International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 11-04-2026, Accepted: 12-05-2026, Published: 27-05-2026
Volume 11, Issue 2, 2026, Page No. 87-97

Acute toxicity of chloramine T to the fry of *Cyprinus carpio* (Linnaeus, 1758) and the post-larvae of *Macrobrachium rosenbergii* (De Man, 1879)

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Abstract
Chloramines are residuals of chlorine. Chloramine T (Cl-T) is a low toxic disinfectant used in aquaculture against external bacterial infections and parasitic infestations. Toxicity of any chemical is species specific. Therefore in order to understand the sensitivity cultivable aquatic organisms, this study dealt with acute toxicity of Cl-T to the common carp *Cyprinus carpio* fry and the giant river prawn *Macrobrachium rosenbergii* post-larvae (PL). The 96 hr LC_{50} of Cl-T was found to be 24.677 mg/l and 7.650 mg/l to the fry of *C. carpio* and the PL of *M. rosenbergii*, respectively. Abnormal behaviors such as erratic swimming, loss of balance, gasping at the water surface, excessive mucus secretion over the gill chamber, jumping at the water surface, lethargy and gill hyperventilation, and morphological changes like loss of scales, ocular opacity and skin darkening were seen in the test fish. In the test prawn erratic swimming, loss of balance, excessive mucus secretion over the gill chamber, jumping at the water surface, lethargy, gill hyperventilation, and whitening of the body were seen. When comparing the LC_{50} values of Cl-T to the fish fry and prawn PL, it is concluded that toxicity of Cl-T is species specific.

Keywords: Chloramine T, acute toxicity, fish, prawn, behaviour

Introduction

Chlorination of water for disinfecting leaves residual chlorine, which include hypochlorous acid (HOCl), hypochlorite ions (OCl^-) that results from the dissociation of HOCl, element chlorine (Cl) as a greenish-yellow poisonous gas and its molecular form (Cl_2), and chloramines. Once chlorine is released in water, it reacts with organic matter to form trihalomethanes, a group of persistent, carcinogenic compounds that remain in the environment long after the chlorine itself has dissipated. Actually, the chlorination product dissolves in water and dispersal of chlorine speciation occurs based on pH. At pH below 2, chlorine gas is dominant. Between pH 2 and 6, HOCl is the only type. As pH increases beyond 6, OCl- appears. At a pH of about 7.5, HOCl and OCl- occur in equal proportions, but at higher pH, OCl- is the most abundant chlorine. Free chlorine residuals are strong oxidants that oxidize organic matter, nitrite and other reduced substances. Free chlorine residuals are reduced to nontoxic chloride and water in the presence of light, and the disinfecting power is reduced. In outdoor systems, the residual action of chlorination lasts only a few hours or at most a few days.

Chloramine T (Cl-T) is a complex organic sodium salt, derivative of toluene-4-sulfonamide with a chloro substituent in place of amino hydrogen. Cl-T (actually an organic *N*-chloramine) is an exception to the organic chloramines because of its considerable value as a disinfectant and sanitizer. It contains a chloro (p-tolylsulfonyl) azanide, therefore this organometallic compound is a *N*-Chloro-*p*-toluenesulfonamide sodium salt/ Tosylchloramide sodium ($CH_3C_6H_4SO_2NClNa$). It degrades in solution to p-toluenesulfonamide and sodium hypochlorite. Cl-T is a mild oxidant in neutral or alkaline medium and functions primarily through its ability to form hypochlorous acid and directly transfer chlorine atoms to substrates. These reactions enable its use as an oxidizing and chlorinating agent in biochemical, microbiological, and analytical applications. In water, Cl-T decomposes to release active chlorine, which acts as a disinfectant, an antifouling biocide (germicide, bactericide, fungicide, algicide etc.) and an allergen (Gilchrist *et al*. 2016; Nayak *et al*. 2022) [32, 59]. It is used as a reagent in organic synthesis. It is commonly used as a cyclizing agent in the synthesis of aziridine, oxadiazole, isoxazole and pyrazoles (Campbell and Graham 1978; Bull *et al*. 2011) [14, 16].

In human chloramine fumes can cause an individual to become congested due to sneezing, sinusitis, coughing, choking, wheezing, shortness of breath and asthma (Wastensson and Eriksson 2020) [86]. These problems are most commonly encountered in swimming pools containing excess chloramines. The excessive chloramines can be removed from water by adding ammonia, and a high grade of granular activated carbon. When removing chlorine from the water, the reducing agents in the dechlorinator use up oxygen, and the reaction could be hazardous in poorly oxygenated tanks. Generally, chlorine and chloramines in drinking water usually between 0.5-2.0 ppm is safe for human consumption, but reacts with fish gill tissues causing necrosis, which lead to respiratory difficulty and asphyxiation, which in turn may be deadly to fish (WHO 1991). However, the recommended minimum residual of chloramines is 1.0 mg/l in water (CDW 2018) [17]. The permissible limits of total residual chlorine in fresh receiving waters are 11.0µg/l (4 days chronic average) and 19.0µg/l (1 hour acute average) (EPA 2001). The relative amounts of residual chlorine formed are dependent on numerous factors, including pH, chlorine:ammonia ratio (Cl_2:NH_3), temperature and contact time (DWCD 1994; CDW 2018; Karthik *et al*. 2022) [45].

Organic chloramines (chlorine atom + dissolved organic matter contains organic nitrogen, which can be converted to ammonia) in general are less toxic to aquatic life than the inorganic chloramines (chlorine atom + added ammonia), [monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine (NCl_3), the Cl-T]. Both aqueous free chlorine ($HOCl + OCl^-$) and the inorganic chloramines are extremely toxic to fish and other aquatic life even at < 10 µg/l (Kalmaz and Kalmaz 1981) [44]. Cl-T has both therapeutic and toxicological effects on aquatic organisms, depending on the concentration and duration of exposure. In aquaculture farms Cl-T is used as a disinfectant to treat external bacterial (ulcers; rot on the fin, tail or mouth; loose scales) and parasitic (Costia, Trichodina, skin or gill flukes) infections in fish (Bowker and Erdahl 1998; Madsen *et al*. 2000; Cruz-Lacierda *et al*. 2000; Altinok 2004; Harris *et al*. 2004; Meinertz *et al*. 2004; El-Atta and El-Tantawy 2008; Bowker *et al*. 2008; Tantry *et al*. 2016; Buchmann 2022; Ahmed *et al*. 2025) [1, 3, 11, 12, 23, 33, 57, 58, 78]. Infection of fish by opportunistic pathogens like *Pseudomonas* spp. and *Flavobacter* spp. caused an erosive dermatitis on the caudal fin (Harris *et al*. 2005; Powell *et al*. 2011) [34, 69]. Cl-T is generally regarded as one of the most effective agents to control mortality of freshwater salmonids due to bacterial gill disease, columnaris caused by *Flavobacterium columnare* (Schroeter *et al*. 2021). According to Slinger *et al*. (2021) [75], the amebic gill disease in fish is associated with bacterial pathogenic genera *Aliivibrio*, *Tenacibaculum* and *Pseudomonas*. However, it has been reported that exposure of carp to 0.15-0.20 mg/l C1-T leads to 25% mortality within 12-16 h and the physiological performance of fish has been reduced (Klyszejko 1996) [50]. Even below this level also Cl-T has been reported to be toxic that trout larvae die at concentrations as low as 0.006 mg/l, and at just 0.1 mg/l, most marine plankton are killed (EPA 1984, 1985). Therefore, the present study was conducted with an aim to assess the 96 h acute toxicity of Cl-T to the fry of the common carp *Cyprinus carpio* and the post-larvae of the giant freshwater prawn *Macrobrachium rosenbergii* as these species contribute much in the fisheries and aquaculture practices in India.

Materials and Methods

1. Toxicant (Chloramine T)

Chloramine-T (Ottokemi, Works-101, Aarkay Ruby Industrial Estate (1B). Opp. Shree Narayan Industrial Estate, Chinchpada, Vasai East, Waliv, Maharastra-401208, India), was purchased from a local chemical supplier. Chloramine-T (Cl-T) is a widely used therapeutic and prophylactic agent in the treatment for amoebic gill diseases in fishes and increase bathing efficacy and reduced amoeba survival (Thorburn and Moccia 1993; Leef *et al*. 2007) [54, 81]. The mechanism of this disinfectant involves the release of hypochlorous acid when dissolved in water, which is an oxidizing agent, quickly destroying microbial cell walls and damaging cell material/components or disrupting essential cellular processes. It is stable, slowly releasing chlorine donor, which is effective against bacteria, viruses, fungi, and spores. Cl-T is also used in organic synthesis for iodination of peptides and proteins. The Cl-T has following chemical properties (Figure 1).

Molecular formula: $C_7H_7CINNaO_2S.3H_2O$
Molecular weight: 281.69
Assay: 98%
Physical state: White or slightly yellow crystals, crystalline powder, or prisms
Odor: Weak chlorine odor (trihydrate)
Boiling point (C): Not applicable
Melting point (c): 167 to 170 ºC; decomposes ca 174 (trihydrate)
Flash point (c): 192 (trihydrate)
Density (kg/m3): 1430
Solubility in water: Soluble
Specificity gravity: 1.43 (trihydrate form)

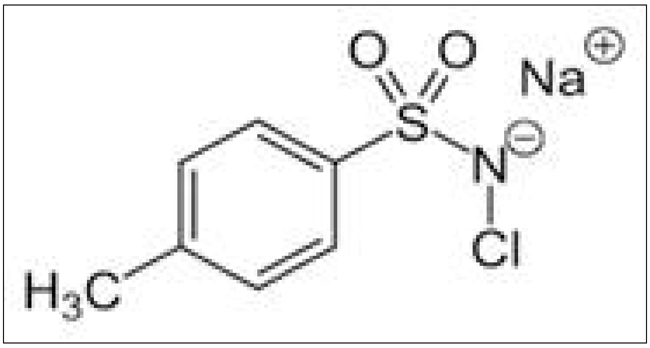

Fig 1: Chemical structure of chloramine T

Cl-T is generally stable under proper storage conditions, but its stability in solution varies with pH and temperature. Aqueous solutions are stable in strongly alkaline media even up to 60°C. However, in acidic solutions (especially hydrochloric acid) and at higher temperatures, it can decompose via oxidation or disproportionation. Cl-T is generally available as a white to slightly yellow crystalline powder or solid. It is also available as a trihydrate, which is also a white powder. Both the anhydrous and trihydrate forms are used as reagents in organic synthesis and as disinfectants. The half-life of Cl-T varies depending on the environment. In water, its half-life is around 75 minutes when reacting with organic chlorine demand. In fish, the half-life of residues is estimated to be between 32.6 and 40.3 hours, depending on the measurement method.

2. Procurement and acclimation of the fish fry

The fry of common carp, *C. carpio*, with an average length of 1.84±0.36 cm and an average weight of 0.290±0.06g were procured from The Tamil Nadu Fisheries Department, which implements various welfare schemes for fishermen in Edappadi, Salem, India, stocked in clean fiberglass aquarium tanks (120 x 80 x 40 cm) irrespective of the sex and acclimatized to laboratory conditions for three weeks (21 days) with groundwater (Temperature, 28 ºC; pH, 7.4; Salinity, 0.54 ppt; total hardness, 135.0 mg/l; total dissolved solids, 0.85 g/L; dissolved oxygen, 7.754 mg/l; BOD, 32.0 mg/l; COD, 135.30 mg/l; ammonia, 0.321 mg/l; nitrate, 1.14 mg/l), during which they were fed with rice bran, groundnut oil cake and corn flouer mix twice a day. In order to maintain a healthy environment, which devoid of accumulated metabolic state, the medium was adequately aerated, every day at least 50% of the tank water was replaced, the feces, and unfed feed if any were removed by siphoning method causing minimum disturbance to the fishes. During acclimation, the fish stock was maintained at natural photoperiod and ambient temperature. This ensures sufficient oxygen for the fish and the environment is devoid of any accumulated metabolic state. These fish fingerlings were served as the stock for the experimental schedule.

3. Procurement and acclimation of the prawn post-larvae

The post-larvae (PL-15) of the giant freshwater prawn, *M. rosenbergii*, with an average length of 0.95±0.24 cm and an average weight of 0.027±0.009 g were procured from Sri Durgai hatcheries P. Ltd, Chengalpattu, Chennai, India, stocked in clean cement aquarium tanks (120 x 80 x 40 cm) and acclimatized to laboratory conditions for three weeks (21 days) with groundwater had the physico-chemical characteristics similar to that of described elsewhere, during which they were fed with egg albumin and *Artemia* nauplii three times each per day. In order to maintain a healthy environment, which devoid of accumulated metabolic state, the medium was adequately aerated, every day at least 50% of the tank water was replaced, the unfed feed, feces, and moult if any were removed by siphoning method causing minimum disturbance to the prawn. During acclimation, the prawn nursery was maintained at natural photoperiod and ambient temperature. This ensures sufficient oxygen for the prawns and the environment is devoid of any accumulated metabolic state. These prawn PLs served as the stock for the experimental schedule.

4. Bioassay (96 h LC_{50})

In this study, the bioassay signifies a test in which *C. carpio*, and *M. rosenbergii* were used as living test organisms for the determination of the toxic potency of chloramine T through static renewal type (APHA, 1998) [11]. The technical grade chloramines T is a white, crystalline powder with a purity of at least 98%. The static renewal type toxicity test was conducted on 10 groups of *C. carpio* fry (2.25 cm size and 0.40 g weight). Each group consisted of 10 fingerlings, which were exposed to 10 different concentrations of chloramines T, ranging from 10-45 mg/l (technical grade) for a duration of 96 hr. The water was renewed daily by siphoning method, causing minimum disturbance to the fish and freshly prepared concentrations of chloramines T were added. During the experiment the animals were neither fed nor aerated. The experiment was repeated at least three times to get concurrent value. Simultaneous controls were also maintained. The 96 h LC_{50} value with 95% confidence limits were assessed using SPSS (v. 16) programme of profit analysis (Finney, 1971) [30].

Similarly, 10 groups of *M. rosenbergii* (PL-35; 1.84 cm size and 0.29 g weight), each with 10 PL were exposed to 10 different concentrations of chloramines T, ranging from 2.0-14 mg/l (technical grade) for a duration of 96 hr. The other conditions were similar to that described elsewhere. The 96 h LC_{50} value with 95% confidence limits were assessed using SPSS.

Results and Discussion

Lethal effect of a chemical to an aquatic organism is determined by conducting toxicity tests. According to Ikefuti *et al*. (2015) [40], an acute toxicity test (LC_{50}) is to determine how toxic an agent is to cause potential mortality to a particular species? In this study, we assessed the 96 h LC_{50} of Cl-T to the fry of *C. carpio* and the PL of *M. rosenbergii*. The 96 hr LC_{50} of Cl-T was found to be 24.677 mg/l to the fry of *C. carpio* (Table 1 and Figure 2). Similarly, the 96 hr LC_{50} of Cl-T to the PL of *M. rosenbergii* was found to be 7.650 mg/l (Table 2 and Figure 3).

Table 1: The 96 hr LC_{50} of chloramine T to the fish *C. carpio* fry (2.25 cm size and 0.40 g weight)

Concentration (mg/l)	Log_{10}	Mortality (%)	Probit	Calculation
5	0.698	0	0.00	Intercept à -6.945
10	1.000	0	0.00	X Variable 1 à8.578
15	1.176	10	3.72	Y=ax+b
20	1.301	30	4.48	Y=8.58x+(-6.945) = 1.392
25	1.397	40	4.75	5=8.58x-6.945
30	1.477	70	5.52	5+6.945=8.58x
35	1.544	80	5.84	x=(5+6.945)/8.58
40	1.602	90	6.28	x=1.392
45	1.653	100	8.09	LC_{50}=antilog 1.392
50	1.698	100	8.09	LC_{50}=24.677 mg/l 95% lower confidence limit = 21.346 mg/l 95% upper confidence limit = 28.393 mg/l

Table 2: The 96 hr LC_{50} of chloramine T to the prawn *M. rosenbergii* PL (1.84 cm size and 0.29 g weight)

Concentration (mg/l)	Log_{10}	Mortality (%)	Probit	Calculation
2	0.301	0	0.00	Intercept à -1.811
4	0.602	10	3.72	X Variable 1 à7.704
6	0.778	30	4.48	Y=ax+b
8	0.903	40	4.75	Y=7.704x+(-1.811) = 0.884
10	1.000	70	5.52	5=7.704x+(-6.945)
12	1.079	90	6.28	5+1.811=7.704x
14	1.146	100	7.33	x=(5+1.811)/7.704 x=0.884 LC_{50}=antilog of 0.884 LC_{50}=7.650 mg/l 95% lower confidence limit = 6.314 mg/l 95% upper confidence limit = 8.920 mg/l

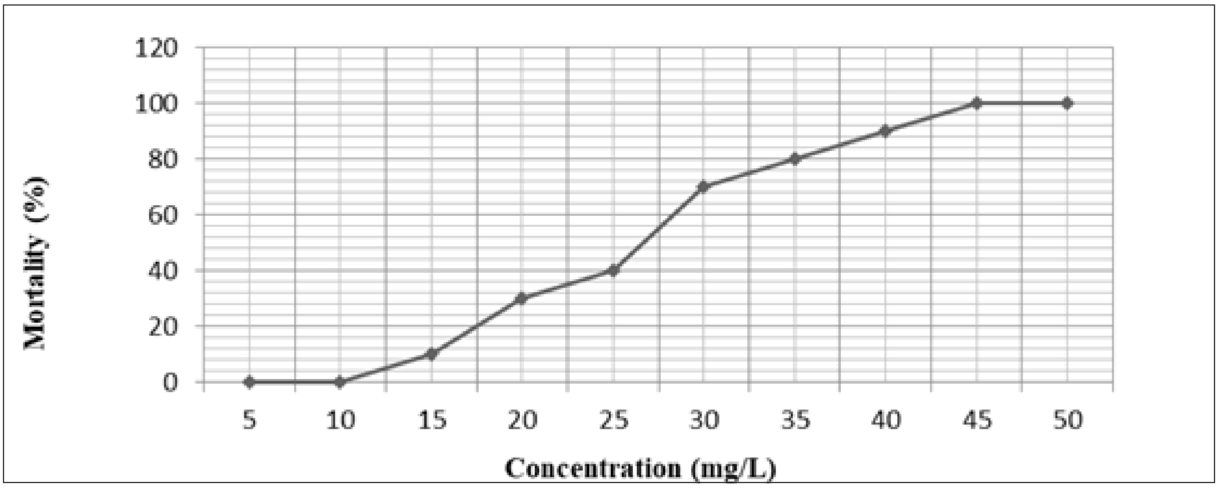

Fig 2: Graphical representation (the slop) of the 96 hr LC_{50} of chloramine T to the fish *C. carpio* fry.

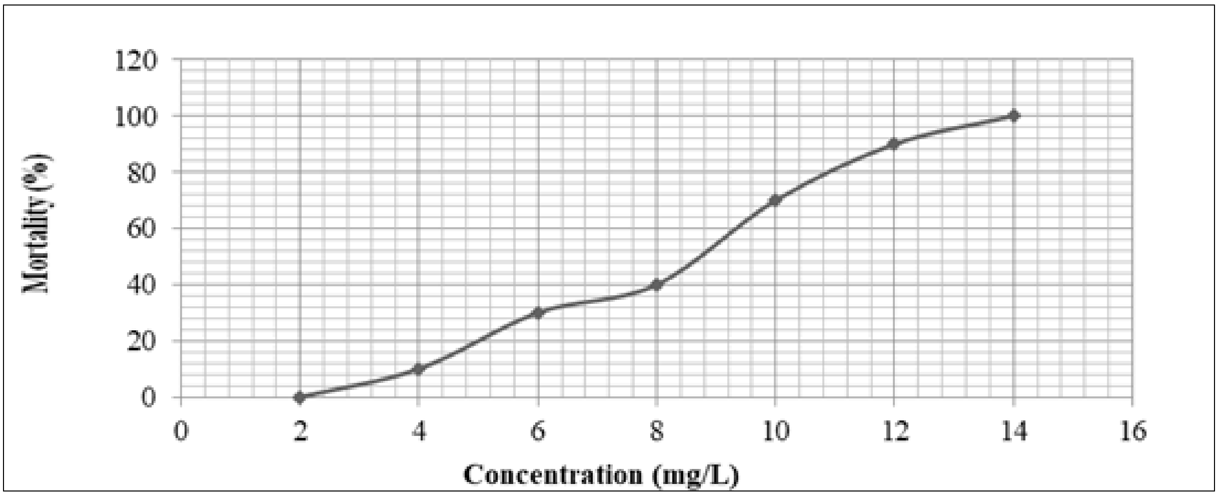

Fig 3: Graphical representation (the slop) of the 96 h LC_{50} of chloramine T to the prawn *M. rosenbergii* PL.

The acute toxicity tests revealed that the toxicity of Cl-T was species specific. Moreover, tolerance to xenobiotics can vary depending on the target species, ontogenetic development, exposure time, test concentrations, water quality, as well as general health status of the test organism (Neves *et al*. 2020) [61]. The reported toxicity values of different disinfectants on aquatic animals have been given under the following categories.

1. Chloramine toxicity

The reported 96 h LC_{50} of Cl-T for the rainbow trout, *Salmo gairdeneri* was 2.80 mg/l, for channel catfish, *Ictalurus punctatus* it was 3.75 mg/l, and for fathead minnows, *Pimephales promelas* it was 7.30 mg/l (Bills *et al*. 1988) [7]; for the Atlantic sturgeon, *Acipenser oxyrinchus* it was 7.73 mg/l (King and Farrell 2002) [47]; for the zebrafish (*Danio rerio*) it was 11.044 mg/l (Soleimani *et al*. 2017); for the juvenile Atlantic sturgeon *A. oxyrinchus* (average weight, 2.1 g) it was 7.73 mg/l (King and Farrell 2002) [47]. The reported 24 h LC_{50} of Cl-T to the goldfish *Carassius auratus* was 24.3 mg/l, and 15 mg/l Cl-T was recommended to use to treat columnaris disease in *C. auratus* (Altinok 2004) [3]. The reported 4 h LC_{50} of Cl-T to *Arapaima gigas* juveniles was 23.8 mg/l (Bentes *et al*. 2022). Cl-T was more acutely toxic to Atlantic salmon, *Salmo salar* in seawater (the median lethal times (LT_{50s}) at 50 and 25 mg/l were 119.1 and 297.3 min, respectively under oxygen supersaturation than to those in freshwater (LT_{50s}) at 50 and 25 mg/l were 166.8 and 474.3 min, respectively under oxygen supersaturation (Powell and Harris 2004) [33]. It is more toxic at 200% air saturation. The primary mechanism of toxicity in both seawater and freshwater appears to be extensive oxidative necrosis of the gill filament and lamellar epithelium, causing acute osmoregulatory dysfunction (Powell and Harris 2004) [33]. The reported average time of death response of monochloramine at the maximum legal residual disinfectant level of 4.0 mg/l was 1.0 hour for the pink shrimp *Farfantepenaeus duorarum*; 11.7 hour for the hard clam *Mercenaria mercenaria*; and 30.18 minutes for the mosquito fish *Gambusia affinis* (Le 2021) [75]. However, it has been reported that 10 mg/l of Cl-T does not affect the crayfish *Astacus leptodactylus* when exposed for 1h (Kuklina *et al*. 2014) [51].

2. Chlorine toxicity

The 96 h LC_{50} of chlorine in swordtail (*Xiphophorus helleri*) and koi (*C. carpio*) were reported to be 1.375 mg/l and 2.4425 mg/l, respectively (El-Bouhy *et al*. 2006) [24]. A 30 days exposure of the clam *Cyclina sinensis* to 60 mg/l chlorine significantly affects the survival rate, growth, immune, and antioxidant performance (Wang *et al*. 2025) [85]. The free residual chlorine in seawater is more toxic to aquatic organisms. For example, at 0.20 mg/l, chlorella growth was greatly affected, but the effect on shellfish was relatively small even at 1 mg/l (Xin *et al*. 2023) [88]. The 96 h LC_{50} of residual chlorine for bluegill were reported from

0.18 to 0.33 mg/L depending on temperature and fish weight; for channel catfish it was about 0.09 mg/1 with temperature not a factor; in the case of ammonia the 96 h LC_{50} were reported from 0.40 to 1.3 mg/1 for bluegill depending on temperature and fish weight; for the bass it was from 0.72 mg/1 (at 22° C) to 1.2 mg/1 (at 30°C); for channel catfish it was from 1.5 mg/1 (at 22 °C) to 3.0 mg/1(at 28 °C) with size not a factor; further it is suggested that the residual chlorine should be below detectable and NH_3-N should not exceed a concentration of 0.04 mg/1(Roseboom and Richey 1977) [74]. It has also been reported that the 24 h LC_{50} of chlorine to the shrimp (2.75 g wt.), *Penaeus monodon* was 2.05 mg/l at residual chlorine of 1.39 mg/L; pH, 8.49; temperature, 27.0 ºC; DO, 7.55 mg/l (Husnah and Lin 2002) [38].

A 3-days LC_{50} of residual chlorine (Cl^-) from STEL (a commercial machine that produces a stream of modified saltwater using an electrolytic process exerts a strong disinfecting effect at low concentration because HOCl can easily penetrate cell walls) water for brood stock and 2-month old shrimp of *Penaeus orientalis* were reported to be 2.3 and 3.2 ppm, respectively (Park *et al*. 2004) [64]. The chemicals discharged from the reverse osmosis desalination plant have a potential toxic impact on the coastal ecosystem. Exposure of these chemicals individually (and combined also) on *Artemia franciscana* nauplii and their reported 24 h LC_{50} were in the following order: 0.97 mg/l for glutaraldehyde ($C_5H_8O_2$, used as non-oxidizing biocide) > 5.92 mg/l for sodium hypochlorite (NaClO, used as disinfectant) > 28.91mg/l for copper sulfate ($CuSO_4$, used as algaecide) > 36.06 mg/l for potassium permanganate ($KMnO_4$, used as oxidant) > 202.77 mg/l for ferric chloride ($FeCl_3$, used as coagulant and flocculant). This indicates that $C_5H_8O_2$ was the most toxic biocide. However, all compounds in combination with $C_5H_8O_2$ exhibited a very strong antagonistic effect, except, in mixtures, NaClO/$FeCl_3$ and $CuSO_4$/KMnO4 resulted in an additive effect (Cortes *et al*. 2018) [19].

Chlorine dioxide (ClO_2) is considered as an alternative to chlorine for drinking water treatment. It does not generate toxic nitrogenous, chloramines or trihalomethanes, carcinogenic organic residuals. The reported 96 h LC_{50} of ClO_2 were 2.2 mg/l for larvae and 8.3 mg/l for adult Rainbow Trout, *Oncorhynchus mykiss*, respectively, but the 96 h LC_{50} of chlorite were 106 mg/l for larvae and 149 mg/l for adult *O. mykiss*, respectively (Svecevicius *et al*. 2005) [77]. The 24 h LC_{50} of ClO_2 to the juvenile white leg shrimp, *Penaeus vannamei* was reported to be <1.2 ppm (Imaizumi *et al*. 2024) [41].

3. Toxicity of other disinfectants

The reported 96 h LC_{50} of a disinfectant, Virkon-S (contains potassium peroxymonosulfate (a peroxygen compound), sodium dodecylbenzenesulfonate (a surfactant/detergent), organic acids (sulfamic acid and malic acid) (organic acids) and sodium chloride) for *Litopenaeus vannamei* juvenile was 43.23 mg/l (Ganjoor 2008) [31]. However, Virkon-S was reported to be safe to the fish *C. carpio* up to 10 ppm for 10 days when treating saprolegniasis (Rahman and Choi 2018) [71]. It has been reported that 48 to 96 h LC_{50} of phenol to several species of freshwater fishes fall between 1.6 to 100 mg/l, and to freshwater crustaceans, these were between 3 to 200 mg/L. Similarly for marine fishes, these were between 5.2 to 44 mg/l, and for the marine crustaceans, these were between 5.8 to 186 mg/l. Among crustaceans a mysid shrimp was the most sensitive species. Its moderate reliability trigger value in freshwater is 320 µg/l, but 400 µg/l in marine water (ANZECC & ARMCANZ 2000). The reported 96 h LC_{50} of Formalin to eggs of the fish, *Odontesthes bonariensis* was 652.14 µl/l (Pacheco-Marino and Salibian 2010) [63] and to juvenile Atlantic sturgeon *A. oxyrinchus* (average weight, 2.1 g) it was 31.00 mL/l (King and Farrell 2002) [47].The 24 h LC_{50} of toluene to rotifers was reported to be in the range of 113.3 - 552.6 mg/l (Ferrando and Andreu-Moliner 1992) [29].

4. Species specificity/sensitivity of organisms against toxicant

Generally, the freshwater invertebrates are more sensitive than vertebrates, and within invertebrates (for example different crustacean species) or vertebrates (different species of fishes), there is a wide range of sensitivity (Lanctot *et al*. 2016) [52]. Some species are highly susceptible while others are more tolerant. For example, cladocerans and planarians are acutely sensitive to toxins, and that tadpoles can be more sensitive than fish. The specific range of sensitivities depends on several factors like body size, physiology, and the type of stressor, leading to a wide spectrum of tolerance levels. Invertebrates (crustaceans, mollusks, insects etc.) often have smaller body sizes, less complex organ systems, have different physiological characteristics, and may be more vulnerable to physical, chemical and biological stresses compared to their larger vertebrate counterparts. Vertebrate species like fishes also exhibit a wide range of sensitivity. For example, Coho salmon (*Oncorhynchus kisutch)* and rainbow trout (*O. mykiss*) are highly sensitive to various chemicals, whereas goldfish (*C. auratus*) and carp (*C. carpio*) are less so (Teather and Parrott 2006; Liewa *et al*. 2013; Treeck *et al*. 2020) [56, 80, 84]. However, it has been stated that invertebrates displayed equal to or greater sensitivity than fish to environmental contaminants (Poirier *et al*. 1986; Hughes *et al*. 2021) [37, 66]. Moreover, exposure to chronic pollution could lead to local adaptation or maladaptation, which could result in high intra-specific variability of sensitivity (Jacquin *et al*. 2020) [43]. Invertebrates have fewer protective layers than some vertebrates, and since their exposure to water pollution is direct, making them more susceptible. In this study, the prawn PL is more sensitive/ susceptible than that of the fish fry for Cl-T toxicity.

5. Influence of water quality parameters on toxicity determination of chemicals

The effects of water quality parameters on determination of toxicity of a chemical can be complex and may depend on the specificity of that chemical and also test species specific. In addition to body size, life stage, and physiology of the individual, the toxicity of a chemical is controlled by various water quality parameters like temperature, pH, salinity and hardness. In which, water pH, salinity and hardness are interconnected, though their relationships can be complex and context-dependent. However, salinity can influence pH, whereas the water hardness can affect both salinity and pH. The relationships between these water quality parameters in determining the toxicity of a chemical can also vary depending on the water type (freshwater, estuarine water, and coastal water). Temperature can interact with salinity, alter the chemical speciation, its solubility and uptake by an organism, thus significantly impacting the determination of acute toxicity of a chemical. Changes in pH and water hardness can also significantly alter the chemical form of a toxicant, influence its solubility, bioavailability, and affect the ability of the organism to uptake or excrete the toxin by disrupting the physiology through altering enzyme activity or ionic balance, all of which can influence the lethal concentration. Some heavy metals may precipitate out of solution at certain pH levels, reducing their toxicity. Generally, increased water hardness makes the toxicant less soluble, leading to reduced bioavailability for uptake/absorption and tends to decrease the toxicity.

Water temperature

In the present study, the water temperature was 28 ºC. Temperature significantly affects determination of acute toxicity of a xenobiotic substance. When water temperature is higher, death of a test organism may occur quickly even at lower toxic concentration (de Figueiredo *et al*. 2020; Nin and Rodgher 2021; Kazmi *et al*. 2022) [21, 46, 62]. A higher temperature can greatly interact with other water quality parameters, like salinity, pH, hardness, ammonia etc., and exacerbate the toxicity of a chemical, leading to quicker death of test organisms (Kir *et al*. 2004; Aktaş 2006[2, 73]; Kir and Oz 2015) [49]. At a higher temperature, a chemical may dissolve more quickly in the water, more readily available for absorption and cause early death due to the increased kinetic energy of toxic molecules as well as the biological molecules of test organisms, because of increased metabolic and respiratory rates. According to Buckley (2022) [13], the temperature changes itself makes the invertebrates sensitive as they are poikilothermic ectotherms, and causes alterations in physiology, growth, distribution, and survival, which in turn can alter metabolic rates, affect development, and reduce their overall fitness.

At 12 ºC, the 12 h LC_{50} of Cl-T for rainbow trout was reported to be 45 mg/l and the 96 h LC_{50} was 10 mg/l in hard water; in soft water it was 38.7 mg/L and 7.35 mg/l, respectively (Bills *et al*. 1988) [7]. These LC_{50} concentrations are beyond the therapeutic range of Cl-T (typically, 10–20 mg/L for 1 h) used in aquaculture. The 1 h LC_{50} of Cl-T for rainbow trout at 12ºC was more than 60 mg/l in either hard or soft water (Bills *et al*. 1988) [7]. At 25 ºC and 20 ppt seawater, the reported 96 h LC_{50} of perfluoro octane sulfonate (PFOS) for larvae of estuarine species as follows: 0.919 mg/L for the sheepshead minnow *Cyprinodon variegatus*; 1.375 mg/l for the mysids *Americamysis bahia*; 1.559 mg/l for the Eastern mud snails *Tritia obsolete*; and 2.011 mg/l for the grass shrimp *Palaemon pugio* (Chung *et al*. 2024) [18]. But at the increased temperature (32 ºC) and decreased salinity (10 ppt) these species have reported to be show varied responses: at higher temperature the toxicity of PFOS for *C. variegates* was reported to be increased, but was not altered at lower salinity; for *P. pugio* and *T. obsolete* the lowered salinity was reported to be increase the toxicity of PFOS (Chung *et al*. 2024) [18].

Water pH

In the present study, the pH and salinity was 7.4 and 0.54 ppt, respectively. In freshwater bodies, the pH and salinity can be influenced by factors like rainfall (the rain water influx may reduce the salinity and pH), bedrock, and the presence of organic matter. The ideal range of pH for healthy freshwater ecosystems is from 6.5 to 9.0**.** Usually, the pH levels will increase with salinity until the water reaches calcium carbonate (CaCO3) saturation. The higher levels of calcium and magnesium ions can lead to alkaline pH. Mostly, toxicants are weak acids or bases. The pH of the environment in which the organism is thriving can influence the uptake and elimination of chemicals, which is determined by the proportion of its neutral and ionized forms to maintain acid-base balance, and it is species specific also (EPA 2021). The neutral form of a toxicant is often more lipid-soluble and bioavailable then it can be readily permeable through plasma membrane and exerting its toxic effect (Baynes and Hodgson 2004) [6].

At pH 6.5, the reported toxicity of Cl-T to fishes was greater than at alkaline (Bills *et al*. 1988) [7]. In contrast to this some fish may be more susceptible to ammonia (NH_3) toxicity at alkaline pH because ammonia is more readily absorbed in its un-ionized form (Ip and Chew 2010; Guan *et al*. 2010) [42]. The LC_{50} of a pesticide 'abamectin' for *A. franciscana* was reported to be 0.145 µg/l in 35-100 g/l salinity and pH, 7.5-10.0; the decrease of the pH from 7.5 and the salinity from 30 g/l was reported to increase the toxicity of 'abamectin', and the simultaneous increase in pH and salinity decreased its toxicity (Toochaei 2023; Toochaei *et al*. 2025; Barani et al. 2025) [5, 82].

Salinity

In the present study, the salinity was 0.54 g/kg or ppt. The freshwater typically has a salinity of 0.5-1.0 g/kg or ppt., (usually >0.5 ppt.), while the seawater has an average salinity of about 3.5% or 35 ppt., (usually, 33-41 ppt.). The changes in salinity can cause osmotic and respiratory stress, making organisms more susceptible to toxicants. The 24 h LC_{50} of salinity for larvae of the striped catfish, *Pangasianodon hypophthalmus* was reported to be 10.63 ppt (LC_{100} in 12.0 ppt); similarly, the reported 48 h LC_{50} was 8.48 ppt (LC_{100} in 12.0 ppt); better survival was reported at lower salinity (Hossain *et al*. 2021, 2022) [35, 36]. It has also been reported that at higher salinities toxicity of Cu increased, its 96 h LC_{50} to the estuarine fish *Terapon jarbua* was reported as 4.26 mg/l at 22‰, but at 32‰, it was 2.24 mg/l; similarly, the reported 96 h LC_{50} of Zn were 14.45 mg/l and 8.70 mg/l at 22‰ and 32‰, respectively (Rao and Nair 2008) [72]. Therefore, toxicity of salinity was dose and time dependent one. However, salinity reacts with toxicants differently, when salinity increases the toxicity of a metal decreases due to abundant chloride ions, which reduces the availability of metal ions for absorption (Riba *et al*. 2004; Hutton *et al*. 2021) [39, 73]. For example, the 96 h LC_{50} of Ni to the shrimp *L. vannamei* was reported to be 41 μmol/l and 362 μmol/l in 5 ppt and 25 ppt, respectively; similarly at these ppt, the 96 h LC_{50} of Ni to the isopod, *Excirolana armata* were 278 μmol/l and > 1000 μmol/l, respectively (Leonard *et al*. 2011) [55]. Therefore, as far as heavy metal is concerned, the animal can tolerate the toxicity of Ni even at higher salinity, but not to the toxicity of Cu and Zn.

Water hardness

In the present study, the total hardness of the water was 135.0 mg/l. The term "hard water" is associated with freshwater with higher calcium and magnesium contents than that of "soft water". The hardness of freshwater generally falls between 15-375 ppm (tap water might have a hardness up to 600 ppm), while the hardness of seawater is typically in the range of 5,800-7,500 mg/L as CaCO3. The water hardness can increase the response of some species while decreasing others against cationic surfactants, sodium chloride etc. For example, in *Daphnia* species the acute NaCl toxicity decreased as calcium concentrations increased (Buren and Arnott 2025) [15]. The 96 h LC_{50} of NaCl varies widely among fish species (3.5–150.0 g/l) depending on other water quality parameters and many fish species are sensitive to concentrations near those required for their development, growth or for controlling and treating parasites (Tavares-Dias 2022) [79]. Similarly, the reported 96 h LC_{50} of NaCl for the Caspian white fish, *Rutilus kutum* was 8.12 g/l at a water temperature of 22 °C and pH of 7.52 (Nazari *et al*. 2021), it was reported to be 3526 mg/l and 7700 mg/l for Limnephilidae (caddisflies) and *Gammarus* (Amphipoda), respectively (Blasius and Merritt 2002) [8] and 9.735 g/l to *A. oxyrinchus* juvenile (King and Farrell 2002) [47]. It has also been reported that a 7-day LC_{50} of NaCl contaminated water for the freshwater amphipod, *Hyalella azteca* was 12.8 g/l (Picinic *et al*. 2022) [65].

6. Behavioural abnormality

In this study, behavioural changes like erratic swimming, loss of balance, gasping and jumping at the water surface, excessive mucus secretion over the gill chamber, lethargy and gill hyperventilation, and morphological changes like loss of scales, ocular opacity and skin darkening were seen in fish exposed to 30-50 mg/l of chloramine T for 96 h (Table 1). These symptoms were mild in fish exposed to 15-25 mg/l of chloramine T for 96 h. In prawn erratic swimming, loss of balance, excessive mucus secretion over the gill chamber, jumping at the water surface, lethargy, gill hyperventilation, and whitening of the body were seen in 8-14 mg/l of chloramine T for 96 h (Table 2). These symptoms were mild in fish exposed to 6 mg/l of chloramine T for 96 h. Similar kinds of behavioural abnormalities, such as abnormal swimming, restlessness, decreased respiratory rate with signs of anoxia, congested gill tissues and excessive mucus secretion on body surface and over the gill chamber were reported in swordtail (*X. helleri*) and koi (*C. carpio*) when exposed to chlorine while conducting the acute toxicity test (El-Bouhy *et al*. 2006) [24]. Since Cl-T generates reactive chlorine and oxygen, and the formation of the hypochlorite ion causes cellular membrane damage due to gill trans-cellular ionic effluxes, which leads to iono-regulatory compromise (Booth and McDonald 1988; Powell *et al*. 1998) [9, 70]. The hypoxia and ionic effluxes ultimately lead to increased mucus secretion onto the gill as a protective mechanism against Cl-T induced toxicity and impairs CO_2 excretion (Powell and Perry 1995) [68].

Conclusion

In this study, Cl-T is toxic to fish and prawn, but it is species specific. The prawn *M. rosenbergii* PL is more sensitive to Cl-T than that of the fish *C. carpio* fry. Both organisms exhibited behavioural abnormalities against Cl-T toxicity. Therefore, it is suggested that the usage of this disinfectant in aquaculture industry (both culture and processing) of fish and prawn can be maintained at least ten times below the concentration levels of its acute toxicity (i.e., 2.46 mg/l and 0.76 mg/l), respectively. Hence, it is warranted that the indiscriminate application of chlorine may be restricted to minimize its residue in the seafood.

Acknowledgements

The authors gratefully acknowledge the Tamil Nadu Fisheries Department, Edappadi, Salem, India, for providing the fish *C. carpio* fry at free of cost, and Sri Durgai hatcheries P. Ltd., Chengalpattu, Chennai, India, for the supply of prawn (*M. rosenbergii*) PL.

Authors' contributions

Work designing, manuscript preparation and formatting: PSB; Experimentation: VP; Data analysis: BS and KG; Content evaluation: TJ.

Competing interests

The authors declare no competing interests.

Data available statement

The data used in this study are provided and included within the article.

Funding statement

No funding exists.

Disclaimer (Artificial intelligence): Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during preparation of this manuscript.

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