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Do TBT free-antifouling booster biocides pose a threat to the marine environment?

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Ευχαριστίες

Ολοκληρώνοντας την παρούσα πτυχιακή εργασία, θα ήθελα να ευχαριστήσω τους ανθρώπους με τους οποίους συνεργάστηκα όλο αυτό το διάστημα, για την αμέριστη βοήθεια και υποστήριξή τους. Εργάστηκα σε ένα εξαιρετικό περιβάλλον, με ανθρώπους που αποτελούν πηγή έμπνευσης. Πιο συγκεκριμένα ευχαριστώ την κ. Μαρία Κωστοπούλου-Καραντανέλλη, Αναπλ. Καθηγήτρια του Τμήματος Επιστημών της Θάλασσας του Πανεπιστημίου Αιγαίου για την ανάθεση της παρούσας εργασίας και την καθοδήγηση της μέχρι ολοκλήρωσή της, η οποία όμως κατά την παρουσίαση της πτυχιακής βρισκόταν σε εκπαιδευτική άδεια.

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Μα πιο πολύ απ' όλους ευχαριστώ την οικογένεια μου και την φίλη μου Ιωάννα για την αμέριστη συμπαράσταση τους και αγάπη που μου δείχνουν σε κάθε μου προσπάθεια.

Abstract

Aim of the study was to investigate the existing literature in order to evaluate the risk associated to the use of TBT free-antifouling booster biocides. More specifically, a preliminary investigation was carried out to assess the potential threat posed to target and non-target marine organisms by the use of nine alternative booster biocides: chlorothalonil, dichlofluanid, diuron, irgarol 1051, sea nine 211, TCMS pyridine, zinc pyrithione (ZnPT), copper pythione (CuPT), and zineb. It is well known that antifouling paints are used to protect any submerged surface from the adhesion of aquatic species, well known as the biofouling phenomena. Although fouling is a natural process, it results to serious operational, economical and safety issues. Booster biocides were introduced into the market as alternatives of TBT and the formulation of the commercial products comprises mostly percentages of close to 5% of booster biocide and $\geq 40\%$ of copper compound, the latter being added to strengthen antifouling efficacy. As copper based compounds leaching of copper from submerged surfaces is expected, thus leading to increased levels of copper (Cu^{2+}) in the marine environment. TBT free-antifouling booster biocides show to be less persistent in the marine environment while they are not characterized by endocrine disrupting properties, as was the case of TBTs. However, it should be mentioned that sea nine 211 has been recently discriminated as an endocrine disruptor indicating the need for further investigation. Research carried out focused mostly on laboratory experiments dealing with the fate and behaviour of booster biocides. Accordingly, most toxicities tests have been carried out with single compounds and effects refer mostly to acute toxicities. However, little information exists on the toxicity of booster biocides on three trophic levels (algae, crustacea, and fish) while data on chronic or sub-lethal effects are limited. Recent research (later than 2010) carried out at different parts of world and the concentrations levels reported suggest the widespread use of TBT free-antifouling paints (irgarol, diuron, metalpyrithiones, etc) in harbours and marinas. Elevated concentrations of the alternative booster biocides irgarol 1051, diuron, sea-nine 211, copper pyrithione, and their degradation metabolites are reported in coastal areas due to their widespread use on ship/recreational and fishery boats while new upcoming monitoring studies reveal their occurrence in coastal areas especially in harbors and marinas. Worldwide, there is still an increasing scientific concern over the environmental occurrence and persistence, the fate and toxicity of these booster biocides as most relevant studies focused on single biocides by neglecting i.e. mixture toxicities and any synergistic, additive or antagonistic combined effects to marine species. More precisely, risks and effects associated with the use of biocides to target and non-target organisms should be further evaluated. Finally, EU established by 2008 and 2017 respectively, that diuron and irgarol 1051 should not be used as active ingredients of antifouling paints. By bearing in mind the 'precautionary principle' in respect to the use of booster biocides, policies can be addressed for pollution mitigation and preservation of marine systems as well as for the protection of human health.

Keywords: TBT free-antifouling booster biocides, potential adverse effects, threat, marine environment

Περίληψη

Σκοπός της παρούσας εργασίας είναι η διερεύνηση της υπάρχουσας βιβλιογραφίας προκειμένου να εκτιμηθεί ο κίνδυνος που σχετίζεται με τη χρήση των νέων εναλλακτικών υφαλοχρωμάτων. Ειδικότερα, διεξήχθη προκαταρκτική έρευνα για να εκτιμηθεί η πιθανή απειλή για τους θαλάσσιους οργανισμούς-στόχους και των μη στοχευμένων από τη χρήση εννέα εναλλακτικών ενισχυμένων βιοκτόνων: chlorothalonil, dichlofluanid, diuron, irgarol 1051, sea nine 211, TCMS pyridine, zinc pyriithione (ZnPT), copper pyriithione (CuPT), and zineb. Είναι γνωστό ότι υφαλοχρώματα χρησιμοποιούνται για την προστασία οποιασδήποτε βυθισμένης επιφάνειας στο νερό από την προσκόλληση ορισμένων υδρόβιων ειδών γνωστό και ως βιοεναπόθεση. Παρόλο που είναι μια φυσική διαδικασία, οδηγεί σε σοβαρά λειτουργικά, οικονομικά και προβλήματα ασφάλειας. Τα ενισχυμένα βιοκτόνα εισήχθησαν στην αγορά ως εναλλακτική λύση μετά την απαγόρευση των TBT και η διαμόρφωση των εμπορικών προϊόντων περιλαμβάνει ως επί το πλείστον ποσοστά περίπου 5% ενισχυμένου βιοκτόνου και $\geq 40\%$ ένωση χαλκού, τα οποία προστίθενται για την ενίσχυση της αποτελεσματικότητάς τους. Ως ενώσεις με βάση το χαλκό αναμένεται η έκπλυση χαλκού από τις βυθισμένες επιφάνειες, οδηγώντας έτσι σε αυξημένα επίπεδα χαλκού (Cu^{2+}) στο θαλάσσιο περιβάλλον. Τα νέα ενισχυμένα βιοκτόνα φαίνεται να είναι λιγότερο ανθεκτικά στο θαλάσσιο περιβάλλον ενώ δεν χαρακτηρίζονται από ιδιότητες ενδοκρινικής διαταραχής, όπως στην περίπτωση των TBT. Ωστόσο, πρέπει να αναφερθεί το sea nine 211 έχει χαρακτηριστεί πρόσφατα ως ενδοκρινικός διαταράκτης, υποδεικνύοντας την ανάγκη για περαιτέρω διερεύνηση. Οι έρευνες που έγιναν επικεντρώθηκαν κυρίως σε εργαστηριακά πειράματα που αφορούσαν την τύχη και τη συμπεριφορά των ενισχυμένων βιοκτόνων. Συνεπώς, οι περισσότεροι έλεγχοι τοξικότητας έχουν διεξαχθεί με μεμονωμένες ενώσεις και τα αποτελέσματα αναφέρονται κυρίως σε οξεία τοξικότητα. Ωστόσο, υπάρχουν λίγα στοιχεία σχετικά με την τοξικότητα των ενισχυμένων βιοκτόνων σε τρία τροφικά επίπεδα (άλγη, καρκινοειδή και ψάρια), ενώ τα δεδομένα σχετικά με τις χρόνιες ή υποθανατηφόρες επιπτώσεις είναι περιορισμένα. Από πρόσφατες έρευνες (μετά το 2010) που πραγματοποιήθηκαν σε διάφορα μέρη του κόσμου, τα επίπεδα συγκεντρώσεων που αναφέρθηκαν υποδεικνύουν την ευρεία χρήση των νέων ενισχυμένων βιοκτόνων (irgarol, diuron, metalpyriithiones, κ.λπ.) σε λιμάνια και μαρίνες. Οι αυξημένες συγκεντρώσεις των εναλλακτικών ενισχυμένων βιοκτόνων irgarol 1051, diuron, sea nine 211, copper pyriithione, και των μεταβολιτών τους αναφέρονται σε παράκτιες περιοχές λόγω της ευρείας χρήσης τους σε πλοία / σκάφη αναψυχής και αλιείας, ενώ νέες επικείμενες μελέτες παρακολούθησης αποκαλύπτουν την εμφάνισή τους σε παράκτιες περιοχές, ιδίως στα λιμάνια και τις μαρίνες. Σε παγκόσμια κλίμακα εξακολουθεί να υπάρχει μια αυξανόμενη επιστημονική ανησυχία σχετικά με την περιβαλλοντική παρουσία και την παραμονή, την τύχη και την τοξικότητα αυτών των ενισχυμένων βιοκτόνων, καθώς οι περισσότερες μελέτες επικεντρώθηκαν σε βιοκτόνα μεμονωμένα παραβλέποντας τις τοξικές ιδιότητες μειγμάτων και οποιωνδήποτε συνεργιστικών, προσθετικών ή ανταγωνιστικών επιδράσεων σε διάφορα θαλάσσια είδη. Πιο συγκεκριμένα, πρέπει να αξιολογηθούν περαιτέρω οι κίνδυνοι και οι επιπτώσεις που συνδέονται με τη χρήση βιοκτόνων σε στοχευμένους και μη στοχευμένους οργανισμούς. Τέλος, η ΕΕ θέσπισε, το 2008 και το 2017 αντίστοιχα, ότι το diuron και το irgarol 1051 δεν πρέπει να χρησιμοποιούνται ως ενεργά συστατικά υφαλοχρωμάτων. Λαμβάνοντας υπόψη την «αρχή της προφύλαξης» όσον αφορά τη χρήση ενισχυμένων βιοκτόνων, μπορούν να αντιμετωπιστούν οι πολιτικές για την μείωση της ρύπανσης και τη διατήρηση των θαλάσσιων συστημάτων καθώς και για την προστασία της ανθρώπινης υγείας.

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1. Introduction

Antifouling paints (AFs) are chemicals that are used constantly to prevent the natural fouling process that occurs on submerged into water surfaces. The general mechanism of AFs is the slow over time release of the chemical (biocide) from the paint matrix into the aquatic environment to avoid any settlement of organisms onto the submerged surfaces. Two main activities are seriously concerned about marine biofouling effects. The first includes ships and boats; biofouling can cause several problems i.e. great fuel consumption, low speed due to increased water resistance, erosion, etc. Another sector is that of aquaculture as fouling can decrease water exchange through net mesh, being by far the cause of severe operational and economic issues (Guardiola et al., 2012; Nikolaou et al., 2014; Cronin et al., 1999).

Organotin compounds have been the first antifouling paints used worldwide. Since the early 1970s, their use has been proven to pose a serious threat to many target (bacteria, fungi and algae) and non-target aquatic species. Research carried out showed high levels of TBT-concentrations in the water column, sediments samples as well in the tissues of marine organisms. Besides, high toxicity at very low TBT-concentrations has been recorded for molluscs, fish behaviour and reproduction, etc. (Dimitriou et al., 2003; Alzieu et al., 1986; Fent, 1996). Since the early 1990s, initiatives have been undertaken to restrict the use of tributyltin (TBT). The use of TBT on large vessels was banned by the International Convention on the Control of Harmful Anti-fouling Systems set by the International Maritime Organization (IMO, 2008). However, it should be stated that TBT use still continuous as a number of countries did not sign the relevant convention (Ansanelli et al., 2017).

Worldwide, new alternative booster biocides have been introduced into the market of antifouling paints. These are generally based on copper metal oxides and booster biocides. Irgarol 1051, diuron, and Zn pyrithione, three among the most used antifouling booster paints, are combined with metallic copper or copper compounds i.e. cuprous oxide (Cu_2O), copper thiocyanate (CuSCN). Inorganic Zn is often included in the formulation to improve leaching process (Watermann et al., 2005). Based on the international literature, the most used to-date antifouling booster biocides are chlorothalonil, dichlofluanid, diuron, irgarol 1051, sea nine 211 (DCOIT), TCMS pyridine, zinc pyrithione (ZnPT), copper pyrithione (CuPT), and zineb, (Guardiola et al., 2012; Thomas and Brooks, 2010; Yamada, 2007). Recent research carried out showed the persistence of some AFs in the marine environment; despite that they seem not to have endocrine disrupting properties, their current environmental concentrations can have severe effects on primary producers. Diuron as active ingredient in AFs has been re-examined and forbidden in some European countries (UK, Netherlands, Sweden, and Denmark) due to its adverse effects (Price and Readman, 2013). Irgarol 1051 was registered for use in vessels > 25m length while gradually was phased out in some countries. Finally, EU established by 2008 and 2017 respectively, that diuron and irgarol 1051 should not be used as active ingredients of antifouling paints (ECHA).

To evaluate the risk associated to the most used new booster biocides an investigation was undertaken by reviewing the existing international literature. Aim of this work was to assess the potential threat posed to target and non-target marine organisms by nine alternative booster biocides: chlorothalonil, dichlofluanid, diuron, irgarol 1051, sea nine 211, TCMS pyridine, zinc pyrithione (ZnPT), and copper pyrithione (CuPT), and zineb. It is well known that AFs are used to protect any submerged surface from the adhesion of some aquatic species (bacteria, fungi, algae). A key issue is their environmental fate and behavior in marine coastal systems as these can predict their persistence in the marine environment. In the marine systems, environmental concentrations of pollutants are mostly low

suggesting the presence of more than one chemicals that could have potential synergistic effects on diverse marine species. The present investigation focused on toxicity research that has been carried out on the effects exerted to marine species by these alternative booster biocides. Based on these, a first assessment will be indicative of the effects on target and non-target marine species that are reported in the international literature and the eventual need for further research to be carried out.

1.1 Environmental fate and behavior

Any chemical released into the marine environment is subjected to a set of processes that are usually complex and multivariable. Booster biocides can undergo several processes which include transport and partition mechanisms, chemical and biotic transformation/degradation, and bioaccumulation.

The behaviour of biocides in the marine environment is closely related to their physicochemical properties (Table 1) as well as to a number of environmental parameters and the conditions prevailing in the study area. Among the biocides considered, diuron presents the higher water solubility (35.6 mg L⁻¹), while sea nine 211 (DCOIT), zineb, zinc-pyrithione and irgarol 1051 are characterized by lower solubilities being respectively 14 mg L⁻¹, 10 mg L⁻¹, 8 mg L⁻¹ and 7 mg L⁻¹. Solubility values lower than 1 mg L⁻¹ are reported in the literature for chlorothalonil, dichlofluanid and TCMS-pyridine. For the considered biocides, logKow values reported in the literature range from 0.8 to 4.0 with the lowest for zineb and zinc pyrithione and the highest for chlorothalonil. The low solubility in water suggests that the corresponding biocides may not bioaccumulate eventually in aquatic organisms. Based on the estimated logK_{OC} zineb and zinc pyrithione present the lowest values suggesting that they will be mostly associated to the dissolved phase. For all others it is suggested that they will exist both in seawater and sediments.

Table 1. Physicochemical properties of biocides, (Thomas and Brooks, 2010; Yamada, 2007).

Biocide	CAS No	Solubility (mg L ⁻¹)	Log Kow	Estimated LogK _{OC}
Chlorothalonil	1897-45-6	0.6-0.9	4.0	3.8
Dichlofluanid	1085-98-9	1.3	3.7	3.5
Diuron	330-54-1	35.6	2.87	2.6
Irgarol 1051	28159-98-0	7.0	3.95	3.7
Sea nine 211	64359-81-5	14	2.8-4.2	2.6
TCMS-pyridine	13108-52-6	< 1	na	na
Zinc pyrithione	13463-41-7	8.0	0.9	0.7
Copper pyrithione	14915-37-8	low	na	na
Zineb	12122-67-7	10	0.8	0.6

Chlorothalonil, (2,4,5,6-tetrachloro isophthalonitrile), a broad-spectrum organochlorine fungicide used widely in agricultural and urban areas, can undergo microbial degradation in seawater demonstrating a half-life ranging from 3-10 days, while hydrolysis is considered insignificant (Caux et al., 1996). Chlorothalonil has been shown to degrade in both fresh and estuarine waters with a half-life of 4 and 150h (Davies, 1985), and 1.8 and 8 days in a natural estuarine/sediment test system

(Walker et al., 1988). A half-life of approximately 8 weeks in seawater at 25°C is reported (Callow and Finlay, 1995; Callow and Willingham, 1996). Degradation in sunlight is rapid with a photolysis half-life of 1-48h depending on water type and metabolites formed are chloro-1,3-dicyanobenzene, dichloro-1,3-dicyanobenzene, dichloro-1,3-dicyanobenzene and benzamide (Sakkas et al., 2002).

Dichlofluanid, (N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulfamide), a broad spectrum fungicide used on crops, undergoes rapidly hydrolysis in seawater (half-life ~18 h) to form N'-dimethyl-N-phenylsulfamide (DMSA). It also photodegrades under sunlight with a half-time ranging from 8-83 h, depending on the presence of dissolved organic matter (DOM), to form three main metabolites, N'-dimethyl-N-phenylsulfamide (DMSA), dichlorofluoromethane, aniline (Sakkas et al., 2001). To-date, very few data exist on the partitioning of dichlofluanid to sediments. The low water solubility of dichlofluanid (1.3 mg L⁻¹) and the high octanol/water partition (logK_{OW}= 3.7) and logK_{OC} suggest strong adsorption on particulate matter. However, the presence of dichlofluanid in marine sediments and sediments associated to paint particles is reported, and its rapid degradation (< 10h and 1.4 d respectively) to form DMSA (Thomas et al., 2002, 2003).

Diuron, (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), a residual herbicide for weeds and mosses control of non-crop and woody areas, is reported to be stable to hydrolysis and sunlight irradiation in seawater (Okamura, 2002), but less persistent in marine sediments (t_{1/2} < 14 d), (Callow and Willingham, 1996; Thomas et al., 2002). However, under aerobic conditions, diuron degrades to 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and 1-(3,4-dichlorophenyl)urea (DCPU), while in anaerobic environments it is transformed to 1-(3-chlorophenyl)-3,1-dimethylurea (CPDU), (Thomas et al., 2003; Ellis and Camper, 1982). The high solubility in water as well as logK_{OW} (2.87) and estimated K_{OC} (2.6) suggest that diuron will be mostly associated to the dissolved phase. This is confirmed in the existing literature; worldwide, high concentrations are reported for seawater, while high levels of diuron in marine sediments could be attributed to their contamination with AF paint particles (Turner, 2010). Diuron degradation when associated to AF paint particles presents a half-life of 42 d (Thomas et al., 2003).

Irgarol 1051, (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine), degradation half-time is reported between 100-350 days in seawater (Scarlett et al., 1997), and is very persistent in anaerobic sediments (Thomas et al., 2003). M1 is the main metabolite produced through dealkylation following biodegradation (Liu et al., 1999), photodegradation (Okamura et al., 1999) or chemical hydrolysis (Liu et al., 1999). Other metabolites are M2 and M3 (Lam et al., 2005). The persistence of metabolites in the environment is largely unknown (Thomas and Brooks, 2010). However, M1/GS26575 is relatively stable in water (t_{1/2} > 200 d) and sediment (t_{1/2}= 260 d), (Okamura, 2002; Thomas et al., 2002). Irgarol 1051 seems to have low affinity to particulate matter (logK_{OW}= 3.95 and logK_{OC}= 3.7), however studies report its presence in sediments (Thomas and Brooks, 2010).

Sea nine 211 or DCOIT, (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), is an effective and broad-spectrum antifouling biocide against a variety of organisms, (bacterial slime, algae, barnacles, etc). DCOIT is reported as a relatively labile compound which degrades rapidly in natural seawater (t_{1/2}=1 d) and sediment (t_{1/2}=0.04 d), (Callow and Willingham, 1996; Jacobson and Willingham, 2000; Thomas et al., 2002, 2003). Biotic degradation in natural seawater, under aerobic and anaerobic conditions, is faster than hydrolysis and photolysis and leads to the formation of N-(octyl)oxamic acid, 4,5 dichlorothiazole, and N-octyl carbamic acid. Despite its high water solubility, values of logK_{OW}= 2.8 and logK_{OC}= 2.8 suggest that the parent compound will bind strongly to marine

sediments. In this case degradation is reported as $t_{1/2} < 12$ h. As with other AFs, it is more persistent when present in sediments with paint particles (Thomas et al., 2003).

TCMS pyridine, (2,3,5,6-tetrachloro-4-methylsulfonyl pyridine), is a chemical used in textile and leather industry that has been introduced in the market of AFPs recently. No available information could be found for this biocide.

Metal pyrithiones (MePTs), zinc pyrithione (ZnPT) and copper pyrithione (CuPT) were introduced in the antifouling paints market in 1990s. Zinc pyrithione, (bis (1 hydroxy-2(1H)-pyridine thionate-O,S) zinc), is rapidly degraded in natural seawater ($t_{1/2} < 24$ h), with photolysis process ($t_{1/2} < 1$ h) being faster than hydrolysis ($t_{1/2}$ range from 96 to 120 h), (Turley et al., 2000). Biotic degradation occurs rapidly under aerobic and anaerobic conditions with a half-life reported of < 2 h. Copper pyrithione (bis (1 hydroxy-2(1H)-pyridine thionate-O,S) copper), shows a similar behaviour despite its lower solubility and higher photodegradation stability when compared to ZnPT (Grunnet and Dahlløf, 2005).

Metal pyrithiones in aquatic media undergo rapid degradation under sunlight; six metabolites [pyridine-N-oxide (PO), 2 mercaptopyridine (HPS), 2-mercaptopyridine-N-oxide (HPT), 2,2'-dithio-bis-pyridine (PS)₂, 2,2'-dithio-bis-pyridine-N-oxide (PT)₂ and pyridine-2-sulfonic acid (PSA), which is suggested to be the final product. Despite the light sensitive of MePTs, they will persist in seawater if solar penetration does not occur in depth which is usually the case in areas of high turbidity i.e. harbours. ZnPT is known to undergo trans-chelation process prior to biodegradation and/or hydrolysis, with free copper and possibly manganese ions (Turley et al., 2000). More precisely, ZnPT reacts with Cu(II) in seawater by changing to CuPT which is more stable (Grunnet and Dahlløf, 2005). It has been shown that HPT is transformed back to the parent compound in the presence of free Cu(II).

Zineb, (zinc ethylenebis-(dithiocarbamate)), is a foliar fungicide used widely in the agricultural and industrial domain. It undergoes rapid degradation by hydrolysis and photolysis ($t_{1/2} = 96$ h, pH= 10) to form 5,6-dihydro-3H-imidazo(2,1-c)-1,2,4-dithiazole-3-thione (DIDT), ethylene diisothiocyanate (EDI) and ethylenethiourea (ETU) (Hunter and Evans, 1991).

1.2 Toxic effects of booster biocides

Antifouling paint components are the organic booster biocides and metals such as copper (Cu^{2+}) and/or zinc which act by strengthening the antifouling action. Despite that copper is a minor nutrient it may affect non-target aquatic species by becoming toxic for both plants and animals. Metal speciation is very important regarding metal bioavailability and toxicity to marine species. Copper (Cu^{2+}) in seawater tends to be bound to inorganic and organic ligands and/or particulate matter.

Phytoplankton species exhibit different sensitivities to copper toxicity; diatoms are the most resistant, coccolithophores and dinoflagellates are of intermediate sensitivity while the most sensitive are cyanobacteria. Copper is stored in bivalves, barnacles and aquatic insects while it is actively regulated in fish, decapod, crustaceans and algae. Copper causes toxicity by impairing the osmoregulation and ion regulation in the gill of numerous aquatic animals. In *Mytilus galloprovincialis*, copper interferes with Ca^{2+} gill homeostasis causing severe alterations in the Na/K ATPase and Ca^{2+} ATPase enzyme activities. Additionally, copper toxicity can be induced by generating also Reactive Oxygen Species (ROS) (Guardiola et al., 2012).

Diuron has been proven to be very toxic for the reproduction of the green freshwater alga *Scenedesmus vacuolatus*. It affects planktonic and periphytic microalgae by reducing chlorophyll a levels. It has been proven to be toxic to certain bacterial species (Guardiola et al., 2012). The growth at the early life stages of macroalgae *Ulva Intestinalis* has been proved to be significantly affected at concentrations as low as 0.010 µg/L of diuron (Girling et al., 2015), (Annex I, Table 5). Diuron shows high stability to hydrolysis and photolysis. Half-life in seawater has been reported to be higher than 30d when associated with antifouling paint particles, while when non-associated degradation occurs in about 14d. Thus, diuron seems to be persistent in seawater, suggesting that in certain areas (harbours, marinas, and touristic coastal areas) with high density of vessels and low water exchange rate, concentrations can reach high levels posing eventually a risk to aquatic life.

Irgarol 1051 toxicity effects showed decrease in alga growth, inhibition in cell number, and a decrease of photosynthetic activity, i.e. green alga *Dunaliella tertiolecta*, *Synechococcus sp.*, and *Emiliania huxleyi*. Acute effects on the growth of algae *Enteromorpha intestinalis* were determined at 10 µg L⁻¹ (Scarlett et al., 1997). The lowest EC₅₀ reported for algae is 0.450 µg L⁻¹. Irgarol 1051 appears to be especially toxic to the freshwater diatom (5 day, EC₅₀ = 136 ng L⁻¹) and the freshwater macrophytes (14 day, EC₅₀= 0.017 ng L⁻¹), (Lambert et al., 2006). Irgarol 1051 seems to have adverse effects to periphyton colonization and phytoplankton species. These effects have been seen in many different marine plants and algae, such as the eelgrass (*Zostera marina*), the brown macroalga (*Fucus serratus*), the green macroalga (*Enteromorpha intestinalis*) and the green macroalga (*Ulva intestinalis*) (Guardiola et al., 2012). Dahl and Blanck (1996) showed chronic effects on periphyton communities at concentrations 0.060-0.250 µg L⁻¹ while Okamura et al. (2000) cites the phytotoxicity of Irgarol 1051 to various seaweeds at concentration ranging from 0.6-5.9 µg L⁻¹.

According to the existing literature (not presented here), metal pyrithiones (MePTs) acute toxicity is reported to vary from 0.54 to 310 µg L⁻¹ depending on the marine algae tested and accordingly the no-observed effect concentrations (NOECs) ranged from 0.20 to 3.2 µg L⁻¹ indicating the high sensitivity of the tested marine algae. Onduka et al., (2010) investigated the acute toxicity of ZnPT and CuPT on the marine algae *S. costatum*, *D. tertiolecta*, *T. tetrathele* and *C. calcitrans*, (Table 2). The low 72-h EC₅₀ values indicate high toxicity while it should be noticed that CuPT is slightly to more toxic than ZnPT. By bearing in mind the transchelation process that could be enhanced in the presence of high concentrations of Cu(II) in seawater leading to the formation of CuPT.

Table 2. Toxicity values (72-h EC₅₀) and no-observed effect concentrations (NOECs) of MePTs on marine algae *S. costatum*, *D. tertiolecta*, *T. tetrathele* and *C. calcitrans*.

MePT	<i>S. costatum</i>	<i>C. calcitrans</i>	<i>D. tertiolecta</i>	<i>T. tetrathele</i>
	72-h EC ₅₀ (NOEC), (µg L ⁻¹)			
CuPT	1.5 (0.70)	3.2 (2.0)	7.3 (3.5)	12 (5.0)
ZnPT	1.6 (1.0)	4.5 (2.8)	8.0 (5.0)	19 (10)

Macroalgae are important organisms in coastal zones, where they serve as primary producers and sheltered rocky shore habitat modifiers (Stagnol et al., 2013). Using the developmental stages (germlings) of macroalgae to determine harmful concentrations of aquatic pollutants could be an extremely useful tool for coastal monitoring

Antifouling paints are designed to affect efficiently in time the target marine species that usually adhere on the submerged surfaces of ships and vessels. However, the best situation would be that non-target marine species would not be affected, which would be the ideal scenario. Research carried out since 1990s shows that even the TBT free-antifouling booster biocides raise environmental concerns regarding the effects on non-target marine organisms.

Irgarol 1051 presents a potential ecological risk to 95% of organisms in the water column at levels above $0.024 \mu\text{g L}^{-1}$ (Dafforn et al., 2011). Acute toxicity to crustaceans is $400 \mu\text{g L}^{-1}$ (96-h, LC50 mysid shrimp) and the 48-h EC_{50} for *Daphia magna* is $8,100 \mu\text{g L}^{-1}$, (Annex I, Table 4,5). Diuron exhibited similar toxicity with Irgarol 1051 in bioassays using *D. magna*, *Zostera capricorni* seagrass, sea urchin eggs and embryos, and suspension of cultured fish cells (Kobayashi and Okamura, 2002; Okamura et al., 2002; Macinnis and Ralph, 2003). As already mentioned, EU established by 2008 and 2017 respectively, that diuron and irgarol 1051 should not be used as active ingredients of antifouling paints (ECHA).

Chlorothalonil showed to be toxic to eastern oysters with an EC_{50} value of $7.3 \mu\text{g L}^{-1}$ (Voulvoulis et al., 2000). According to OECD classification guidelines chlorothalonil has been classified as “very toxic” as acute toxicities are significant to crustacean and fish species (Yamada et al., 2007). Chlorothalonil can accumulate in fish tissue while it can be acutely toxic depending on the fish species with 96h-LC50 ranging from 8.2 to $76 \mu\text{g L}^{-1}$ (Davies and White, 1985). For dichlofluanid few studies have investigated its toxic effects, such as embryotoxicity in sea urchin, and *Glyptodidaris crenularis* (Guardiola et al., 2012).

Sea nine 211 concentrations of 32 and 100 nM are able to exert a long-term effect on natural phytoplankton communities (Larsen et al., 2003). The maximum environmental concentration that causes adverse effects to non-target species has previously been established to be $0.063 \mu\text{g L}^{-1}$ (Shade et al., 1994 cited in Konstantinou and Albanis, 2004). Even low concentrations of sea nine 211 may be sufficient to harm sea urchins (Kobayashi and Okamura, 2002) and algae (Fernandez-Alba et al., 2002). A wide number of studies investigated the toxicity, and effects of sea nine 211 on marine species, demonstrating the larval mortality in crustaceans, the embryolarva immobility and embryotoxicity in molluscs, the embryotoxicity in echinoderms, and the embryotoxicity and inhibition of larval settlement in tunicates and mortality in teleosts (Guardiola et al., 2012). According to Mochida et al., (2015) the ecological risk in the case of the sediments was difficult to evaluate because sediment toxicity data were available for only one species, the polychaete *Perinereis nuntia* (Onduka et al., 2013). The 14-d LC50 and 14-d NOEC of growth as end points are reported to be 110 mg kg^{-1} dry weight and 9.7 mg kg^{-1} dry weight, respectively. Finally, the calculated EPAF (Environmental Potential Accumulation Factor) suggests that approximately up to a maximum of 0.45% of marine species are influenced by the toxicity of sea nine 211. It showed stronger acute toxicities to phytoplankton and fish species than to bivalves. Its chronic toxicity is equivalent to the toxicity of other antifouling compounds and it is considered "very toxic-toxic" by the OECD (Yamada et al., 2007).

Sea nine 211 acute toxicities have been recorded in diverse species across different trophic levels (bacteria, phytoplankton, zooplankton, teleosts). Results showed that sea nine 211 is the most toxic among alternative booster biocides with toxicities comparable or higher than organotin (Fernández-Alba et al., 2001; Kobayashi and Okamura, 2002; Bellas, 2006; Fernández-Alba et al., 2002; Tsunemasa and Okamura, 2011). After the proteomic analysis of DCOIT-treated brain tissues from *O. melastigma* results showed that sea nine 211 mainly triggers the differential expressions of proteins associated with mitogen-activated protein kinase (MAPK) signalling pathway in males. Also results

showed that sea nine 211 effects was mainly manifested as DNA and RNA processing, metabolic process, and cytoskeletal assembly in female brains (Chen et al., 2014a).

Disruption of the endocrine system and impairment of the reproductive performance can be the results of exposure to sea nine 211 at environmentally worst-case concentrations. Unlike the other booster biocides sea nine 211 has been determined as an endocrine disruptor in Chen et al., (2014b, 2015b, 2016). It has been found that moderate estrogenic activity is demonstrated in both sexes of marine medaka (*O. melastigma*) in characteristic of inducing vitellogenin (VTG) and eggshell proteins, both being sensitive indicator of endocrine disruptors. The very high toxicity to non-target marine organisms, the accumulation of sea nine 211 in environmental matrices will pose a threat to the health of marine organisms and thus result in non-negligible threats to the safety of marine ecosystem. As a lesson from the incidents of organotin, there is an urgency to comprehensively evaluate the environmental risks of sea nine 211 to local ecosystems (Chen and Lam, 2017).

Zinc pyrithione (ZnPT) was found more toxic than irgarol 1051, diuron and sea nine 211 by using suspension of cultured fish cells, and sea urchin eggs and embryos (Kobayashi and Okamura, 2002; Okamura et al., 2002). ZnPT can cause teratogenic effects, such as spinal cord deformities in embryos and on the larvae of zebra fish (*Danio rerio*) at very low-sublethal concentrations. It has also been found to be toxic to the Japanese medaka fish (*Oryzias latipes*). Acute toxicity of CuPT to fish species is greater than that of ZnPT. Onduka et al., (2010) reported that CuPT exhibited toxicity to non-target organisms of different trophic levels; algae *Skeletonema costatum* (72-h EC₅₀=1.5 µg L⁻¹), crustacean *Tigriopus japonicus* (24-h EC₅₀=23 µg L⁻¹), fish *Pagrus major* (96-h LC₅₀=9.3 µg L⁻¹). Bao et al., (2014) have reported acute toxicity and synergistic effects of CuPT and Cu on the marine copepod *Tigriopus japonicus*. Bao et al., (2012) reported the acute toxicity of CuPT and ZnPT to the marine amphipod *Elasmopus rapax* where the 96-h median LC₅₀ were 11.5 and 21.5 µg L⁻¹ respectively. Both are considered to be highly toxic to aquatic organisms and they are classified as “very toxic” by the OECD (Yamada et al., 2007). Their toxicity decreased as degradation process occurred indicating that metabolites were less toxic than the parent compound. Furthermore, both compounds might exhibit high chronic toxicity to aquatic organisms, mostly species.

The resuspension of coastal sediments contaminated with APPs could produce deleterious effects on planktonic (and/or pelagic) local biota. Relevant studies have shown toxic effects of elutriate from sediment contaminated with APPs on different organisms, including: i) a decrease in growth of the green macroalga *Ulva intestinalis* in the presence of irgarol 1051 derived from APPs, and ii) bioluminescence inhibition in *Vibrio fischeri*, growth inhibition in *Ceramium tenuicorne*, and larval development decrease in *Nitocraspinipes* in the presence of Cu and Zn from APPs (Soroldoni et al., 2017). Soroldoni et al., (2017) reported that sediments spiked with antifouling painting particles (APPs) presented high toxicity to the epibenthic copepod *Nitokra sp.*, reducing fecundity and causing mortality. In sediments spiked with lower levels of APPs (0.01% by mass of sediment), a significant decrease in *Nitokra sp.* fecundity was observed. Toxicity recorded may be attributed to the presence of metals such as Cu and Zn which were detected in the tested sediments. A 36% reduction in *Nitokra sp.* fecundity was observed at 30 µg g⁻¹ and 6.9µg g⁻¹ of Cu and Zn in the tested sediments, respectively.

Ytreberg et al., (2010) showed that on the adult crustacean *N. Spinipes* Zn appears to be more toxic than Cu, presenting a 96-h LC₅₀ of 900 µg Zn L⁻¹, while for Cu 96-h LC₅₀ is 2,100 µg Cu L⁻¹. Although, Wang et al., (2011) found a 96-h LC₅₀ of 350 µg Cu L⁻¹ whereas for Zn no effect in survival was observed in concentrations up to 500 µg L⁻¹. Binary mixture of Cu-Zn produced either additive effects or synergism toxicity effect in *Daphnia magna*. APPs may contain high levels of toxic

metals and organic compounds that may be released into sediments and the water column, acting as a local source of metal and organic contaminants and, consequently, affecting epibenthic organisms. During recent decades, it has been observed that chemicals never occur alone in the marine environment, but mostly as complex mixtures at various concentrations. Synergistic effects of two or more chemicals could be exerted to biota even if each chemical has been tested and proven to be not potentially toxic to aquatic species. Research carried out to AFs showed that some of these chemicals do not pose a risk when acting separately, while when they co-exist or in the presence of other pollutants (heavy metals), they can exert toxic effects.

Koutsaftis and Aoyama (2006) investigated the toxic effects on the marine algae *Chaetoceros gracilis* of three common antifouling biocides, irgarol 1051 diuron and Zn-pyrithione (ZnPT). Inhibition concentration (IC50) at 72h of the combined biocides showed synergistic effects when compared to the corresponding IC50 values of each biocide separately.

Table 3. Inhibition concentration (IC50) at 72h of irgarol 1051 diuron and Zn-pyrithione and the combined biocides.

Biocide	72-h IC50 ($\mu\text{g L}^{-1}$)	Binary mixture	72-h IC50 ($\mu\text{g L}^{-1}$)
Diuron	36.0	Diuron-Irgarol 1051	0.10
Irgarol 1051	1.1	Diuron-Zn-pyrithione	0.44
Zn-pyrithione	3.2		0.41

The synergistic action of irgarol 1051 and diuron against *Zostera marina* has been reported by Chesworth et al., (2004). It is stated that the simultaneous use of irgarol and diuron in AFs improves dramatically the efficacy towards the settled on the surface algae as well as the non-target seagrass meadows. Irgarol 1051 was more toxic than diuron with 10-days observable EC50 values being 1.1 and 3.2 $\mu\text{g L}^{-1}$, while photosynthesis showed to be affected at significantly lower concentrations, at 0.5 and 1.1 $\mu\text{g L}^{-1}$ respectively. However, mixtures of different concentrations of these biocides showed different response depending on the ratio of biocides used during the laboratory test.

2. Alternative booster biocides: a threat to the marine environment ?

Alternative tin-free booster biocides have been introduced to the market to replace the tributyltin antifouling paints as it was necessary to overcome the natural fouling phenomena. TBT showed to be especially toxic to molluscs, with chronic toxicity in oysters and clams at fractional mg L^{-1} concentrations (Alzieu, 2000). TBTs are known to be toxic to over 100 species of gastropods; at low ng L^{-1} , TBT activates a mechanism in female gastropods which leads to the development of male reproductive organs (Fent, 1996). Induction of imposex to *Nucella lapillus* occurs at the very low levels of 0.5 ng L^{-1} of Sn while sterile effects of females occur at 3-5 ng L^{-1} of Sn. Acute toxicity of TBT to some fish species was reported at a few mg L^{-1} , while chronic toxicity at slightly higher concentrations. TBTs are poorly biodegradable ensuring their presence in the aquatic environment and their bioaccumulation.

The formulation of the commercial products comprises mostly percentages of close to 5% of booster biocide and $\geq 40\%$ of copper compound, the latter being added to strengthen antifouling efficacy. As copper based compounds are used leaching of copper from submerged surfaces is expected, thus leading to increased levels of copper (Cu^{2+}) in the marine environment. TBT free-antifouling booster biocides show to be less persistent in the marine environment while they are not characterized by endocrine disrupting properties, as was the case of TBTs. However, it should be mentioned that sea

ine 211 has been recently discriminated as an endocrine disruptor indicating the need for further investigation.

Worldwide, since the late 1980s, the new generation booster biocides irgarol 1051, diuron, sea nine 211, ZnPT and CuPT, etc have been used widely mostly in combination with Cu compounds (cuprous oxide, cuprous thiocyanate, etc) to ensure boost efficacy against fouling phenomena. Another issue related to the booster biocides use is the input of Cu to coastal areas as the percentage of Cu in the product accounts $\geq 40\%$. Therefore, potential interactions between booster biocides and Cu(II) in seawater and sediment should further investigated.

Elevated concentrations of the alternative booster biocides irgarol 1051, diuron, sea nine 211 and their degradation metabolites are reported in coastal areas due to their constant use on submerged surfaces (Konstantinou and Albanis, 2004) while new upcoming monitoring studies reveal their occurrence in coastal areas. Worldwide, there is still an increasing scientific concern over the environmental occurrence and persistence, the fate and toxicity of these booster biocides as most relevant studies focused on single biocides by neglecting i.e. mixture toxicities and any synergistic, additive or antagonistic combined effects to marine species.

Metal pyrithiones, ZnPT and CuPT are reported as highly toxic to aquatic autotrophic species i.e. microalgae and cyanobacteria (Bao et al., 2012; Onduka et al., 2010; Turley et al., 2000).

Despite the MePTs high rate of degradation, zinc pyrithione was reported to accumulate into marine sediments while it has been shown that is highly persistent when associated to AF paint particles.

In certain areas of the world (Japan, Vietnam), MePTs are the most used antifouling booster biocides cuprous oxide where concentrations in sediments ranged from $< 2 \mu\text{g kg}^{-1}$ to $420 \mu\text{g kg}^{-1}$ (Harino et al., 2007, 2006, 2005).

Research carried out on the potential toxicity of the most used AFs biocides indicates a serious threat that seems to affect many non-target species and especially plant species. According to OECD, irgarol 1051, sea nine 211, ZnPT, CuPT, chlorothalonil, dichlofluanid etc., are classified as “toxic or very toxic”. However, when compared to TBT’s they have smaller environmental impacts and they are not so stable.

Marine environmental assessment should be based on well documented and experimental information relevant to the environmental fate behavior of antifouling booster biocide and to its potential effects to marine organisms. According to the Organization for Economic Co-operation and Development (OECD, 2001) chemicals toxicity should be investigated on three trophic levels: algae, crustaceans and fish. Over the last decades, it has been observed that chemicals occur mostly as complex mixtures at various concentrations. Synergistic effects of two or more chemicals could be exerted to biota even if each chemical has been tested and proven to be not potentially toxic to aquatic species. Some chemicals do not pose a risk when acting separately, but when they co-exist or in the presence of other pollutants (heavy metals), they can exert toxic effects.

➤ Irgarol 1051 presents a potential ecological risk to 95% of organisms in the water column.

Toxicity effects:

- decrease in algal growth
- inhibition in cell number
- decrease of photosynthetic activity.

Appears to be especially toxic to the freshwater diatom and the freshwater macrophytes.

➤ Diuron exhibited similar toxicity with irgarol 1051. According to OECD classification guidelines chlorothalonil has been classified as “very toxic” (as acute toxicities are significant to crustacean and fish species).

➤ Sea nine 211 toxicity effects in marine species:

- larval mortality
- embryolarva immobility
- embryotoxicity
- inhibition of larval settlement

Sea nine 211 toxicity affects 0.45% of marine species in Hiroshima bay according to EPAF (Mochida.,2015), which shows stronger acute toxicities to phytoplankton and fish species than to bivalves. It is also considered "very toxic- toxic' by the OECD.

➤ Zinc pyrithione (ZnPT) was found to be more toxic than irgarol 1051, diuron and sea nine 211. Acute toxicity of copper pyrithione (CuPT) to fish species is greater than that of ZnPT. Both are classified as “very toxic” by the OECD and considered to be highly toxic to aquatic.

➤ APPs can act as a local source of metal and organic contaminants consequently, affecting epibenthic organisms. Sediments (spiked with antifouling painting particles) present high toxicity to the epibenthic copepod *Nitokra sp.*, reducing fecundity and causing mortality.

➤ Zn appears to be more toxic than Cu, on certain marine organisms such as the adult crustacean (*N. Spinipes*).

EU established by 2008 and 2017 respectively, that diuron and irgarol 1051 should not be used as active ingredients of antifouling paints. By bearing in mind the ‘precautionary principle’ in respect to the use of booster biocides, policies can be addressed for pollution mitigation and preservation of marine systems as well as for the protection of human health.

Risks and effects associated with the use of biocides to target and non-target organisms should be evaluated. Further research need to be carried out focusing on physicochemical properties, toxicology on three different trophic levels, also to evaluate the risk associated to the use of new booster biocides, however scientific community should be aware of the introduction of copper in the marine environment.

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Annex I

Table 4. Most used TBT free-antifouling booster biocides and their indicative effects on non-target species (in Guardiola et al., 2012).

Chemical	Application	Mode of Action	Species	Effects
Chlorothalonil	Fungicide	Mitochondrial e-transport inhibition	Crustaceans	
			<i>Cancer magister</i>	Behaviour, larval mortality
			<i>Penaeus duoramun</i>	Mortality
			Molluscs	
			<i>Crassostrea virginica</i>	Growth Embryotoxicity
			<i>Mytilus edulis</i>	Embryotoxicity Mortality
			Tunicates	
			<i>Ciona intestinalis</i>	Embryotoxicity & inh. larval settlement
			Teleosts	
			<i>Anguilla japonica</i>	Mortality
			<i>Cyprinodon variegates</i>	Mortality
			<i>Galaxias auratus</i>	Mortality
			<i>Galaxias maculatus</i>	Mortality
<i>Leiostomus xanthurus</i>	Mortality			
<i>Pseudaphritis urvillii</i>	Mortality			
Dichlofluanid	Fungicide	PS II e-transport inhibition	Echinoderms	
			<i>Glyptocidaris crenularis</i>	Embryotoxicity
			Molluscs	na
			Tunicates	na
			Teleosts	na
TCMS pyridine	Fungicide	Mitochondrial e-transport inhibition	Crustaceans	na

Chemical	Application	Mode of Action	Species	Effects
			<i>Molluscs</i>	na
			<i>Tunicates</i>	na
			<i>Botryllus schlosseri</i>	Immunotoxic
			<i>Teleosts</i>	
Zineb	Fungicide	Multi-site inhibitor (metabolic process)	<i>Crustaceans</i>	na
			<i>Molluscs</i>	na
			<i>Tunicates</i>	na
			<i>Teleosts</i>	
			<i>Salmo gairdneri</i>	Embryotoxicity
Diuron	Herbicide	PS II e-transport inhibition	<i>Crustaceans</i>	na
			<i>Molluscs</i>	na
			<i>Teleosts</i>	
			<i>Carassius auratus</i>	Acetylcholinesterase inhibition
Irgarol 1051	Herbicide	PS II e-transport inhibition	<i>Crustaceans</i>	na
			<i>Molluscs</i>	na
			<i>Teleosts</i>	
Sea nine 211	Herbicide	PS II e-transport inhibition	<i>Crustaceans</i>	
			<i>Balanus amphitrite</i>	Larve mortality
			<i>Molluscs</i>	
			<i>Crassostrea virginica</i>	Embryo-larva immobility
			<i>Mytilus edulis</i>	Embryo-larva immobility & embryotoxicity
			<i>Echinoderms</i>	
			<i>Hemicentrotus pulcherrimus</i>	Embryotoxicity
			<i>Anthocidaris crassispina</i>	Embryotoxicity
			<i>Tunicates</i>	

Chemical	Application	Mode of Action	Species	Effects
CuPT	Microbiocide	Multi-site inhibitor (metabolic process)	<i>Ciona intestinalis</i>	Embryotoxicity & inh. larval settlement
			Teleosts	
			<i>Cyprinodon variegates</i>	Mortality
			Crustaceans	
			<i>Artemia salina</i>	Inh. Na/K ATPase & Mg(II) ATPase enzyme activities
			Molluscs	
			<i>Mytilus galloprovincialis</i>	Alterations Na/K ATPase & Ca(II) ATPase enzyme activities
			Teleosts	
			<i>Fundulus heteroclitus</i>	Alterations gill osmoregulation
			<i>Oncorhynchus kisutch</i>	Alterations gill osmoregulation
ZnPT	Microbiocide	Multi-site inhibitor (metabolic process)	Crustaceans	na
			Molluscs	na
			Tunicates	na
			Teleosts	
			<i>Oryzias latipes</i>	Embryotoxicity
			<i>Danio rerio</i>	Embryo-larva

Table 5. Toxic effects of booster biocides on target and non-target species according to the reviewed literature.

	Booster Biocides	Toxic effects	Marine species
	Diuron	acute toxicity	<i>Daphia magna</i> , <i>Zostera capricorni</i>
	Chlorothalonil	accumulates in fish tissue	
	Dichlofluanid	embryotoxicity	<i>Glyptocidaris crenularis</i>
Non-target species	Sea-Nine 211	larval mortality embryolarva immobility and embryotoxicity embryotoxicity and inhibition of larval settlement embryotoxicity mortality	crustaceans molluscs tunicates echinoderms teleosts
	Zinc Pyrithione	teratogenic effects (spinal cord deformities in embryos and larvae) toxic toxic toxic toxic	<i>Danio rerio</i> <i>Oryzias latipes</i> <i>Skeletonema costatum</i> <i>Tigriopus japonicus</i> <i>Pagrus major</i>
	CuPT and Cu	acute toxicity	<i>Tigriopus japonicus</i>
	CuPT and ZnPT	acute toxicity	<i>Elasmopus rapax</i>
	APPs (Irgarol 1051)	decreases growth	<i>Ulva intestinalis</i>
	APPs (Cu and Zn)	bioluminescence inhibition	in <i>Vibrio fischeri</i> ,

	growth inhibition larval development decrease	<i>Ceramium tenuicorne</i> , <i>Nitocra spinipes</i>
APPs	reduces fecundity, mortality	<i>Nitokra sp.</i> ,
Cu-Zn	additive effects or synergism toxicity effect	<i>Daphnia magna</i>
Irgarol 1051-Diuron	synergistic action	<i>Zostera marina</i>
	Booster Biocides	Toxic effects
	Booster Biocides	Marine species
	Diuron	reduces chlorophyll a levels affects growth
		<i>Scenedesmus vacuolatus</i> <i>Ulva Intestinalis</i>
Target species	Irgarol 1051	algal growth/ inhibition in cell number/ decrease of photosynthetic activity alga growth
		<i>Dunaliella tertiolecta</i> , <i>Synechococcus sp.</i> , <i>Emiliania huxleyi</i> . <i>Enteromorpha intestinalis</i>
		adverse effects to periphyton colonization and phytoplankton species
		<i>Zostera marina</i> , <i>Fucus serratus</i> , <i>Enteromorpha intestinalis</i> , <i>Ulva intestinalis</i>

