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Review: Ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for Environmental Hazard and Risk Assessments in aquatic ecosystems

Samantha Eslava Martins[1,2*] , Gilberto Fillmann[1] , Adam Lillicrap[2], Kevin V. Thomas[2,3]

1Universidade Federal do Rio Grande - FURG. Av Itália km 8, 96203-900, Rio Grande/RS, Brazil, samanthamartins@furg.br*, +55(53)32935162; gfillmann@gmail.com, +55(53)32336738

2Norwegian Institute for Water Research (NIVA). Gausatadalléen 21, 0349, Oslo, Norway, Adam.Lillicrap@niva.no, +4798215407

3Queensland Alliance for Environmental Health Sciences (QAEHS) - The University of Queensland. Block 3, Queensland Health Precinct, 39 Kessels Road, Coopers Plains QLD 4108, Australia, kevin.thomas@uq.edu.au, +61 7 34432444

*Corresponding author: samanthamartins@furg.br

Hazard assessments of Irgarol 1051, diuron, 2-(thiocyanomethylthio)benzothiazole (TCMTB), dichloro-octylisothiazolin (DCOIT), chlorothalonil, dichlofluanid, thiram, zinc pyrithione, copper pyrithione, triphenylborane pyridine (TPBP), capsaicin, nonivamide, tralopyril and medetomidine were performed to establish robust Environmental Quality Standards (EQS), based on predicted no effect concentrations (PNECs). Microalgae, zooplankton, fish and amphibians were the most sensitive ecological groups to all evaluated antifoulants, especially in early life stages. There were no differences identified between freshwater and seawater species. The use of toxicity tests with non-standard species is encouraged because they increase the datasets allowing EQS to be derived from probabilistic-based PNECs whilst reducing uncertainties. The global ban of tributyltin (TBT) has been heralded as a major environmental success; however, substitute antifoulants may also pose risks to aquatic ecosystems. Environmental Risk Assessments (ERAs) have driven decision-makings for regulating antifouling products, but in many

countries there is still a lack of regulation of antifouling biocides which should be addressed.

Introduction

Biofouling is the settlement and attachment of organisms on the external surfaces of submerged or semi-submerged objects (Lewis 1998). Hulls of boats are one example of biofouling, which can increase frictional drag and thereby fuel consumption (Abbott et al. 2000), or can impair navigation devices. Antifouling coatings were developed to reduce biofouling to increase the performance of ships. The use of antifouling coatings greatly reduces the emission of carbon dioxide and sulphur dioxide, with annual reductions estimated to be 384 and 3.6 million tonnes, respectively (http://www.foulxspel-antifouling.com/, access in August 1st 2017). Moreover, antifouling coatings prevent the introduction of invasive species that might threaten indigenous aquatic biodiversity (Drake & Lodge 2007).

Antifouling coatings have been used for centuries to reduce or prevent the settlement of organisms on hull surfaces. The Phoenicians and Carthaginians were the first to be credited with the use of pitch, and possibly copper sheeting, on the bottom of ships, probably as an attempt to prevent bioencrustation during their expeditions to Africa and the west coast of Europe (WHOI 1952; Hellio & Yebra 2009). The use of heavy metals in coatings increased from the late 18th century and metals (particularly copper) are still incorporated into certain modern coatings (Dafforn et al. 2011).

A major milestone in antifouling technology was the discovery of the high efficacy of tributyltin (TBT), used in combination with copper-based algicides in paint formulations (Yebra et al. 2004). Organotin-based paints were introduced as marine antifoulants in the early 1960s and at the time were believed to be the solution to preventing biofouling. However, severe impacts on the marine environment occurred following the introduction of TBT. Ecological effects of TBT on growth, development, survival and reproduction have been reported in a wide range of species from bacteria to

mammals (Antizar-Ladislao 2008, Dafforn et al. 2011). A well-documented example of this was the imposex-driven decline of the marine gastropod *Nucella lapillus* in coastal areas of Southwest England (Gibbs & Bryan 1996). These adverse ecological effects led authorities to gradually restrict and then ban the use of TBT as an antifouling biocide. In 2008 there was a global prohibition of TBT as an active ingredient in antifouling paints (IMO 2000, Dafforn et al. 2011).

Following the ban of organotin-based paints, tin-free technologies dominated the antifouling paint market, with paint formulations being composed mainly of inorganic biocides (typically cuprous oxide) and one or more organic or organo-metallic co-biocides (Hellio & Yebra 2009), which boost the biocidal efficacy of the paint. Examples of co-biocides in widespread use are Irgarol 1051, diuron, DCOIT, chlorothalonil, dichlofluanid, TCMTB, thiram, zinc pyrithione (ZnPT) and copper pyrithione (CuPT) (Hellio & Yebra 2009, Castro et al. 2011). Booster co-biocides usually make-up 0.1 - 10% of the paint formulation (International 2013, 2014; Renner 2016a, 2016b) and are anticipated to not leach into the environment at sufficiently high concentrations to trigger acute toxic effects on non-target species (Hellio & Yebra 2009). However, some lower trophic ecological groups or early life stages may be very sensitive to such co-biocides (Lambert et al. 2006; Zhang et al. 2008; Okamura et al. 2009; Onduka et al. 2010; Wendt et al. 2016). Furthermore, the sublethal effects and modes of action of antifouling co-biocides, after chronic exposure, are largely unknown (Hellio & Yebra 2009). The environmental behavior of some co-biocides is still to be fully elucidated, especially with regard to degradation products (Thomas & Brooks 2010).

Many studies have been conducted to understand the sensitivity of different groups of organisms to antifouling co-biocides and these have been critically reviewed

herein. The aim of this review was to define potential Environmental Quality Standards (EQS) for selected antifouling co-biocides. Different approaches to derive predicted no effect concentrations (PNECs), acute vs. chronic ecotoxicity assays, non-standard vs. standard ecotoxicity assays, freshwater vs. estuarine/marine ecosystems were all compared and critically discussed.

Structure and methodology

In this study, PNECs were derived according to the Technical Guidance Document (TGD) on Risk Assessment (ECB 2003), for acute and chronic exposure, in either freshwater or estuarine/marine systems. A PNEC is defined as the concentration below which an unacceptable effect is unlikely to occur (ECB 2003). Assessment factors (AF), defined as numerical adjustments used to extrapolate from experimentally determined relationships to estimate the agent exposure below which an adverse effect is not likely to occur (https://www.opentoxipedia.org/index.php/Assessment_factor, access in September 14th 2017), were applied according to the type and amount of data available (ECB 2003, van Wezel & van Vlaardingen 2004). Based on the calculated PNECs, Environmental Quality Standards (EQS) were then determined according to the European Guidance Document (EC 2011). As summarized in Figure 1, an EQS for both freshwater and seawater has been calculated for each co-biocide.

Antifouling co-biocides selection

Not all compounds with biocidal activity used in antifouling paint formulations were addressed in this review. The most commonly used organic and organo-metallic antifouling co-biocides (Irgarol 1051®, diuron, dichloro-octylisothiazolin (DCOIT), 2-(thiocyanomethylthio)benzothiazole (TCMTB), chlorothalonil, dichlofluanid, thiram, zinc pyrithione (ZnPT) and copper pyrithione (CuPT)), that are more likely to occur in

aquatic environments, were selected (Tornero & Hanke 2016; Chen & Lam 2017). Emerging biocidal compounds, with restricted regional markets (ie medetomidine, tiphenylborane pyridine (TPBP) and tralopyril) were also included to evaluate their suitability (Oliveira et al. 2017). Furthermore, capsaicin naturally extracted from chilli peppers, and its synthetic derivative nonivamide, are potential candidates to be used as environmentally-friendly antifouling biocides and these were also included (Table 1).

Irgarol 1051 and diuron are herbicides that act by inhibiting the transport of electrons during photosystem II (Hall et al. 1999), affecting mainly non-target photosynthetic organisms. Irgarol 1051 and diuron have been widely applied as antifouling co-biocides (Castro et al. 2011; Ferrer et al. 1997) and exhaustive ecotoxicity datasets exist for these compounds (Table S1 in Supplementary Information).

Chlorothalonil, dichlofluanid and thiram are mainly used as fungicides in antifouling coatings. Chlorothalonil is a broad-spectrum fungicide used for over 30 years in agriculture, but its use in antifouling paints increased after the TBT ban. Chlorothalonil acts through the inhibition of glycolysis or depleting glutathione (Caux et al. 1996), causing effects in animals and plants (WFD 2012). The presence of multiple reactive electrophilic centers makes chlorothalonil extremely toxic to aquatic organisms (Castro et al. 2011) (Table S2 in Supplementary Information). Dichlofluanid is a potent inhibitor of fungal spore germination (PPDB 2007-2017) (Table S2). However, its toxicity might be caused by its degradation products, since dichlofluanid rapidly undergoes hydrolysis in water (Hamwijk et al. 2005), even when incorporated in paint particles (Thomas et al. 2003). Thiram is a dithiocarbamate fungicide designed to inhibit spore germination and mycelial growth (PPDB 2007-2017). Thiram is a multi-site inhibitor and can affect a wide range of organisms (KEMI 2015) (Table S2).

DCOIT and TCMTB are regarded as broad-spectrum biocides used as either herbicides or fungicides (Fernández-Alba et al. 2002). DCOIT undergoes rapid degradation in natural seawater and binds strongly to sediments, reducing its bioavailability and hence its potential to bioaccumulate (Castro et al. 2011). These characteristics led to DCOIT being considered as one of the environmentally safest antifoulants (Jacobson & Willingham 2000; Castro et al. 2011). However, DCOIT prevents fouling by reacting with proteins of organisms that encounter the coating surface, resulting in interruption of metabolic processes and disruption of the physiological processes involved in the attachment of the organism to solid surfaces. Thus, ecotoxicity studies have reported high toxicity of DCOIT to non-target organisms, especially zooplankton and microalgal species (Table S3 in Supplementary Information). TCMTB acts through inhibition of the electron transport chain in mitochondria (Fernández-Alba et al. 2002), so a wide range of non-target organisms can be affected (Table S3).

The pyrithione salts, such as zinc pyrithione (ZnPT) and copper pyrithione (CuPT), were introduced on the market in the 1990s. Due to broad antimicrobial activity, low water solubility and high degradability, they have been used in marine antifouling paints as replacements for tributyltin (TBT) (Mochida et al. 2006). It has been reported that pyrithiones disrupt the proton motive force in target organisms (KEMI 2014). Pyrithiones act by catalyzing the electroneutral exchange of H^+ and other ions with K^+ across cell membranes, resulting in a collapse of ion gradients important to cell function. This process may inhibit membrane transport of nutrients and lead organisms to starvation and eventual death (KEMI 2014) (Table S4 in Supplementary Information).

Emerging compounds (Table S5 in Supplementary Information) have been used as antifouling biocides on a smaller scale, typically in regional markets. TPBP is a broad-spectrum antifoulant used mainly in Japan, where it has been the predominant biocide in 40 antifouling products since 1995 (Mochida et al. 2012). There is a lack of reported studies on the occurrence and ecotoxicity of TPBP and its mode of action is largely unknown (Wendt et al. 2016). Tralopyril is a broad-spectrum biocide used to boost antifouling potential of copper-free antifouling formulations by uncoupling mitochondrial oxidative phosphorylation (EU 2014b; International 2014). Medetomidine is designed to protect against hard fouling (shell-building) marine organisms, acting via the activation of analogous octopamine leading to an anti-settling effect (EU 2015).

The natural co-biocide capsaicin and its derivative nonivamide have been introduced in the formulation of antifouling paints in China (Oliveira et al. 2014, Liu et al. 2016). Both act on the nervous systems through several different mechanisms and also disrupt metabolism and damage membranes (Gervais et al. 2008).

Survey of ecotoxicity information

Data on the ecotoxicity of antifouling co-biocides was obtained from previously published papers and reviews, technical reports and datasets from Environmental Protection Agencies (EPAs). The ecotoxicity data that were used in this review required that the following criteria were achieved: the test was performed under laboratory conditions; exposure was to a single compound; the endpoint and exposure time were clearly indicated; and the environment (ie freshwater or seawater) was described.

Ecotoxicity datasets were analysed according to being either: (1) acute and (2) chronic ecotoxicity in freshwater systems; (3) acute and (4) chronic ecotoxicity in estuarine/marine systems. The endpoints considered for acute tests were the lethal

(LC$_{50}$) or effective (EC$_{50}$) concentration which caused a response in 50% of the test-population. For chronic exposures, valid endpoints were NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration). In this study, only effects related to population dynamics were used to compose the datasets, such as development/growth, reproduction, and survival (van Wezel & van Vlaardingen 2004). For those compounds where more than one toxicity value were reported for the same test species, the most sensitive endpoint was used (ECB 2003). Tests conducted with salinity lower than 0.5 were considered freshwater, all others were considered seawater.

In total, ecotoxicity data were derived from 101 marine and 132 freshwater species (Tables S6-S7 in Supplementary Information), belonging to different ecological groups, which provided a comprehensive overview of the ecosystem, yielding more reliable results. Both standard and non-standard species for ecotoxicity tests were included. The SciRAP tool (Moermond et al. 2016) was used to check the reliability of the non-standard tests, and only tests with a reliability > 70% were used to compose the datasets. Each dataset was assessed for its internal variability and all data fitted a log-normal distribution ($p > 0.01$). Differences in the toxicity between the antifouling co-biocides, across ecological groups (within the same dataset), and between freshwater and marine test organisms, were statistically assessed using Kruskal-Wallis test ($\alpha = 0.05$), with Statistica 13.0. All datasets were used to calculate PNECs as described below.

Calculating Predicted No Effect Concentrations (PNECs)

PNECs for each antifouling co-biocide were derived according to either a deterministic approach with the application of assessment factors (AFs) or, when sufficient data were available, a probabilistic approach using species sensitivity distributions (SSDs) (ECB 2003; EC 2011).

Whenever possible, PNECs were estimated for each antifouling co-biocide for each exposure condition: acute PNEC for seawater (PNEC$_{ASW}$), chronic PNEC for seawater (PNEC$_{CSW}$), acute PNEC for freshwater (PNEC$_{AFW}$) and chronic PNEC for freshwater (PNEC$_{CFW}$), using both the deterministic and the probabilistic approaches.

Deterministic approach

A deterministic approach is used especially when a limited dataset is available and then AFs are applied to calculate a PNEC. The criterion for choosing the size of the AF was following the European TGD on Risk Assessment (ECB 2003, EC 2011).

Probabilistic approach

The hazard assessments to derive PNECs were performed by statistical extrapolation when the datasets were robust enough to allow the use of SSDs, accounting for several ecological groups in a dataset containing at least 10 different species (ECB 2003). SSDs were performed following the TGD on Risk Assessment (ECB 2003) and in accordance with the Guidance on the Biocidal Products Regulation (ECHA 2015).

Ecotoxicity datasets were analysed using a probability distribution of the ranked log-normal toxicity, and PNECs were derived following the equation (Aldenberg & Slob 1993), based on the confidence intervals (c.i.):

$$PNEC = \frac{5\%SSD(50\% \text{ c.i.})}{AF} .$$

Plotting positions on the SSD curves were calculated based on the rank of the datum (i) and the total number of points (n) in the dataset, following the formula (i - 0.5)/n (EC 2011). Due to the high robustness of the datasets, the size of the AFs (usually 5-1) is lower than those applied in the deterministic approach (ECB 2003, EC 2011).

Within each dataset, the organisms were sorted into ecological groups as follows: (1) Microalgae, including cyanobacteria; (2) Macrophyta, encompassing

macroalgae and all other aquatic plants; (3) Zooplankton, encompassing all holoplankton animals and the meroplankton invertebrates in their planktonic phase; (4) Benthic invertebrates, encompassing juvenile/adult organisms that have already settled to their substrates; (5) Fish; (6) Amphibia, encompassing tadpoles of anuran amphibians; (7) Bacteria; and (8) Fungi.

Whenever differences ($p < 0.05$) were seen in the sensitivity to an antifouling co-biocide between ecological groups within the same dataset, a refined SSD was performed based on the most sensitive group or groups to reduce variability in the dataset (van Wezel & van Vlaardingen 2004).

The size of the AFs was chosen also following the TGD on Risk Assessment (ECB 2003, EC 2011), except when SSDs were created from acute toxicity data. In these cases, an AF from 20 to 10 was assigned, accounting for the extrapolation from acute to chronic toxicity and the reliability of the dataset (SciRAP score, Moermond et al. 2016).

Estimating Environmental Quality Standards (EQS)

The most robust PNEC for each condition was set as the Environmental Quality Standard (EQS), defined as the maximum acceptable concentration for certain substances in a water body (EC 2011). One EQS for seawater (EQS_{SW}) and another for freshwater (EQS_{FW}) were determined. EQS estimated from SSDs were preferred over those estimated from deterministic values (EC 2011). Likewise, EQS derived from chronic studies were preferred over those from acute studies. Determination of EQS based on acute SSDs was included when the resulting value was sufficient to protect 95% of the ecosystem from chronic effects. This was evaluated using the acute: chronic ratio (ACR). The acute SSDs were divided by the lowest chronic value reported, which resulted in an ACR <10 in all cases (data not shown).

Data analysis

Resulting PNECs and EQS

Since a reasonable number of ecotoxicity studies have been performed with Irgarol 1051, diuron (Table S1) and chlorothalonil (Table S2), deterministic and probabilistic-based PNECs were derived, in either freshwater and seawater, for both acute and chronic exposures. For dichlofluanid (Table S2) and ZnPT (Table S4), datasets for acute exposure in both seawater and freshwater were adequate to derive probabilistic-based PNECs. For DCOIT (Table S3), CuPT (TableS4) and TPBP (Table S5), only acute ecotoxicity data in seawater was robust enough to derive PNECs based on SSD, while for thiram (Table S2) and TCMTB (Table S3), only the acute dataset in freshwater was robust enough.

For medetomidine and tralopyril (Table S5), only one value could be calculated using the deterministic-based approach. It was not possible to derive PNECs for capsaicin and nonivamide due to the lack of data (Table S5). All PNECs and the size of their respective assigned AFs are summarized in Table 2.

Deterministic-based PNECs were derived mostly by assigning an AF to the lowest reported value for the endpoints evaluated, according to the European guidance documents (ECB 2003, EC 2011). Exceptions were made for diuron under chronic exposure in seawater, where the most sensitive species was the embryo of the bivalve *Crassostrea gigas* (24 h-NOEC = 4 x 10^{-3} µg l^{-1}; Mai et al. 2013). However, microalgae species were overall more sensitive to diuron than all other ecological groups, and therefore chronic toxicity to the cyanobacteria *Synechococcus* sp. (72 h-NOEC = 0.21 µg l^{-1}; Devilla et al. 2005) was used to derive the deterministic PNEC$_{CSW}$ (Table 2). The high sensitivity of the bivalve might have been due to binding to sulphydryl groups on molecules that control sperm activation and motility, resulting in impairment of

fertilization and embryonic development (Dinnel et al. 1987). For DCOIT, ZnPT and

CuPT, exceptions were made to derive deterministic $PNEC_{CSW}$. Both co-biocides were

extremely toxic to the embryo of the marine echinoid *Anthocidaris crassispina*

(Kobayashi & Okamura 2002) (Tables S3-S4). However, when compared to embryo of

Paracentrotus lividus, which is another sensitive echinoid species from the same order

(Bellas et al. 2005, Bellas 2006), the latter was much less sensitive, with the range of

toxicity within the same level to early life stages of other zooplankton. Furthermore, *A.

crassispina* is only known to occur in Japanese rocky shores habitats (GBIF 2016), and

subsequently may not be representative for other ecosystems. Therefore, deterministic

$PNEC_{CSW}$ for DCOIT, ZnPT and CuPT were derived from other sensitive marine

species.

Probabilistic-based PNECs for Irgarol 1051 and diuron, were first derived using

the whole ecotoxicity dataset for each exposure condition, however primary producers

were more sensitive than all other ecological groups. Therefore, for the reliability of the

datasets, chronic PNECs based on refined SSDs (AF = 5) were proposed as EQS for

Irgarol 1051 and diuron, in either seawater and freshwater. Previous studies proposed a

$PNEC_{water}$ of $1.6 \times 10^{-2} \, \mu g \, l^{-1}$ (Yamada 2007), $5.8 \times 10^{-3} \, \mu g \, l^{-1}$ (NZEPA 2012), and $2.4 \times$

$10^{-2} \, \mu g \, l^{-1}$ (van Wezel & van Vlaardingen 2004) for Irgarol 1051, the latter being based

on the refined chronic SSD to only microalgae and macrophytes. In the present work,

due to the addition of more sensitive species in the datasets, more protective $EQS_{SW} =$

$1.4 \times 10^{-3} \, \mu g \, l^{-1}$ and $EQS_{FW} = 2.2 \times 10^{-4} \, \mu g \, l^{-1}$ were calculated (Table 3).

For diuron, $EQS_{SW} = 2.2 \times 10^{-2} \, \mu g \, l^{-1}$ and $EQS_{FW} = 1.6 \times 10^{-4} \, \mu g \, l^{-1}$ were

calculated. These values were respectively less and more protective than the previously

proposed $PNEC_{water}$ of $5.48 \times 10^{-3} \, \mu g \, l^{-1}$ by the New Zealand Environmental Protection

Agency (NZEPA 2012). Although the New Zealand EPA also derived the PNEC based

on SSD for primary producers, their data were based on acute toxicity datasets, so an AF = 1000 was assigned due to the extrapolation from acute to chronic. Conversely, in the present study, EQS were derived from chronic SSDs and a smaller AF = 5 was used.

For chlorothalonil, refined SSDs were performed by excluding the most tolerant ecological groups in each dataset. Benthic invertebrates were removed to derive $PNEC_{ASW}$, benthic invertebrates and macrophytes to derive $PNEC_{AFW}$, and fungi to derive $PNEC_{CFW}$, even though chlorothalonil is a fungicide. The high toxicity of chlorothalonil to freshwater fish has been reported (van Wezel & van Vlaardingen 2001; Sherrard et al. 2002). In the present review, amphibian tadpoles showed the lowest threshold values for chronic toxicity among all groups (Table S2), showing that freshwater vertebrates can be very sensitive to this antifouling co-biocide. To date, limited information is available on the mechanism of action of chlorothalonil (Gallo & Tosti 2015). It has been hypothesized that chlorothalonil binds strongly to enzymes involved in cellular respiration (Caux et al. 1996), which may explain the slight higher toxicity to animals.

EQS for chlorothalonil were based on chronic SSDs with an AF of 2, resulting in $EQS_{SW} = 8.5 \times 10^{-2}$ µg l^{-1} and $EQS_{FW} = 2.7 \times 10^{-3}$ µg l^{-1}. Conversely, the New Zealand EPA found $PNEC_{SW}$ (8.3×10^{-3} µg l^{-1}) more protective than $PNEC_{FW}$ (8.3×10^{-2} µg l^{-1}) (NZEPA 2012). However, the agency extrapolated the $PNEC_{SW}$ from the freshwater dataset (AF =10), while in the present study, a robust dataset for seawater was used (Table S2). Previous studies have derived $PNEC_{water}$ of 0.69 µg l^{-1} (Yamada 2007) and of 0.53 µg l^{-1} (van Wezel & van Vlaadinger 2004), much less protective than those proposed herein.

For dichlofluanid, probabilistic-based PNECs were performed only for the acute assays, in both seawater and freshwater. Resultant EQS_{SW} was 0.2 µg l^{-1} (AF = 20), but

this value should be used with caution as it is recommended that more chronic tests be performed with marine organisms to derive a more reliable $PNEC_{SW}$. A previous study suggested a $PNEC_{SW}$ of 2.65×10^{-2} µg l^{-1}, using the deterministic approach extrapolated from a freshwater dataset (NZEPA 2012). For freshwater, a refined SSD, without benthic invertebrates, resulted in an EQS_{FW} of 0.18 µg l^{-1} (AF = 20), similar to the previously estimated $PNEC_{FW}$ of 0.27 µg l^{-1} (NZEPA 2012). Because dichlofluanid is very unstable, van Wezel and van Vlaardingen (2004) did not calculate PNECs and recommended to use the metabolites to estimate Environmental Risk Limits (ERLs).

Fish and zooplankton showed the lowest toxicity threshold values in freshwater and seawater for Thiram, respectively. A previously reported $PNEC_{FW}$ of 0.1 µg l^{-1} (NZEPA 2012) is similar to the EQS_{FW} of 0.18 µg l^{-1} proposed in the present study. The same value is recommended for marine ecosystems (EQS_{SW} of 0.18 µg l^{-1}), considering that all tested marine species are protected by this value, estimated using an acute freshwater SSD.

The EQS for ZnPT was derived from probabilistic acute PNECs with an AF of 10, resulting in EQS_{SW} of 1.4×10^{-2} µg l^{-1} and EQS_{FW} of 7.1×10^{-2} µg l^{-1}. This was in line with a previous estimated $PNEC_{water}$ of 2.6×10^{-2} µg l^{-1} (Yamada 2007). For CuPT, EQS_{SW} was estimated from the probabilistic acute PNEC, resulting in EQS_{SW} of 1.9×10^{-2} µg l^{-1}, while EQS_{FW} was derived from the lowest chronic value resulting in EQS_{FW} = 2.4×10^{-3} µg l^{-1}. Previous $PNEC_{water}$ were found to be 2.5×10^{-2} µg l^{-1} for CuPT (Yamada 2007), and $PNEC_{SW}$ of 4.6×10^{-2} µg l^{-1} and $PNEC_{FW}$ of 0.11 µg l^{-1} for both ZnPT and CuPT, considering that similar toxicity is expected for both compounds (NZEPA 2012). In this study, although similar EQS_{SW} were derived for both pyrithione salts, the resultant CuPT EQS_{FW} was one order of magnitude lower than ZnPT EQS_{FW}. This can be explained because an AF of 100 was applied to estimate EQS_{FW} for CuPT

using the deterministic approach, which might be overprotective. In this regard, more data on chronic toxicity is needed to estimate a more reliable $PNEC_{FW}$ for CuPT.

For DCOIT, probabilistic-based PNECs for freshwater could not be derived due to lack of data. Therefore, an EQS_{FW} of 2.7×10^{-2} µg l^{-1} was estimated based on an AF of 100 assigned to the lowest acute value, corroborating with previous calculated $PNEC_{water}$ of 2.7×10^{-2} µg l^{-1} (Yamada 2007) and $PNEC_{FW}$ of 3.4×10^{-2} µg l^{-1} (NZEPA 2012). Conversely, resultant $EQS_{SW} = 6.7 \times 10^{-4}$ µg l^{-1} was based on probabilistic $PNEC_{ASW}$. The New Zealand EPA estimated DCOIT $PNEC_{SW}$ as 6.8×10^{-3} µg l^{-1}, derived from the lowest NOEC of the freshwater microalgae *F. pelliculosa* (0.34 µg l^{-1}) (NZEPA 2012). Herein, the $PNEC_{ASW}$ was derived using an acute SSD, resulting in a lower value, mainly because of the addition of recent results for a very sensitive copepod species (Table S3).

For TCMTB, previous studies have proposed $PNEC_{water}$ of 0.38 µg l^{-1} (van Wezel & Vlaardingen 2004), $PNEC_{FW}$ of 1.8×10^{-2} µg l^{-1} and $PNEC_{SW}$ of 1.8×10^{-3} µg l^{-1} (Londesborough 2005). Herein, due to a lack of data with marine species, the same EQS_{FW} of 8.6×10^{-2} µg l^{-1} was derived and extended to seawater (EQS_{SW}), since this value showed to be protective to all species tested. However, there is a clear need for chronic tests with marine zooplankton and microalgae for TCMTB.

In a previous evaluation, Mochida et al. (2012) estimated Hazardous Concentrations ($HC_5 = 0.79$ µg l^{-1} and $HC_1 = 0.17$ µg l^{-1}) for TPBP based on SSD, and attributed low risk of TPBP to the coastal area of Hiroshima Bay, where the study was conducted. In the present review, acute data for the copepod *Acartia tonsa* was added to the dataset (48 h-$LC_{50} = 0.16$ µg l^{-1}; Wendt et al. 2016), the reported most sensitive species to TPBP. Based on acute SSD, an $EQS_{SW} = 6.2 \times 10^{-3}$ µg l^{-1} was estimated, which is more protective than the values derived in the previous study. The lack of

chronic data (Table S5) calls for more conservative values to better protect the ecosystem. $PNEC_{FW}$ was not estimated due to a lack of data.

For other emerging antifoulants, such as capsaicin, nonivamide, medetomidine and tralopyril, there is a need for more ecotoxicity data (Table S5) before robust PNECs can be estimated. Despite that, an EQS_{FW} of 6.5 µg l^{-1} was estimated for medetomidine, based on the acute toxicity to the microalgae *Desmodesmus subspicatus* (72h-EC50 = 650 µg l^{-1}; ECHA 2014); and an EQS_{FW} = 2 x 10^{-3} µg l^{-1} for tralopyril, based on the chronic toxicity to *Daphnia magna* (21 d-NOEC = 0.2 µg l^{-1}; ECOTOX 2000-2017). However, more robust chronic datasets are needed to reduce uncertainties in deriving quality standards.

In summary, EQS were based on chronic toxicity whenever there was enough quality data (Table 3). The continuous release of antifouling biocides into the aquatic environment, especially seawater, may lead to chronic effects in wildlife, in which they display less severe effects (other than mortality) developed by continuous exposure to low levels of pollutants (Walker et al. 2012). EQS based on chronic ecotoxicity data were therefore preferred since they are more protective on a long-term basis. Furthermore, a probabilistic approach was preferred because SSDs consider many representative groups of the ecosystems, generating more robust PNECs and EQS. When toxicity was clearly associated with one or a few ecological groups, SSDs curves were further refined to protect the most sensitive groups of organisms (van Wezel & van Vlaardingen 2004), ensuring the protection of the whole ecosystem.

Comparative ecotoxicity

Freshwater vs. seawater

Most studies reported in literature have calculated EQS and PNECs by collating ecotoxicological results from freshwater and marine species in the same dataset (Del Signore et al. 2016). This approach is supported by the European technical guidance for ERA, which establishes criteria to use data from freshwater species to derive PNECs for seawater (ECB 2003, EC 2011). Depending on the chemical analysed and the biological group exposed, derived PNECs and EQS for a certain compound can be higher in freshwater than seawater or vice-versa. Conversely, they can be similar in both ecosystems, as has been demonstrated from SSDs resulting from different studies (Del Signore et al. 2016).

In the present study, tests performed in freshwater (FW) and seawater (SW) were analysed separately to investigate the influence of the salinity in the toxicity of the selected antifouling co-biocides. Overall, the results were not statistically different ($p > 0.05$) between FW and SW, suggesting that both datasets could be combined for an integrated analysis. In fact, the influence of dissolved salts on the toxicity of organic compounds is not very well understood and may vary depending on the group of contaminants (Wright & Welbourn 2002).

On the other hand, despite the lack of differences between the FW and SW datasets, resultant EQS for some biocides differed between SW and FW. For example, estimated EQS_{FW} for Irgarol 1051, diuron and chlorothalonil were lower than EQS_{SW} (Table 3). This might be explained by the differences in the individual toxicity value of the sensitive species in the dataset, which may shift the SSD curve to the left resulting in lower PNECs. For example, the most sensitive freshwater species to chlorothalonil (amphibian tadpoles of *Hyla cinerea* and *Rana sphenochephala*, 10 d-LOEC = 0.0164 µg l^{-1}) were one order of magnitude more sensitive than the seawater species

(microalgae *Thalassiosira pseudonana*, 96 h-NOEC = 0.57 µg l^{-1}), resulting in PNEC$_{CFW}$ more conservative than PNEC$_{CSW}$.

Conversely, EQS$_{SW}$ was lower than EQS$_{FW}$ for DCOIT, unlike previous data reported by Mochida & Fujii (2009). Herein, marine organisms showed lower acute toxicity threshold values when compared individually. The EC$_{50}$ or LC$_{50}$ for the most sensitive freshwater microalgae, zooplankton and fish were 32, 12.7 and 2.7 µg l^{-1}, respectively. For marine species, these values were 0.35, 0.02 and 20.5 µg l^{-1}, respectively (Table S3). As a result, the EQS was more sensitive to marine species, even when assigning a lower AF (10) compared to EQS$_{FW}$ (AF =100). The large variance of the datasets can be a confounding factor making the results difficult to interpret. Nevertheless, the resulting EQS suggest that marine ecosystems may be considered overall more sensitive to DCOIT.

Antifouling co-biocides

Since there was no difference between FW and SW ecotoxicity datasets, antifouling co-biocides were grouped according to their mode of action and groups were compared with regard of their toxicity. Only co-biocides for which there were both FW and SW datasets were used in the comparative analysis. Herbicides (Irgarol 1051 and diuron) were clearly the most toxic group to aquatic ecosystems ($p < 0.01$), followed by microbiocides (ZnPT and CuPT) and broad-spectrum co-biocides (DCOIT and TCMTB). The group of fungicides (chlorothalonil, dichlofluanid and thiram) was the least toxic group to aquatic organisms (Figures 2-3). As expected, herbicides were more toxic to primary producers, however it is important to highlight that Irgarol 1051 and diuron were toxic for many species at environmentally relevant concentrations (Thomas et al. 2001; Carbery et al. 2006; Ali et al. 2013; Diniz et al. 2014) (Table 4).

Fungicides tend to be more toxic to animal groups, and very toxic to seawater zooplankton and freshwater vertebrates (Table S2). Indeed, chlorothalonil was toxic for freshwater fish and amphibians in concentrations in which it has been detected across the world (Saakas et al. 2002; Lee S et al. 2011; Lee M et al. 2015) (Table 4), indicating its potential risk to water bodies. Broad-spectrum biocides and microbiocides showed similar toxicity, affecting primary producers and consumers, even at low concentrations. In Asian and European countries, DCOIT was reported in levels above thresholds to trigger toxicity to microalgae and zooplankton species (Martínez & Barceló 2001; Thomas et al. 2002; Harino et al. 2007; Tsunemasa 2013), indicating also potential risks brought by these group of co-biocides.

In the scope of the above, it can be noted that distinct biological groups respond differently according to the group of antifouling co-biocides. This is a major challenge for selecting compounds that could be applied to eliminate undesired fouling organisms, but are not very harmful to other biotic components of the ecosystem. Research performed so far has shown that different contaminants undergo various processes into water bodies, resulting in different degrees of bioavailability (Thomas & Brooks 2010) and environmental concentrations mainly in the orders of pico and nanograms per liter (Yamada 2007; Castro et al. 2011; Dafforn et al. 2011; Lee S et al. 2011). As exemplified above, such environmental concentrations are sometimes high enough to trigger sublethal and even lethal effects to sensitive groups.

Ecological groups

As discussed above, deriving PNECs and EQS using the probabilistic approach reduces uncertainties because it considers representative organisms that occupy different niches in the ecosystems, generating more realistic results which requires lower AF. Most of the published studies on SSD categorized organisms into three groups, algae (primary

producer), crustacean (primary consumer) and fish (secondary consumer) (ie DeLorenzo & Fulton 2012; Mochida et al. 2012). However, other ecological groups play crucial roles in ecosystems. In this sense, the European TG for deriving EQS requires at least 8 taxonomic groups to perform SSD for aquatic ecosystems (EC 2011). Still, they are categorized according to their taxonomic classification and not by their ecological group, as has been done in the present work.

Herein, the test species were grouped according to the expected sensitivity in different life stages, along with their representativeness. Thus, zooplankton and benthic invertebrates were treated as two different ecological groups, even if they belong to the same taxonomic group. For example, embryo and larvae of oyster species were included in the zooplankton group, whilst settled adults were treated as benthic invertebrates. Life stage is one of the most important biotic parameter that can influence toxicity, since organisms are usually more sensitive to chemicals during their early life stages (Grosell et al. 2002, Mohammed 2013). Crustaceans and mollusks, for example, have different sensitivities to pollutants throughout their ontogeny (Mohammed 2013). Indeed, zooplankton were more sensitive than benthic invertebrates to all antifoulants analysed in the present study (Figures S1-S10 in Supplementary Information), with the early life stages of cladocerans, copepods, mysids, bivalves and sea urchins the taxa having the lowest toxicity trigging values. Moreover, estuarine-dependent and estuarine-opportunist species tend to inhabit areas usually in close proximity to sources of antifoulants, during their initial life stages (DeLorenzo & Fulton 2012). This reinforces the importance of treating organisms in their zooplankton phase with great attention.

It is known that fish also exhibit different sensitivities along their ontogeny, with the early life stages usually being the most sensitive (Gagnon & Rawson 2009).

However, they were grouped together in the present study, because most of published studies did not provide the information on the life stage tested. Publications lacking in information on the life stage of the test organism may either over or underestimate the actual toxicity of pollutants. Thereby care should be taken when reporting ecotoxicity data, especially when standard guidelines are not followed.

In freshwater systems, tadpoles of amphibians were very sensitive to antifouling co-biocides. However, data on amphibian toxicity are available only for chlorothalonil, diuron and thiram. Nearly one-third of the amphibian species are currently threatened with extinction, and environmental pollution, such as the use of pesticides, is known to be one of the threats to amphibian biodiversity (Alza et al. 2016). Although the use of antifoulants is more often associated with estuaries and seas, they are also used to some extent in freshwater bodies (Arai et al. 2009). Therefore, it is important to elucidate how amphibians respond to exposure to other antifouling co-biocides.

The ecological groups used in the present study included representative organisms of many trophic levels and niches of aquatic ecosystems, encompassing 101 marine and 132 freshwater species (Tables S6-S7 in Supplementary Information). Out of these, only 21 freshwater and 16 marine species were evaluated according to standardised ecotoxicity tests, showing a limited number of standard test species. Despite that, the use of results from non-standardised test species to derive EQS must be carefully considered. Herein, the data quality was assessed using the SciRAP tool, and only reliable assays were included (Moermond et al. 2016).

Comparing EQSs based on standard species only (Tables S8-S9 in Supplementary Information) with EQSs for the whole datasets (standard + non-standard species; Table 3) resulted in low differences (< 10) in most cases. Thus, the use of non-standard species does not necessarily reduce the reliability of toxicity test data, if

performed under adequate and reliable conditions. Exceptions were EQS$_{SW}$ for chlorothalonil, ZnPT and thiram, and EQS$_{FW}$ for TCMTB and diuron, which were 50, 15, 78, 25 and 16 times, respectively, more protective when datasets were based on standard species only. These differences may be explained because EQS for standard species were calculated from deterministic PNECs with the application of higher AFs, which represents a certain overestimation of the toxicity. The addition of non-standard species to the datasets, on the other hand, greatly increased the number of ecological groups allowing the calculation of probabilistic-based PNECs using much lower AFs for many of the antifoulants analysed.

Furthermore, most standard species are not as widely distributed or as ecologically representative as they should be (GBIF 2016). For example, zooplankton were very sensitive to fungicides, but all standardised test species of freshwater zooplankton originate from North America and Europe (OECD 2004; ISO 2008, 2012; GBIF 2016). Hence, deriving EQS$_{FW}$ based on these species might not be a good strategy to protect aquatic ecosystems in South America, Africa or Oceania, where distinct physicochemical properties of water bodies may result in different toxicity to native zooplankton. Subsequently species from these different geographical locations may exhibit different sensitivities than standard species to antifoulants. In this sense, the suitability of using non-standard species is of great importance for deriving site-specific EQS, in which locally abundant species can greatly aid on a case-by-case basis.

Implications for Environmental Risk Assessment (ERA)

Risk is the probability of harmful effects to human health or to ecological systems resulting from exposure to an environmental stressor (U.S.EPA 1992). Thereby, ERA is an essential tool to estimate the risks associated with the release of antifoulants into aquatic ecosystems, since ERAs consider both the exposure scenario and the hazard to

aquatic wildlife. Importantly, industry have been required to perform ERAs to register or reregister biocidal products (including antifoulants) in many countries, especially in Europe, North America and Oceania (U.S.EPA 1998; EU 2012; NZEPA 2013).

Environmental exposure scenarios to the antifouling co-biocides was not evaluated in the present study, except for freshwater/seawater conditions. However, whenever checking the reliability of the ecotoxicity reports, it was noted that not all authors provide information about the exposure duration and how often the exposure medium is renewed during the test. Describing such information is imperative since half-lives of chemicals are determined by the rate in which their transformation processes in water occur, which in turn depends on the geochemical and physicochemical properties of the ecosystem (Wright & Welbourn 2002; Walker 2009). In this sense, water quality parameters such as temperature, dissolved organic carbon and pH should also be clearly described due to the direct influence on the bioavailability and toxicity of contaminants (Wright & Welbourn 2002).

Following the global ban of TBT, the use of alternative antifouling co-biocides has increased throughout the world, despite there being limited knowledge about the potential deleterious effects associated to their use. Assessing risks in complex ecosystems is a difficult task, and there are still many gaps to be filled. Considering that the behavior and effects of most single chemicals are still not fully understood, uncovering the overlapping effects of many antifoulants along with several other types of contaminants is an even harder task (van Gestel et al. 2010). In addition, quite specific uptake routes must be considered for antifouling biocides, such as the ingestion of paint particles, which may be aggravated by the improper use and removal of antifouling paints (Thomas & Brooks 2010, Soroldoni et al. 2017). On top of that, there

are many uncertainties of extrapolating laboratory observations to real ecosystems (Chapmen 1995; Lombardo et al. 2015; Forbes & Galic 2016).

Nevertheless, deriving reliable PNECs and EQS are of utmost importance to protect wildlife and ensure ecological equilibrium in aquatic ecosystems (EC 2011). Regulatory science has been increasingly refined towards the protection of ecosystems, and ERAs have been helpful to identify acceptable risks of contaminants (ECHA 2015). Considering that some antifouling co-biocides have been found in nature at concentrations above PNECs/EQS, adverse effects may take place in the aquatic ecosystems. Thus, some protective measures have been implemented by authorities to reduce the potential risks in some countries (Tornero & Hanke 2016).

Irgarol 1051 and diuron have been detected in water and sediment samples worldwide (Thomas & Brooks 2010; Dafforn et al. 2011). They are among the most persistent co-biocides (Thomas et al. 2002, 2003), increasing the risk to ecosystems. Previous risk assessments have identified Irgarol 1051 and its metabolite M1 as hazardous to coastal waters, specifically marinas and fishing harbours (Yamada 2007; Fernandez & Gardinali 2016). Governments from Europe, Asia, North America and Oceania have already restricted or forbidden the use of Irgarol 1051 and/or diuron (Bannink 2004; Cresswel et al. 2006; DEPA 2008; Thomas 2009; Dafforn et al. 2011).

The current European legislation includes diuron as a priority substance in the water framework directives and establishes Annual Average EQS (AA-EQS) of 0.2 µg l^{-1} and Maximum Allowable Concentration EQS (MAC-EQS) of 1.8 µg l^{-1} for this herbicide (Directive 2013/39/EU) (OJEU 2013). However, the estimated EQS in the present study showed that the autotrophic community is affected by concentrations below that, as previously reported by Sjollema et al. (2014), who concluded that

selected microalgal species are not protected by the current legislation and suggested the continuation of monitoring programs for diuron.

The fungicides thiram and chlorothalonil are not registered for use as antifoulants in European countries, but are authorized in Australia, New Zealand (thiram only) and some Asian countries (Thomas 2009; Dafforn et al. 2011; NZEPA 2013). Chlorothalonil has restricted use in Canada, with an established water quality criteria (WQCs) for fresh ($0.18\ \mu g\ l^{-1}$) and marine waters ($0.36\ \mu g\ l^{-1}$) (CCME 1999). On the other hand, dichlofluanid has been regarded as a low-risk antifouling co-biocide, being approved in Oceania, Asian countries, UK and European Union (NZEPA 2013; APVMA 2017; OJEU 2017). In the present review, similar EQS values were attributed to thiram and dichlofluanid, which were higher than the EQS for chlorothalonil (Table S2). In addition to toxicity endpoints, the exposure scenario should also be considered before concluding on the ERA. Since dichlofluanid is very unstable in the environment (Hamwijk et al. 2005), it is unlikely to occur at toxic levels in the ecosystems (Table 4).

The broad-spectrum antifoulant DCOIT is highly toxic to zooplankton and microalgae (Table S3). However, the rapid biodegradation and adsorption to sediment effectively limit its concentration to levels below toxic thresholds (Jacobson & Willingham 2000), resulting in a low risk to the environment. However, some authors have concluded that hazardous impacts of DCOIT might appear in areas where boats are moored due to continuous inputs (Madsen et al. 2000; Yamada 2007; Chen & Lam, 2017), and should be given priority for further work (Mochida et al. 2015). Indeed, despite the relatively low DCOIT EQS_{SW} of 6.7×10^{-4} and EQS_{FW} of $2.7 \times 10^{-2}\ \mu g\ L^{-1}$, especially for seawater, aquatic ecosystems are under risk since concentrations above these thresholds have already been measured in the environment (Table 4). Even so, the use of DCOIT in paint formulations is authorized in many countries, including

Australia, Japan, China, UK and the European Union (NZEPA 2013; OJEU 2014; APVMA 2017). DCOIT is under review in the U.S. (U.S. 2014).

For the microbiocidal pyrithione salts, Madsen et al. (2000) indicated a risk of chronic effects of ZnPT from pleasure crafts in Denmark, although the risk is low from seaborne vessels. ZnPT is pending approval in the European Union, while CuPT is already approved (OJEU 2015). Japan, Hong Kong, China, Australia and New Zealand authorize the use of both pyrithione salts in antifouling paint formulations (NZEPA 2013; APVMA 2017).

Emerging compounds with regional markets have also been risk assessed. Mochida et al. (2012) concluded that TPBP posed a low risk to the Hiroshima Bay (Japan), suggesting an HC_1 of 0.17 µg l^{-1} (estimated from SSD) as a cutoff value. Herein, a more protective EQS_{SW} of 6.2 x 10^{-3} µg L^{-1} was suggested, considering that only two reports of chronic toxicity for TPBP are available. Thus, more chronic studies are necessary to provide more reliable PNECs/EQS. Yamada (2007) also recommended further studies before evaluating the hazardous impacts of TPBP.

For medetomidine, Wendt et al. (2013) concluded that this antifoulant poses a low risk to the macroalgae *Ulva lactuta* because the maximum predicted environmental concentration (PEC) is low (0.057 µg l^{-1}, Ohlauson et al. 2012). Conversely, the EC_{10} for egg production of the copepod *A. tonsa* was reported as 0.16 µg l^{-1} (Wendt et al. 2016), suggesting that medetomidine might pose a risk to the marine environment, depending on the exposure conditions. Since medetomidine is an octopamine-receptor agonist, mimicking the action of this neurotransmitter (Lind et al. 2010), animals are expected to be more sensitive than algae and plants. This reinforces the importance of understanding the response of a pollutant to a wide range of ecological groups. Hilvarsson et al. (2007) indicated medetomidine as a promising candidate for use as a

safer antifouling biocide, but stated that its actual risk to the environment would not be sufficiently understood until its leaching and degradation rates in the environment are better known. Risks can be reduced using paints with controlled leaching to minimise concentrations in the marine environment, and consequently their effects on non-target organisms (Krång & Dahlström 2006).

Wang et al. (2014) predicted the environmental risk of capsaicin, a natural compound extracted from chili peppers, and concluded that this antifoulant poses a relatively low risk to marine environments. This is because it undergoes rapid biodegradation, has a low potential for bioconcentration and is present in the environment at concentrations below toxic thresholds. Similarly, in a preliminary ERA of nonivamide, Liu et al. (2016) found a low risk of this antifoulant to marine microalgal communities due to its easy and rapid degradation. However, limited data on the ecotoxicity of capsaicin and nonivamide is currently available (Table S5).

In summary, the regulation of antifouling co-biocides is within the environmental policies of some regions, especially Oceania, some Asian countries and Europe. ERAs have been used to make final decisions to register, restrict, revoke or ban the application of the compounds, based on acceptable risks or otherwise. Conversely, there are many countries with no regulations addressing the issue of antifouling biocides in natural environments. South America, for example, has a large coast line facing both Atlantic and Pacific Oceans, which shelter a great biodiversity of aquatic life (MMA 2004). However, to our knowledge there is no regulation concerning antifouling biocides, except for the ban of organotin paints (IMO 2000).

In the present study, ecological effects of the selected antifouling co-biocides were assessed, bringing important contributions towards the refinement of hazard assessments: (1) ecotoxicity datasets were updated (Tables S1-S5 in Supplementary

Information); (2) EQS were mostly derived from probabilistic-based PNECs, allowing the reduction of the size of AF and ensuring protection to ecosystems in their entirety (Tables 2-3); (3) it was shown that freshwater and seawater are overall equally sensitive to the analysed antifouling co-biocides; and (4) the use of non-standardised species was supported since they resulted in similar EQSs when compared to standardised species only (Tables 3, S8-S9 in Supplementary Information).

In this sense, the present study determined reliable EQSs for many antifouling co-biocides that have been applied worldwide, using a methodology with fewer uncertainties, and accounting for important variables that may influence toxicity. Therefore, the present work can better guide hazard and environmental risk assessments, serving as a benchmark to drive future directions in ERAs of antifouling co-biocides, especially in regions where no such policies are available.

Conclusions

A probabilistic method (SSD) for estimating PNECs and EQS is preferred over the simple use of assessment factors (AFs), because SSDs account for different representative ecological groups of an aquatic ecosystem, reducing uncertainties and the need of assigning large AFs. However, there are still insufficient ecotoxicity data to construct reliable SSD curves for many antifoulants, thus requiring the application of overprotective AFs. In this regard, effort should be made to increase reliable chronic ecotoxicity datasets for certain antifouling co-biocides, using either standardised or non-standardised representative species, to estimate more accurate PNECs and EQS.

Among the ecological groups tested to date, algae, zooplankton, fish and amphibians were the most sensitive and are therefore very important for deriving more realistic PNECs and EQSs. Zooplankton were much more sensitive than benthic

invertebrates, highlighting the advantage of categorizing groups for SSDs according to their ecology instead of taxonomic classification.

The derived EQS$_{FW}$ for Irgarol 1051, diuron and chlorothalonil, as well as the EQS$_{SW}$ for Irgarol 1051, DCOIT and TPBP, were more restrictive than previously estimated EQSs. Due to lack of data, it was not possible to estimate EQS for most of emerging antifoulants. However, even based on a few studies, it seems that some emerging compounds eg medetomidine, pose low risks to the environment, although more robust datasets are necessary for a thorough appraisal.

Overall, among the assessed antifouling co-biocides, herbicides were more toxic to the aquatic ecosystems, followed by microbiocides and broad-spectrum biocides. Fungicides were the least toxic, but still of some concern. Since many antifouling co-biocides seem to be toxic in concentrations below those already detected along water bodies, they pose a real risk to the aquatic ecosystems. Thus, the more frequently an antifoulant is used, the more likely it is to cause environmental impacts due to continuous input. Despite their widespread use, regulations on antifouling co-biocides are still restricted to a few countries. Effort should be made by national authorities to increase the number of other nations to adopt policies for regulating antifouling co-biocides based on risk assessments. In this regard, the present study brings important contributions to address hazard assessments of antifouling co-biocides, by estimating reliable EQS based on a wide range of ecological groups.

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Table and Figure Captions

Table 1. Physicochemical properties of antifouling co-biocides

Table 2. Derived PNECs to antifouling co-biocides following different approaches. Assessment factors applied are shown in brackets. Proposed EQS are bold highlighted

Table 3. Summary of the proposed EQS for different antifouling co-biocides. Values in bold (chronic) or bold-italic (acute) were derived from SSD curves. Other were extrapolated from the lowest toxicity threshold value

Table 4. Reported world maximum concentration of organic antifouling co-biocides in coastal environments

Figure 1. Workflow diagram for the present study. Dashed rectangles - used tools; light gray ellipses – compared data in each block.

Figure 2. Comparative ecotoxicity of antifoulants to marine species. Bac = Bacteria

Figure 3. Comparative ecotoxicity of biocides to freshwater species. B = Bacteria; F = Fungi