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Aurelia aurita as a Model for Assessing Food Additives: Comparative Results Across Trophic Levels

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Abstract: The industry currently generates numerous substances, such as food additives, whose environmental impacts, particularly in marine environments, remain inadequately assessed. This study employs *Aurelia aurita* for the first time as a model organism to evaluate the toxicity of such compounds. For this, the main goal is to evaluate the toxicity of two food additives, 2-Methyl-1Phenylpropan-2-ol (S1) and 1-Phenylethan-1-ol (S2), on *A. aurita* ephyrae, comparing the results with other organisms representing different trophic levels, specifically the alga *Phaeodactylum tricornutum* and the crustacean *Artemia salina*. Acute toxicity tests were conducted on each organism. Mortality rates were measured for Artemia, growth inhibition for the algae, and mortality and pulsation frequency for ephyrae. LC_{50} values were determined using probit regression analysis with R software. Tests for algae and Artemia followed ISO guideline and international standardized protocols. For *A. aurita*, a modified protocol by Mercado et al. (2023) was used. The tested additives showed a toxicity around 600 mg/L for *A. salina*. For the algae, S1 was significantly more toxic (LC_{50} ≈ 100 mg/L) compared to S2 (LC_{50} ≈ 450 mg/L). Similarly, in *A. aurita*, S1 exhibited high toxicity (LC_{50} ≈ 10 mg/L) while S2 had a lower toxicity (LC_{50} ≈ 80 mg/L). Pulsation frequency data for *A. aurita* revealed that S1 initially increased pulsation rates at lower concentrations (maximum at 10 mg/L), followed by a significant decrease at higher concentrations. Conversely, S2 showed a steady decrease in pulsation rates up to 10 mg/L, with a slight increase at concentrations of 15, 20, and 25 mg/L. The results demonstrate varying sensitivity to the toxic effects of the two compounds across different trophic levels, with *A. aurita* ephyrae being the most sensitive. This suggests the potential efficacy of jellyfish as novel ecotoxicological models due to their heightened sensitivity, enabling detection of lower contaminant concentrations in test samples. Further research is required to enhance the efficiency of ecotoxicological assays using these models.

Keywords: cnidarian; ecotoxicology; additives; trophic levels; bioassays

1. Introduction

The 21st century is marked by exponential growth in industrial and technological development, leading to a significant increase in the production and use of chemical compounds. This, coupled with the unsustainable rise in the global human population, has resulted in a concerning consequence: the excessive increase of anthropogenic pollutants in the environment [1]. Among these pollutants, chemical additives stand out, which are widely used across various industrial and domestic sectors [2].

Additives are compounds designed to control potentially harmful organisms such as bacteria, fungi, algae, and insects among other applications. They are present in everyday products such as pesticides, herbicides, disinfectants, and preservatives, and are used in various fields including agriculture, human medicine, veterinary medicine, the food industry, and environmental

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management. All of them are also used to improve the physical, chemical, or biological properties of different products. They are found as we mentioned earlier in food, cosmetics, plastics, and many other daily-use items [3].

Modern additives are designed to target specific organisms; however, they often are toxic to many other living organisms that are not the intended target. Moreover, the target organisms often coexist in their natural habitat with other more sensitive species [4]. Chemical additives can have carcinogenic, toxic, or endocrine-disrupting properties. Additionally, some of them are not biodegradable, posing a danger to the environment in cases of emissions, spills, and leaks [5].

The marine ecosystems are probably the most affected ecosystems by the current concerning situation, due to the incessant increase in anthropogenic pressure, resulting from the increased release of new pollutants through various activities, many of which are directly discharged into seas and oceans, in addition to indirect emissions such as pollutant discharges into non-endorheic river basins. In this context, which poses a serious threat to the biodiversity and functionality of these systems both locally and globally, the lack of adequate infrastructure for wastewater treatment and irresponsible disposal of industrial and urban waste are key factors in the accumulation of this pollution in the waters [6,7]. When these substances enter the waters, due to their high resistance to biodegradation, they disperse through seas and oceans, not limited to direct discharge areas, but also transported over long distances by ocean currents [8]. Furthermore, the accumulation of chemicals and biocides in marine sediments and water columns has emerged as a critical problem, with potentially severe impacts endangering the health of multiple organisms and disrupting marine ecosystems [9]. Moreover, there is growing concern about the secondary effects of these chemicals on human health, especially through bioaccumulation and biomagnification in the marine food chain [3].

Due to the presence and potential impact of marine pollutants on both humans and wildlife, the importance of monitoring and testing the fate and effects of these chemicals has long been recognized [10,11]. This is one of the main goal of ecotoxicology assays. However, in ecotoxicology there is always an urgent need to identify new model organisms for their use in the development of sensitive and reliable test methods for laboratory testing. Gelatinous zooplankton are not typically included in routine ecotoxicological studies, despite the growing recognition over the past few decades of their crucial role in maintaining the balance of marine ecosystems.

Jellyfish, which form part of this gelatinous zooplankton, play a critical role in marine ecosystems by preying on planktonic organisms such as crustaceans, copepods, and fish larvae. They, in turn, are consumed by species like sea turtles, sunfish, and seabirds. As predators, jellyfish can significantly alter plankton composition and affect fishery yields through dietary overlap and direct predation. Jellyfish blooms are episodic events driven by complex physical, behavioral, and physiological processes, often exacerbated by human activities like overfishing, eutrophication, and changes in estuarine circulation. These factors have prompted the exploration of using the ephyra stage of the Scyphozoan jellyfish *Aurelia aurita* (common moon jellyfish) as an innovative model organism in marine ecotoxicology [12].

Given the reasons mentioned above, the ephyrae of *A. aurita* have been selected to test the compounds 2-Methyl-1-Phenylpropan-2-ol (S1) and 1-Phenylethan-1-ol (S2), which are commonly used as additive in the food industry as well as in the perfume industry. To compare the effects and sensitivity of these food additives on scyphomedusae, the compounds will also be tested on two additional marine organisms, *Phaeodactylum tricornutum* (diatom) and *Artemia salina* (crustacean). The primary objective of these assays is to evaluate the toxicity of the tested compounds in marine invertebrates and their potential impact on the marine food chain.

2. Materials and Methods

To evaluate the toxicity of the tested substances, an acute toxicity test will be conducted. This method involves exposing test organisms to varying doses of the substance over a short duration, typically 24, 48, and/or 72 hours. Following this exposure period, the survival, mortality and other parameters like the pulsation frequency for *A. aurita* will be recorded, in order to calculate the lethal

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concentration 50 (LC_{50}), which represents the concentration of the compound that affects 50% of the test organisms [13]. The LC_{50} value will be expressed as the weight of the substance per unit volume (mg/L or ppm). A lower LC_{50} value indicates higher toxicity, signifying that the species is more sensitive to the tested compound. To ensure standardization of the toxicity assays, specific protocols as outlined in the ISO (International Standarization Organisation) guidelines for various species will be followed for *A. salina* and *P. tricornutum*. For *A. aurita* will be used the methodology proposed by Faimali et al. (2014) and Mercado et al. (2023) [12,14].

2.1. Artemia salina Acute Test

The assay was based in the commercial Artoxkit M™ (Microbiotests Inc., Gent, Belgium). and conducted according its standard operational procedure. All tests used cysts from a single batch. Artificial seawater was prepared using the preconcentrated media supplied with the kit. This water was used for the incubation of the cysts, the dilution of the compounds, and the execution of the toxicity assays. All tests were conducted with Artemia nauplii at the instar II-III stage, aged between 24 to 48 hours post-hatching. A small quantity of cysts was placed in a beaker with standard saline water at 25 °C and incubated in a chamber at 25 °C (± 2 °C) for 30 hours, ensuring continuous aeration via an air stone and constant illumination provided by a 4000-lux growth lamp.

Serial dilutions of the test compounds were prepared in the artificial seawater in concentration series of 1000, 700, 500, 300, and 100 ppm. Depending on the initial test results, further dilutions or new concentrations were prepared to achieve mortality results close to the LC_{50}, thus aiming for more precise results. Once the different concentrations of the compounds were prepared, they were placed in 24-well microplates (Nunc®) with a capacity of 3 mL per well. The plates were covered to prevent evaporation and sealed with Parafilm™ to ensure airtightness. One plate was used for each compound, and a control blank was included for each plate.

Each well was filled with 1 mL of dilution, using the first two wells as washing wells to avoid over-diluting the sample and thus minimize errors when transferring the Artemia nauplii to the plates. Twenty nauplii were added to each concentration using a transfer pipette and incubated at 25 °C (± 2 °C) in darkness for 24 hours. After this period, an initial mortality count was conducted using a binocular magnifying glass, followed by a subsequent incubation period to reach 48 hours, at which point a second mortality count was performed. In all cases, control mortality did not exceed 10%.

2.2. Phaeodactylum Tricornutum Acute Test

The assay was conducted following the Marine Algaltoxkit™ protocol by Microbiotests Inc. (Gent, Belgium), designed according to the ISO 10253:2017 guideline. The kit includes all necessary materials for the assays, including vials with the inoculum of *Phaeodactylum tricornutum*, which were stored at +5 °C (± 2 °C) in darkness until use. Algal growth medium was prepared from the pre-concentrates provided in the kit (final concentrations in the medium: NaCl 6.4 g/L, KCl 840 mg/L, $CaCl_2{\cdot}2H_2O$ 1670 mg/L, $MgCl_2{\cdot}6H_2O$ 4600 mg/L, $MgSO_4{\cdot}7H_2O$ 5580 mg/L, $NaHCO_3$ 170 mg/L, H_3BO_3 30 mg/L). This medium was enriched with a nutrient solution provided in the kit to facilitate algal growth. The medium was used for incubation, compound dilution, and toxicity testing. Prior to the assay, a pre-incubation of the *Phaeodactylum tricornutum* inoculum was conducted. The 25 mL vial was emptied into a 50 mL beaker, and the vial was rinsed twice with 7.5 mL of the medium to retrieve as much inoculum as possible. The beaker was sealed with Parafilm® and the final 40 mL dilution was incubated for 72 hours at 20 °C (± 2 °C) under 4000 lux illumination as specified by the ISO standard. After the incubation period, measurements were taken with a benchtop UV/VIS spectrophotometer (Jenway™ model 6305) at 670 nm using 100 mm glass cuvettes, ensuring a 10-second agitation prior to measurement, confirming a cell concentration of $1{\cdot}10^6$ cells/mL. With the culture at the required density, 75 mL Erlenmeyer flasks were used, each containing 50 mL of the toxicant dilution and algal inoculum to achieve an initial density of $1{\cdot}10^4$ cells/mL.

Serial dilutions of the toxicants were prepared in the artificial seawater for algal growth, with concentration series of 500, 250, 125, 62.5, and 31.25 mg/L Based on the initial test results, further

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dilutions or concentrations were adjusted to achieve mortality results close to the LC_{50}, aiming for more precise outcomes.

2.3. Aurelia aurita Acute Test

As we mentioned earlier, this assay is an adaptation of the guidelines from the protocol by Faimali et al. (2014) and Mercado et al. (2023) [12,14]. The ephyrae of *A. aurita* were supplied by the Oceanogràfic of Valencia (Valencia, Spain). They were used 24 hours post-strobilation; thus, no prior preservation was necessary. All tests utilized ephyrae generated from the same polyp population and within the same size range (see Figure 1).

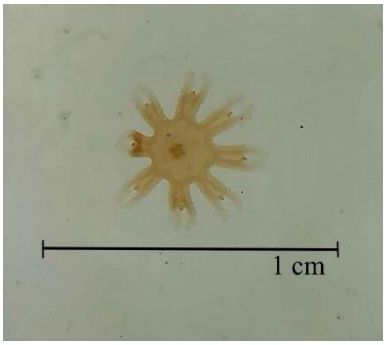


Figure 1. *Aurelia aurita* ephyrae after the strobilation.

Dilutions were prepared based on the LC_{50} values obtained from *A. salina* assays. However, due to the high sensitivity of *A. aurita* to the compounds, lower concentrations (below 50 mg/L) were tested 50, 25, 15, 10 and 2 mg/L. The dilutions were tested using 20 mL capacity cell culture multiwell plates with lids to prevent evaporation. The plates were sealed with Parafilm® to ensure airtightness and prevent salinity variations. Each well was filled with 10 mL of the compound dilution, and two ephyrae were transferred to each well. The wells were sealed with Parafilm® and maintained in a thermostatic refrigerator at 20 °C (± 2 °C) under dark conditions.

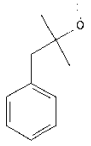
After 24 and 48 hours two parameters were evaluated: On one hand, the pulsation frequency. Defined as the number of pulsations performed by an ephyra within a defined time unit (60 seconds) and measured as the percentage alteration of pulsations compared to the control. On the other hand, the mortality rate being the percentage of ephyrae mortality at each concentration relative to the control.

Finally, a Probit analysis was performed on the obtained data to determine the LC_{50} and LC_{10} values [15].

2.4. Tested Additives

Both additives were provided by Enamine Ltd (Latvia) with a certified purity of 95%. To ensure the stability of the compounds across time and concentrations, the prepared dilutions were analyzed using high performance liquid chromatography (HPLC) as Chen et al. (2014) and Trenholm et al. (2008) suggested [16,17]. Main chemical characteristics are presented in Table 1.

Table 1. Chemical characteristics of each compound.

ID	Substance	CAS	Formula	Chemical structure
S1	2-Methyl-1-phenylpropan-2-ol	100-86-7	$C_{10}H_{14}O$	

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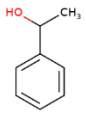
S2	1-Phenylethan-1-ol	98-85-1	$C_8H_{10}O$	

The compound S1 (2-Methyl-1-phenylpropan-2-ol) has demonstrated functionalities as a food additive, cleaning product, and in perfumery. Currently, despite being considered a toxic compound in cases of inhalation and ingestion in humans (H322 and H302), it is in the process of registration under the ecotoxicity section of the REACH regulation, due to insufficient and inconclusive information for classification and, therefore, authorization. Currently, there is no consensus on the classification of compound S1. According to Article 10 of REACH, ECHA records indicate that it is synthesized or imported into the EU in quantities between 10 to 100 tonnes per year and its use in food, medicines, pesticides, or biocidal products is not recommended.

The compound S2 (1-Phenylethan-1-ol) as we mentioned above, was designed as an additive for perfumes, cleaning products, cosmetics, and pharmaceuticals, according to ECHA.

The toxicity ranges selected for classifying the degree of toxicity for the three models were chosen based on the Regulation (*EC*) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (*CLP* Regulation). The EC_{50} values were ranked into four toxicity categories (cat): cat 1, high toxic substances with $E(L)C_{50} \leq 1$ mg/L; cat 2, from >1 to ≤ 10 mg/L (medium toxicity); cat 3, from >10 to ≤ 100 mg/L (low toxicity); and cat 4 for non-toxic substances with $E(L)C_{50}$ >100 mg/L. This classification has already been used by other authors in similar studies [18]. Results were summarized in Table 2.

Table 2. *LC_{50} and LC_{10} and the 95% confidence limit (C.L.) of each organism model for each tested compound.*

Compound	Organism	LC_{50} (mg/L)	C.L. 95%	
			Lower	Upper
S1	*A. salina*	577.9	338.8	985.8
	P. tricornutum	89.0	72.95	108.4
	A. aurita	2.5	1.4	4.3
S2	*A. salina*	623.3	557.3	697.0
	P. tricornutum	452.9	155.1	1323.3
	A. aurita	77	46.3	128.2
Compound	**Organism**	**LC_{10} (mg/L)**	**C.L. 95%**	
			Lower	**Upper**
S1	*A. salina*	130.7	66.6	194.0
	P. tricornutum	69.2	56.76	84.3
	A. aurita	0.29	0.17	0.51
S2	*A. salina*	140.9	77.9	254.7
	P. tricornutum	28.8	9.9	84.3
	A. aurita	22	13.2	36.7

2.5. Environmental Risk Assesment (ERA)

The environmental risk characterization for chemicals in the EU relies on the ratio of the predicted environmental concentration (PEC) to the predicted no-effect concentration (PNEC). This approach is detailed in the guidance on information requirements and chemical safety assessment (ECHA, 2008) and formalized within the European Union System for the Evaluation of Substances (EUSES) (EU, 2008) [19,20]. Considering the environmental concentrations, we assess the risk following the mentioned approach. The PNECs were calculated from the LC_{10} values using an assessment factor of 1000. This analysis could be considered as a firsts approach for the environmental risk assessment [21].

3. Results

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The toxicity assay results revealed distinct sensitivity levels among the three tested organisms. The LC_{50} values were determined for each species, providing insight into their respective tolerances to the compounds studied. The LC_{50} for A. salina was calculated at 577.90 mg/L for the S1 and 623.30 mg/L for the S2, while P. tricornutum exhibited an LC_{50} of 89.0 mg/L and 452.9 mg/L for the S1 and S2 respectively. Notably, A. aurita showed the highest sensitivity with an LC_{50} of 2.5 mg/L for the S1 and 77 mg/L. These findings highlight the varying toxicological impacts on different marine organisms. The different concentrations of each additive tested with HPLC remained constant over time, ensuring that the amount of the studied compound was as calculated. This consistency confirms the reliability of the measured concentrations throughout the study.

The results of the analysis of variance (ANOVA) showed in Table 3 indicated that there are significant differences in the LC_{50} among the three tested organisms for the S1 and S2 compounds ($p < 2e\text{-}16$). The sum of squares (Sum Sq) and the mean squares (Mean Sq) for the Organism factor were high for both additives while mean square was close to 0. These results suggest that the observed differences in LC_{50} among the organisms are not due to chance, with a highly significant p-value ($p < 0.001$).

Table 3. Analysis of variance for the LC_{50} of each compound.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
S1	Organism	2	1926530	963265	3295947	$<2 \cdot 10^{-16}$ ***
	Residuals	27	8	0		
S2	Organism	2	1562053	781027	5199973	$<2 \cdot 10^{-16}$ ***
	Residuals	27	4	0		

0*** 0.001** 0.01* 0.05•

As shown in Figure 2, the LC_{50} values for three model species exposed to the additives, higlights the highest sensitivity of *A. aurita* to both additives, demonstrating the lowest LC_{50} values. In contrast, *A. salina* showed the least sensitivity, with the highest LC_{50} values among the three species. On the contrary, *P. tricornutum* exhibited intermediate sensitivity. These findings indicate that the jellyfish is the most vulnerable to the toxic effects of the additives, whereas crustacean is the most resistant.

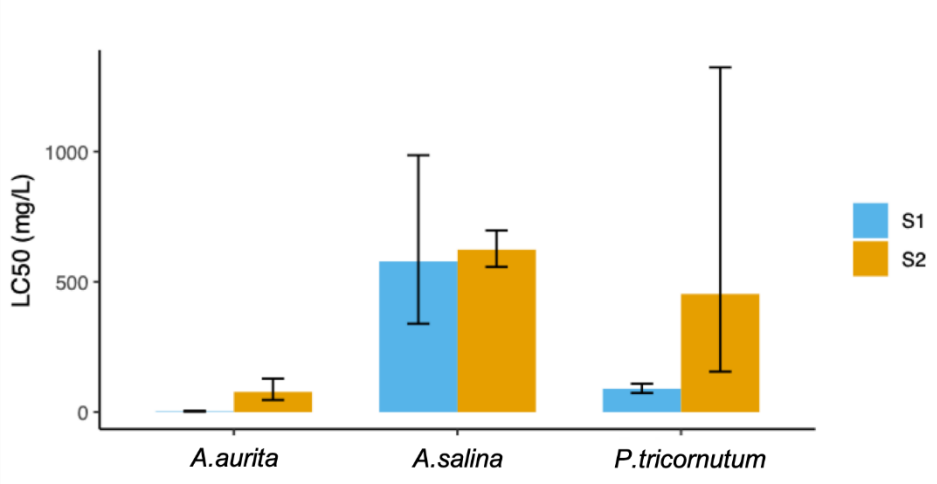

Figure 2. values of LC_{50} for all the organisms tested in each additive.

Comparing the effects of the compounds on the two-model species, *A. salina* and *A. aurita*, at exposure times of 24 and 48 hours shows no differences between the 24-hour and 48-hour time points for *both* compounds in each organism. This indicates that the toxic effects of the compounds are primarily observed within the first 24 hours of exposure. Therefore, the toxic effects of the compounds are established predominantly within the initial 24-hour period, and prolonging exposure to 48 hours does not significantly alter the toxicity (Figure 3).

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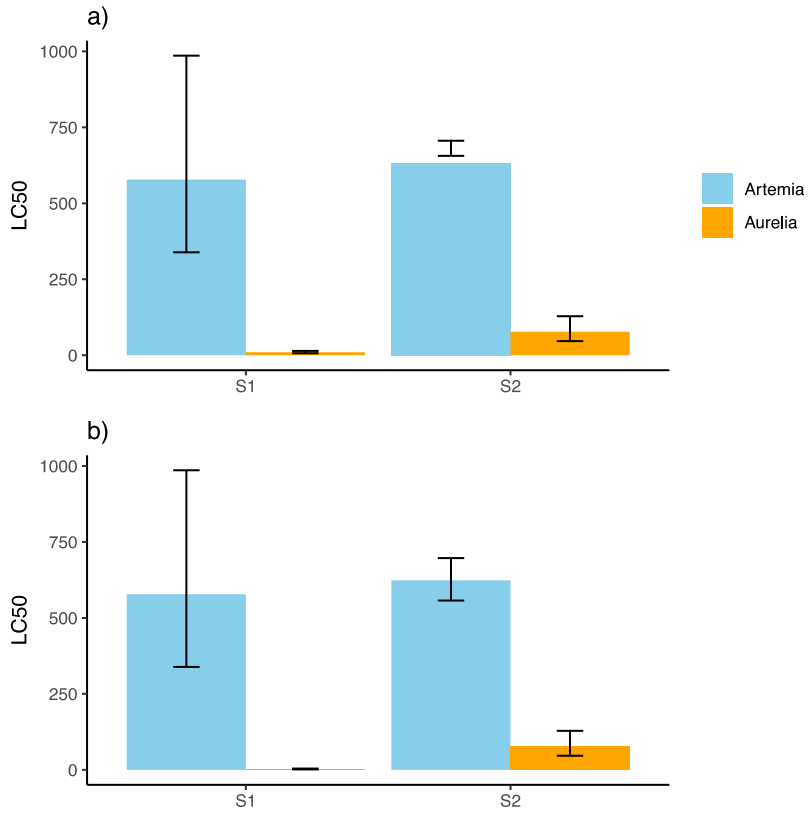

Figure 3. values of LC_{50} for *A. salina* y *A. aurita* at a) 24h and b) 48h for the two additives.

However, the LC_{50} values for *P. tricornutum* were not compared to the 24 and 48-hour LC_{50} values of *A. salina* and *A. aurita* because the testing protocol for the algal species uses a 72-hour endpoint. Consequently, the effect of time was not considered for the algae in this study. The protocol's focus on a 72-hour exposure period for algae precluded a direct comparison with the shorter exposure times used for the other species.

According to the adapted classification, the species that experienced some level of toxicity from both compounds was *A. aurita*, while the studied alga showed toxicity to compound S1. In contrast, for the crustacean *A. salina*, neither of the two compounds showed toxicity (see Tables 2 and 4).

Table 4. Toxicity classification of each additive according each LC_{50} and the categorical criteria used. Colour codes for the different toxic categories: cat 1, high toxic substances with $E(L)C_{50} \leq 1$ mg/L (red); cat 2 (orange), from >1 to ≤ 10 mg/L (medium toxicity); cat 3 (yellow), from >10 to ≤ 100 mg/L (low toxicity); and cat 4 for non-toxic substances (green) with $E(L)C_{50}$ >100 mg/L.

Ref	Compound	CL50 – 48-hour *A. salina*	CL50 – 72-hour *P. tricornutum*	CL50- 48-hour *A. aurita*
S1	2-Methyl-1-phenylpropan-2-ol	Non-toxic	Low Toxic	Medium-toxic
S2	1-Feniletan-1-ol	Non-toxic	Non-toxic	Low Toxic

The analysis of the pulsation rate (pr) in relation to each concentration and exposure time for compounds S1 and S2 is illustrated in the boxplot diagrams (Figure 4). For compound S1, differences in were observed between different concentrations at both 24 hours and 48 hours of exposure. The medians of pr exhibit a decreasing trend with increasing concentrations reaching de minimum values from concentrations above 5 mg/L. Similarly, for compound S2, notable differences were detected between various concentrations over the two exposure times, with the pr generally declining as concentration increases. The ANOVA test results recorded in Table 4, indicate that both concentration and time have significant main effects. Specifically, the concentration factor showed a highly

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significant effect ($F = 13.059$, $p < 0.001$), suggesting that variations in concentration lead to substantial differences in the outcome. Similarly, time had a significant impact ($F = 14.133$, $p < 0.001$), indicating that changes over time significantly affect the dependent variable. However, the interaction between concentration and time was not significant ($F = 0.954$, $p = 0.472$), implying that the combined effect of these two factors does not differ significantly from the effects of each factor individually. This analysis underscores the importance of both concentration and time in influencing the results, while suggesting that their interaction does not contribute additional variance in the dependent variable.

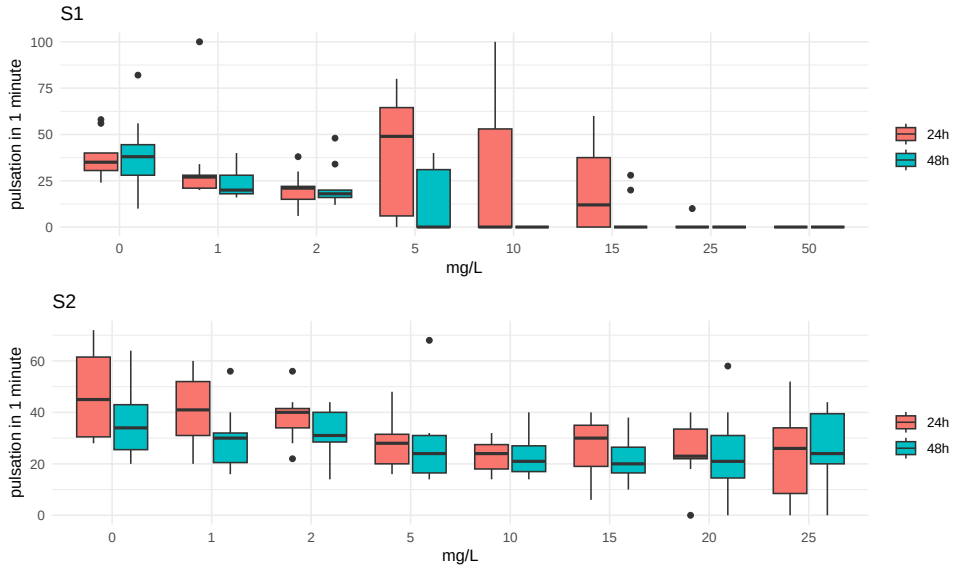

Figure 4. Pulsation data for each compound (S1 and S2) at both exposure time (24-hour and 48-hour).

Table 5. Analysis of variance for the pulsation rate.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	8	29191	3649	13.059	$<4.08 \cdot 10^{-16}$ ***
Exposure time	1	3949	3949	14.133	0.000205 ***
Concentration:time	8	2134	267		

0*** 0.001** 0.01* 0.05•

4. Discussion

The compound S1 is recognized for its established applications as a food additive, cleaning product, and fragrance ingredient. Currently, despite being classified as toxic upon inhalation and ingestion in humans (H322 and H302) [22]. it is undergoing registration under the ecotoxicity section of the REACH regulation due to a lack of sufficient and conclusive information for a definitive classification and authorization . In our study, the tests conducted suggests that S1 could be classified as "Low toxic" to *P. tricornutum* and "Medium-toxic" to *A. aurita*. However, *A. salina* was not affected by this compound. These results highlight that the toxicity of S1 is highly dependent on the susceptibility of individual species, suggesting we can be underestimating the toxicity of this additive if only is tested with the more resistant organisms. For small mammals such as rats and guinea pigs, the LD_{50} ranges from 980 to 1300 mg/L, which is higher than the concentrations recorded for invertebrates [22]. As other authors have noted in their works, our findings support the need for more comprehensive ecotoxicity studies assessing organisms from different trophic levels within the food chain [23,24]. As we have previously mentioned, there is a possibility that we have underestimated the toxic potential of certain compounds, such as the studied additives, which are currently present in the marine environment and ecosystems in general.

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On the contrary for compound S2 there are several studies related with. It has demonstrated varying levels of toxicity across different organisms and this is supported by our tests results. For *A. aurita*, the LC_{50} value was found to be 77 mg/L, indicating "Low toxic". In contrast, S2 was "Non-toxic" to *A. artemia* and *P. tricornutum*, with LC_{50} values of 623 mg/L and 452 mg/L, respectively. However, other researchers reported LC_{50} values of 100 mg/L and 345 mg/L in the fish species *Danio rerio* and *Leuciscus idus*. For invertebrates, the mean LC_{50} value was 45.3 mg/L, while cyanobacteria exhibited a mean LC_{50} value of 200 mg/L. Notably, a separate study involving *P. tricornutum* recorded an LC_{50} value of 257.14 mg/L, which is significantly lower than our finding of 452 mg/L [25].This substantial difference in the toxicity values for the additive suggests that there are species-specific and methodological factors influencing the outcomes.

Considering that the additive S2 is an intermediate product in the petrochemical industry, with concentrations in wastewater ranging from 114 to 237 mg/L, the importance of establishing toxicity limits becomes even more critical (see Figure 5). The use of 1-phenylethanol in the perfume industry, coupled with its presence as an intermediate in the petroleum industry, increases the possibility to be present in the marine environment [26–29]. The discrepancies about its toxicity highlight the necessity of establishing standardized toxicity limits for better classification and assessment of substance toxicity in marine environments. A comprehensive evaluation encompassing a diverse array of marine organisms is crucial for accurately determining the ecological risks posed by chemical compounds like S2. Following the precautionary principle, the release of this compound into the environment should be restricted or minimized until definitive results are obtained.

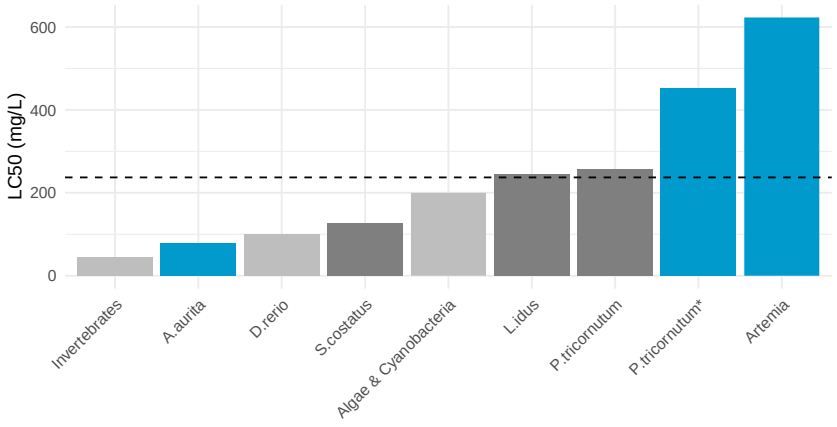

Figure 5. Comparison of LC_{50} values for the S2 compound. The grey bars represent data extracted from Dou et al. (2019) [25], while the blue bars indicate data obtained in this study. The dashed lines correspond to the maximum environmental concentration values reported by Dao et al. (2014) [27].

From a chemical standpoint, both compounds have a phenyl group attached to a carbon chain with alcohol groups as substituents. The difference between them is that S2 has the phenyl and alcohol groups attached to the same carbon atom (C1 of the ethane). On the other hand, S1 has the substituents attached to adjacent atoms (phenyl group on C1 of the propane and alcohol group on C2). The proximity of the electron-donating alcohol group to the aromatic ring may reduce the reactivity of compound S2. In contrast, in compound S1, the alcohol group being further away from the phenyl group has a lesser effect on the reactivity of the phenyl group. Therefore, *a priori* we can reason that compound S1 is more reactive than compound S2. And thus, our data support that S1 is more toxic compared to the S2 compound.

The analysis of ERA reveals that S2 compound, the Hazard Quotient (HQ) values are significantly greater than 1, indicating a high risk to the environment [19–21]. Nevertheless, we have used the PEC from Dao et al. (2014) [27] which estimated the concentration from a pretreatment wastewater and thus, we can assume less concentration in the marine environment. Given the potential environmental impact of these compounds, it is crucial to further study their reactivity and

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toxicity. Understanding these properties is essential to ensure the environmental health of our ecosystems, as even slight variations in chemical behavior could lead to significant ecological consequences. Continued research in this area will help mitigate any potential risks these compounds may pose.

Table 5. Data from ERA analysis.

Organism	LC_{10} (mg/L)	Average PEC (mg/L)*	PNEC (mg/L)**	HQ (PEC/PNEC)
A. salina	140.9	175.5	0.1409	1266.85
P. tricornutum	28.8	175.5	0.0288	6232.63
A. aurita	22	175.5	0.022	8204.54

*Data obtained from Dao et al. (2014)[27] 237-114 mg/L of S2. ** PNEC calculated using assessment factor of 1000.

In any case, both additives have exhibited toxic effects on the secondary consumer model *A. aurita*. It is crucial to consider these findings for the protection of our marine ecosystems. As highlighted in several studies, there is a direct correlation between trophic diversity and the diversity of ecosystem services. Therefore, the loss of secondary consumers due to underestimation of the effects of any pollutant or compound in our biosphere may result in the loss of intermediate trophic levels and, consequently, the degradation of ecosystem services.

To better understand the toxic effects of these additives or any other compound, we have explored the potential of using the scyphozoan *A. aurita* as a model for ecotoxicology assays. As noted by Faimaly et al. (2014), *A. aurita* was more sensitive to Sodium Dodecyl Sulphate than *A. salina*, *Amphibalanus*, and *Americamysis* [12]. Additionally, Mercado et al. (2023) further supports the work of Faimaly by testing the potential of *A. aurita* as a new model for ecotoxicology [14].

5. Conclusions

Our study has demonstrated that 2-Methyl-1-phenylpropan-2-ol (S1) is toxic or medium-toxic to the alga *P. tricornutum* and the scyphozoan *A. aurita*, but not to *A. salina*. In contrast, 1-Phenylethan-1-ol (S2) was only toxic to the cnidarian and non-toxic to the other two organisms. In both cases, the scyphozoan proved to be the most sensitive organism, corroborating findings from previous studies. This suggests that *A. aurita* could serve as an important model for toxicity testing with secondary consumers, enhancing assay sensitivity and preventing the underestimation of the toxic potential of substances. Therefore, our work supports the validity of the ephyras as a model for toxicity assays.

Additionally, our results highlight the urgent need to establish universal toxicity criteria to reduce the environmental impact of industrial substances that enter wastewater and subsequently the marine environment. Implementing these criteria is crucial for better protecting marine ecosystems from potentially harmful chemicals.

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