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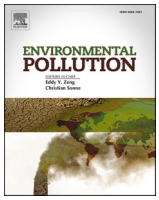

Applying quantitative structure-activity relationship (QSAR) models to extend the mixture toxicity prediction of scrubber water☆

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ABSTRACT

Marine organisms are constantly exposed to complex chemical mixtures from natural and anthropogenic sources. One source that has raised concerns is the discharge water from ships equipped with exhaust gas cleaning systems, commonly known as scrubbers. During operation, ships with scrubbers discharge large volumes of scrubber water, known to adversely affect marine organisms, into the environment. Scrubber water is highly acidic and contains a complex mixture of contaminants, including metals and polycyclic aromatic hydrocarbons (PAHs), at high concentrations. To assess the effect from these mixtures, critical values for individual mixture components can be determined from ecotoxicological studies and then compared to measured exposure concentrations. However, for several substances identified in scrubber water, for instance many alkylated PAHs, ecotoxicological studies are unavailable, preventing the determination of critical values. In this study, Quantitative Structure-Activity Relationship (QSAR) models have been used to amend and complement experimental data to estimate the mixture toxicity of scrubber water. Our results show that the combined predicted ecotoxicological response of an amended list of 50 substances measured in scrubber water from the substance groups metals (n = 10), PAHs (n = 16) and their alkylated derivatives (n = 24), still underestimates the response observed in whole effluent toxicity tests. This suggests that there are additional substances and/or synergistic effects in the scrubber water mixtures that contribute to the overall toxicity. Thus, to accurately describe the toxicity of scrubber water, measurements and toxicity assessments must extend far beyond the usual suspects of 16 PAHs and a limited selection of metals. Here, QSAR models and advanced chemical screening-based methods are valuable tools for identifying substances of concern.

1. Introduction

Marine organisms are constantly exposed to complex chemical mixtures from natural and anthropogenic sources. Examples of anthropogenic sources are discharges of wastewater and oil residues from shipping (Jalkanen et al., 2021; Ytreberg et al., 2022), produced water from oil platforms (de Vries et al., 2022) and pharmaceuticals and biocides in effluent water from wastewater treatment plants (Ghekiere et al., 2013; Gustavsson et al., 2017; Gustavsson et al., 2023). In comparison to stationary land-based activities, ships operate in more remote areas that are otherwise less affected by human activities. However, although a commercial ship in operation can be compared to a mobile process industry, or in the case of cruise ships even a small city, the monitoring and reporting of discharges of hazardous substances from ship generated waste streams are generally not mandatory, resulting in large knowledge gaps (Magnusson et al., 2018; Lunde Hermansson and Hassellöv, 2020; Ytreberg et al., 2020; Moldanová et al., 2022).

A relatively new liquid waste stream from ships that has raised environmental concerns is the discharge water from exhaust gas cleaning systems, commonly known as scrubbers (e.g., Turner et al., 2017; Teuchies et al., 2020; Ytreberg et al., 2021a). Scrubbers have become widely used in shipping with over 5000 ships, constituting

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approximately 25% of the global bunker fuel demand (IEA, 2021), having installed a scrubber system by 2024 (DNV, 2024). Ship scrubbers are installed in response to stricter global regulations on sulphur content in marine fuels which aim to reduce sulphur oxide emissions to the atmosphere (IMO, 2020; DNV, 2024). The reduction of the maximum allowable sulphur content requires shipowners to switch to more expensive low sulphur fuels (marine gas oil (MGO) or very low sulphur fuel oil (VLSFO)), or to install a scrubber if they want to continue to use cheaper high sulphur fuels (heavy fuel oil (HFO)).

With scrubbers, the ship's exhausts are led through a continuous spray of water that cause the sulphur oxides to dissolve, forming sulphuric acid. The sulphur oxide is, thus, removed from the exhaust and the ship fulfil the requirements in the emission regulations. However, other contaminants, such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) are also washed out during the scrubbing process. The process results in large volumes of contaminated water, that is both heavily acidified and contains a complex mixture of chemicals, to be discharged directly into the marine environment (Lunde Hermansson et al., 2021; Moldanová et al., 2022). For the most common scrubber system, the open loop, seawater is continuously pumped through the scrubber, producing a discharging of 100–1000 m^3 scrubber water per hour (Lunde Hermansson et al., 2021; Ytreberg et al., 2021b). In contrast, closed loop scrubber systems recirculate the water and thus discharge lower volumes. However, due to the recirculation, the contaminants are then found at much higher concentrations in the resulting discharge water (Lunde Hermansson et al., 2021; Thor et al., 2021; Achten et al., 2024).

Whole effluent toxicity (WET) tests of scrubber discharge water have shown adverse effects on several different marine organisms, that also fulfil different ecosystem functions (Koski et al., 2017; Thor et al., 2021; Ji et al., 2023; Picone et al., 2023; Monteiro et al., 2024). Similar results have been obtained from WET tests of mixtures similar to scrubber water. For example, from exposure of the water accommodated fraction of crude oils and fuels (Nahrgang et al., 2016; Jönander and Dahllöf, 2020; Almeda et al., 2023), exposure of burnt oil residues in the aquatic environment (Almeda et al., 2023), exposure of discharges of produced water from oil platforms (de Vries et al., 2022) and exposure of oil polluted samples from the marine environment (e.g., Hicken et al., 2011; Turcotte et al., 2011, da S. Moreira et al., 2024).

Assessing the exposure and effect of mixtures can be complex (e.g. Posthuma et al., 2018) and the combined effect of mixtures is often different and difficult to predict based on the toxicity of known individual substances. The two most commonly used methods to assess the toxicity of mixtures with known constituents are concentration addition (CA) and independent action (IA) (de Zwart and Posthuma, 2005; Nys et al., 2017). The two models differ in their assumptions on the modes of action of the substances, where CA assumes that all substances have a similar mode of action and IA assumes that all substances have individual modes of action (de Zwart and Posthuma, 2005; Nys et al., 2017). Several studies have shown that CA is a suitable and conservative approach when predicting the toxicity of mixtures and, that for environmentally realistic mixtures, the difference between CA and IA is generally small (Escher and Hermens, 2002; Silva et al., 2002; Escher et al., 2009; Backhaus and Faust, 2012; Altenburger et al., 2018; Bopp et al., 2019; Escher et al., 2020; Jakobs et al., 2020; Lai et al., 2022).

A substance-based approach, such as CA, requires that the presence and concentrations of all the substances in the mixture are known. However, there are often several unidentified chemicals that contribute to the total effect (Escher et al., 2009; Tang et al., 2014; Brack et al., 2018; Meador and Nahrgang, 2019; Escher et al., 2020; Lai et al., 2022). WET test, on the other hand, can be applied without knowledge of the mixture content and accounts for interactions within the mixture, yielding a total toxicity response (Chapman, 2000). The toxic response of the WET tests can therefore often not be fully explained by the substance-based approach (de Vries et al., 2022). This discrepancy can be either due to unknown constituents of the effluent, formation of transformation and degradation products or due to possible synergistic or antagonistic effects (Escher et al., 2009; Escher and Fenner, 2011; de Vries et al., 2022; Hong et al., 2023). Thus, substances that are present in concentration below their effect threshold, or that are not measured at all, can contribute to the overall toxicity (Escher and Hermens, 2002; Silva et al., 2002; Kortenkamp and Faust, 2018).

The current guidelines regarding risk and impact of scrubber water discharge (MEPC, 2022) propose to apply a substance-based approach where the risk ratios of a selection of nine metals and the 16 US-EPA PAHs are summarised, analogous to the CA concept (MEPC, 2022). However, recent studies show that many additional substances, for example alkylated PAHs, are present in scrubber water at high concentrations (Du et al., 2022; Achten et al., 2024; García-Gómez et al., 2024) and that observed effects from WET tests occur at concentrations lower than expected from the individual substances (Koski et al., 2017; Thor et al., 2021; Picone et al., 2023). Alkylated PAHs are generally considered more toxic than their parent homologues (Turcotte et al., 2011; Achten and Andersson, 2015; Cong et al., 2021; Wassenaar and Verbruggen, 2021; Donald et al., 2023) and for example the alkylated 3-ring PAHs anthracene and phenanthrene are more persistent, have higher bioaccumulation potential and can be more toxic than their parent PAHs (Wassenaar and Verbruggen, 2021). However, there is a lack of experimental ecotoxicological tests of alkylated PAHs (Andersson and Achten, 2015), excluding them from risk and impact assessments. Non-testing methods such as Quantitative Structure-Activity Relationship (QSAR) models provide one possibility to fill the experimental data gap.

QSAR models relate the properties of a substance to a toxicity endpoint (Hermens, 1989; Muratov et al., 2020). Traditionally, in order to account for the large variability in toxicity between different chemical classes and durations of exposure, QSAR models are developed from highly specific groups of data. The data is then grouped based on chemical class, (eco)toxicological effects, species and exposure scenarios and regression models are then fitted to the data (Wright et al., 2022). More recent developments use different machine learning algorithms and train on larger, more diverse, datasets (Sheffield and Judson, 2019; Martin, 2020). QSAR models that use AI-methods commonly used in large language models to interpret chemical structures and predict toxicity have also been developed (Gustavsson et al., 2024).

In Europe, land-based industries have an obligation to monitor and report their emissions and discharges, if they exceed annual limits, through the Pollutant Release and Transfer Register (EC, 2006; EU, 2010). No such obligation exists for the shipping industry, creating a larger knowledge gap on what is released to the marine environment and how that will affect the marine ecosystems. The objective of this study is to contribute with information that narrows this gap, to explore the usefulness of QSAR models as a complement to experimental data, and to assess the possibility to predict toxicity of scrubber water from extended knowledge of the mixture components. This is done by comparisons with observed response from previously reported ecotoxicological tests of scrubber water exposure. For the predictions, the current available experimental data is mainly limited to the 9 metals and 16 US-EPA PAHs listed in the risk and impact assessment guidelines (MEPC, 2022). The use of QSAR models allowed for an extended list of substances identified in scrubber water to be included in the mixture toxicity prediction.

2. Materials and methods

The first step was to collect experimental and modelled ecotoxicological test results, i.e. effect concentrations of different substances (data available in Appendix B). In the second step, the outcomes of experimentally measured and modelled effect concentrations were compared to evaluate the suitability of applying QSAR models to estimate effect concentrations. For further evaluation of the QSAR models (ECOSAR, VEGA, T.E.S.T and TRIDENT) and comparison of model performance,

see e.g. Gustavsson et al. (2024). In the third step, the effect concentrations were used to determine critical values (CV) for all substances identified and quantified in scrubber water or scrubber sludge. The QSAR model results were used as complements to the experimental data, and all modelled effect concentrations were treated as unique test results. The critical values for the identified substances were then used to calculate toxic units (TUs) for measured constituents of scrubber water (Eq. (1)).

$$TU_i = \frac{c_i}{CV_i} \quad \text{Eq.1}$$

Herein, the TU_i is defined as the ratio between the measured concentration of the known substances (i) in the mixture (c_i) and the critical value (CV_i) of that the same substance (Escher et al., 2009; de Vries et al., 2022). The critical value is based on the lowest chronic effect concentration determined from ecotoxicological tests (deterministic approach), or be based on a probabilistic approach (i.e. a species sensitivity distribution (SSD) curve from where a hazardous concentration for 5% of the selected species is derived (HC05 value)) (ECHA, 2008; EC, 2018). For mixtures, the TUs was then summarised (TU_{sum}), according to the CA concept, to provide an approximation of the total toxicity (Backhaus and Faust, 2012; Nys et al., 2017). Finally, the predicted response was compared to observed response from WET tests where scrubber water constituents had been quantified.

2.1. Collection of effect concentration data of single substances

The 69 substances included in the analysis (Table A.1) were selected based on their presence in at least one scrubber water or scrubber sludge sample, as reported in a set of previous studies (Lunde Hermansson et al., 2021; Marin-Enriquez et al., 2023; Achten et al., 2024; García-Gómez et al., 2024). The substances were grouped as inorganic (i.e. metals, n = 9), 16 US-EPA PAHs (n = 16), alkylated PAHs plus benzo[*c*]phenanthrene (n = 25), PCBs & dioxins (n = 7) and organics with either sulphur (n = 9), nitrogen (carbazole, n = 1) or oxygen (dibenzofuran, n = 1) derivatives. For all organic substances (n = 60), the effect concentrations were collected from experimental data (section 2.1.1) and computed by four different QSAR models (section 2.1.2). Metal (n = 9) critical values were only included in the final step where TUs were calculated and summarised to be compared to WET test results.

To harmonize the datasets, and to ensure that updated and correctly spelled names were used, all species names were checked using Tree of Life (R-package Taxize v0.9.99) and all species were assigned to an organism group (n = 13) (listed in Appendix A). The datasets were assessed using an in-house developed script in MATLAB (version 2023b, MathWorks Inc., Natick, Massachusetts, USA) that allowed for further filtration (e.g. selection of effects and/or endpoints), separation (e.g. selection based on organism groups, i.e. fish, crustacean/invertebrate and algae) and harmonization (recalculation of units) and endpoint transformation in accordance with Eq (2).

$$NOEC = \frac{LOEC \text{ or } LOEL}{2} = \frac{MATC}{\sqrt{2}} = \frac{ChV}{\sqrt{2}} \quad \text{Eq. 2}$$

Where LOEC and LOEL is the lowest observed effect concentration (level), MATC is the maximal acceptable toxicant concentration, defined as the geometric mean of the NOEC and the LOEC. The chronic effect value (ChV) is the output given by the QSAR model ECOSAR and has the same definition as MATC.

2.1.1. Experimental ecotoxicological data of single substances

Experimental ecotoxicological data for 27 out of the 60 relevant organic substances (Table A.1) was available through the US EPA ECOTOX knowledgebase and downloaded in March 2024 (US EPA, 2024) (n = 1367). The dataset was amended with ecotoxicological data for PAHs from Verbruggen (2012) and four EU commissioned dossiers on the derivation of EQS values for use in the Water Framework Directive (anthracene, fluoranthene, naphthalene and 5–6 ringed PAHs) (European Commission, 2022) (n = 634). Aquatic tests with both freshwater and marine species were included. The measured effects included were; mortality, growth, reproduction, development, morphology, intoxication, immobilization, population and physiology. Both chronic (EC05-E(L)C10 and NOEC(L)) and acute (E(L)C50) endpoints (Appendix C) were included. Raw data is listed inAppendix B.

2.1.2. Modelled ecotoxicological data of single substances

The SMILES (Simplified Molecular Input Line Entry System) for each unique registry number (CAS) were retrieved from the American Chemical Society's database SciFinder) in January 2023 (https://scifinder-n.cas.org) and used to generate toxicity predictions with four different QSAR models; ECOSAR v2.2 (Wright et al., 2022), VEGA v1.1.5 (Benfenati et al., 2013), T.E.S.T. v5.1.1.0 (Martin, 2020) and TRIDENT (Gustavsson et al., 2024). ECOSAR predicts effect concentrations based on physiochemical properties. VEGA runs an assemblage of different models with underlying methods ranging from linear regression to machine learning algorithms. T.E.S.T. is an ensemble model based on four different machine learning algorithms that in turn interpret chemical fingerprints. TRIDENT bases its predictions on interpretations of chemical structures performed by a transformer and a deep neural network. Test results for representatives of both marine and/or limnic fish, crustacean and algae were included in the analysis (grouped according to Table 1).

For ECOSAR (n = 431), for each individual chemical and organism group (fish, daphnia, mysid and algae), the prediction with the most toxic effect concentrations were selected, as suggested in the user manual (Wright et al., 2022). Both chronic (ChV) and acute (E(L)C50) effect concentrations were given for four different species/trophic levels. In accordance with current REACH guidance, the chronic effect values (ChVs) were divided by $\sqrt{2}$ to rescale them to a value representing a NOEC (Eq. (2)) (ECHA, 2008). Predictions flagged with "LogKowCutOff" and "DomainOfApplicability" were removed, as the substances in both cases are out of the applicability domain. For VEGA (n = 517), both chronic (NOEC) and acute (EC50) effect concentrations were provided for three different organism groups and acute effect concentrations were provided for two additional fish species (Table 1). Predictions labelled with reliability 'low' were removed as this indicates that the substances are outside the applicability domain of the model. T.E.S.T (n = 124) only predicts acute effect concentrations (LC50) for fish and daphnia. For TRIDENT (n = 708), the most sensitive effect concentration per organism group and endpoint (EC50, EC10) was gathered from the online service where three trophic levels were represented (https://trident.serve.scilifelab.se/). In TRIDENT, the median distance to the data used to develop TRIDENT was >0.3 for 25 substances, between 0.2 and 0.3 for 18 and below 0.2 for 17 substances. These similarity levels to the trained model is reported as high, intermediate and low, respectively (Gustavsson et al., 2024).

2.2. Comparison of modelled and experimental effect concentrations

The model outputs were compared to the experimental dataset where data was available. The evaluation was done at 8 different levels (Table 2), where the datasets were split into two groups: one representing acute endpoints (LC50, EC50, IC50) and one representing chronic endpoints (NOEC, LOEC, NOEL, LOEL, EC10 and EC05). Where possible, the minimum and median effect concentrations of three different organism groups (fish, crustacean, and algae) for each substance were evaluated independently. Finally, in line with common chemical risk assessment procedures, one dataset containing data from all organism groups was constructed and the minimum and median effect concentrations from experimentally measured and modelled datasets were compared.

The variability in the experimentally measured and modelled data

Table 1
Summary of the comparison of the four QSAR models. The organism group column indicates to what organism group each test species have been assigned. ChV is the chronic value, LC is the lethal concentration, NOEC is the no observed effect concentration and EC is the effect concentration.

QSAR model	Trophic level/ species	Organism group	Endpoint	Reliability assessment within the separate model tool
ECOSAR v2.2	Fish (FW and SW)	Fish	ChV and LC50	Omitted predictions flagged "LogKowCutOff" or "DomainOfApplicability" (Molecular mass over 1000)
	Daphnia	Crustacean	ChV and LC50	
	Mysids (FW and SW)	Crustacean	ChV and LC50	
	Green algae	Algae	ChV and LC50	
VEGA v1.1.5	Fish	Fish	NOEC and LC50	Experimental, good, moderate (low is omitted)
	Guppy	Fish	LC50	
	Fathead minnow	Fish	LC50	
	Daphnia	Crustacean	NOEC, EC50 and LC50	
	Green algae	Algae	NOEC and EC50	
T.E.S.T v5.1.1.0	Fish	Fish	LC50	Experimental or prediction
	Daphnia	Crustacean	LC50	
TRIDENT	Fish (pooled)	Fish	EC50 and EC10	High, (n = 25)
	Aquatic Invertebrates	Crustacean	EC50 and EC10	Intermediate (n = 18)
				Low (n = 17)
	Algae	Algae	EC50 and EC10	

Table 2
Number of substances and effect concentration data points included at the different comparisons. The data was labelled as acute or chronic and evaluated for fish, crustacean, algae as well as for the combination of all data. Within parenthesis show the percentage of datapoints that are experimentally measured.

	Acute		Chronic	
	Substances	Datapoints	Substances	Datapoints
Fish	18	368 (61%)	11	249 (81%)
Crustacean	20	688 (76%)	13	383 (78%)
Algae	15	142 (71%)	11	132 (80%)
All	27	1566 (70%)	18	1074 (77%)

for each organism group and endpoint was compared at the interquartile range (Figure A.2) and the median absolute deviation factor (ADF) was calculated for each group (i.e. fish, crustacean, algae and all). The ADF (Eq. (3)) for each group and substance is the absolute deviation of the measured and predicted effect concentrations.

$$\mathrm{ADF} = \max\left(\frac{\mathrm{EC_{measured}}}{\mathrm{EC_{predicted}}}, \frac{\mathrm{EC_{predicted}}}{\mathrm{EC_{measured}}}\right) \quad \text{Eq. 3}$$

ADF is calculated for each substance, group and effect concentration (EC) containing both measured and modelled data. Note that the ADF is calculated independently for the median and minimum value of each dataset (Table A.2).

2.3. Determination of critical values

In accordance to the European Commission Technical Guidance for Deriving Environmental Quality Standards (Document No. 27), critical values should be determined from reliable and relevant ecotoxicological tests (EC, 2018). If effect concentrations of at least eight taxonomic groups and ten species are available, the critical value can be determined from a species sensitivity distribution (SSD) curve where a hazardous concentration for 5% of the selected species (HC05 value) can be derived (EC, 2018). For the probabilistic approach, the open access program SSDToolbox (v1.1 by US EPA) was used to determine the SSD curves and derive a HC05 (Figure A.4). When less data is available, the deterministic approach is applied. If the most sensitive organism group from chronic tests differed from the most sensitive organism groups from the acute tests, or if no chronic studies were available, the lowest acute or chronic effect concentration was then selected as the critical value (REACH, 2008; EC, 2018).

The critical values were determined based on 1) only including experimentally measured values (section 2.1.1), providing critical values of 27 substances, 2) only including modelled QSAR output (section 2.1.2) providing critical values for 60 substances and 3) combining all effect concentrations, considering measured and modelled data equally, to compare the resulting critical values from deterministic (n = 57) and probabilistic (n = 3) approaches. In addition to determining critical values with all species included, specific critical values for the organism groups algae, crustacean and fish were determined separately (Table A.5). The lowest critical value, from experimental and modelled results, were used in the calculations of substance-based TUs (Eq. (1)) and the toxicity prediction of scrubber water (section 2.4). As the probabilistic approach is considered less uncertain, the lowest HC05 value was given precedence when available (in the case of phenanthrene, fluoranthene and benzo[a]pyrene (Figure A.4)).

2.4. Predicting ecotoxicological response from scrubber water exposure

TU_{sum} were calculated for 9 different scrubber waters previously used in ecotoxicological WET tests (Table A.4 (Koski et al., 2017; Thor et al., 2021; Ji et al., 2023; Picone et al., 2023; García-Gómez et al., 2024; Monteiro et al., 2024);. For some alkylated PAHs, the separation of every homologous alkyl-PAH isomers was not possible (García-Gómez et al. (2024) and isomers were then grouped in accordance toTable A.2. To ensure a conservative toxicity estimate, each isomer group was then assigned the lowest critical value from their respective group. (24 alkylated PAHs were represented by 12 isomer groups).

The dilution ratios ($DR_{predicted}$) needed to reach the respective critical concentration (i.e. when the mixture containing all identified substances are diluted to the expected no effect concentrations) were determined from the TU_{sum}. $DR_{predicted}$ is here defined as the dilution required to reach $TU_{sum} = 1$. The $DR_{predicted}$ was also recalculated to the corresponding predicted NOEC (as % scrubber water) for comparisons with WET test results (Eq. (4)).

$$\mathrm{NOEC_{predicted,Id}} = \frac{100}{\left(\sum_{i=1}^{n} \mathrm{TU_i}\right)_{\mathrm{Id}}} = \frac{100}{\mathrm{DR_{predicted,Id}}} \quad \text{Eq. 4}$$

The $NOEC_{predicted}$ is the predicted NOEC for a specific scrubber water (Id) that was then compared to the observed NOEC from the corresponding WET test. Together, WET tests were performed using eleven different species from the groups algae, crustaceans, fish, molluscs, worms (polychaetes) and echinoderms. For algae, crustaceans and fish the predicted NOEC was based on TU_{sum} calculations only including critical values for the respective organism group. For molluscs, worms and echinoderms, effect concentrations where not available for the

specific organism groups andthe predicted NOEC was based on TU_{sum} calculations when the critical value was determined from all available data.

3. Results and discussion

3.1. Comparing modelled and experimentally measured effect concentrations

The experimentally measured effect concentrations, both acute (LC50 and EC50) and chronic (NOEC, EC10), was compared to the predicted effect concentrations from the QSAR models for three different organism group levels (fish, crustacean and algae) (Fig. 1 and Figure A1). In the modelled data (n = 60), crustacean is the most sensitive organism group for 95% of the substances. Exceptions are the three substances anthracene, 2-methyldibenzothiophene and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-tetraCDF) where fish is the most sensitive organism group. In the experimentally measured dataset (n = 27), crustaceans are the most sensitive species for 52% of the substances (n = 14), followed by fish for 22% (n = 6). The remaining 26% contains several other organism groups, e.g. algae, molluscs and echinoderms. When measured and modelled data are combined, crustaceans have the lowest effect concentrations for 53 out of 60 substances.

Within each of the three organism groups large differences, sometimes several orders of magnitude, can be seen for individual substances both in the modelled and experimental data (Figure A.2). However, there is seldom no overlap between the measured and modelled effect concentrations. In Fig. 1 only the critical values, i.e. the lowest reported effect concentrations, are compared (see Figure A.1for a comparison of median effect concentrations).

For the chronic data (Fig. 1A and B), when comparing the minimum modelled and the minimum measured effect concentration per substance, the median ADF and percentage of deviations outside a factor 10 was 7.4/45%, 5.8/23%, 4.2/27% and 4.3/28% for fish, crustaceans, algae and all organism groups respectively (Table A.3), with 95% of all deviations within a factor 100. When comparing at the organism group level (Fig. 1A), the models more often underestimate the effect on algae and fish and more often overestimate the effect on crustaceans. When considering all organisms the model results show better agreement with the measured dataset, but the effect of retene and carbazole are overestimated (Fig. 1B and Figure A.3A). The largest deviation was seen for retene (ADF = 2455) where the only measured effect concentrations are from tests on fish (Billiard et al., 1999). While the models can predict the effect concentration of retene for fish almost within a factor 10 (Fig. 1A), the deviation become larger when all organisms are combined as the modelled effect concentration for crustaceans is > 100 times lower than for fish. As no measured data exist for retene exposure on crustacean, it is not possible to evaluate if the model overestimate toxicity or if the

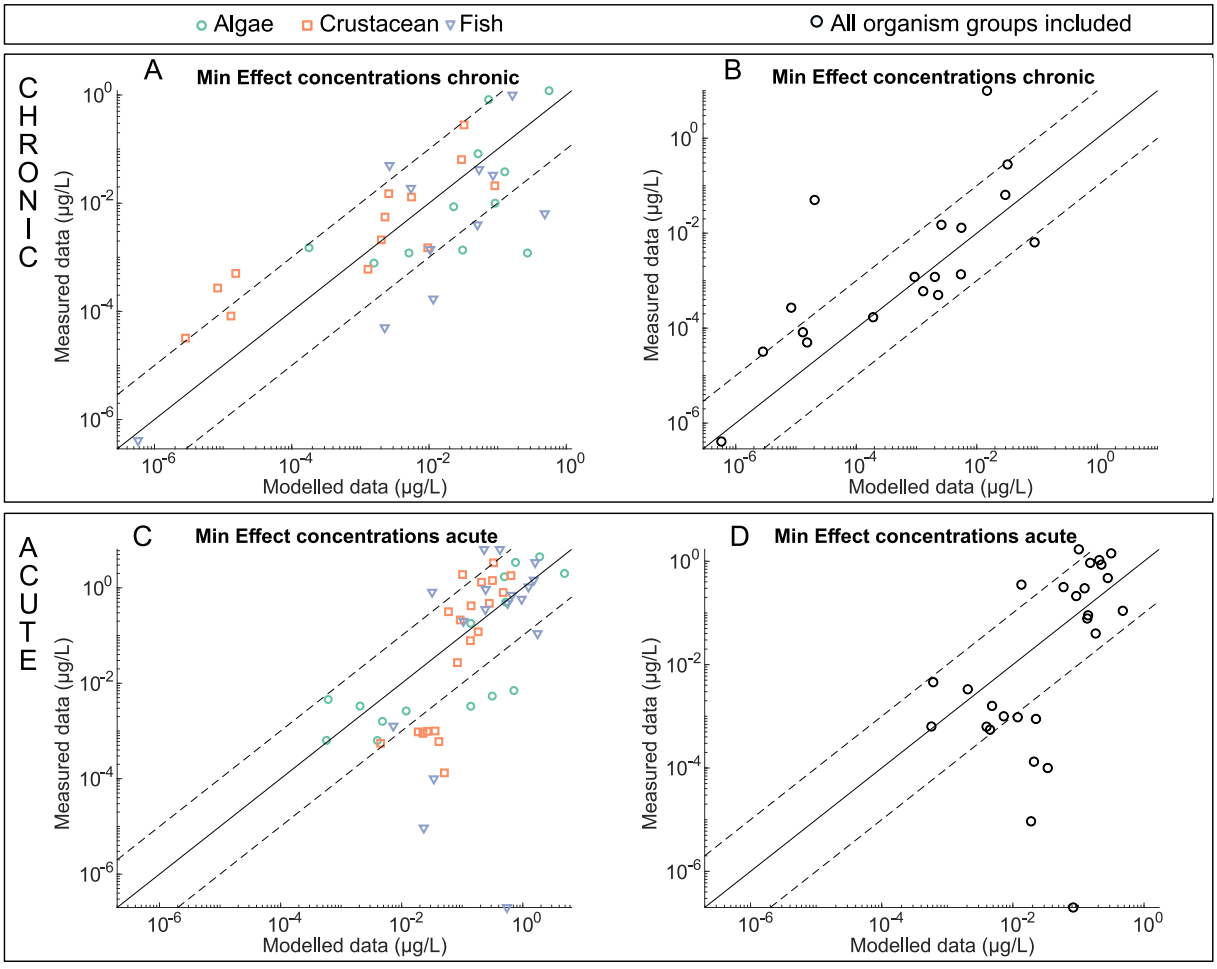

Fig. 1. Comparison of the minimum effect concentration of the measured and modelled datasets. The upper panel show the chronic data and the lower panels the acute data. The plots to the left (A and C) show the effect concentrations for fish (blue triangles), crustacean (red squares) and algae (green circles) separately and the plots to the right (B and D) show the minimum effect concentration when all available data is combined (black circles). The full line shows the 1:1 relationship, i.e. when the measured and modelled data set show the same minimum effect concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

deviation is explained by the lack of tests of the most sensitive species. For carbazole (ADF = 680), the only available chronic test is from a population growth test on ciliate *Tetrahymena thermophila* (Pauli et al., 1993) and the reported chronic NOEC is > 10 times higher than the reported acute LC50 value of fish (Brooke, 1991) (Appendix B).

For the acute data, a comparison between modelled and measured data show that the models tend to underestimate the toxic response (Fig. 1C and D). The median ADF and percentage of deviations outside a factor 10 for acute data was 3.8/41%, 5.8/40%, 3.4/20% and 5.3/30% for fish, crustaceans, algae and all organism groups, respectively (Table A.3). 75% of the deviations that are larger than a factor 10 have lower effect concentrations in the measured dataset (Fig. 1C and D). When considering all organism groups together (Fig. 1D and Figure A.3B), phenanthrene (ADF = 416000), 2,3,7,8-tetraCDF (ADF = 2030), fluoranthene (ADF = 340) and benzo[*g,h,i*]perylene (ADF = 158) have the most underestimated acute effect concentrations. For phenanthrene and 2,3,7,8-tetraCDF, fish have the lowest measured effect concentrations and the largest deviations for the modelled acute effect concentrations. This deviation could be due to specifically acting modes of action for the substances that might not be captured by the selected models. For example, several studies have shown high fish sensitivity to phenanthrene exposure resulting from heart deformities and cardiac malfunction (Zheng et al., 2020; Donald et al., 2023).

The lowest measured acute effect concentrations are lower than the lowest chronic effect concentrations for anthracene, pyrene, benzo[*a*]anthracene and carbazole. For each of these substances, the lowest chronic effect concentrations were measured using algae/protozoans, while the lowest acute effect concentrations are measured using crustacean (n = 3) or fish (n = 1). For anthracene, several organism groups are represented both in the acute and chronic test battery and the lowest experimental effect concentrations for acute and chronic tests are similar (minimum NOEC = 0.001 mg/L, minimum EC50 = 0.0014 mg/L). However, some of the lowest effect concentrations are reported for tests including UV radiation (from a lamp or natural sunlight, Appendix B) and the response can thus be driven by photoinduced toxicity (Oris and Giesy, 1985; Pelletier et al., 1997; Weinstein and Polk, 2001). Sunlight stimulation of anthracene also resulted in genotoxic transformation products with increased toxicity in *Vibrio fischeri* (Escher and Fenner, 2011).

For three substances (benzo[*a*]pyrene, fluoranthene and phenanthrene), sufficient data (i.e. chronic effect concentrations of at least eight taxonomic groups and ten species) was available to construct SSD curves and derive HC05 values as critical values (Figure A.4). The inclusion of modelled effect concentrations did not affect the fit of the curve significantly, but the most sensitive species for fluoranthene and benzo[*a*]pyrene were represented by mysid from the QSAR output, yielding larger effects on the HC05 determination when the number of species are lower (i.e. benzo[*a*]pyrene (n = 22–27) versus fluoranthene (n = 38–42)).

A full review of the respective models and model performance is beyond the scope of this study, (see e.g. Gustavsson et al. (2024) for performance comparisons). However, based on the available data, the modelled effect concentrations are deemed sufficiently accurate to be used when estimating critical values of data-poor substances.

3.2. Predicted and observed ecotoxicological response from scrubber water exposure

Comparison between the observed effects from 16 WET test on open loop scrubber water, and the predicted effects based on measured concentrations in the corresponding scrubber waters (Table A.6), show that the substance-based predictions often underestimate the effects (Table 3). This is true even when the substances normally measured in scrubber waters are amended with an extended list of alkylated PAHs. The ratio between the predicted and observed NOEC vary by more than 3 order of magnitude and the largest observed difference is a predicted NOEC that is 2000 times higher than the observed NOEC (Table 3 (Monteiro et al., 2024),).

Scrubber water content differ depending on several parameters connected to the operation of the ship, the construction of the scrubber system and the type of fuel used (Lunde Hermansson et al., 2021). For example, when comparing onboard open loop scrubber samples from García-Gómez et al. (2024) to open loop samples from Du et al. (2022), the total 16 US-EPA PAH concentration are both approximately 15 μg/L. However, the concentration of alkylated PAHs, i.e. alkylated derivatives listed in Table A.2, differed substantially with approximately 40 μg/L in the samples reported by García-Gómez et al. (2024) versus 80 μg/L in the samples reported by Du et al. (2022). Also, other substances of potential concern, that are not included in the NOEC prediction (Table 3 and Eq. 4), but that have been identified in scrubber water screenings include e.g. phenylphenanthrenes, *4H*-cyclopenta[*4,5-def*]phenanthrene (Achten et al., 2024), benzothiophenes and carbazoles (Thor et al., 2021; Achten et al., 2024). Another important aspect of whole effluent toxic response is the low pH of concentrated scrubber water (Koski et al., 2017; Lunde Hermansson et al., 2021; Thor et al., 2021). In addition to having an acidic effect on exposed organisms, the low pH also increases the bioavailability of toxic substances like metals. However, as the ecotoxicological effects presented in table 3 almost always are recorded at low exposure concentrations, the pH is of less relevance (shown in e.g. Koski et al., 2017; Thor et al., 2021) and here this effect is not considered further.

There are large variations in sensitivity to scrubber water both between and within species, with early life stages development generally being the most sensitive endpoint (Table 3). According to the predicted NOEC, between WET test, the highest dilution required to reach the NOEC is for the closed loop scrubber water. However, this is not reflected in the observed NOEC (Thor et al., 2021; Ji et al., 2023). In some of the tests, the effects were observed already at the lowest tested concentration, i.e. no NOEC could be reported, and the predicted NOEC is then instead compared to 0.5 times the reported LOEC.

The concentration of ten metals in the scrubber water are available for all 35 WET tests. For 33 tests, the concentrations of the 16 US-EPA PAHs are also reported and for 25 tests the concentrations of alkylated PAHs are available. Based on characterisation of open loop scrubber water from García-Gómez et al. (2024) (Id A, C and D in Table 3) and critical values derived in this study, the relative contribution to the TU_{sum} for metals, 16 US-EPA PAHs and alkylated PAHs in open loop scrubber water is approximately 28%, 15% and 57%, respectively. However, if the TU_{sum} of open loop scrubber water characterised by García-Gómez et al. (2024) (Id A, C and D in Table 3) is compared to observed ecotoxicological NOEC of 0.1%, the predicted NOEC only accounts for less than 20% of the observed response. This suggest that other substances that are not accounted for in the estimation, but present in the samples, may contribute more than 80% to the response (Fig. 2). Substances currently not monitored being responsible for the observed effects is also supported by the fact mixtures with many compounds present at low concentrations (such as diluted scrubber discharge water) rarely demonstrate synergistic and antagonistic effects (Escher et al., 2020; Lai et al., 2022). Thus, broadened measurements, that allow the analysis of more compounds than PAHs and alkyl derivatives, are required for a more precise risk and impact assessments of scrubber water.

In addition to the alkylated PAHs, previous studies on marine organisms exposed to water samples in contact with oil or combustion products, have proposed alkylphenols (Barron et al., 1999; de Vries et al., 2022), alkylbenzenes (Uhler et al., 2016) and naphthenic acids (de Vries et al., 2022) to be present and contributing to the observed effect. In addition, both transformation and degradation products might be of importance (Escher and Fenner, 2011; Lai et al., 2022; Hong et al., 2023). Previous studies have also shown that, for alkylated phenanthrenes and benzo(a)anthracene, photoinduced toxicity could exceed the expected toxicity with one order of magnitude (Boese et al., 1998;

Table 3
Comparison between the predicted and observed response. Observed NOEC (%) shows the determined NOEC from WET tests of a specific species (organism group in parentheses), exposed to a scrubber water (origin of scrubber water indicated by Id A-I) for a specific endpoint. The predicted NOEC is indicated as a concentration (in %) based on the calculated dilution ration (Eq. (4)) required to achieve $TU_{sum} = 1$. The analytes included in the predictions are grouped as metals (Me), 16 US-EPA PAHs (PAH), alkylated PAHs (Alk-PAH) and benzothiophenes (BTP). A predicted-to-observed response ratio >1 means that the prediction underestimates the toxic response while a ratio <1 means that it overestimates the toxicity. F0 is the parent generation while F1 is the offspring of F0. C-III and C-V indicate the copepodite (juvenile copepod) stage (3 vs. 5).

Id	Species tested	Observed NOEC (%)	Endpoint	Predicted NOEC (%) (Eq. (4))	Predicted-to- observed response ratio	Analytes	Reference
Chronic ecotoxicological tests. Open loop scrubber.							
A	*A. tonsa* (crustacean)	0.01[a,b]	Egg production (F0)	0.37	37	Me, PAH, Alk-PAH	Picone et al. (2023)
A	*A. tonsa* (crustacean)	1	Larval development (F0)	0.37	0.37	Me, PAH, Alk-PAH	Picone et al. (2023)
A	*A. tonsa* (crustacean)	0.1[a,b]	Larval development (F1)	0.37	3.7	Me, PAH, Alk-PAH	Picone et al. (2023)
A	*A. tonsa* (crustacean)	10	Larval mortality (F0)	0.37	0.04	Me, PAH, Alk-PAH	Picone et al. (2023)
C	*Artemia* sp. (crustacean)	0.001	Feeding inhibition	1.0	1000	Me, PAH, Alk-PAH	Monteiro et al. (2024)
D	*Artemia* sp. (crustacean)	0.001	Feeding inhibition	0.27	270	Me, PAH, Alk-PAH	Monteiro et al. (2024)
E	*C. helgolandicus* (crustacean)	1[a]	Mortality (C-III)	1.6	1.6	Me, PAH	Thor et al. (2021)
C	*M. galloprovincialis* (mollusc)	1	Feeding inhibition	1.3	1.3	Me, PAH, Alk-PAH	Monteiro et al. (2024)
D	*M. galloprovincialis* (mollusc)	0.0005[a]	Feeding inhibition	0.43	860	Me, PAH, Alk-PAH	Monteiro et al. (2024)
A	*M. galloprovincialis* (mollusc)	0.1	Larval development	0.5	5	Me, PAH, Alk-PAH	Picone et al. (2023)
C	*P. lividus* (echinodermata)	0.005[a]	Fertilization	1.3	260	Me, PAH, Alk-PAH	Monteiro et al. (2024)
C	*P. lividus* (echinodermata)	0.0005[a]	Larval development	1.3	2600	Me, PAH, Alk-PAH	Monteiro et al. (2024)
D	*P. lividus* (echinodermata)	0.01	Fertilization	0.43	43	Me, PAH, Alk-PAH	Monteiro et al. (2024)
D	*P. lividus* (echinodermata)	0.01	Larval development	0.43	43	Me, PAH, Alk-PAH	Monteiro et al. (2024)
C	*S. alveolate* (worm)	0.001	Larval development	1.3	1300	Me, PAH, Alk-PAH	Monteiro et al. (2024)
D	*S. alveolate* (worm)	0.0005[a]	Larval development	0.43	860	Me, PAH, Alk-PAH	Monteiro et al. (2024)
Chronic ecotoxicological tests. Laboratory scrubber.							
F	*Artemia* sp. (crustacean)	0.001	Feeding inhibition	0.83	830	Me, PAH, Alk-PAH	Monteiro et al. (2024)
F	*M. galloprovincialis* (mollusc)	0.1	Feeding inhibition	0.83	8.3	Me, PAH, Alk-PAH	Monteiro et al. (2024)
F	*P. lividus* (echinodermata)	0.005[a]	Fertilization	0.83	166	Me, PAH, Alk-PAH	Monteiro et al. (2024)
F	*P. lividus* (echinodermata)	0.001	Larval development	0.83	830	Me, PAH, Alk-PAH	Monteiro et al. (2024)
F	*S. alveolate* (worm)	0.001	Larval development	0.83	830	Me, PAH, Alk-PAH	Monteiro et al. (2024)
Chronic ecotoxicological tests. Closed loop scrubber.							
G	*C. helgolandicus* (crustacean)	0.02[a]	Mortality (C-V)	0.07	3.5	Me, PAH, Alk-PAH, BTP	Thor et al. (2021)
H	*C. helgolandicus* (crustacean)	0.05[a]	Mortality (C-III)	0.15	3	Me, PAH	Thor et al. (2021)
I	*D. salina* (algae)	0.32[a]	Growth	0.56	1.8	Me, PAH	Ji et al. (2023)
I	*M. bahia* (crustacean)	0.63	Mortality	0.13	0.21	Me, PAH	Ji et al. (2023)
I	*M. bahia* (crustacean)	0.63	Growth (body weight)	0.13	0.21	Me, PAH	Ji et al. (2023)
I	*M. bahia* (crustacean)	5	Reproduction	0.13	0.03	Me, PAH	Ji et al. (2023)
I	*M. chulae* (fish)	2.5	Mortality	0.41	0.16	Me, PAH	Ji et al. (2023)
I	*M. chulae* (fish)	5	Growth (body weight)	0.41	0.08	Me, PAH	Ji et al. (2023)
Acute ecotoxicological endpoints. Open loop scrubber.							
A	*A. tonsa* (crustacean)	5	Survival	0.37	0.07	Me, PAH, Alk-PAH	Picone et al. (2023)
A	*A. fischeri* (algae)	10	Bioluminescence	1.1	0.11	Me, PAH, Alk-PAH	Picone et al. (2023)
A	*P. tricornutum* (algae)	20	Growth	1.1	0.06	Me, PAH, Alk-PAH	Picone et al. (2023)
A	*D. tertiolecta* (algae)	10	Growth	1.1	0.11	Me, PAH, Alk-PAH	Picone et al. (2023)
B	*A. tonsa* (crustacean)	1	Adult mortality	2.2	2.2	Me	Koski et al. (2017)

(*continued on next page*)

Table 3 (*continued*)

Id	Species tested	Observed NOEC (%)	Endpoint	Predicted NOEC (%) (Eq. (4))	Predicted-to- observed response ratio	Analytes	Reference
B	*A. tonsa* (crustacean)	50	Adult mortality	2.2	0.04	Me	Koski et al. (2017)

[a] The lowest tested concentration resulted in effect, i.e. no NOEC was reported and the observed NOEC is represented by 0.5 times the reported LOEC reported from the study.
[b] U-shaped curve.

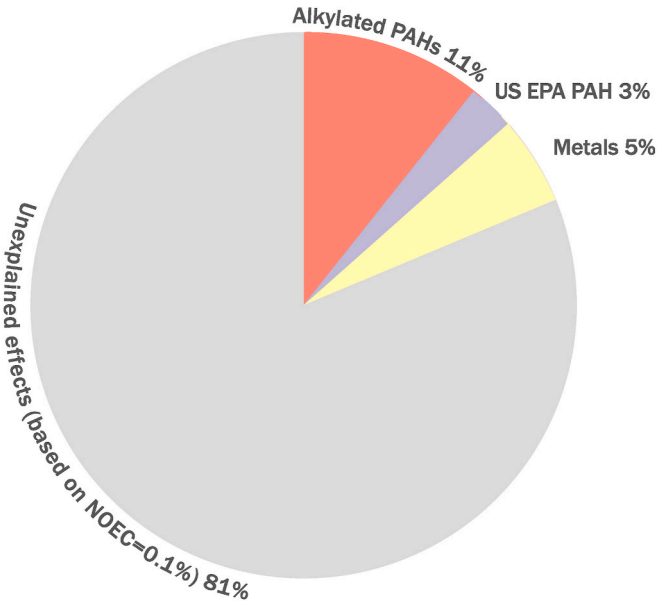

Fig. 2. Relative contribution to observed response based on toxic units (TUs) of alkylated polycyclic aromatic hydrocarbons (PAHs), 16 US-EPA PAHs and metals. Reported concentrations were retrieved and averaged from measurements in open loop scrubber water as reported in García-Gómez et al. (2024). The critical values are determined in this study based on all available measured and modelled data. Additional, unexplained, toxicity is required to reach a NOEC of scrubber water corresponding to a dilution down to 0.1%.

Wassenaar and Verbruggen, 2021). Photoinduced toxicity could thus also explain some of the discrepancy between the predicted and observed effects.

QSARs are generally developed to fit the average species within a taxonomic group while a critical value aims to protect the most sensitive species and endpoint measured. Thus, large differences between QSAR predictions and measured results are likely to occur when, for individual substances, especially sensitive species or endpoints exist and are used in exposure experiments. The species for which WET tests are performed (Table 3) spend either their entire or sensitive developmental life stages in the water column, with the risk of being exposed to scrubber water. These species also represent different biologically relevant functions in the marine environment (e.g. mussels are filter feeders, copepods as primary consumers and the tested polychaetes is reef-forming). Thus, if exposures of low concentrations of scrubber water show adverse effects in these species, this might seriously affect other trophic levels (e.g. Beaugrand et al., 2003).

The EU guidance documents for deriving environmental threshold values within the scope of the Water Framework Directive (EC, 2018) states that QSAR models "should not be used to generate critical data to derive an EQS; however, predicted data can play a role in reducing uncertainty". In this study, QSAR models were used to produce effect concentration values that were then used to determine critical values and to estimate substance-based TUs of scrubber water constituents. Including alkylated PAHs improved the accuracy of the predicted scrubber water toxicity compared to assessments based solely on metals and the US EPA 16 PAHs. However, despite the inclusion of these additional substances, a large proportion of the effects observed in the WET tests remains unexplained (Fig. 2). Identifying and predicting the toxicity of other components (e.g. sulphurated, oxygenated and nitrated PAHs) could further improve these estimates.

QSAR models are helpful tools for identifying substances of concern or prioritizing substances in need of additional attention. This is exemplified in Fig. 3, where the average concentration of each identified substance in scrubber water from García-Gómez et al. (2024) is compared to their respective lowest critical value and the cumulative TU_{sum} is plotted for the ten largest contributors to the predicted toxicity. Together, alkylated phenanthrenes and naphthalenes contribute almost 45% of the TU_{sum} and the top ten substances contribute almost 80% (Fig. 3). Although many unknowns still remain (e.g. Fig. 2), the understanding of how anthropogenic pressures may impact the marine environment can be improved by combining WET tests and an extended chemical analysis of waste streams with additional ecotoxicological effect concentrations from experiments or QSAR models.

The identification of ecologically relevant but less conventionally studied effects, e.g. crude oil induced metamorphosis (Almeda et al., 2023), are important to understand the environmental impact from fossil fuel use. Studies have also showed that low levels of petroleum hydrocarbons result in cardiac abnormalities in early life stages of fish and decreased swimming performance one year after exposure (e.g. Hicken et al., 2011). Similar delayed effects of phenanthrene exposure were observed by Donald et al. (2023), who also saw cardiotoxic effects after exposure during early life stages of Atlantic haddock (*Melanogrammus aeglefinus*). Zheng et al. (2020) observed adverse effects such as decreased and delayed hatching and increased deformity and mortality in marine medaka after early life stage exposure. As the chemical constituents of scrubber water are similar to those of produced water and burnt oil, these findings put new light on the potential impact of scrubber water discharge. Thus, despite the physiological mechanisms not yet being fully understood this emphasizes the need for broader chemical measurements and increased efforts to determine effects from scrubber water.

Current guidelines (MEPC, 2022) propose a list of 26 standard substances (10 metals and 16 US-EPA PAHs) to be included and support decisions regarding use and approval of ship scrubber technology. However, our results show that this type of assessment must extend beyond these usual suspect substances as 1) there are other identified substances, e.g. alkylated PAHs, that are present at relevant concentrations in scrubber water and 2) that even if alkylated PAHs are included in the toxicity prediction, the detected constituents can only explain a fraction of the observed response. When risk and impact assessments of scrubber water discharges are discussed, these aspects must therefore be considered to ensure well-informed decision-making.

4. Conclusions

Compared to the observed ecotoxicological response the mixture toxicity prediction, using a substance-based approach and including both measured and/or modelled data, substantially underestimates the toxicity of open loop scrubber water. Despite now including alkylated PAHs in the toxicity prediction, our study still shows that nearly 80% of the measured scrubber water toxicity remains unexplained, likely

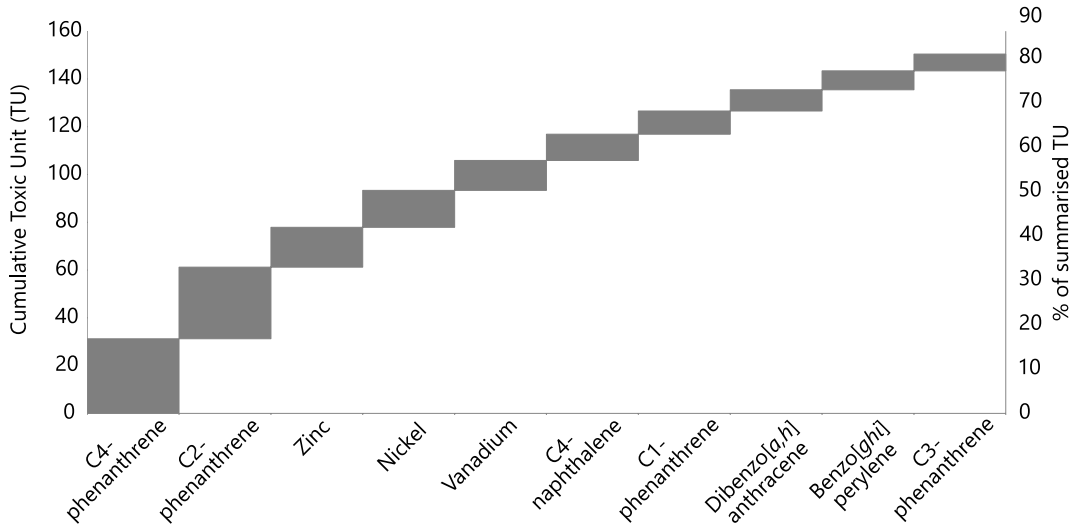

Fig. 3. The cumulative toxic units (TUs), i.e. the ratio of measured concentrations in scrubber water and the lowest critical value based on the dataset containing measured and modelled data for all organism groups. A ratio >1 means that the concentration exceeds the respective critical value, and a higher ratio indicate higher impact. Reported concentrations were retrieved and averaged from García-Gómez et al. (2024) and the critical values are derived herein. The top ten substances correspond to approximately 80% of the total summarised TUs (TU_{sum}) (right y-axis).

because of the presence of additional substances of concern. The discrepancy between predicted and observed response in WET tests emphasizes the importance of measuring effects directly from the scrubber water, especially using species that are likely to be exposed in the environment. Finally, as decision-makers and risk managers rely on predictions to estimate impacts from scrubber-use, it is vital that not only the substances currently listed in the guidelines are included in the assessments. Doing so would severely underestimate the environmental impacts from scrubber water discharges.

CRediT authorship contribution statement

A. Lunde Hermansson: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **M. Gustavsson:** Writing – review & editing, Methodology, Formal analysis, Data curation. **I.-M. Hassellöv:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **P. Svedberg:** Writing – review & editing, Data curation. **E. García-Gómez:** Writing – review & editing, Resources, Investigation. **M. Gros:** Writing – review & editing, Resources, Investigation. **M. Petrović:** Writing – review & editing, Resources, Investigation. **E. Ytreberg:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125557.

Data availability

Data will be made available on request.

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