Pakistan J. Zool., pp 1-4, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20200623160651

Short Communication

Acute Toxicity of Four Disinfectants to Brine Shrimp, *Artemia* (Crustacea: Anostraca)

Song Jiang[1,2,3], Zhenhua Ma[1,2], Falin Zhou[1,2], Xu Chen[1], Jing Hu[1], Rui Yang[1], Shengjie Zhou[1], Yundong Li[2] and Qibin Yang[1]*

[1]Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Sanya 572018, China
[2]Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture and Rural Affairs, P.R. China; South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China 510300
[3]Shenzhen Base of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shenzhen, 518108,China

ABSTRACT

In order to determine the impact of glutaraldehyde, dibromohydantoin, methionine iodine and bleaching powder on brine shrimp *Artemia* (crustacean, Anostraca), acute toxicity parameters were assessed. The analysis revealed a highest level of toxicity for bleaching powder followed by dibromohydantoin, methionine iodine and glutaraldehyde. The median lethal concentrations (LC_{50}) of bleaching powder, dibromohydantoin, methionine iodine and glutaraldehyde were 14.24 mg/L, 28.21 mg/L, 55.01 mg/L and 68.51 mg/L, respectively after 24 h of treatment. However, 48 h post-treatment, the LC_{50} were 11.26 mg/L, 10.15 mg/L, 30.24 mg/L and 36.71 mg/L, respectively. The safe concentration (SC) of tested disinfectants were recorded to be 1.93 mg/L, 0.41 mg/L, 2.73 mg/L and 7.81 mg/L for bleaching powder, dibromohydantoin, methionine iodine and glutaraldehyde, respectively. These results highlight that glutaraldehyde can safely be used as disinfectant against brine shrimp *Artemia* and bleaching powder appeared to be toxic whereas dibromohydantoin and methionine iodine can be applied with caution.

Article Information
Received 23 June 2020
Revised 05 September 2022
Accepted 24 September 2022
Available online 14 June 2023
(early access)

Authors' Contribution
SJ and FZ conceived the study and designed the experiments. SJ performed the bioinformatics analysis and prepared the manuscript. ZM, XC, JH and RY conducted the experiment. RY, SZ and YL collected the samples. All authors have read and agreed to the published version of the manuscript.

Key words
Brine shrimp *Artemia*, Acute toxicity, Disinfectant

The brine shrimps *Artemia* (Crustacea, Anostraca) are distributed in salt lakes and ponds worldwide with the exception of Antarctica (Stappen *et al*., 2001). The *Artemia* is widely used in laboratory toxicology studies due to its small body size, short lifespan and its availability from dry cysts (Litvinenko *et al*., 2015). Due to expanding aquaculture, the water quality in offshore areas is aggravating and the breeding of aquatic animals are becoming increasingly unstable. As a result, large-scale bacterial diseases are routinely being observed Speer *et al*., 2018). Therefore, various disinfection and sterilization drugs are being applied to inhibit breeding of pathogens and to ensure the success of aquatic animals breeding and cultivation (Yukihira *et al*., 2006; Liu *et al*., 2009; Fan *et al*., 2014). Consequently, there are emerging evidence that these drugs may influence aquatic animals. Zhao *et al*. (2014) studied the acute toxicity of benzalkonium bromide, dibromohydantoin, methionine iodian and glutaraldehyde on *Babylonia areolate*. Liu and Wang (1994) studied the impact of SC on vitality, ingestion rate and daily growth rate of D-larvae and the umbo-larvae of *Pinctada martensii*. Fan *et al*. (2014) determined acute toxicity of Hg^{2+} and Cd^{2+} on juvenile *Pinctada maxima* (Fan *et al*., 2014). Here, we aim to determine the acute experimental toxicity, safe concentration, and tolerance of glutaraldehyde, dibromohydantoin, methionine iodian and bleaching powder against *Artemia*. These findings provide scientific basis for the rational use of disinfectants in the cultivation of *Artemia*.

Materials and methods

Artemia's cysts (purchased from Aquamaster Company) were hatched in a funnel shaped plastic container filled with synthetic seawater. Newly hatched *Artemia* were processed following the procedure described by Litvinenko *et al*. (2015). Four kinds of disinfectants were used including glutaraldehyde (20% effective concentration, Beijing Zhongnong Huazheng Veterinary Drug Limited Liability Company), dibromohydantoin

* Corresponding author: yangqibin1208@163.com
0030-9923/2023/0001-0001 $ 9.00/0

(20% effective concentration, Beijing Biological and Fisheries Technology Limited Liability Company), methionine iodine (50% effective concentration, Shanxi Shenlong Tianyi Science and Technology Limited Company) and bleaching powder (50% effective chlorine, Guangxi Nanning Chemical Industry Group Corporation). These disinfectants were prepared into mother liquor before application. The experimental container (glass beakers) were filled with 2 L of sea water was first filtered by sand and then inflated with protein skimmer. During the experiment, the dissolved oxygen contents in the water was kept at more than 5 mg/L and the temperature of water during the experiment was maintained at 29±0.5°C, pH at 8.4±0.2, salinity at 30~33ppt.

A total of 100 *Artemia* were kept in 1 L of seawater containing glass beaker. Different volume of disinfectant mother liquor was supplemented in each glass beaker. Throughout the experiment, the water was kept inflated. The *Artemia* was kept off-feed, and disinfectant solution was changed every 12 h. The death of the *Artemia* was recorded to determine the highest survival zero lethal concentrations(LC_0) after 48 h of treatment and the LC_{100} was determined when all *Artemia* were dead after 24 h.

Based on the experimental results of pre-test, five treatment groups of different mass concentrations and one control group were set according to the numerical arithmetic interval method. Each treatment was applied in triplicates and each group of 100 *Artemia* were placed in one experimental unit. The survival of *Artemia* was observed at 48 h and any dead Artemia was removed swiftly. Artemia were considered dead when the swimming foot stopped moving and the body sang to the bottom of the glass beaker.

The mortality rates after 24 h and 48 h in each group were calculated according to the following formula:

$$\text{The mortality rate (\%)} = \frac{\text{number of death}}{\text{total number of experimental } \textit{Artemia}} \times 100$$

The regression equation of the probability unit of the mortality rate and the usual logarithm of drug mass concentration, the death concentration of the drugs (LC_{50}) was obtained by using the linear interpolation method. The X-axis of the regression equation was the usual logarithm of drug mass concentration, and the Y-axis of the regression equation was the probability unit of the mortality rate. Then the safety quality concentration (SC) was obtained according to the following formula:

$$SC = 48\text{ h } LC_{50} \times 0.3/(24\text{ h } LC_{50}/48\text{ h } LC_{50})^2$$

Results

Based on the analysis of the pre-test data, the LC_0 and LC_{100} for glutaraldehyde, dibromohydantoin, methionine iodine and bleaching powder were 14.00 mg/L and 142.00 mg/L, 4.00 mg/L and 97.00 mg/L, 18.00 mg/L and 105.85 mg/L, 5.60 mg/L and 18.58 mg/L, respectively. Five mass concentration of four chemicals were determined according to two mass concentrations by equal spacing method. The results of the test of the toxicity of four chemicals to the larvae of *Artemia* are shown in Table I. During the entire analysis period, there was no death recorded in the control group. Analysis revealed that regression curve equations between the unit of mortality after 24 h and the four chemicals concentration were Y=4.74X-3.33, R^2=0.98, Y=2.66X+0.88, R^2=0.95, Y=5.17X-4.51, R^2=0.99, Y=6.28X-2.26, R^2=0.96, respectively (Fig. 1). It was calculated that at 24 h post treatment, LC_{50} was 68.51 mg/L, 28.21 mg/L, 55.01 mg/L and14.24 mg/L with the method of linear interpolation. The confidence limit of 95% was 62.35 ~ 76.50 mg/L, 25.14 ~ 32.53 mg/L, 50.31 ~ 58.62 mg/L and

Table I. Acute toxicity test of four disinfectants to *Artemia*.

Disinfectant	Drug concentration (mg/L)	Mortality (%)	
		24h	48h
Glutaraldehyde	0.00	0.00	0.00
	14.00	0.00	3.75
	26.38	3.48	41.40
	49.17	17.62	81.17
	85.94	70.47	94.62
	142.00	93.71	100.00
Dibromohydantoin	0.00	0.00	0.00
	4.00	0.00	5.62
	7.24	6.37	47.54
	18.25	18.21	72.05
	43.71	66.25	98.72
	97.00	98.61	100.00
Methionine iodine	0.00	0.00	0.00
	18.00	0.00	2.56
	28.73	3.63	45.37
	45.72	15.54	95.26
	67.66	59.51	97.61
	105.85	98.03	100.00
Bleaching powder	0.00	0.00	0.00
	5.60	0.00	3.53
	7.13	6.51	28.51
	9.66	25.35	54.51
	13.16	55.22	67.48
	18.58	73.59	83.26

13.15 ~ 15.26 mg/L, respectively. The regression equation of the medicine bath after 48 h as Y=4.07X-1.22, R^2= 0.96, Y= 2.88X + 2.30, R^2= 0.99, Y= 5.04X-2.61, R^2= 0.94, Y= 4.18X + 0.55, R^2= 0.94, respectively. The 48 h LC_{50} were 36.71 mg/L, 10.15 mg/L, 30.24 mg/L and 11.26 mg/L with the method of linear interpolation. The 95% confidence limit were 40.07 ~ 61.18 mg/L, 8.42 ~ 11.36 mg/L, 27.54 ~ 32.81 mg/L, 10.03 ~ 11.96 mg/L and the SC were 7.81 mg/L, 0.41 mg/L, 2.73 mg/L and 1.93 mg/L, respectively.

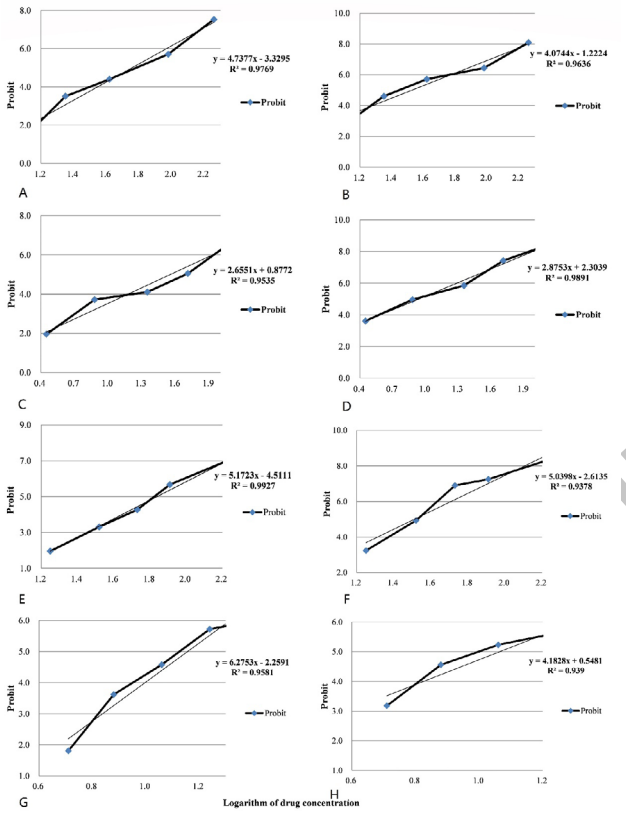

Fig. 1. Effect of four disinfectants treatment for 24 (left) and 48 h (right) on mortality of *Artemia.*

Discussion

Artemia was the most sensitive to bleaching powder with concentration of 5.60-18.58 mg/L followed by dibromohydantoin, methionine iodine and glutaraldehyde with the concentration was 4.00-97.00 mg/L, 18.00-105.85 mg/L, 14.00-142.00 mg/L, respectively. These results were comparable to Zhao *et al*., who have studied juvenile *B. areolata* (Zhao *et al*., 2014). The analysis of four kinds of disinfectants to *Artemia* showed that after 24 h of treatment, the LC_{50} value of bleaching powder against *Artemia* was 14.24 mg/L, however, 48 h post-treatment the LC_{50} was 11.26 mg/L. It was also noticed that the difference between two data was the least, which illustrated the toxicity of bleaching powder was stronger than others against larvae. The toxicity of three kinds of disinfectants in descending order was for dibromohydantoin, methionine iodine and glutaraldehyde. By calculating the safe concentration (SC), the sensitiveness of *Artemia* to four kinds of disinfectants in descending order was concluded to be bleaching powder, dibromohydantoin, methionine iodine and glutaraldehyde.

Chlorine reacts with water to produce atomic oxygen which acts as sterilizing agent. The sterilization efficacy of bleaching powder against the bacteria in aquaculture water has been studied. It was found that the minimum bactericidal concentration (MBC) of bleaching powder to marine vibrio was 8-19 mg/L; the minimum inhibitory concentration (MIC) of vibrio was 4.6 mg/L (Jiang *et al*., 2009; Yang *et al*., 1999). According to our results analysis, the SC of bleaching powder to *Artemia* was inferior to the MIC, which was only 1.93 mg/L. It highlights that the sterilizing effect will not be obvious if safe concentration of bleaching powder is used. Therefore, the use of bleaching powder is not recommending for disinfection of the water of the *Artemia*.

The reports about the research on bactericidal effect of dibromohydantoin in aquaculture can be easily found in shrimps and crabs, fish, *Stichopus japonicas*, *B. areolata* and the SC to the animals above ranged from 0.6 mg/L to90 mg/L, however, there were few reports on the bactericidal effect of dibromohydantoin to *Artemia* (Zhao *et al*., 2014; Shi *et al*., 2008; Zha *et al*., 2010; Sun *et al*., 2008). Compared with the aquatic animals reported above, the *Artemia* were more sensitive to the toxicity of dibromohydantoin and the SC was only 0.41 mg/L. Therefore, in the production, it should not use dibromohydantoin disinfect aquatic water, avoiding the damage to *Artemia*.

A previous study has found that the five species of fish, such as *Megalobrama amblycephala*, *Carassius auratus*, *Ctnopharyngodon idellus*, *Hypophthalmichthys molitrix*, *Spinibarbus sinensis* were sensitive to methionine iodine and the SC of methionine iodine was 2.03-2.38 mg/L in these fish (Ye and Tu, 2009). Our study identified that the SC of methionine iodine was 2.73 mg/L against *Artemia*, which was slightly higher than the manufacture's recommended SC of 2.0 mg/L. However, during production, farmers are accustomed to use several times higher than the recommended dosage of disinfection drugs. Therefore, the dosage of disinfectant should be strictly controlled when using methionine iodine in the aquatic water for *Artemia*.

Glutaraldehyde is a broad-spectrum sterilizing agent to kill microorganisms (Thorn *et al*., 2013; Denyer and Stewart, 1998). Several studies have showed that MBC (1.6-4.2 mg/L) and MIC (0.9-3.2 mg/L) of glutaraldehyde

varied against the Vibrio and Aeromonas and other bacteria in the aquaculture water (Kaleta, 2013). The results of our study showed that the SC of the glutaraldehyde against *Artemia* was 7.81 mg/L, which was much higher than the minimum bactericidal concentration against some pathogens. Therefore, glutaraldehyde is safe and effective to prevent and treat bacterial diseases in the water used for the production of *Artemia*.

Funding

This study was funded by the Youth Fund of Hainan Natural Science Foundation (321QN351) Industrial Technology System of Modern Agriculture (CARS-48), Special fund project for scientific and technological innovation and industrial development in Dapeng New Area (KJYF202101-08), National key research and development plan project (2022YFD2400104), Fangchenggang Science and Technology Plan Project (Fang ke AB22013015), Sanya Science and Technology Project (2018YD11).

IRB approval

The experimental protocol was approved by the animal ethics committee of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Ethical statement

The whole experiment was conducted according to the guidelines established by the National Institutes of Health.

Statement of conflict of interest

The author have declared no conflict of interest.

References

Denyer, S.P., and Stewart, G.S., 1998. *Int. Biodeterior. Biodegrad.*, **41**: 261-268. https://doi.org/10.1016/S0964-8305(98)00023-7

Fan, S.G., Huang, G.J., Guo, Y.H., Liu, B.S., and Yu, D.H., 2014. *Mar. environ. Sci.*, **33**: 351-355.

Jiang, Y.E., Du, L.N., Chen, X.Y., and Yang, J.X., 2009. *Sichuan J. Zool.*, **28**: 85-88.

Kaleta, E.F., 2013. *Lohmann Inf.*, **48**: 3-16.

Litvinenko, L., Litvinenko, A., Boiko, E., and Kutsanov, K., 2015. *Chin. J. Oceanol. Limnol.*, **33**: 1436-1450. https://doi.org/10.1007/s00343-015-4381-6

Liu, J.Y., Zeng, G.Q., Yu, D.H., Li, X.M., Gu, L.C., Shen, M.H. and Wu, K.C., 2009. *J. Anhui Agric. Sci.*, **37**: 3573-3575.

Liu, Z.G. and Wang, B.X., 1994. *J. Zhanjiang Fish. Coll.*, **14**: 7-13.

Shi, H.D., Xu, W.J., Xue, L.J. and Shi, H., 2008. *Mod. Fish. Inf.*, **23**: 24-27.

Speer, F.W. and Weider, L.J., 2018. *Hydrobiologia*, **805**: 391-397. https://doi.org/10.1007/s10750-017-3326-9

Stappen, V.G., Fayazi, G., and Sorgeloos, P., 2001. *Hydrobiologia*, **466**: 133-143. https://doi.org/10.1023/A:1014510730467

Sun, Z.X., Zhao, Y.C., Chen, Y.N., Du, Y.Y., and Zhang, J.Y., 2008. *Mar. Sci.*, **32**: 68-72.

Thorn, R.M., Robinson, G.M., and Reynolds, D.M., 2013. *Antimicrob. Agents Chemother.*, **57**: 2210-2216. https://doi.org/10.1128/AAC.02589-12

Yang, F., Chen, W., Shen, C.G., Yang, W.D., and Guan, J.M., 1999. *J. Dalian Fish. Univ.*, **24**: 29-35.

Ye, Z.H. and Tu, B., 2009. *J. Hydroecol.*, **2**: 118-121.

Yukihira, H., Lucas, J.S., and Klumpp, D.W., 2006. *Aquaculture*, **252**: 208-224. https://doi.org/10.1016/j.aquaculture.2005.06.032

Zha, Z.H., Xu, W.J., Xie, J.J., Zhang, J., and Chai, X.J., 2010. *J. Hydroecol.*, **3**: 66-71.

Zhao, W., Wu, K.C., and Ye, L., 2014. *J. Guangdong Agric. Sci.*, **10**: 106-110.