Research Article

Toxicological assessment of binary mixtures and individually of chemical compounds used in reverse osmosis desalination on *Artemia franciscana* nauplii

Alondra A. Cortés¹, Sebastián Sánchez-Fortún², Martha García¹
Héctor Martínez¹ & María Carmen Bartolomé¹

¹School of Chemistry-Pharmacobiology, Universidad Michoacana de San Nicolás de Hidalgo Morelia, Michoacán, México
²Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine Universidad Complutense de Madrid, Madrid, Spain

Corresponding author: María Carmen Bartolomé (carbarcam@hotmail.com)

ABSTRACT. The Reverse Osmosis desalination has become a technological option to guarantee an adequate drinking water supply in zones with water scarcity. Nevertheless, the process is accompanied by potential adverse impacts on the coastal ecosystem, mainly due to chemical discharges. Taking into account the environmental risk presented by these chemical mixtures, in this work we used a short-term toxicity bioassay with *Artemia franciscana* nauplii expressed in lethality to exposures individually and their combinations. Results showed that toxicity degree from single exposures was C₅H₈O₂ > NaClO > CuSO₄ > KMnO₄ > FeCl₃, indicated that C₅H₈O₂ was the most toxic biocide. However, all compounds in combination with C₅H₈O₂ exhibited a very strong antagonism. Except, in mixtures, NaClO/FeCl₃ and CuSO₄/KMnO₄ resulted in an additive effect. This environmental assessment will allow reducing risk significantly on the concentrate considering the sensitivity of the marine ecosystem to the application of chemical agents during the desalination process.

Keywords: *Artemia franciscana*, chemical hazards, desalination process, mixture analysis, combination index.

INTRODUCTION

Most of the acute toxicity bioassays in the aquatic ecosystems are focused on the individual effects of individual pollutants (Forget et al., 1999). In this context, there are key factors which influence the environmental toxicity of different chemical compounds the dose-response relationship, mode(s) of action, evaluation criteria, environmental factors (such as organic carbon, pH, and temperature), and potential for additive or interactive effects of pollutant mixtures (Nowell et al., 2014). Within this context, the marine and estuarine environments are composed of a variety of biological communities, which are exposed to complex chemical residual mixtures, which varies in concentration ratios (Arrhenius et al., 2004; Cruzeiro et al., 2017).

These chemical pollutants enter into marine environment indirectly through municipal, industrial wastewater or directly through fertilizer application to agriculture and aquaculture farm agents (Schlusener & Bester, 2006; Yang et al., 2008). Desalination of seawater involves the use of complex chemical treatments associated with the application of two or more chemicals simultaneously. However, this technology has disadvantages on the discharge of the brine rejection corresponding which is then returned to the sea containing chemical residuals used in water treatment systems (Einav & Harussi, 2002). This spill is continuous and accumulative in the discharge zone (mainly in coastal areas), which are at constant risk of discharge of saline and chemical concentrates, thereby affecting the performance and integrity of the biota of coastal ecosystem and causing additive and synergistic toxicity in conjunction with other chemical spills (Lattmann & Höpner, 2008).

As a consequence, risk assessment techniques for contaminants that affect aquatic ecosystems (in a particular marine environment), should no longer be restricted to single toxicants and instead have to consi-
under the combined effects resulting from multiple chemical exposures (Backhaus et al., 2003). In most cases, the toxicity of chemicals in mixtures is additive. Even many types of interactions on aquatic toxicology may co-occur, and it is possible that any potentiation or synergism that occurs will be masked by antagonism (Könemann & Pieters, 1996; Silva et al., 2002; DeLorenzo & Fleming, 2007). Exposure to mixtures involving multiple components may induce toxicological responses even if the dose levels of the individual compounds are at or below their no-effect levels (NOEC). The greatest difficulty in analyzing and evaluating the mixture toxicity of is the high concentration variability in both temporal and spatial scales, especially when marine environments are involved in the process. Thus, the assays of the all possible mixtures of environmental pollutants, even when focusing on only one group of chemicals, is merely impossible (Backhaus et al., 2004).

The effects due to the chemical mixture (e.g., heavy metals) can be bioaccumulated and transferred through to aquatic food chains (Wilson et al., 2003). Given the importance of this link in the marine food chain, it is necessary to establish the environmental hazards of the pollutant mixtures to predict the bioavailability and toxicity of various chemicals associated with their use in water treatment and their presence in the salt concentrate used in desalination plants. In this scenario, mutual interactions (synergistic/antagonistic) among the co-occurring compounds can also take place, further complicating environmental assessment (Ginebreda et al., 2013). Thus, bioassays offer the possibility of monitoring the overall response from multiple chemicals in an environmental sample by assessing the impact on different levels of biological organization, such as individuals, community, and populations (Carvalho et al., 2014).

Artemia sp. (brine shrimp) is found in estuarine ecosystems (saline water environments). It has gained popularity as a test organism because of its ease culture, short life-cycle, resistance to manipulation, cosmopolitan distribution and the commercial availability of its latent eggs (cysts). Artemia sp. is used in standardized bioassays mainly due to the reduced test variability since test animals hatching from cysts are of similar age, genotype and physiological condition (Vanhaecke et al., 1981; Persoone et al., 1989; Barahona & Sánchez-Fortún, 1999; Nunes et al., 2006). However, their use in predictive hazard assessments of multicomponent mixtures is limited. Indeed, this kind of studies requires modeling approaches using the available knowledge regarding the toxicities of single substances.

In the literature, there are many models and theories in substance combination analysis, which has been exhaustively described in many review articles such as those by LePelley & Sullivan (1936), Plackett & Hewlett (1948), Loewe (1953), and Greco et al. (1990). These theories have often been defined as confusing. However, Chou & Talalay (1984), and subsequently Chou (2006) proposed a simple way to determine synergism or antagonism by introducing an explicit mass-action law based on enzyme kinetics models and receptor binding theories. This method also uses mathematical induction and deduction, through automated computerized simulation of synergism and antagonism involving the Median Effect Principle (MEP) and Combination Index (CI).

This research aims to predict the potential interactions of the different binary chemical mixtures used in the desalination process by Reverse Osmosis (RO) on the lethality of Artemia franciscana nauplii.

**MATERIALS AND METHODS**

**Test chemicals**

Five chemicals were selected, based on their use for different maintenance work on desalination plants, to carry out this study (Table 1). All chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). In all assays, the working solutions were freshly prepared each time.

**Experimental organisms**

The Artemia franciscana cysts were purchased from Argent Chemical Laboratories, Washington, USA (Argentemía Silver Grade). The method of Persoone et al. (1989) to obtain Artemia nauplii for the test was modified according to the following procedure: A. franciscana cysts were hydrated in distilled water at 4°C for 12 h, followed by washing to separate the cysts that float from those that sink. The hydrated cysts were collected and incubated in a graduated glass cylinder in 100 mL of seawater medium. The synthetic seawater was prepared by mixing 35 g L⁻¹ synthetic sea salts (Sera Premium, Germany) with distilled and deionized (Milli-Q) water, stirring for 24 h with suitable aeration and filtering through 30 µm Millipore cellulose filters. The parameters for this study were: temperature at 28°C, pH 8.4 ± 0.2, the light intensity was 18.5 µmol m⁻² s⁻¹ and as light aeration was maintained by a small tube in contact with the bottom of the cylinder. Under these conditions, the time required for the cysts to hatch was 24 h.
Table 1. Desalination by Reverse Osmosis (RO): Chemical products and their function.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular formula</th>
<th>Function on desalination process by RO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite solution 40%</td>
<td>NaClO</td>
<td>Disinfectant and oxidant chemical</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>KMnO₄</td>
<td>Oxidant chemical</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>FeCl₃·6H₂O</td>
<td>Coagulant and flocculant chemical</td>
</tr>
<tr>
<td>Copper sulfate pentahydrate</td>
<td>CuSO₄·5H₂O</td>
<td>Algaecide</td>
</tr>
<tr>
<td>Glutaraldehyde solution (Grade I, 25% in H₂O)</td>
<td>C₅H₈O₂</td>
<td>Non-oxidizing biocide</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>K₂Cr₂O₇</td>
<td>As reference substance</td>
</tr>
</tbody>
</table>

Assessment of single toxicities

To determine lethal concentrations at 50% of the population of nauplii Artemia franciscana was according to the methodology proposed by Sánchez-Fortún et al. (1995) and the Mexican Norm NMX-AA-110-1995-SCFI of each compound individually, assays were performed in sterile 24-well polystyrene tissue culture plates (Sarstedt Inc., USA). In each well, ten (n = 10) Artemia nauplii were included and exposed to exponential concentrations of each of the chemicals used in the various desalination treatments in a total volume of 1000 µL. Preliminary 24 h static toxicity tests were performed to define the range of concentrations that include 0% and 100% lethality. Test concentrations, chosen on the basis, preliminary range finding tests, covered the range of 1-100 mg L⁻¹ for five chemical products. Test series consisted of five dilutions of the test compound and a seawater control (no compound). As a reference substance K₂Cr₂O₇ (Sigma-Aldrich Chemical Co. St. Louis, Mo, USA) was used in individual exposures on Artemia franciscana nauplii.

At least four replicate test series were performed to each concentration of the compound. Assay plates were placed in an incubator under standard conditions for a period of 24 h. Larvae were considered dead if they did not exhibit any internal or external movement during 10 s of observation in a stereoscope (Zeiss, Carl Zeiss Microscopy GmbH, Germany).

Assessment of mixture toxicities

The assessment of binary mixture toxicity was performed using the Median-Effect/Combination Index (CI)-equation described by Chou & Talalay (1984). This quantitative assessment allows evaluating the combination of two substances taking into account the potency of each product. If two xenobiotics having similar mechanisms of action are presented in a Combination Index (CI) CI <1, they show a synergistic effect. However, if they have completely different mechanisms, they will exhibit an antagonistic effect, and the CI will be >1. When the combination index is CI = 1, it means that they have an additive effect. This analysis was performed through the software package CompuSyn v 1.0 (CombiSyn Inc., Paramus, NJ, USA) developed by Chou & Martin (2005) and Chou (2010).

Data analysis

The concentration causing 50% of lethality on Artemia franciscana nauplii was expressed as LC₅₀(24) which was estimated with 95% confidence limits for each compound. Linear regression analysis determined the LC₅₀(24) values, and all the results were expressed as mean ± SD values. The LC₅₀ estimates were subjected to a one-way analysis of variance (one-way ANOVA) followed by Dunnett’s multiple comparisons test in contrast with reference substance K₂Cr₂O₇. Differences were considered significant with a probability level of P < 0.05. Statistical analysis was performed using the computer software package GraphPad Prism version 5.0 (Graph-Pad Software Inc., USA).

RESULTS

Single toxicity assays

Obtained results show that the acutest toxicity was exhibited by glutaraldehyde (GA) showing close to 1 mg L⁻¹ LC₅₀(24) values (Table 2), followed by sodium hypochlorite with an LC₅₀(24) of 5.92 mg L⁻¹; while the ferric chloride was the compound less toxic with a median lethal concentration above 100 mg L⁻¹ (202.77 mg L⁻¹) on Artemia franciscana nauplii. However, the 24 h-NOEC value of copper sulfate was seven times lower than that of the LC₅₀(24), i.e., the highest concentration of this metal that which had no lethality was 4.15 mg L⁻¹. Analysis of LC₅₀(24) values showed the following rank order for acute single toxicity on A. franciscana: C₅H₈O₂ > NaClO > CuSO₄ > KMnO₄ > FeCl₃. However, there are no statistically significant differences (P > 0.05; P = 0.7264) respect to the reference substance K₂Cr₂O₇ which exhibited an LC₅₀(24) value 19 mg L⁻¹ (15.99-22.44) and 24 h-NOEC value of 4.03 mg L⁻¹ (2.76-5.35).

According to the Environmental Protection Agency (EPA, 1986) classification for acute toxicity, which determines the environmental risk of pollutants...
Table 2. Twenty-four-hour Median Lethal Concentration (LC50(24)) values with 95% confidence limits (CL 95%; n = 4 bioassays) and the highest concentration of each compound tested which has no effect (24 h-NOEC) on 24 h-Artemia franciscana. Values are mean 24-h LC50 (95% C.L.; n = 4 bioassays) in mg L−1, ns = P > 0.05.

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>LC50(24) mg L−1 (CL 95%)</th>
<th>24 h-NOEC mg L−1 (CL 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde</td>
<td>4</td>
<td>0.97 (0.86-1.08)</td>
<td>0.48 (0.37-0.57)</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>4</td>
<td>5.92 (5.44-6.30)</td>
<td>3.50 (2.76-4.03)</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>4</td>
<td>28.91 (23.23-36.14)</td>
<td>4.15 (2.43-6.04)</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>4</td>
<td>36.06 (27.86-43.25)</td>
<td>12.05 (6.12-17.49)</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>4</td>
<td>202.77 (171.40-224.90)</td>
<td>167.50 (98.17-188.79)</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>4</td>
<td>19.00 (15.99-22.44)</td>
<td>4.03 (2.76-5.35)</td>
</tr>
</tbody>
</table>

Mixture toxicity assays

The results of the toxicity data for chemical binary mixtures (Table 3, Fig. 1) showed that combinations between NaClO and FeCl3 using the dosage of desalination plants, resulted in the sum of their individual effects in the mixture (CI: 1.46), i.e., an additive effect. This same behavior resulted for the mixture between CuSO4/KMnO4 according to the reverse osmosis (RO) doses (Table 3). Interesting, NaClO/CuSO4 mixture showed a tendency to an enhancement in toxicity producing synergism at higher concentrations (Figs. 1b, 2). Nevertheless, in reverse osmosis, this does not occur in the mixture with CuSO4 showing an antagonism (CI > 1) (Table 3). The mixture of NaClO/KMnO4 showed a CI = 2.37, manifesting a slight antagonistic interaction between oxidizing agents.

The rest of combinations showed antagonistic effects (CI > 1) on the different magnitude, but remaining in the same plane of antagonistic force to the doses used in reverse osmosis (Figs. 1-2, Table 3). Even all inorganic compounds in combination with glutaraldehyde (GA) exhibited a very strong antagonism (CI >>> 1) showed in the Fig. 2. This result means that the individual toxic response of this biocide is stronger than binary combinations (Table 2).

**DISCUSSION**

**Single substance toxicity**

The effluent discharges from RO plants of seawater have a high concentration of chemical discharges which may adversely impair the coastal water quality and the functioning of marine ecosystems (Areiqat & Mohamed, 2005; Lattemann & Höpner, 2008). The acute single toxicity assays performed in this study showed that A. franciscana is more sensitive to GA exposures than the other inorganic chemicals tested. These results agree to those reported in previous assays by Bu-Olayan & Thomas (2006) seem to indicate that this compound induced the lethal effect in approximately LC50 range (0.01534 mg L−1 at 96 h) for Artemia franciscana. However, it is clear that their estimate was more sensitive, which agrees with that indicated by Barahona-Gomariz et al. (1994) and Sánchez-Fortún et al. (1995) where the naupliar stage of Artemia at 72 and 96 h are more sensitive to various xenobiotics. Sodium hypochlorite was also considered as highly toxic. In RO plants, chlorine is added to the intake water to reduce biofouling and as a disinfectant, and many toxicological studies have confirmed its toxicity. In previous research, on Brachionus plicatilis and A. franciscana, researchers obtained an LC50 at 24 and 96 h of 1.23 mg L−1 and 0.00696 mg L−1 respectively (Bu-Olayan & Thomas, 2006; López-Galindo et al., 2010). Furthermore, this residual disinfectant has synergistic effects with high temperatures resulting from stream rejection (EU, 2007). Thereby, its toxicity is based by the production of trihalomethanes (THM’s) as persistent organic pollutants and represent a severe hazard to aquatic life (EPA, 1991; Sorlini & Collivignarelli, 2005; Belluati et al., 2007).

Concerning the response produced by the CuSO4, on previous data, the effect of metal on the hatching of A. franciscana was evaluated getting an EC50(48) of 0.0118 mg L−1 on the cysts significantly below that the dose needed to cause death stage naupliar (Brix et al., 2006). Copper concentrations in the brine of desalination plants are expected to be in the range of 0.015-0.1 mg L−1, and it does not necessarily mean that it will adversely affect the environment, but in a continuous discharge, copper can be accumulated in sediments and consequently benthic organisms (Lee et al., 2010; Zhong et al., 2012). As to the ferric ion (Fe3+) from FeCl3, A. franciscana was less sensitive to this...
Table 3. Combination Index (CI) values of chemicals binary mixtures with respect to LC50(24) on A. franciscana of each chemical and the RO dosage. *CI: CI > 1 Antagonism, CI < 1 Synergism and CI = 1 Addition.

<table>
<thead>
<tr>
<th>Binary mixture</th>
<th>*CI according to LC50(24)</th>
<th>RO dosage in mg L⁻¹</th>
<th>*CI according to the dose in RO</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaClO/FeCl₃</td>
<td>1.08</td>
<td>1.5/5.0</td>
<td>1.46</td>
</tr>
<tr>
<td>NaClO/CuSO₄</td>
<td>1.11</td>
<td>1.5/1.0</td>
<td>5.61</td>
</tr>
<tr>
<td>NaClO/KMnO₄</td>
<td>1.54</td>
<td>1.5/5.0</td>
<td>2.37</td>
</tr>
<tr>
<td>CuSO₄/KMnO₄</td>
<td>1.25</td>
<td>1.0/5.0</td>
<td>1.63</td>
</tr>
<tr>
<td>FeCl₃/KMnO₄</td>
<td>7.00</td>
<td>5.0/5.0</td>
<td>108.43</td>
</tr>
<tr>
<td>FeCl₃/CuSO₄</td>
<td>2.29</td>
<td>5.0/1.0</td>
<td>489.43</td>
</tr>
<tr>
<td>C₅H₈O₂/NaClO</td>
<td>43.22</td>
<td>10.0/1.5</td>
<td>146.11</td>
</tr>
<tr>
<td>C₅H₈O₂/KMnO₄</td>
<td>81.10</td>
<td>10.0/5.0</td>
<td>530.32</td>
</tr>
<tr>
<td>C₅H₈O₂/CuSO₄</td>
<td>466.94</td>
<td>10.0/1.0</td>
<td>20128.30</td>
</tr>
<tr>
<td>C₅H₈O₂/FeCl₃</td>
<td>321.55</td>
<td>10.0/5.0</td>
<td>273.11</td>
</tr>
</tbody>
</table>

Figure 1. Fa-Log CI plot. Effects of the chemicals binary mixtures on Combination Index (CI). a, b, c), represent the combination of sodium hypochlorite with ferric chloride, copper sulfate, and potassium permanganate, d, e), both symbolize the mix of potassium permanganate with copper sulfate and the ferric chloride, f) represent de combination of ferric chloride and copper sulfate, g, h, i, j, denote combinations of glutaraldehyde with sodium hypochlorite, potassium permanganate, copper sulfate and ferric chloride. Fa (Fraction affected): represent the effect on Artemia franciscana. CI values represent the doses customarily handled in desalination plants.

metal. It is believed that iron salts have low acute toxicity due to rapid oxidation to insoluble forms (FeOH₃) and can be readily precipitated (Van Anholt et al., 2002). Iron is not a toxic component to marine life by itself, but at high concentrations might impact the environment (e.g., anoxia, turbidity, high-suspended solids and harmful algae bloom) (Safrai & Zask, 2006; Lattemann & Höpner, 2008). On the other hand, the
KMnO₄ used as an oxidizing agent of organic matter, for instance, the deposition of algae on the membranes, and in the removal of certain metals (Sorlini & Gialdini, 2010). Unfortunately, the partial oxidation of contaminants may result in the formation of intermediates more toxic than the parent compound (Rizzo, 2011). The toxicity exhibited was less toxic on *A. franciscana* compared to the toxicity in freshwater organisms (*Daphnia magna* LC₅₀(96) 2.13 mg L⁻¹) (Hobbs et al., 2006). In other studies, this agent is toxic to some marine fish species (Kori-Siakpere, 2008).

**Mixture toxicity assays**

It is well known that in the environment, aquatic organisms are exposed to mixtures of pollutants, and very seldom to single substances (Backhaus *et al.*, 2003; Breitholtz *et al.*, 2008). Initially, in desalination plants, chemical mixtures are employed at concentrations in which the individual compounds are believed to be safe, even though if their mixtures can represent a danger for aquatic organisms belonging to the first trophic levels (Lattemann & Höpner, 2008). Also, EPA (1986) recommends an evaluation of whether toxicological interactions among the components are likely to result in higher (or lesser) hazard or risk than would be expected from additivity alone. Nevertheless, in the scientific literature, the knowledge about the toxicological potential of interactions is limited as well as their direct applicability to mixtures associated with hazardous wastewater sites (ATSDR, 2004). Within of the tested mixtures, the combination between NaClO/C₅H₈O₂ showed a great antagonism at the dosage used in RO (CI: 146.11). These results seem to be in contradiction to the findings of Bu-Olayan &
Thomas (2006) who reported a synergistic effect in the same mixture on A. franciscana (CI: 0.91). Moreover, the other substances here tested, that was mixed with GA exhibited higher antagonism. In other similar studies, Emmanuel et al. (2005) and Wang et al. (2010) investigated the effect of other chemicals on the toxicity of GA and did not affect the toxicity of this biocide. Indeed, it has been demonstrated that mixtures, could have different modes of action and different target sites. The FeCl₃/CuSO₄ and NaClO/CuSO₄ mixtures had a high antagonistic behavior. However, the RO brine may contain traces of iron, copper, nickel, lead, cadmium, zinc, chromium, interacting together and will antagonize or enhance the effects of other metals both in vitro and in vivo (Wong et al., 1982; Enserink et al., 1991; Sagripanti et al., 1991; Lattemann & Höpner, 2008; Xu et al., 2011). The effect of these mixtures was evident as the redox-active metals Cu²⁺/Fe³⁺ could enhance the sensitivity of cells to other toxicants (Xu et al., 2011). The following mixtures: NaClO/FeCl₃ and CuSO₄/KMnO₄ (IC 1.46 and 1.63 respectively), indicated slight additive effects in both cases. Bao et al. (2008), reported that the combined effects of copper with other substances like antifouling agents resulted in a synergistic effect. So, the mixtures of metal compounds with oxidizing agents could be highly reactive, and the additive effect is related to an enhancement in the production of reactive oxygen species (ROS), oxidative stress, and reduction of the cellular antioxidant capacity (Chen & Yeh, 2005; Wu et al., 2009). The former agrees with the finding by Sotero-Santos et al. (2007), were mentioned that the sludge from the treatment containing metals combined with other substances might have an additive or synergistic effect on the ecosystem where they are discharged. In other studies, found that metal mixtures (e.g., copper and zinc) gave additive toxicity that influenced the tendency of recolonization of estuarine sediments (Fukunaga et al., 2010; Beyer et al., 2014). In the same context, Vijver et al. (2011) and Cedergreen (2014) reported synergy among Cd+Zn, Cd+Cu, and Cd+As on Daphnia magna and the shrimp Penaeus setiferus. In recent researches, it was reported that salinities of 35 g L⁻¹, the binary mixture of Zn and Ni was additive, however, at low concentrations of salinity between 10 and 17 g L⁻¹ this metal mixture presented an antagonistic response (Damasceno et al., 2017).

Hence, the effects of chemical mixtures on the aquatic ecosystem should consider concepts such as the bioavailability of the mixture on the environment together with its exposure on nontarget species. Additionally, it is important to take into account the mixture accumulation by its toxicokinetics and its toxicodynamics which is related to the receptor interactions and the chemical interactions. Also include cases where one chemical may affect speciation, binding, and transport of the other (Spurgeon et al., 2010). Also, low salinity levels influence the decrease in toxicity of mixtures of different xenobiotics (Damasceno et al., 2017). Overall, the main differences between this study and other investigations found in the literature are the difference between the trophic level of organisms and the biological complexity; the concentrations ratios which are different and also the chemical nature of the chemical agents. The development of methods for modeling and predicting the toxicity of various ratios of mixtures at different concentrations on Artemia franciscana produce reliable results which can be extrapolated to other aquatic biological communities.

CONCLUSIONS

In summary, the results of the present study indicate a correct dynamic use of these compounds, based on the combined application of selected items at certain times of system maintenance can significantly reduce the ecotoxicological risk of discharge points derived from these routine practices in desalination plants by the reverse osmosis. Nevertheless, the effects of these combinations used in desalination should directly evaluate its presence in coastal water samples to determine and achieve a better assessment of the ecotoxicological potential of waste on the marine aquatic environment. So, several other questions remain to be investigated regarding mixture toxicity of these chemicals and their effects on marine communities.

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