Assessment on Biocides Bioaccumulation in Mullet Liza klunzingeri in Kuwaiti Waters, off the Arabian Gulf

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Abstract: Biocides, such as formaldehyde (HCHO), sodium hypochlorite (NaOCl) and glutaraldehyde (C5H8O2) that are commonly used in thermal, desalination and power plants and industries were tested on the commercially important mullet fish, Liza klunzingeri to determine the environmental contamination in the stressed ecosystem of Kuwait Bay sites. Multi-factor Probit analysis toxicity tests (96 h) on L. klunzingeri showed the lowest observed effective concentration (LOEC) and median lethal concentration (LC50) with NaOCl (0.019 and 0.027 µg L-1) followed by HCHO (0.058 and 0.157 µg L-1) and C5H8O2 (0.056 and 0.072 µg L-1). Site-wise analysis in the absence of feed showed high biocides toxicity in L. klunzingeri reared in seawater from Site I when compared to Sites II-III. Experiments were conducted (2-9 months) by rearing fish separately in seawater collected from three Kuwait Bay sites to test the bio-accumulated toxicity levels at LOEC of biocides fed fish using Feed Conversion Ratio (FCR) calculation. The lowest FCR was observed in fish fed with biocides in the sequence of NaOCl (0.40-1.1) followed by C5H8O2 (0.91-1.2) and HCHO (0.92-1.3) as well as with fish reared in seawater from Site I followed by Site II and Site III. High FCR was recorded in control (1.2-1.6) without the addition of biocides. These results exemplify the use of L. klunzingeri as an indicator species and may characterize a better quantification of biocides bioaccumulation using FCR calculation in mullet fish.

Key words: FCR, toxicity, biocides, mullet fish

INTRODUCTION

Environmental concern over the use of biocides stems from its use in desalination, power and thermal plants, cooling water disinfectant and anti fouling paints. An exhaustive literature has been made on biocides toxicity in the marine environment[1-3]. Unregulated discharges mainly from the thermal, power and desalination plants as well as from sewages caused deleterious effects to organisms in Kuwait marine environment[4]. Biocides such as glutaraldehyde (C5H8O2), formaldehyde (HCHO) and sodium hypochlorite (NaOCl) used mainly in the thermal and desalination plants as biocide, disinfectant, in medical application, oil and as solvents in various industries were discharged in the aquatic environment without any safety precautions[5-10]. Probit analysis and bioaccumulation factor (BAF) determined the acute toxicity tests on pollutants in fish[11]. In nature, the biocides toxicity varied in fish subjected to feeding. Therefore, experiments were conducted to determine the accumulated biocide levels using feed conversion ratio (FCR). Over the recent years, biocides such as glutaraldehyde (C5H8O2), formaldehyde (HCHO) and sodium hypochlorite (NaOCl) were used in thermal, power and desalination plants (Sites I-III) in the Kuwait Bay respectively. These biocides added to the stressed ecosystem Kuwait Bay sites which was primarily affected as a result of untreated effluent discharges, fluctuating seawater variables (specifically observed in sites I-II), upwelling of water current, inorganic pollutants, influence of anthropogenic activities and harmful algal blooms (as observed in Sites II-III). The above reasons were also suspected to be the cause for mass mortality of L. klunzingeri during the years 1999 and 2001 in Kuwait Bay sites[12,13]. However, publications on L. klunzingeri as a tool to determine the bio-accumulated biocide toxicity that could be supported by Feed Conversion Ratio (FCR) calculation were limited for Kuwait Bay sites which developed differential site-specific stressed ecosystem and hence the study.

MATERIALS AND METHODS

Sampling sites: Abundantly available mullet fish, L. klunzingeri and seawater were collected from three commercially important Kuwait Bay sites (Sites I-III) that represented stressed ecosystem over the recent years (Fig. 1).

Site I (Subiyah) is in the Northern part of Kuwait Bay. The profile is run from shell hash beach with a very large tidal flat having Eolithic bed rock, calcareous sands pro-grading into silts and clays. This site is subjected to effluent discharges from the thermal plant.
Site II (Doha) has a power plant. It has been affected by major oil spills discharged during the Gulf War by the Iraqis. The seabed is shallow. This site has power plant and various industries and thus demarcated as an industrialized zone of Kuwait.

Site-III (Kuwait Towers) has a desalination plant, few beach rocks and inter-tidal flats. This site is prone to domestic and industrial effluents. This location is one of the centers for recreational activities and thus found polluted with sewage wastes in the aquatic system.

**Fish rearing in the laboratory:** Fish (10 replicates), each weighing 20±3 g and total length 9±3 cm were acclimated separately in three aquarium tanks in the laboratory containing filtered disinfected seawater collected from three Kuwait Bay sites that served as controls. Toxicity and bioaccumulation using FCR experiments were carried out separately in fish reared in disinfected and filtered seawater (250 l) collected from three Kuwait Bay sites (I-III). The fish were fed (2 % body weight) with Grower Feed (Tetra Marin (Tetra Werke, Germany) comprising of crude protein (45%), dry matter-dry yeast, Oat meal, shrimp meal, wheat gluten (30%) crude fat (6%), oil (4.8%), crude fiber (2%), vitamins (0.2%), moisture (6%) and ash (5.1%). Seawater (5 %) was exchanged every two days. Antibiotics and fungicides were used in both test samples (before the initiation of toxicity tests) and in control.

**Formaldehyde determination in seawater:** Based on the concentration ranges of formaldehyde (HCHO) employed for treatment plants, test solutions ranging 0.09-0.40 µg L\(^{-1}\) was prepared and added to seawater in the aquarium tanks. After the preparation of the test solution, samples of 10 ml were subjected to 4-amino-3-hydrazine-5mercapto-1, 2, 4-triazole (AHMT) method\(^{[5]}\) using a HP Spectrophotometer. Standard curves of HCHO (37% v/v) reagent was used to calculate their concentrations (µg L\(^{-1}\)) in seawater.

**Glutaraldehyde determination in seawater:** Stock solution of glutaraldehyde (C\(_5\)H\(_8\)O\(_2\)-26.57% v/v) ranging 0.06-0.10 µg L\(^{-1}\) was prepared based on their usage of biocide in desalination and power plants. The test method employs a titrimetric chemistry using sulfuric acid and sodium sulfite with an end point indicator phenolphthalein\(^{[14]}\).

**Sodium hypochlorite determination in seawater:** Test solution of sodium hypochlorite with concentrations range of 0.023-0.042 µg L\(^{-1}\) were prepared based on their levels (5%) used as disinfectant and biocide in desalination and power plants. Following DPD (N-N Diethyl-P-Phenylenediamine) method\(^{[15]}\), direct determination of hypochlorite concentrations were made as DPD compounds reacted with hypochlorite ions\(^{[15,16]}\).

**RESULTS AND DISCUSSION**

**Seawater analysis:** The present study chose biocides since they were utilized abundantly in thermal, desalination and power plants and discharged into the Bay without much treatment. Biocides concentrations of NaOCl, HCHO and C\(_5\)H\(_8\)O\(_2\), were below detectable levels in the sampled seawater collected from the three classified Kuwait Bay sites (Fig. 1). Reasons could be attributed to: (1) their highly photo and biodegradable nature and upwelling water current and (2) the effect of seawater treatment used in the industrial plants before.
Table 1: Estimated exposure concentrations of biocides to *Liza klunzingeri* (10 replicates) using Probit Program (USEPA 1993)

| Biocides/Conc. (µg/l) at LC point | Site | 15 | 50 | 99 |
|----------------------------------|------|----|----|----|
|                                  | Subiyah-I |    |    |    |
| C₅H₈O₂                           | 0.056 | 0.072 | 0.128 | 0.212* |
| C.I (0.035-0.064)                 | (0.061-0.079) | (0.104-0.255) |      |
| HCHO                             | 0.066 | 0.157 | 1.092 | 1.339* |
| C.I (0.018-0.103)                | (0.101-0.222) | (0.540-11.780) |      |
| NaOCl                            | 0.019 | 0.027 | 0.053 | 1.717* |
| C.I (0.010-0.024)                | (0.020-0.030) | (0.043-0.112) |      |
|                                  | Doha-II |    |    |    |
| C₅H₈O₂                           | 0.058 | 0.074 | 0.130 | 0.044* |
| C.I (0.039-0.066)                | (0.065-0.082) | (0.106-0.248) |      |
| HCHO                             | 0.058 | 0.174 | 2.059 | 0.824* |
| C.I (0.005-0.101)                | (0.098-0.284) | (0.740-346.87) |      |
| NaOCl                            | 0.020 | 0.028 | 0.066 | 0.276* |
| C.I (0.008-0.024)                | (0.021-0.033) | (0.049-0.235) |      |
|                                  | Kuwait Towers-III | | | |
| C₅H₈O₂                           | 0.061 | 0.083 | 0.165 | 0.293* |
| C.I (0.036-0.070)                | (0.074-0.100) | (0.123-0.640) |      |
| HCHO                             | 0.077 | 0.222 | 2.430 | 0.824* |
| C.I (0.011-0.124)                | (0.145-0.424) | (0.848-380.267) |      |
| NaOCl                            | 0.027 | 0.038 | 0.081 | 1.384* |
| C.I (0.015-0.031)                | (0.033-0.048) | (0.057-0.436) |      |

C.I: Confidence interval (upper and lower limits); LC: Lethal concentration; χ²: Calculated Chi square for heterogeneity; *: Chi square significance

Table 2: FCR in *Liza klunzingeri* (10 replicates) reared in three Kuwait Bay sites and fed with and without biocides

| Months/ Biocides/ | 2nd Month | 3rd Month | 4th Month | 6th Month | 9th Month |
|------------------|-----------|-----------|-----------|-----------|----------|
|                  | Site – I  | Site – II | Site – III| Site – III| Site – III|
| C₅H₈O₂           | 0.40      | 0.45      | 0.47      | 0.52      | 0.59      |
| NaOCl            | 0.42      | 0.45      | 0.48      | 0.54      | 0.62      |
| HCHO             | 0.45      | 0.45      | 0.49      | 0.49      | 0.64      |

FCR: Feed conversion ratio; Control: FCR without biocides addition; Site-I-III: Subiyah, Doha, Kuwait Towers, C₅H₈O₂: glutaraldehyde, NaOCl: sodium hypochlorite, HCHO: formaldehyde

they were let out in the Bay. This was also validated in the present study in fish reared in seawater and subjected for 96 h without biocides (control) from the three Kuwait Bay sites.

**Acute toxicity tests:** Using Probit program[^18^], the 96-h LC₅₀ of *L klunzingeri* in seawater collected from Kuwait Bay sites (Fig. 1) revealed the sequence of NaOCl (0.027-0.038 µg L⁻¹) < C₅H₈O₂ (0.072-0.083 µg L⁻¹) < HCHO (0.157-0.222 µg L⁻¹) (Table 1). Observation revealed *L. klunzingeri* sensitive to NaOCl at LOEC (0.019µg L⁻¹) than other biocides described in this study (Table 1). This may be attributed to the effect of chlorine compounds in the body tissues of fish when compared with other biocides and supports the earlier findings[^20^]. Statistical analysis revealed significance in all the biocides by Chi-square heterogeneity test (Table 1).

Biocides were found highly effective at LOEC (observed at LC₁₅) and LC₅₀ to *L. klunzingeri* tested with seawater collected from thermal plant (Site-I) than with power plant (Site II) and desalination (Site III) plant in the Bay (Table 1 and 2). This may be attributed to the fluctuating seawater: (a) salinities (24-30 ‰) and
(b) temperatures (30°C-40°C) from the thermal plant discharges into the Bay when compared to the other sites. Nevertheless, moderate and detrimental effect of biocides was observed when *L. klunzingeri* was subjected to toxicity tests with seawater collected from desalination and power plants. This could be attributed to (1) the discharge of untreated brine water back into the Bay, (2) high nutrient deposition and its interaction with biocides and (3) installation of mega desalination plants causing impingement and entrainment effect to marine organisms in seawater and supports the earlier observations [21,22]. However, as these chemicals were photo and biodegradable in seawater, mass mortality of this fish was observed only when large quantities of these biocides were untreated and accidentally discharged into the Kuwait Bay.

Food conversion ratio (FCR): Calculating toxicity levels or bioaccumulation factor (BAF) determined results on acute toxicity tests and bioaccumulation of pollutants in fish [3]. However, results from such studies were limited to the accumulated effect of biocides in post-fed fish. Such limitations were overcome by determining the FCR of fish to biocide toxicity results. FCR calculations also supported fish without being killed and results validated at LC15 toxicity tests. The least FCR was found in fish treated with NaOCl followed by C2H5O2 and HCHO. This could be attributed to the lethal and sustainable effects of chlorine and its residues to sensitive fish than other photo and biodegradable biocides and thus, corroborates the findings of [3]. Furthermore, observations revealed low FCR in fish reared in seawater collected from Sites I-II when compared to FCR values from Site III samples. This clearly indicated high pollution levels in seawater where thermal and desalination plants are installed.

The above findings deduce that the selected biocides can give more toxic effects to the fish caught from the Kuwait Bay. Discharges from the thermal, desalination and power plants plant and rapid industrialization could attribute to the lethal effects on such fish and their consumers. Therefore, future toxicological investigations are recommended that characterize the use of *L. klunzingeri* as biocide indicator and to implement remedial measures to untreated pollutant discharges into the sea.

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