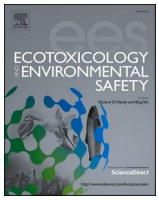
Ecotoxicology and Environmental Safety 313 (2026) 120023

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Contrasting toxicity between explosives– and chemical warfare agents–related compounds to the marine primary producer *Phaeodactylum tricornutum*

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ARTICLE INFO

Edited by Muhammad Zia-ur-Rehman

Keywords:
Explosives
Chemical warfare agents
Munition dumpsites
Acute toxicity
Marine microalgae

ABSTRACT

Vast amounts of munitions dumped in the marine environment, have been reported to leak chemicals due to munition corrosion. The subsequent increased levels of explosives and related chemicals (E&RC), as well as chemical warfare agents and related chemicals (CWA&RC), raise risks for environmental and human health Yet, ecotoxicity data on primary producers is currently scarce. Therefore, this study investigated the acute effects of three CWA&RC (1,4-oxathiane, 1,4-dithiane, and thiodiglycol) and four E&RC (2,4,6-trinitrotoluene (TNT), tetryl, 1,3-dinitrobenzene (1,3-DNB), and picric acid) on *Phaeodactylum tricornutum*, a key marine diatom at the basis of the aquatic food web. Results showed that none of the three CWA&RC significantly inhibited the growth rate of *Phaeodactylum tricornutum* at the tested concentrations. Interestingly, picric acid stimulated growth up under the experimental conditions, suggesting a hormetic effect. TNT, tetryl, and 1,3-DNB strongly inhibited growth, with experimentally derived EC10 and EC50 values approaching environmentally relevant concentrations near dumpsites. Consequently, diatom biomass may be significantly affected by TNT, tetryl, and 1,3-DNB, potentially disturbing primary production and ocean chemistry. Future research should examine potential synergies between munition compounds and other marine pollutants, which may aggravate toxic effects, as well as consider long-term toxicity tests.

1. Introduction

The rapid expansion of human activities into the oceans, such as offshore infrastructure development driven by the growth of the blue economy, has generated significant interest in addressing the environmental impacts caused by the disturbance of munition dumpsites from World War I (WWI) and World War II (WWII) (Beck, 2025; Raupers, 2023). Previous studies have highlighted the escalating risks associated with these dumpsites to human health, fish, bivalves and macrophytes, including effects of arsenic chemicals and explosives (Binder, 2025; Sanderson and Fauser, 2015; Zalewska, 2023). Munition-related chemicals are frequently detected in environmental samples collected in the vicinity of munition dumpsites, as well as WWI and WWII shipwrecks (Barbosa et al., 2023; Maser, 2022). Concentrations of these chemicals can range from tens to hundreds, and in some cases even thousands, of micrograms per liter (μg/L) in water samples, and micrograms per kilogram (μg/kg) in sediment (Barbosa et al., 2023; Fauser, 2023). As a result, research efforts have been directed at understanding the impact, mainly following acute exposure. This included toxicity data for various explosives and related chemicals (E&RC), including nitroaromatic explosives, to fish and human cell lines (Barbosa, 2025; Koske et al., 2019; Liang, 2017; Lotufo et al., 2010) and toxicity data for chemical warfare agents and related chemicals (CWA&RC) to bacteria and crustaceans (Christensen, 2016; Czub et al., 2020). With regards to primary producers, previous studies have specifically addressed the impact of E&RC in macro- and microalgae yet focusing on species with limited distribution in the marine environment, e.g. *Ulva fasciata* (Nipper, 2001), or the freshwater species, e.g. *Pseudokirchneriella subcapitata* (van der Schalie, 1983; Liu, 1983). Tto the best of our knowledge, no recent experimental data is available. Most recent literature, particularly for

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https://doi.org/10.1016/j.ecoenv.2026.120023
Received 13 October 2025; Received in revised form 17 February 2026; Accepted 11 March 2026
Available online 13 March 2026

E&RC, has primarily focused on the effects of primary consumers and seafood sources (Maser et al., 2024). Studies report toxic effects of E&RC including TNT on bivalves, crustaceans and fish (Lotufo, 2016; Maser et al., 2024; Nipper, 2001). In addition to lab exposure, a number of studies have focused on field data, measuring E&RC in seafood including blue mussels (Maser et al., 2024) and fish (Kamman, 2024) captured close to munition dump sites. Simulated data using QSARs is available for both CWA& RC and E&RC, as well as for the broader group of nitroaromatics (Barbosa, 2025; Sanderson, 2007; Schmitt, 2000) but is limited to mainly freshwater environments. As a result, further information on the acute and chronic toxicity of munition-related chemicals to primary producers, including microaglae, is essential to fully understand the impact of dumpsites on the marine environment (ECHA, 2016).

Diatoms are a significant group of microalgae that form the base of the marine and estuarine food webs and contribute approximately 20% of the world's primary photosynthetic production (Guo, 2020). These organisms also synthesize important biomolecules, including fatty acids such as omega 6 (ω-6) linoleic acid and omega 3 (ω-3) linolenic acid, which are essential precursors of long-chain polyunsaturated fatty acids (LC-PUFAs). Vertebrates typically obtain LC-PUFAs from their diet since they cannot synthesize them efficiently from EFAs, emphasizing the importance of their production at lower trophic levels (Parrish, 2009). Furthermore, diatoms serve as major marine carbon sinks and are significant generators of oxygen (Benoiston, 2017).

Phaeodactylum tricornutum is a diatom species with a fully sequenced genome (Bowler, 2008) and widespread distribution (Falkowski, 2002) frequently used in ecotoxicological studies to evaluate the impact of various stressors such as temperature (Dodson, 2013; Feijão, 2018), trace metal pollution (Cabrita, 2018; Ma, 2025), nutrient depletion (Abida, 2015), and emerging pollutants (Liu, 2019; Xia, 2024). The biochemical and physiological responses studied in these assessments include growth inhibition (Feijão, 2020; Xia, 2024), changes in pigment content and electron transport chain efficiency (Duarte, 2019), antioxidant enzyme activity (Liu, 2019; Ma, 2025), and membrane fatty acid saturation (Abida, 2015; Feijão, 2018; Ma, 2025). Together with fish and daphnia toxicity data, microalgae toxicity data is essential in environmental risk assessment procedures (ECHA, 2016). Despite this extensive body of ecotoxicological research for diatoms, toxicity data for munition-related compounds—including chemical warfare agent-related chemicals (CWA&RC) and explosive-related chemicals (E&RC)—remain scarce or absent for this model diatom species. Hence, this study aims to fill the data gap for toxicity data with this species for CWA&RC and E&RC, which is currently lacking.

Given its environmental relevance, this study investigated the potential impact of three CWA&RC (1,4-oxathiane, 1,4-dithiane, and thiodiglycol) and four E&RC, i.e. 2,4,6-trinitrotoluene (TNT), tetryl, 1,3-dinitrobenzene (1,3-DNB), and picric acid, on the growth rate of *Phaeodactylum tricornutum*. The compounds investigated in this study were selected based on their documented occurrence and persistence in marine environments impacted by dumped munitions (Barbosa et al., 2023). While parent chemical warfare agents are seldom detected due to rapid hydrolysis and transformation in seawater, their degradation products—such as thiodiglycol, 1,4-oxathiane and 1,4-dithiane—are frequently reported in sediment and porewater samples (Barbosa et al., 2023). Likewise, conventional explosives including TNT, tetryl, picric acid and nitroaromatic metabolites (e.g. 1,3-dinitrobenzene) are among the most commonly detected munition-related contaminants in water and sediment near dumpsites and shipwrecks (Barbosa et al., 2023). Despite their widespread detection, ecotoxicological data for marine primary producers remain scarce, particularly for CWA-related compounds. The present study therefore contributes to the understanding of the toxicity of seven munition-related chemicals frequently detected in environmental samples near munition dumpsites and shipwrecks, while establishing aquatic toxicity thresholds relevant for the hazard and risk assessment of munition dumpsites.

2. Materials and methods

2.1. Test chemicals, stock, and exposure solutions

Stock solutions of 1,4-oxathiane, 1,4-dithiane, thiodiglycol, and picric acid were prepared in ultra-pure water, while TNT, tetryl, and 1,3-DNB stock solutions were prepared in methanol. The stock solutions of all seven chemicals were prepared and provided by the Belgian Department of Defense (DLD) and the Royal Maritime Academy (RMA).

Exposure solutions of the seven tested chemicals were prepared via serial dilution in the test media, i.e. artificial seawater, following a 1:2.5 ratio. Exposure concentrations range from 0.256 to 25 mg/L for TNT, tetryl, and 1,3-DNB, and 1.6–100 mg/L for picric acid, 1,4-oxathiane, 1,4-dithiane, and thiodiglycol. The maximum concentration of 100 mg/L was established to comply with internal safety measures. A list of the tested chemicals and corresponding relevant physio-chemical properties, specifically the molecular weight and octanol-water partition coefficient (log Kow) is provided in Table 1.

2.2. Algae growth inhibition assay

The marine diatom *Phaeodactylum tricornutum* Bohlin was obtained from the Culture Collection of Algae and Protozoa (CCAP 1052/1 A). The organisms were cultured according to ISO 10253 (International Organization for Standardization, 2016) in terms of culture medium and conditions. Artificial seawater was used to ensure standardized exposure conditions and to avoid background contamination by trace metals or organic pollutants that may be present in natural seawater. Salinity, temperature, and light conditions were kept constant throughout the exposure to minimize variability between treatments. All media chemicals were purchased from Sigma Aldrich. Four days prior to the test start, a pre-culture was prepared by inoculating fresh growth medium with 10 000 cells mL^{-1}. The pre-culture was allowed to grow under continuous white light (100–120 μmol m^{-2} s^{-1}) at 20 ± 1 °C.

Experiments were conducted using a static exposure design in multi-flask setups, with each concentration tested in three replicate flasks alongside solvent and control treatments. Test compounds were grouped into two exposure series (CWA&RC and E&RC) to ensure safe handling

Table 1
Physicochemical properties of the tested chemical warfare agents and related chemicals (CWA&RC), and explosives and related chemicals (E&RC), relevant for environmental fate and toxicity assessment.

Chemical name or abbreviation	CASNR	Class	Molecular Weight (g/mol)[a]	Octanol-water partition coefficient (log Kow)[a]
1,4-oxithiane	15980–15–1	Sulfur mustard metabolite	104.17	0.742
1,4-dithiane	505–29–3	Sulfur mustard metabolite	120.23	0.77
Thiodiglycol	111–48–8	Sulfur mustard metabolite	122.19	-0.630
TNT	118–96–7	Parent compound	227.13	1.6
Tetryl	479–45–8	Parent compound	287.17	1.69
Picric acid	88–89–1	Tetryl metabolite	229.10	1.33
1,3-DNB	99–65–0	TNT metabolite	168.12	1.55

NA – not available.
[a] – Data retrieved from US EPA CompTox Chemical Dashboard (https://comptox.epa.gov/dashboard/), PubChem (https://pubchem.ncbi.nlm.nih.gov/).

and analytical consistency. Initial cell densities were standardized across treatments to allow direct comparison of growth inhibition responses. Blanks, i.e. media without algae were added to control for confounding factors during measurements. For each test concentration, flasks were filled with 50 mL and spiked with *Phaeodactylum tricornutum* Bohlin at an initial algal cell density of 25 000 cells mL^{-1}.

To fulfill ISO 10253 validation criteria, during the tests, all flasks were shaken manually twice a day and the algae cells were measured after 24, 48, and 72 h using the flow cytometer CytoFLEX (Beckman Coulter, USA). The pH of the controls was measured at the beginning and end of the tests and the temperature (in one reference Erlenmeyer flask filled with deionized water for the whole test setup) was recorded daily.

Analytical verification (see Section 2.4) of exposure concentrations was performed at the start and end of the exposure to account for potential degradation, adsorption, or loss of test compounds during the assay. This was particularly important for nitroaromatic explosives, which are known to exhibit variable stability in aqueous media.

2.3. Data analysis

The algal growth rate after 72 h exposure was recorded for each replicate of the controls and different treatments. Growth inhibition was selected as the primary endpoint as it represents a regulatory-relevant apical response for marine primary producers and enables direct comparison with existing risk assessment frameworks. Growth inhibition (%) was calculated relative to the control. following the ISO 10253. In brief, growth inhibition was calculated as the average growth rate of the controls minus the growth rate of the individual flask exposed to the compound, divided by the average growth rate of the control. Growth rates were determined using ln transformed cell densities and subseqnuetly applying the slope function in excel on these transformed cell densities from day 0 to day 3 (Moeris, 2019). Concentration–response relationships for growth inhibition were analyzed using non-linear regression by fitting sigmoidal dose–response models. Effect concentrations causing 10% (EC_{10}) and 50% (EC_{50}) growth inhibition were derived from the fitted models using the *drc* package in R (function *drm*; (R Development Core Team, 2022).

In addition to model-based endpoints, no-observed-effect concentrations (NOECs) were determined using a hypothesis-testing approach. Differences between treatments and controls were assessed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test (*aov* and *TukeyHSD* functions in R). A significance level of $p < 0.05$ was applied throughout. The combined use of model-based (EC_{10}, EC_{50}) and hypothesis-based (NOEC) endpoints follows current OECD and ISO recommendations and allows complementary interpretation of concentration–response data for environmental risk assessment.

2.4. Chemical extraction and quantification

Chemical analysis was performed on samples collected from the Erlenmeyer flasks at the beginning ($C_{0\ h}$) and end of exposure ($C_{72\ h}$) and immediately stored at −20°C. Two biological replicates, i.e. from different experimental flasks, of the highest concentrations were analyzed per chemical (as all concentrations were obtained through serial dilution of the highest concentration). Intermediate concentrations were estimated based on the used dilution factor (1:2.5). We used gas chromatography-mass spectrometry (GC-MS) for 1,4-oxathiane and 1,4-dithiane and liquid chromatography-mass spectrometry (LC-MS) for thiodiglycol and picric acid, and triple quadrupole gas chromatography-tandem mass spectrometry triple quad GC-MS/MS) for TNT, tetryl and 1,3-DNB. Further details on the analytical instruments and procedures, including internal standards and recovery standards, are provided by Barbosa (2025), which formed the basis for the chemical analyses of this study.

3. Results

All tests fulfill the validity criteria for the 72 h growth inhibition assay with *Phaeodactylum tricornutum* (International Organization for Standardization, 2016) with regards to pH and temperature variation. The pH varied maximally by 0.96 (7.87–8.83) in the exposure batch tests with CWA&RC and 0.71 (7.91–8.68) in the exposure batch tests with E&RC. Additionally, the temperature varied by 0.2 °C (20.0–20.2 °C, CWA&RC batch) and 0.1 °C (20.7–20.8 °C, E&RC batch) throughout the duration of the experiments. Concentration-response curves depicting the effects of the seven tested munition-related chemicals on algal cell densities and subsequently on algal growth inhibition (%) will be presented below. The derived NOEC, EC_{10} and EC_{50} values for the seven tested munition-related compounds are summarized in Table 2 and are discussed below in the context of previously reported aquatic toxicity data.

Analytically measured concentrations at the start and end of exposure are reported in Table S1. For several nitroaromatic explosives, particularly TNT, tetryl, and picric acid, measured concentrations were lower than nominal. However, it is important to note that these chemicals are known to be difficult to analyze chemically, as some may for example undergo complexation in the exposure medium, leading to a lower measured concentration which may not necessarily mean a lower exposure concentration. Notably, TNT showed the highest relative degradation over 72 h (~10%). Despite these differences, concentration–response relationships remained robust, and toxicity estimates are therefore reported based on nominal concentrations, in line with standard ecotoxicological practice.

Exposure to the sulfur mustard degradation products thiodiglycol, 1,4-oxathiane, and 1,4-dithiane did not result in large effects on cell densities over the 72 h exposure period (Fig. 1 – top panel). Consequently, no significant growth inhibition of *Phaeodactylum tricornutum* over the 72 h exposure period was observed for thiodiglycol and 1,4-dithiane (Fig. 1 – bottom panel). For 1,4-oxithiane, growth inhibition was statistically significant from the control for the highest concentrations, but effects were low, i.e. less then 5% growth inhibition (Fig. 1 – bottom panel). Hence, the estimation of EC10 or EC50 values within reasonable confidence intervals was not possible for these compounds (Table 2). Consequently, the estimated NOEC for thiodiglycol and 1,4-dithiane is equal to the highest tested concentration, 100 mg/L (measured concentration: 73.98 ± 6.9 mg/L), while the NOEC for 1,4-oxathiane is estimated to be 25 mg/L (average measured concentration deviates less than 10% from nominal concentration) (Tukey's post-hoc HSD test $p < 0.05$ for nominal concentrations of 62.5 and 100 mg/L) (Table 2). However, the NOEC is inherently dependent on the experimental design and the spacing of tested concentrations. Consequently, NOEC values should be interpreted with caution. Model-based effect

Table 2
Summary of NOEC, EC10, and EC50 values (mg/L), with corresponding 95% confidence intervals (in brackets), describing the effects of the seven tested munition-related chemicals on the growth rate of the marine diatom *Phaeodactylum tricornutum* based on nominal concentrations. Analytically measured concentrations are reported in Table S2. CWA&RC: Chemical warafe agents and related chemicals, E&RC: explosives and related chemicals.

	NOEC (mg/L)	EC10 (mg/L)	EC50 (mg/L)
CWA&RC			
Thiodiglycol	100	ND	ND
1,4-oxathiane	25	ND	ND
1,4-dithiane	100	ND	ND
E&RC			
TNT	0.64	0.51 (0.28 – 0.91)	2.21 (1.71 – 2.87)
Tetryl	0.26	0.26 (0.14 – 0.48)	0.52 (0.41 – 0.67)
1,3-DNB	0.64	0.75 (0.46 – 1.24)	2.07 (1.73 – 2.47)
Picric acid	4[a]	ND	ND

ND – not determined.
[a] Growth stimulation.

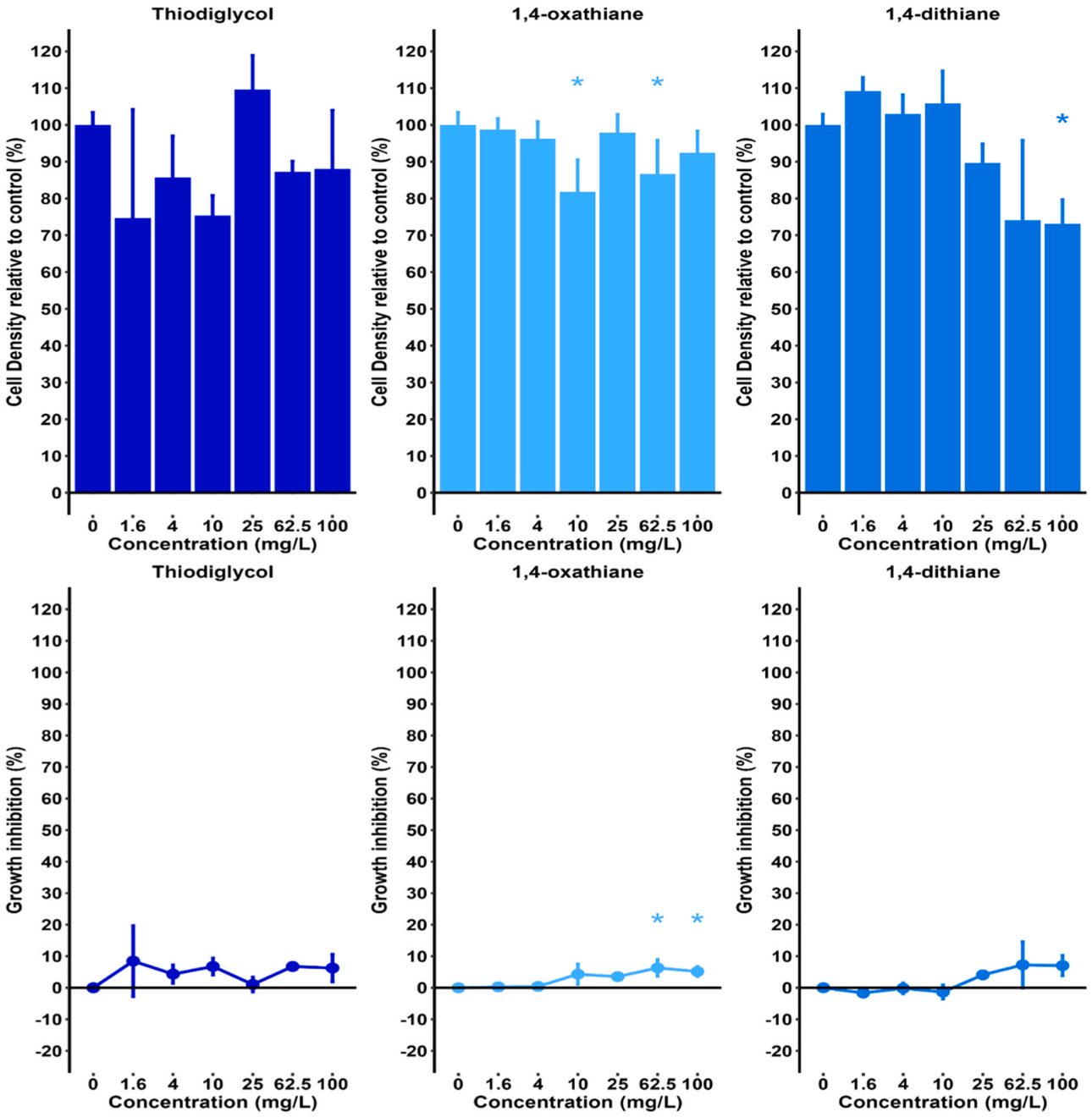

Fig. 1. Top panel: Bar plots of the mean cell densities of Phaeodactylum tricornutum relative to the control cell density (100%) in function of increasing concentrations of the chemical compound after 72 h of exposure to the thiodiglycol, 1,4-oxathiane and 1,4-dithiane respectively. Bottom panel: The mean growth inhibition (%) of Phaeodactylum tricornutum in function of increasing concentrations of the chemical compound after 72 h of exposure to exposure to the thiodiglycol, 1,4-oxathiane and 1,4-dithiane respectively. In all graphs error bars represent standard error (SE) of three independent replicates per concentration. Growth inhibition was calculated relative to the average growth rate of the controls using ln transformed cell densities to determine the growth rates in each flask. Asterix indicate treatments that are statistically significant from the control treatment using a Tukey HSD test (p-value < 0.05).

concentrations (e.g. EC50), which are less sensitive to test design and provide a continuous estimate of low-effect levels, are increasingly considered more robust and informative for environmental risk assessment.

In contrast to the limited effects observed here for CWA&RC (Fig. 2, Table 2), TNT, tetryl, and 1,3-dinitrobenzene induced pronounced effects on cell densities with increasing exposure concentration and thus concentration-dependent growth inhibition in *P. tricornutum*. Within the tested concentration range, the three chemicals were able to fully induce 100% growth inhibition at the highest concentrations, with only limited viable cells (<5% viable cells relative to control) remaining after 72 h of exposure (Fig. 2). Specifically, TNT, tetryl, and 1,3-DNB caused 50% reduction in growth rate (EC50) at nominal concentrations of 2.21 mg/L, 0.52 mg/L, and 2.07 mg/L, respectively (Fig. 2, Table 2). The EC10 based on these experimental results, are below the units of mg/L (0.51 mg/L for TNT, 0.26 for tetryl, and 0.75 for 1,3-DNB) (Table 2).

In contrast to the other explosive-related compounds, picric acid resulted in increased algal cell densities relative to control across all tested concentrations (Fig. 3 – left panel). This increase was significant for the higher concentrations. As such, picric acid did not inhibit algal growth but rather stiumalated it. This translates to a negative growth inhibition of 10–20% or in other words significant growth stimulation,

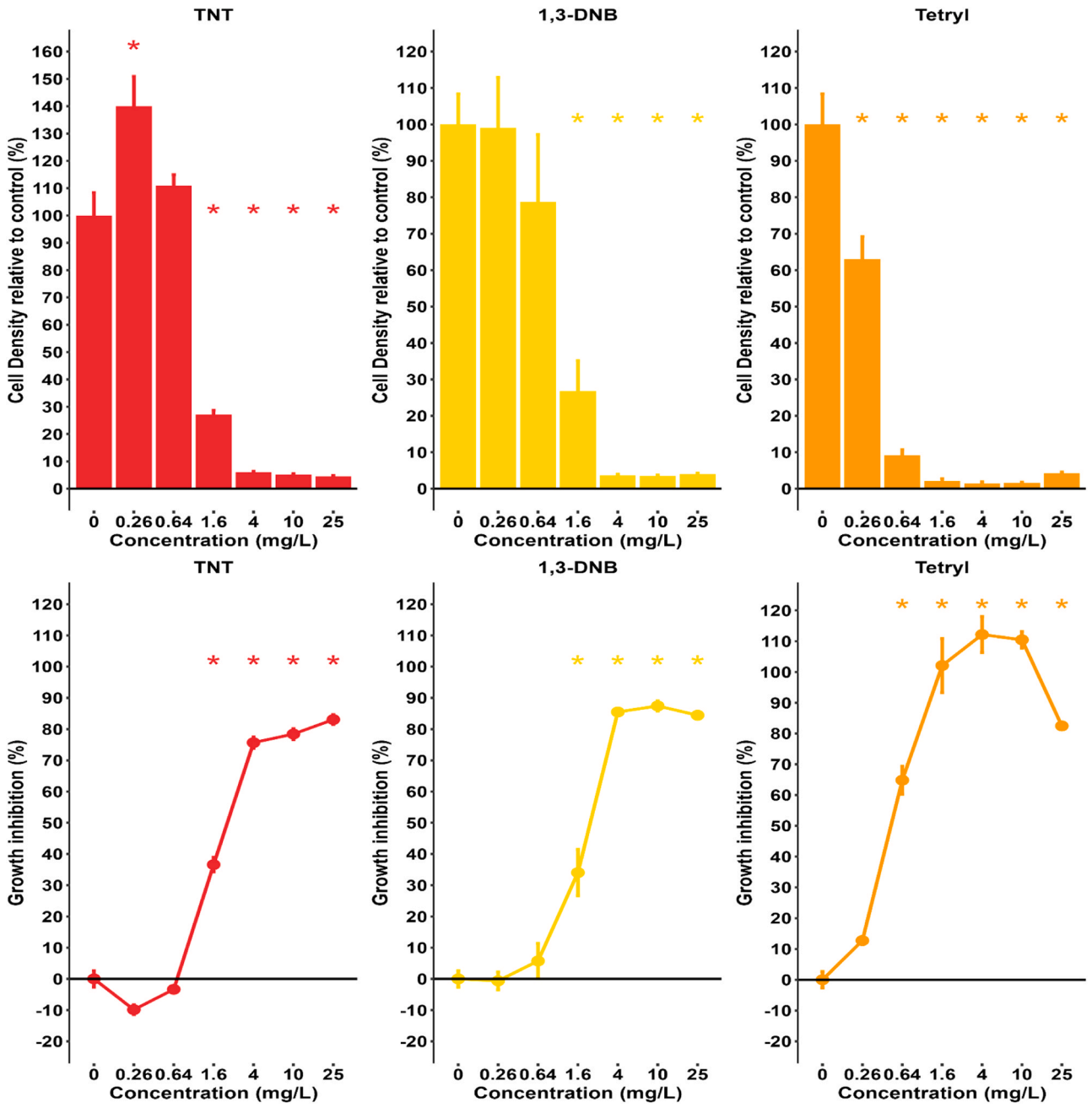

Fig. 2. Top panel: Bar plots of the mean cell densities of Phaeodactylum tricornutum relative to the control cell density (100%) in function of increasing concentrations of the chemical compound after 72 h of exposure to the TNT, 1,3-DNB and tetryl respectively. Bottom panel: The mean growth inhibition (%) of Phaeodactylum tricornutum in function of increasing concentrations of the chemical compound after 72 h of exposure to exposure to the TNT, 1,3-DNB and tetryl respectively. In all graphs error bars represent standard error (SE) of three independent replicates per concentration. Growth inhibition was calculated relative to the average growth rate of the controls using ln transformed cell densities to determine the growth rates in each flask. Asterix indicate treatments that are statistically significant from the control treatment using a Tukey HSD test (p-value < 0.05).

with 10–20% across the tested concentration range. (Fig. 3 – right panel). This non-monotonic response suggests a hormetic effect rather than classical toxicity.

4. Discussion

The present experimental results demonstrate that the sulfur mustard degradation products thiodiglycol, 1,4-oxathiane, and 1,4-dithiane exhibit low acute toxicity to the marine diatom *Phaeodactylum tricornutum*, even at concentrations exceeding those typically reported in the environment (Amato, 2006; Barbosa et al., 2023; Briggs, 2016). For 1,4-dithiane, these results contrast the estimated LC50 of previous QSAR models (Barbosa, 2025). For 1,4 oxathiane and forthiodiglycol, the effects obtained here supported the estimated LC50s by previous QSAR Models (Barbosa, 2025; Sanderson, 2007).

In contrast, the pronounced growth inhibition observed here for TNT, tetryl, and 1,3-DNB at low mg/L concentrations (Fig. 1), and the subsequent experminetally derived effect tresholds (Table 2), are in line with those reported in previous studies with different algae species and in line with LC50 estimates by QSAR models (Barbosa, 2025), estimates EC50 of 1–1.8 mg/L). Specifically, Nipper (2001) determined the lowest-observed effect concentration (LOEC) in the low mg/L range for

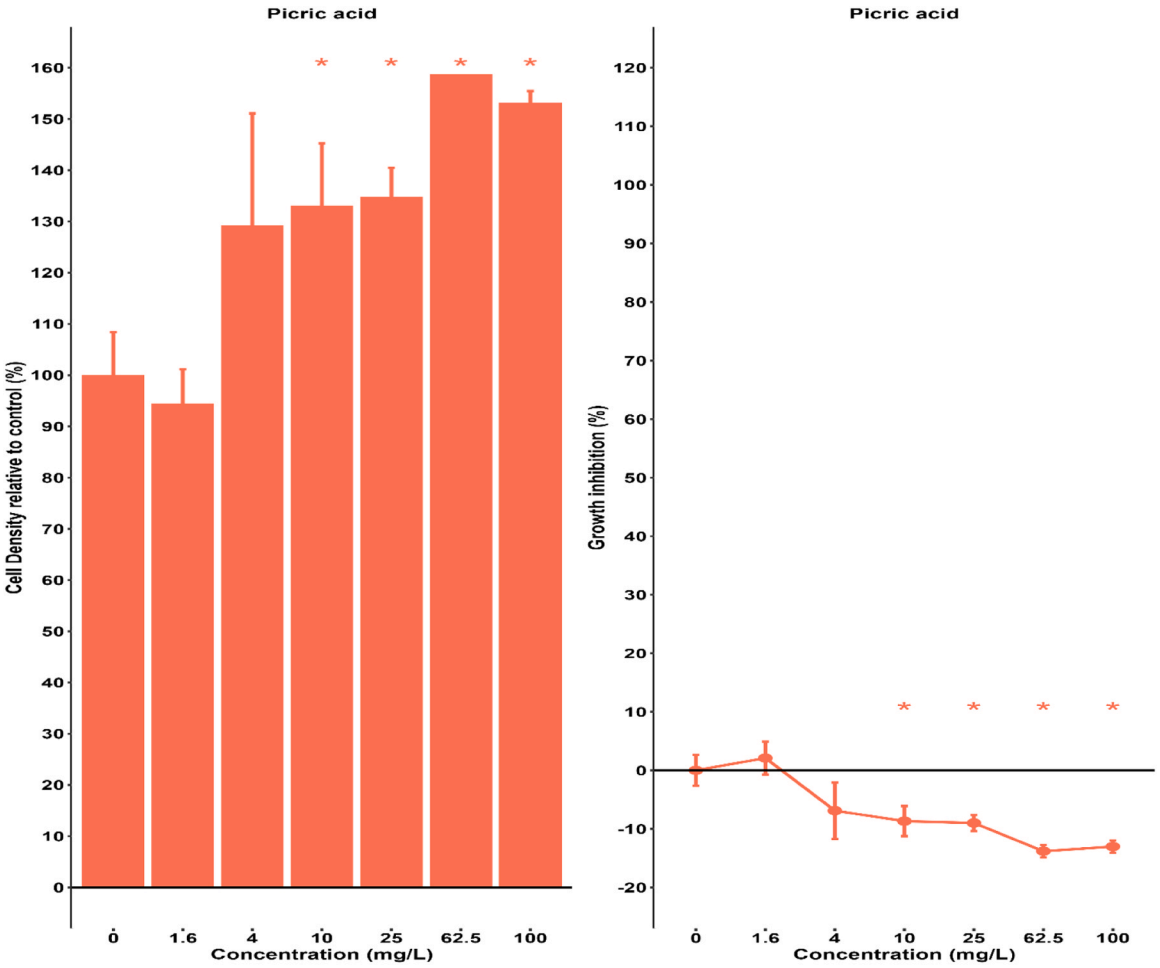

Fig. 3. Left: Bar plots of the mean cell densities of Phaeodactylum tricornutum relative to the control cell density (100%) in function of increasing concentrations of the chemical compound, picric acid, after 72 h of exposure. Right: The mean growth inhibition (%) of Phaeodactylum tricornutum in function of increasing concentrations of picric acid after 72 h of exposure. In all graphs error bars represent standard error (SE) of three independent replicates per concentration. Growth inhibition was calculated relative to the average growth rate of the controls using ln transformed cell densities to determine the growth rates in each flask. Asterix indicate treatments that are statistically significant from the control treatment using a Tukey HSD test (p-value < 0.05).

the zoospore germination of *Ulva fasciata* when exposed to same three E&RC. Similarly, earlier studies using *Pseudokirchneriella subcapitata* estimated LOECs in the lower mg/L for both TNT and 1,3-DNB in terms ofpopulation growth, (van der Schalie, 1983; Liu, 1983). The small differences in reported effect concentrations with *P. tricornutum* may reflect species-specific sensitivity as well as differences in exposure duration and measured endpoints. In contrast to other explosives, including nitroaromatics, our results indicate that TNT seems more toxic to primary producers. A previous study with the freshwater algae *Scenedesmus obliquus,* exposed to wastewater from munition facilities (Abraham, 2018), indicated no toxic effects at concentrations of 24–28 mg per liter of other toxic explosives. Future research is needed however to determine whether these are species-specific differences, differences in marine versus freshwater environments or effectively attributed to differences in toxicity of these chemicals.

Picric acid uniquely induced growth stimulation rather than inhibition, suggesting a hormetic response in *P. tricornutum*. This observation is consistent with QSAR predictions and previous experimental evidence indicating comparatively low acute toxicity of picric acid among nitroaromatics (Barbosa, 2025; Schmitt, 2000). Mechanistically, picric acid is known to be an effective inhibitor of photosynthetic electron transport at the reducing side of photosystem II,(Oettmeier and Masson, 1982). The observed growth stimulation here may indicate that, at low effects levels, partial and transient inhibition of photosystem II may induce compensatory physiological responses. Such stress-induced overcompensation is a well-recognized mechanistic basis of hormesis in photosynthetic marine diatoms (Lavaud et al., 2016). Hormesis has been observed in plants and (micro)algae, both freshwater and marine species, following exposure to mixtures of pharmaceuticals (Backhaus, 2011), herbicides (Belz and Duke, 2017; Cutler and Guedes, 2017), and nanoparticles (Agathokleous, 2019; Erofeeva, 2025). Further, specifically considering marine diatoms, Feng et al. (Feng, 2024) observed hormesis in *Skeletonema costatum* and *Phaeodactylum tricornutum* when exposed to sulfamethoxazole (SMX). While the mechanisms leading to the hormetic response are yet to be completely revealed (e.g., (Zhang, 2023; Erofeeva, 2025), studies suggest that oxidative stress, photosynthetic pigments, and carbohydrate and protein levels may be associated with such a response (Tombuloglu, 2019; Zhou, 2024). Franco et al. (2019) put forward evidence that oxidative stress may occur in the mitochondria when the electron transport chain is impacted.

With regards to the tested CWA&RC, this work reports, to the authors' best knowledge, the first estimations of thiodiglycol, 1,4-oxathaine, and 1,4-dithiane toxicity to marine algae, hence providing a unique insight into the potential toxicity of these chemicals to marine primary producers. Taken together, this suggests that under acute exposure conditions the short-term toxicity of the tested CWA&RC may be comparatively low, particularly when contrasted with energetically reactive nitroaromatic explosives such as TNT. However, the absence of acute growth effects does not preclude sub-lethal or longer-term impacts. From a monitoring and risk assessment perspective, this supports

prioritization of substances with demonstrated higher ecotoxicological potency, such as TNT and related nitroaromatic compounds, while recognizing that sulfur mustard metabolites may still warrant attention under chronic exposure scenarios.

As the munition shells further corrode under marine conditions, continuous leakage and distribution of E&RC and CWA&RC in the water column is expected (e.g., (Silva and Chock, 2016; den Otter, 2024; Beck, 2025). Exposure can be further intensified by the residues from controlled blast-in-place detonations of submerged munitions, which are known to greatly increase munition levels in the short term and cause additional harm through sound and shock waves. Given that the experimentally derived EC_{10} and EC_{50} values for TNT, tetryl, and 1, 3-DNB overlap with concentrations measured near munition dumpsites (Barbosa et al., 2023; Porter et al., 2011; US Army Corps of Engineers (USACE), 2013), the present findings indicate that primary production may be directly affected in contaminated marine areas. Such impacts at the base of the food web could propagate to higher trophic levels and alter biogeochemical cycling., This includes effects on water oxygenation and carbon harvesting, and may also disrupt marine food webs by significantly impacting the primary producers (Benoiston, 2017; Feijão, 2020). Additionally, the described negative impacts can be further potentiated by the persistent and synergistic effects of munition-related chemicals (Beck, 2022; Cabral, 2019) as well as other pollutants, such as heavy metals, pharmaceuticals, pesticides, and microplastics (Mezzelani, 2021; Serra, 2020; Strehse, 2023), and climate change-related stress factors, such as shifts in water temperature (Cabral, 2019; Maser, 2022; Scharsack, 2021; Serra, 2020). Lastly, also more long-term toxicity tests should be conducted, partly because some chemicals may exhibit higher chronic toxicity than would be expected from acute toxicity tests, as also noted by e.g., Wilczynski et al. (Wilczynski, 2024).

While the present study focused on acute, single-compound exposure, several aspects warrant further investigation. Given the pronounced toxicity of TNT observed in this study and its frequent co-occurrence with other marine pollutants, a high-priority next step for future research would be to investigate the combined effects of TNT with a ubiquitous co-contaminant. such as a heavy metal (e.g. copper or cadmium). This may be highly relevant given the composition of the munition shells or a commonly detected persistent pollutant. Such research may determine whether additive, synergistic, or antagonistic effects occur at environmentally realistic concentrations. An interesting combination may be the joint toxicity of TNT metabolites with microplastic pollution in the sediment, as both stressors occur in the sediment. Microplastic pollution in sediments has been reported to reach high concentrations with potential risks already for benthic species (Everaert, 2018). As such, combined effects of this global stressor together with TNT metabolites may be a crucial next step.

5. Conclusion

Marine microalgae such as the diatom *Phaeodactylum tricornutum* play a key role at the base of the marine food web, and algal toxicity data are a cornerstone of environmental risk assessment frameworks. Using an ISO-standardized marine algae growth inhibition assay, this study provides experimentally derived baseline toxicity data of sulfard mustard degradation products thiodiglycol, 1,4-oxathiane, and 1,4-dithiane. Resulsts indicated that these compounds pose a comparatively low acute hazard to marine primary producers at environmentally relevant concentrations. In contrast, the generated experimental data for TNT, tetryl, and 1,3-DNB indicate that these explosive-related compounds may cause severe acute effects on algal populations at concentrations as low as 0.52 mg/L, which fall within the range reported near munition dumpsites, highlighting their potential ecological relevance. Unexpectedly, picric acid stimulated algal growth over the tested concentration range, suggesting a hormectic response at the tested concentrations.

A key strength of this study lies in the comparitive assessment of experiementally derived toxicity thresholds for munition-related chemicals using a standardized marine primary producer assay. Therefore, this study addresses a critical knowledge gap for hazard characterization at the basis of the marine foodweb. At the same time, the study is limited to acute exposure conditions, a single test species, and one apical endpoint, and does not capture potential chronic, sub-lethal, or mixture effects. The ecotoxicity data on the seven environmentally relevant munition-related chemicals reported here provide a robust first-tier screening that supports risk-based prioritization of contaminants associated with munition dumpsites and shipwrecks.

7. CRediT authorship contribution statement

João Alves Barbosa: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Marijke Neyts:** Writing – review & editing, Validation, Resources. **James De Backer:** Writing – original draft, Visualization, Validation. **Colin R. Janssen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Philippe François:** Writing – review & editing, Validation, Resources. **Jana Asselman:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Koen Parmentier:** Writing – review & editing, Validation, Resources. **Kris Geukens:** Writing – review & editing, Validation, Resources. **Frédéric Laduron:** Writing – review & editing, Validation, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Financial support is acknowledged from the Research Foundation Flanders (FWO) through the DISARM project (Dumpsites of munitions: Integrated Science Approach to Risk and Management - https://disarm.be/en; S003719N).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2026.120023.

Data availability

Data will be made available on request.

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