was detected for the other test concentrations along the concentration gradient. At 96 hours, mortality rates were 20%, 26.67%, 43.333%, and 60% for 2, 4, 8, and 16 mg/l concentrations, respectively. As illustrated in Figure 5, this trend revealed that death was time and concentration dependent. At 24, 48, 72, and 96 hours after exposure to Chloroxylenol, the LC_{50} values for *Orectogyrus alluaudi* were 21.587 (15.114-22.490) mg/l, 16.744 (12.608-17.206) mg/l, 11.638 (2.976-15.359) mg/l, and 7.819 (2.172-32.197) mg/l, respectively (Table 1). After 24, 48, 72, and 96 hours, the lowest observable effect concentration (LOEC) was estimated to be 13.507, 5.578, 2.307, and 1.097 mg/l, respectively (Table 1). At 24, 48, 72, and 96 hours, the NOEC values were 6.754, 2.789, 1.1535, and 0.5485 mg/l, respectively (Table 1)

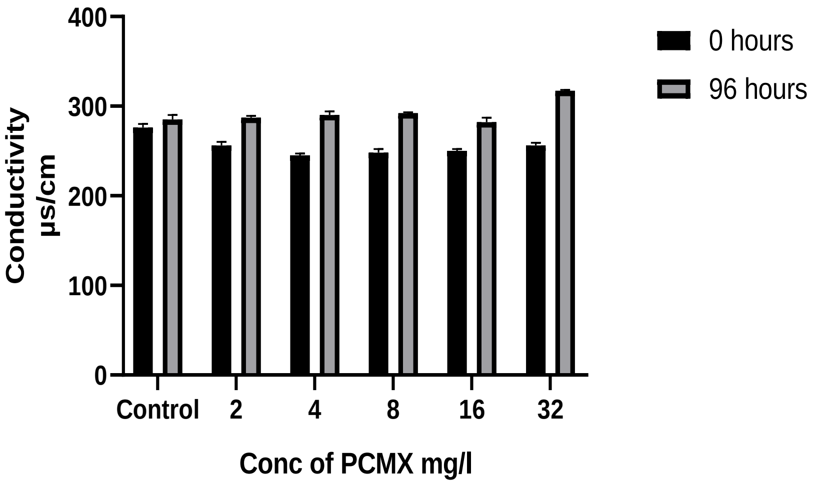

Figure 1: Conductivity in control and different concentrations of chloroxylenol at the 0 h and 96 h.

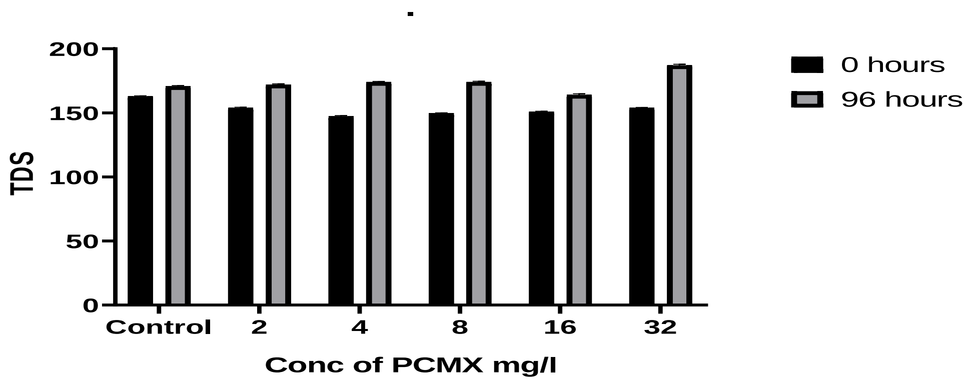

Figure 2: Total dissolved solids in control and different concentrations of chloroxylenol at the 0 h and 96 h.

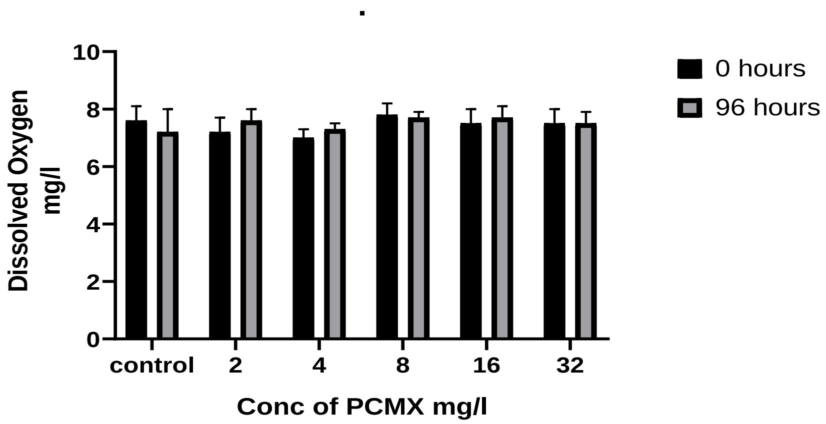

Figure 3: Dissolved Oxygen in control and different concentrations of chloroxylenol at the 0 h and 96 h.

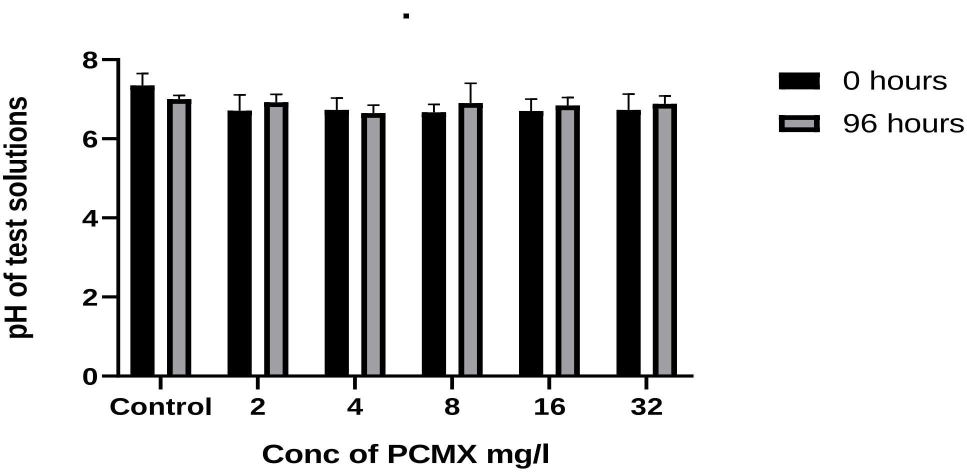

Figure 4: pH of control and different concentrations of chloroxylenol at the 0 h and 96 h.

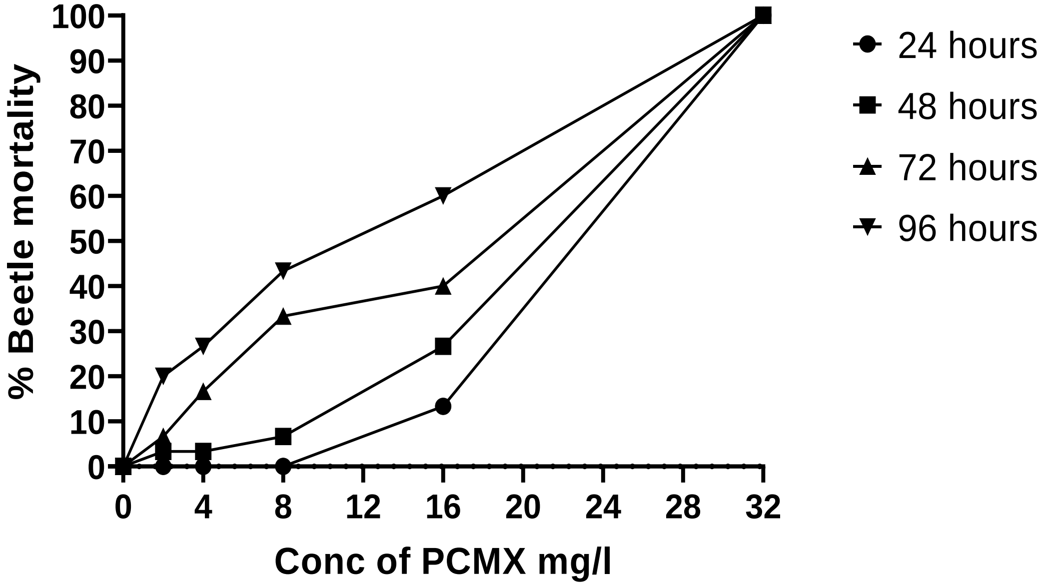

Figure 5: % Mortality of *Orectogyrus alluaudi* Regimbart, 1889 in control and different concentrations of chloroxylenol.

Table 1: Chloroxylenol LC50, LOEC, and NOEC for *Orectogyrus alluaudi.*

Toxicity parameters	24 hours	48 hours	72 hours	96 hours
LC_{50} (mg/l)	21.587 (15.114-22.490)	16.744 (12.608 - 17.206)	11.638 (2.976-15.359)	7.819 (2.172-32.197)
LOEC (mg/l)	13.507	5.578	2.307	1.097
NOEC (mg/l)	6.754	2.789	1.1535	0.5485

*Data in parentheses indicate 95% lower and upper confidence interval.

DISCUSSION

Many xenobiotics are considered hazardous to organisms that are exposed to them. However, toxicity depends to a large extent on the concentration, characteristics of the organisms, period of exposure and the nature of the environment as well (Embrandiri et al., 2016). PCMX caused significant changes in TDS and Conductivity (P = 0.05) but no significant change in pH and DO of the test solutions in this study. In fact, the pH of the control and treated groups approached neutrality at 0h and 96h of the test period. This observation is similar to the report of Capkin et al. (2017) in which similar non-significant change in the pH was observed on the exposure of rainbow trout to PCMX concentrations as well as other antimicrobials. The temperature varied insignificantly in both the treated and control groups throughout *O. alluaudi*'s exposure to the test chemical. The observed variations in TDS and Conductivity level might have been brought about by increased free ions from the addition of PCMX to the diluent (dechlorinated tap water) (Fajana et al., 2017)

PCMX LC_{50} values for these beetles were higher after 24 hours (21.587mg/l) than after 48, 72, and 96 hours (16.744, 11.638 and 7.819) respectively. This trend revealed that the toxicity of PCMX to *O. alluaudi* increased with the duration of exposure. The calculated LC_{50} values in this study suggested a relatively higher sensitivity of *O. alluaudi* to PCMX as compared with other invertebrates that have been used recently in similar studies. For instance, the 24h LC_{50} of PCMX to the rotifer *Brachionus koreanus* was reported to be 24.264 (20.892-28.243) (Won et al., 2022), 31.8 (26.5– 36.8 mg/l) for the nematode *Caenorhabditis elegans* (Sreevidya et al., 2018), 48.6 (45.8–52.0) for the mite *Dermatophagoides pteronyssinus* and 60.9 (46.7–89.1) for the mite *Dermatophagoides farina* (Suhaili et al., 2016). However, the sensitivity of *O. alluaudi* to PCMX was lower than what was reported for a crustacean, *Thermocyclops oblongatus*, which had a 24h LC_{50} of 0.17 mg/l (Chippaux et al., 1996). There is a paucity of information on the acute effect of PCMX on invertebrates exposed for more than 24 hours, but the 48 hour LC_{50} for *Daphnia magna* was reported to be 8.78 mg/l (Novak et al., 2021).

The variations in the sensitivity of aquatic invertebrates to the test ingredient may be attributed to differences in exposure route, physiological sensitivity, and biochemical responses (Aguigwo, 2002; Pickford, 2003). According Van den Berg et al. (2019), differences in sensitivity can also be attributed to the selectivity of the mechanism of action in relation to the species, which may sometimes be responsible for the unpredictability of chemicals' uptake. The whirligig beetles developed behavioural abnormalities such as disorientation in movement and an avoidance mechanism after being exposed to PCMX. This unusual behaviour could be explained by disruption in nervous system coordination or biochemical dysfunction as observed by Won et al. (2022), which reported a similar disruption of *B. koreanus* activities due to PCMX exposure and Amusan (2020) who reported a similar change in behavioural pattern in whirligig beetles exposed to the pesticide paraquat dichloride. In some cases, these behavioural changes may be accompanied by physiological and histopathological changes in the test organisms. For instance, Sreevidya et al. (2018) discovered a change in hatch rate and growth malformations in the zebrafish *Danio rerio* after sub-lethal PCMX exposure. However, other essential physiological responses of beetles to PCMX, such as growth and development and physiological biomarker exposure, were not assessed in this investigation. As such, further studies using biochemical tests that could further reveal the biological response of the organism to the test chemical is necessitated.

Conclusion

Findings in this study have revealed that PCMX is toxic to the Whirligig beetle *O. alluaudi.* This was reflected in the test organisms' mortality pattern, as mortality was found to increase with increasing concentrations of the test substance. Furthermore, the LC_{50} values determined for the beetles were lower than those recently determined for PCMX, thus indicating higher sensitivity of the insect to the chemical.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study, data analysis and interpretation, and critically reviewed and revised the manuscript. GER conducted the experiments and collected the data. AFK performed the statistical analysis. BOA and AFK drafted the manuscript. All authors approved the final version of the manuscript for submission and agree to be accountable for all aspects of the work.

REFERENCES

Aguigwo JN. 2002. The toxic effect of cymbush pesticide on growth and survival of African catfish, *Clarias gariepinus* (Burchell). Journal of Aquatic Sciences, **17**(2): 81-84. DOI: https://doi.org/10.4314/jas.v17i2.19916

Akeredolu OE, Ekele SA, Olaleru F, Egonmwan RI. 2022. Acute and sub-lethal toxicity in African mud catfish (*Clarias gariepinus*, Burchell, 1822) exposed to some pesticides. *Zoologist (The),* **20**(1): 51-60. DOI: http://dx.doi.org/10.4314/tzool.v20i1.7.

American Public Health Association (APHA). 1995. Water and Wastewater, Washington, DC.

American Society for Testing and Materials (ASTM). 2000. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, in 2000 Annual Book of ASTM Standards West Conshohocken 11(5): 213-233.

Au CK., Chan KJ, Chan W, Zhang X. 2023. Occurrence and stability of PCMX in water environments and its removal by municipal wastewater treatment processes. *Journal of Hazardous Materials,* **445**: 130550. DOI: https://doi.org/10.1016/j.jhazmat.2022.130550

Baby J, Raj JS, Biby ET, Sankarganesh P, Jeevitha MV, Ajisha SU, Rajan SS. 2010. Toxic effect of heavy metals on aquatic environment. *International Journal of Biological and Chemical Sciences,* **4**(4): 939-952. DOI: https://doi.org/10.4314/ijbcs.v4i4.62976

Biel-Maeso M, Corada-Fernández C, Lara-Martín PA. 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. *Water Research,* **150**: 129-139. DOI: https://doi.org/10.1016/j.watres.2018.11.045

Brinck P. 1955. A Revision of the Gyrinidae: (Coleoptera) of the Ethiopian Region.

Capkin E, Ozcelep T, Kayis S, Altinok I. 2017**.** Antimicrobial agents, triclosan, chloroxylenol, methylisothiazolinone and borax, used in cleaning had genotoxic and histopathologic effects on rainbow trout. *Chemosphere*, **182**: 720-729. DOI: http://dx.doi.org/10.1016/j.chemosphere.2017.05.093

Chippaux JP, Yovo KP, Soakoude Y, Akogbeto M. 1996. Efficacy of selected compounds on Thermocyclops oblongatus, one of the main intermediate hosts of guinea worm in Africa. *Acta Hydrochimica et Hydrobiological,* **24**(6): 283-285. DOI: https://doi.org/10.1002/aheh.19960240606

Connor R. 2015. The United Nations world water development report 2015: water for a sustainable world. UNESCO publishing.

Dsikowitzky L, Hagemann L, Ariyani F, Irianto HE, Schwarzbauer J. 2017. Complex organic pollutant mixtures originating from industrial and municipal emissions in surface waters of the megacity Jakarta—an example of a water pollution problem in emerging economies. *Environmental Science and Pollution Research,* **24**(35): 27539-27552. DOI: https://doi.org/10.1007/s11356-017-0164-2

Dsikowitzky L, Sträter M, Ariyani F, Irianto HE, Schwarzbauer J. 2016. First comprehensive screening of lipophilic organic contaminants in surface waters of the megacity Jakarta, Indonesia. *Marine Pollution Bulletin,* **110**(2): 654-664. DOI: http://dx.doi.org/10.1016/j.marpolbul.2016.02.019

El-Badawy FM, El-Desoky HS. 2018. Quantification of chloroxylenol, a potent antimicrobial agent in various formulations and water samples: environmental friendly electrochemical sensor based on microwave synthesis of graphene. *Journal of The Electrochemical Society,* **165**(14): B694–B707. DOI: https://doi.org/10.1149/2.0391814jes

Embrandiri A, Kiyasudeen SK, Rupani PF, Ibrahim MH. 2016. Environmental xenobiotics and its effects on natural ecosystem. Plant responses to xenobiotics, 1-18. DOI: https 10.1007/978-981-10-2860-1_1

Fajana HO, Amusan BO, Koleosho A, Owojori OJ. 2017. Acute toxicity of household bleach, sodium hypochlorite, to a whirligig beetle *Orectogyrus alluaudi* Régimbart, 1889 (Coleoptera: Gyrinidae). *Aquatic Insects,* **38**(1-2): 93-100. DOI: http://dx.doi.org/10.1080/01650424.2017.1326614

Finney DJ. 1971. *Probit Analysis* (3rd edn). Cambridge University Press: London; pp 187.

Gavrilescu M, Demnerová K, Aamand J, Agathos S, Fava F. 2015. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New Biotechnology*, **32**(1): 147-156. DOI: https://doi.org/10.1016/j.nbt.2014.01.001

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra-performance liquid chromatography–electrospray tandem mass spectrometry. *Analytical and Bioanalytical Chemistry,* **391**(4): 1293-1308. DOI: https://doi.org/10.1007/s00216-008-1854-x

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Research*, **42**(13): 3498-3518. DOI: https://doi.org/10.1016/j.watres.2008.04.026

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research,* **43**(2): 363-80. DOI: https://doi.org/10.1016/j.watres.2008.10.047

Neerukonda SN, Katneni U. 2020. A review on SARS-CoV-2 virology, pathophysiology, animal models, and anti-viral interventions. *Pathogens,* **9**(6): 426. DOI: https://doi.org/10.3390/pathogens9060426

Nowak M, Zawadzka K, Szemraj J, Góralczyk-Bińkowska A, Lisowska K. 2021. Biodegradation of chloroxylenol by *Cunninghamella elegans* IM 1785/21GP and *Trametes versicolor* IM 373: Insight into Ecotoxicity and Metabolic Pathways. *International Journal of Molecular Sciences,* **22**(9): 4360. DOI: https://doi.org/10.3390/ijms22094360

Pickford KA, Thomas-Jones RE, Wheals B, Tyler CR, Sumpter JP. 2003. Route of exposure affects the oestrogenic response of fish to 4-tert-nonylphenol. *Aquatic toxicology,* **65**(3): 267-279. DOI: https://doi.org/10.1016/s0166-445x(03)00149-8

Polhemus DA. 2011. New distributional records for Gyrinidae (Insecta: Coleoptera) on New Guinea and nearby islands, with a checklist of the New Guinea species. *Zootaxa,* **2900**(1):51-68. DOI: https://doi.org/10.11646/zootaxa.2900.1.3

Seibert D, Quesada H, Bergamasco R, Borba FH., Pellenz L. 2019. Presence of endocrine disrupting chemicals in sanitary landfill leachate, its treatment and degradation by Fenton based processes: A review. *Process Safety and Environmental Protection,* **131**: 255-267. DOI: https://doi.org/10.1016/j.psep.2019.09.022

Sreevidya VS, Lenz KA, Svoboda KR, Ma H. 2018. Benzalkonium chloride, benzethonium chloride, and chloroxylenol-Three replacement antimicrobials are more toxic than triclosan and triclocarban in two model organisms. *Environmental Pollution,* **235**: 814-824. DOI: https://doi.org/10.1016/j.envpol.2017.12.108

Sreevidya VS, Lenz KA, Svoboda KR, Ma H. 2018. Benzalkonium chloride, benzethonium chloride, and chloroxylenol-Three replacement antimicrobials are more toxic than triclosan and triclocarban in two model organisms. *Environmental Pollution,* **235**: 814-824. DOI: https://doi.org/10.1016/j.envpol.2017.12.108

Suhaili ZA, Mariana A, Baharudin O, Ho TM, Azima LH. 2016. Research Note Laboratory contact and topical evaluations of household disinfectants against house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae). *Tropical Biomedicine,* **33**(4): 847-852.

Tan J, Kuang H, Wang C, Liu J, Pang Q, Xie Q, Fan R. 2021. Human exposure and health risk assessment of an increasingly used antibacterial alternative in personal care products: Chloroxylenol. *Science of the Total Environment,* **786**: 147524. DOI: https://doi.org/10.1016/j.scitotenv.2021.147524

Ugwu LLC, Mgbenka BO, Ekanem NB .2005. Toxicity of chromium, copper and zinc to freshwater clam (*Galatea paradoxa*) (Born, 1778) (*Bivalvai, Donacidae*). *Bio-Research,* **3**(1): 72-76. DOI: https://doi.org/ 10.4314/br.v3i1.28574

Umar DM, Dantata AG, Mbaya LA, Mbimbe EY, Umar AM, Wasa AA. 2018. A survey of physicochemical characteristics and Macro invertebrates communities of three contrasting streams in Dadin Kowa, Gombe-State, Nigeria. *Bima Journal of Science and Technology,* **2**: 240-248.

Van den Berg SJ, Baveco H, Butler E, De Laender F, Focks A, Franco A, Van den Brink PJ. 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. *Environmental Science & Technology,* **53**(10): 6025-6034 DOI: https://doi.org/10.1021/acs.est.9b00893

Wilson M, Mowad C. 2007. Chloroxylenol. *Dermatitis,* **18**(2): 120-121.

Won EJ, Byeon E, Lee YH, Jeong H, Lee Y, Kim MS, Jo HW, Moon JK, Wang M, Lee JS, Shin KH. 2022. Molecular evidence for suppression of swimming behavior and reproduction in the estuarine rotifer *Brachionus koreanus* in response to COVID-19 disinfectants. *Marine Pollution Bulletin,* **175**: 113396. DOI: https://doi.org/10.1016/j.marpolbul.2022.113396

Wu YC, Chen CS, Chan YJ. 2020. The outbreak of COVID-19: An overview. *Journal of the Chinese Medical Association,* **83**(3): 217. DOI: https://doi.org/10.1097/jcma.0000000000000270