Ecotoxicity Study Using Dibenzothiophene and Mercury Chloride in "Brine Shrimp"

Melise Lemos Nascimento[1,2*], Madson Moreira Nascimento[2,3], Gisele Olímpio da Rocha[1,2], Jailson Bittencourt de Andrade[2,3]

[1]Interdisciplinary Center of Energy and Environment (CIEnAm), Federal University of Bahia (UFBA),
[2]National Institute of Science and Technology in Energy and Environment (INCT), Federal University of Bahia (UFBA);
[3]SENAI-CIMATEC University; Salvador, Bahia, Brazil

This study investigated the ecotoxicity of Dibenzothiophene (DBT) and mercuric chloride ($HgCl_2$) using *Artemia franciscana* (brine shrimp) as a model organism. Acute toxicity tests were conducted to individually determine the LC_{50} values for DBT and $HgCl_2$ at concentrations of 1, 2, 4, 8, and 10 mg L^{-1}. Results from the probit model revealed that the LC_{50} of $HgCl_2$ was 33.11 mg L^{-1}, which is approximately ten times higher than that of DBT (3.89 mg L^{-1}), indicating that DBT is significantly more toxic. When combined, these contaminants exhibited a pronounced lethal synergy, resulting in total mortality of *Artemia franciscana* at all tested concentrations. These findings underscore the critical need to understand the synergistic effects of environmental contaminants and call for further research into the chronic toxicity and long-term sublethal impacts of polycyclic aromatic sulfur heterocycles (PASHs) on marine ecosystems.

Keywords: Ecotoxicological Test. Brine Shrimp. Dibenzothiophene. Mercuric Chloride. Synergistic Contamination.

The species *Artemia franciscana*, commonly known as brine shrimp (BS), is a saltwater microcrustacean with five life stages, of which the nauplii stage is the most frequently used in ecotoxicity tests [1,2]. These tests provide valuable insights into the toxic effects of contaminants on aquatic biota in marine environments [3]. Consequently, *Artemia franciscana* is widely used as a model organism *in vivo* ecotoxicity studies, mainly through median lethal dose (LD_{50}) or lethal concentration (LC_{50}) assessments, which measure mortality when the species is exposed to substances such as heavy metals (e.g., cadmium, copper, zinc, and nickel) [4] or organic compounds (e.g., methylparaben, dicamba herbicide, and polypropylene microplastics) [5-7]. Beyond ecotoxicological studies, BS has been employed in reproductive toxicity evaluations, mutagenicity assessments (e.g., the Ames test), and chronic toxicity studies [8,9], highlighting its extensive and diverse applications in high-impact research. Ecotoxicity tests traditionally focus on isolated contaminants, such as nanomaterials, microplastics, cosmetics, pharmaceuticals, and pesticides [11-5]. However, contemporary environmental contamination often involves multiple pollutants, necessitating revised testing methodologies to assess the effects of combined contaminants [16,17]. Recent studies, such as those by Albarano and colleagues (2023) [18], have explored the interactions of polycyclic aromatic hydrocarbons (PAHs) mixtures on BS, revealing gene expression changes in both nauplii and adult stages. These findings emphasize the ecological importance of studying mutual contamination and its potential impacts on biological processes in marine invertebrates.

In 2019, a significant oil spill on the Brazilian coast released a mixture of contaminants, including polycyclic aromatic sulfur heterocycles (PASHs), into marine ecosystems [19]. PASHs, such as mercaptans, thiophenes, benzothiophenes, and dibenzothiophenes (DBTs), are natural constituents of crude oil [20] and are gaining attention due to their carcinogenic and mutagenic properties and widespread presence in depositional environments [21]. Recent studies have identified DBT derivatives,

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Address for correspondence: Melise Lemos Nascimento. Avenida Milton Santos, s/n, Campus Ondina, Salvador, Bahia, Brazil. Zipcode: 40170-115. E-mail: melise.lemos@gmail.com.

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such as 2,3-dimethyl benzothiophene (2,3-DMBT), 4-methyl benzothiophene (4-MBT), and 4,6-methyl dibenzothiophene (4,6-MDBT) [22], in marine organisms like ascidians, which were contaminated by maritime transport activities [22]. Similar findings were observed in *Lepas anatifera*, raising concerns about potential risks to marine organisms and their predators [23]. Concurrently, toxic metals like mercury have been documented in polychaetes from regions such as Todos os Santos Bay, Brazil [24]. Despite these findings, the specific effects of PASHs and their interaction with heavy metals on marine biota remain poorly understood.

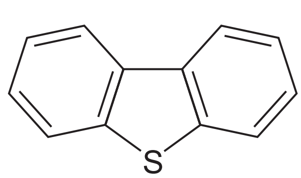
This study evaluates the toxicity of DBT and mercuric chloride ($HgCl_2$) on *Artemia franciscana* through *in vivo* ecotoxicity tests, considering both individual and combined exposure scenarios. The co-contamination test (DBT + $HgCl_2$) was designed to investigate potential synergistic effects, providing a deeper understanding of the ecological risks posed by the interaction of organic and metallic contaminants in marine environments.

Materials and Methods

Hatching of Nauplii

Newly hatched nauplii of *Artemia franciscana* were obtained from commercially dried cysts (Yepist, Bahia, Brazil). Approximately 100 mg of cysts were hatched in artificial seawater (37 g L^{-1}) at 28 °C under continuous illumination and aeration conditions. After 24 hours, the newly hatched nauplii and unhatched cysts were separated from their shells using a phototaxis-based method. The hatched nauplii were immediately transferred with a Pasteur pipette to tubes containing artificial seawater and maintained at 25 ± 1 °C until use in toxicity tests.

Figure 1. Dibenzothiophene compound.



Acute Toxicity of DBT, $HgCl_2$, and DBT + $HgCl_2$

A 24-hour acute toxicity test was conducted to determine the LC_{50} values for:

- Dibenzothiophene (DBT) in ethanolic solution,
- Mercuric chloride ($HgCl_2$) in aqueous solution, and
- Combined exposure to DBT + $HgCl_2$.

The LC_{50} value represents the concentration of a substance that causes mortality in 50% of the test population after a specified exposure period. The concentrations tested for each toxicant were 1, 2, 4, 8, and 10 mg L^{-1}. The control group consisted of artificial seawater with ethanol (the solvent used for DBT). All tests were performed in triplicate, each replicate containing 10 *Artemia nauplii*, resulting in 30 nauplii per concentration and control group (Table 1).

After 24 hours of exposure, mortality was assessed. Dead nauplii were identified as those exhibiting no movement for 10 seconds under continuous observation.

Statistical Analysis

The LC_{50} values were calculated using the probit model in Microsoft Excel. Mortality data were plotted on a $\log_{10}$ concentration scale against the percentage of mortality to determine the relationship between concentration and toxicity. Graphs of mortality data were generated using GraphPad Prism 5D.

Results and Discussion

LC_{50} values represent the concentration of a substance that causes mortality in 50% of a population and provide a measure of the substance's toxicity. The results for LC_{50} showed that the control group containing ethanol did not result in any mortality among the nauplii, confirming ethanol's low toxicity as a solvent. In the tests with DBT, the LC_{50} was 3.89 mg L^{-1}, indicating significant toxicity (Figure 2). Comparatively, $HgCl_2$ showed a higher LC_{50} value of

Table 1. Experimental design.

Toxicity Tests	Composition solution	Replicates	Concentrations	Total BS naupliis
Control Group	Artificial seawater + Ethanol	3	-	30
DBT	DBT + Water + Ethanol	15	1, 2, 4, 8, 10 mg L^{-1}	150
$HgCl_2$	$HgCl_2$ + Water	15	1, 2, 4, 8, 10 mg L^{-1}	150
DBT + $HgCl_2$	$HgCl_2$ + DBT Water + Ethanol	15	1, 2, 4, 8, 10 mg L^{-1}	150

33.11 mg L^{-1} (Figure 3), suggesting lower immediate toxicity within the 24-hour exposure period (Table 2). However, mercury's known bioaccumulative properties could explain the reduced short-term mortality, as its effects may intensify over time. When DBT and $HgCl_2$ were combined, total mortality was observed at all concentrations tested, including the lowest (1 mg L^{-1}) (Figure 4). This highlights a lethal synergistic interaction between the two contaminants, where their combined toxicity far exceeded their individual effects.

Results and Discussion

The solubility of DBT (1.47 mg L^{-1}) is significantly lower than that of $HgCl_2$ (73.30 mg L^{-1}). An ethanolic solution was used to improve DBT's solubility in water. Despite this, the common ion effect in artificial seawater likely reduced the solubility of both compounds due to the presence of Na^+ and Cl^- ions, which may have influenced mortality rates.

Mortality increased gradually with DBT concentrations up to 8 mg L^{-1} but decreased at 10 mg L^{-1}, potentially due to precipitation of DBT as solubility limits were reached. $HgCl_2$ demonstrated higher mortality rates than DBT at equivalent concentrations, reflecting the greater sensitivity of *A. franciscana* to $HgCl_2$.

The co-contamination tests with DBT and $HgCl_2$ revealed a synergistic effect, resulting in 100% mortality across all concentrations. This highlights the importance of studying combined pollutant effects, as they can magnify toxicity and pose significant ecological risks.

Conclusion

Marine environments are increasingly exposed to contamination from diverse sources, such as industrial discharge, agricultural runoff, and household waste. This study's acute toxicity tests demonstrated that $HgCl_2$ is significantly more toxic than DBT, with

Figure 2 Mortality of BS in DBT.

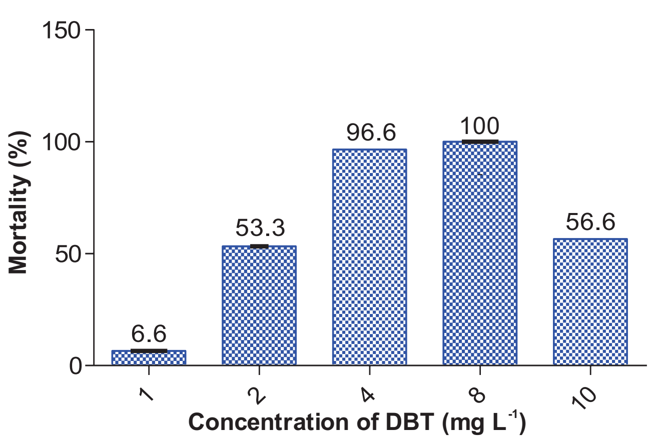


Figure 3. Mortality of BS in $HgCl_2$.

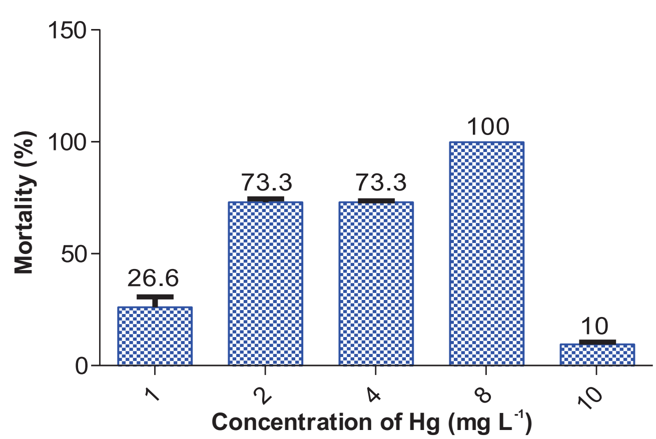


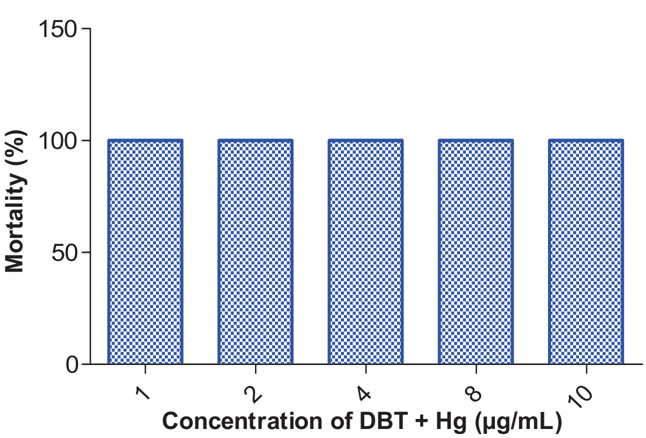
Table 2. Mortality and LC_{50}.

Toxicity Tests	Concentrations ($mg\ L^{-1}$)	Mortality (%)	LC_{50}	Solubility
Control Group	0	0	---	---
DBT	1	6.6	3.89	1.47 $mg\ L^{-1}$
	2	53.3		
	4	96.6		
	8	100		
	10	56.6		
$HgCl_2$	1	26.6	33.11	73.302 $mg\ L^{-1}$
	2	73.3		
	4	73.3		
	8	100		
	10	10		
DBT + $HgCl_2$	1	100	---	---
	2	100		
	4	100		
	8	100		
	10	100		

Source: SciFindern (CAS Chemical Abstracts Service).

an LC_{50} value approximately ten times higher. Furthermore, when combined, these contaminants exhibited a lethal synergistic effect, causing total mortality at all tested concentrations. These findings underscore the critical need to investigate the combined effects of environmental contaminants. Future research should focus on chronic toxicity and sublethal effects to understand the long-term implications of exposure to PASHs and other pollutants in marine ecosystems.

Figure 4. Mortality of BS in DBT + $HgCl_2$.



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