Booster Biocides Levels in the Major Blood Cockle (Tegillarca granosa L., 1758) Cultivation Areas along the Coastal Area of Peninsular Malaysia

Aqilah Mukhtar 1, Syaizwan Zahmir Zulkifli 1,2,*, Ferdaus Mohamat-Yusuff 2,3, Hiroya Harino 4, Mohammad Noor Amal Azmai 1 and Ahmad Ismail 1

1 Department of Biology, Faculty of Science, Universiti Putra Malaysia, Serdang 43400 UPM, Selangor, Malaysia; aqilahmukhtar90@gmail.com (A.M.); mnamal@upm.edu.my (M.N.A.A.); aismail@upm.edu.my (A.I.)
2 International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, Lot 960, Jalan Kemang 6, Port Dickson 71050, Negeri Sembilan, Malaysia; ferdius@upm.edu.my
3 Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, Serdang 43400 UPM, Selangor, Malaysia
4 Department of Human Sciences, Kobe College, Nishinomiya, Hyogo 662-8505, Japan; harino@mail.kobe-c.ac.jp

* Correspondence: syaizwan@upm.edu.my; Tel.: +60-3-9769-6614

Received: 2 April 2020; Accepted: 18 May 2020; Published: 5 June 2020

Abstract: Booster biocides have been rapidly growing in use, mainly in the shipping industry and in agricultural activities. The use of booster biocides is known to cause adverse effects on marine ecosystems, such as by inhibiting the photosynthesis process in marine plants, and they have the potential to accumulate in marine organisms. In the present study, booster biocides of Irgarol 1051, diuron, 3,4-dichloroaniline (3,4-DCA) and chlorothalonil were measured in the major blood cockle (Tegillarca granosa) cultivation areas along the west coast of Peninsular Malaysia. The highest Irgarol 1051 mean was found in the blood cockle with a value of 98.92 ± 13.65 µg/kg in Kapar, Selangor, while the means of diuron and its metabolites and 3,4-DCA showed the highest values of 40.31 ± 7.61 and 41.42 ± 21.58 µg/kg in Kapar, Selangor and Sungai Ayam, Johor, respectively. Sungai Ayam, Johor also exhibited the highest amount of chlorothalonil of 29.76 ± 8.80 µg/kg. By referring to sediment quality guidelines, about 72% and more than 90% of sediment samples exceeded the environmental risk limits (ERLs) and maximum permissible concentration (MPC) for Irgarol 1051 and diuron, respectively. However, referring to the risk characterization ratio (RCR), none of the blood cockle samples exceeded 1, which means that there is no potential for adverse effects to occur. Thus, the contaminants in the marine ecosystem caused by booster biocides are highlighted as a serious issue, mainly in sediment.

Keywords: booster biocides; antifouling paint; blood cockle; sediment; health risk assessment

1. Introduction

A marine organism attached to a submerged surface is a natural phenomenon that increases the hydrodynamic drag and affects the maneuverability of boats and increases the fuel consumption [1]. In the shipping industry, the process of the settlement of organisms on the immersed surface—called biofouling—is a serious problem. It can be controlled by using chemical biocides and non-biocidal technologies [2]. Thus, antifouling paints are used in the shipping sector to avoid the colonization of marine organisms such as algae, barnacles and mussels. Previously, an organotin (OT) compound
was used widely in most antifouling paints. OT compounds such as tributyltin (TBT) and triphenyltin (TPT) have adverse effects on non-target marine organisms [3,4]. Regarding these environmental effects, in October 2001, the International Maritime Organization (IMO) held a convention on “Control of Harmful Antifouling Systems on Ship” (IMO-AFS 2001) with the total restriction of OT compounds starting in January 2008, and this encouraged the manufacture of alternative, tin-free antifouling paints.

The commercial tin-free compounds which have been widely used in recent years are Sea-nine 211, Irgarol 1051, zineb, ziram, diuron, chlorothalonil, dichlofluanid, tolyfluanid, copper omadine, zinc omadine and pyridine–triphenylborane. The concentration of these compounds therefore significantly increased in the marine environment, mainly in marinas and harbors with heavy shipping activities. The leached biocides which were readily transported into the marine environment then settled in the sediment, which created “hotspots” for the contaminants. This is likely to pose a longer-term threat to various aquatic organisms [5]. Thus, there is increasing concern regarding booster biocides due to their increasing application in shipping paints and also as herbicides. Although booster biocides are less toxic than TBT, they are toxic to microalgae and have the potential to destruct the primary producer on the base of the food web [6].

Malaysia’s coastline is a fertile breeding ground for blood cockles, *Tegillarca granosa* (Linnaeus, 1758). The cultivation of blood cockles at present is considered economically important in the fisheries industry. The total production of blood cockles in Malaysia in 2005 was about 59,520 tons. The production of the blood cockle was at its peak in 2010 when the total production increased to 78,024 tons. However, the production values decreased to 16,866 tons in 2015, and only about 9,596 tons in 2016 [7]. This problem probably occurred due to the release of the pollutants into the marine ecosystem and inadequate management of the wild blood cockle population, which resulted in the high mortality of blood cockle and spat [8].

High levels of booster biocides including Irgarol 1051, diuron, Sea-nine 211, chlorothalonil and dichlofluanid are found in several countries’ marinas, such as Japan [9], Korea [10] and the United States of America [11], as well as Spain [12]; large amounts of shipping activities have been conducted in these particular areas with low water exchange. In the present study, booster biocides—namely Irgarol 1051, diuron and its metabolites 3,4-dichloroaniline (DCA), and chlorothalonil—were investigated in the blood cockle, and sediment was collected from major blood cockle cultivation areas of Peninsular Malaysia. A health risk assessment of these compounds on the Malaysian populace was also conducted.

2. Materials and Methods

2.1. Description of the Study Area

The study was carried out at major blood cockle cultivation areas in Peninsular Malaysia. The areas were located at Bagan Pasir in Perak, Sungai Buloh in Selangor, Kapar in Selangor and Sungai Ayam, Batu Pahat in Johor (Figure 1). The location of cultivation areas in the Malacca Straits is protected from monsoon winds. Previously, the cultivation areas were surrounded by wild mangrove trees. The mangrove ecosystem is crucial for protecting the blood cockle cultivation areas. The mangrove provides muddy shelter for blood cockle growth, stabilizes the coastline, prevents waves and storms and also provides sufficient dietary uptake to the marine organism [13].

With the continuous development in this region, the land use in these areas has changed to oil palm plantations and residential areas. Other human activities, including fisheries and industrial activity, also occur in these areas (Table 1). Both changes of land use and human activities have the potential to affect the cultivation areas of blood cockles and release many pollutants into the marine ecosystem, threatening the aquatic organisms and their ecosystem.
**Figure 1.** Sampling sites of major blood cockle cultivation areas in Peninsular Malaysia.

**Table 1.** Sampling locations of blood cockle and sediment in major cultivation areas in coastal waters of Peninsular Malaysia.

| Location              | Site Code | Area Description                      |
|-----------------------|-----------|---------------------------------------|
| Bagan Pasir, Perak    | BP1       | Fisheries, palm oil plantation        |
|                       | BP2       |                                       |
|                       | BP3       |                                       |
|                       | BP4       |                                       |
|                       | SB1       | Urban, paddy field, fisheries, ecotourism |
|                       | SB2       |                                       |
|                       | SB3       |                                       |
|                       | SB4       |                                       |
|                       | SB5       |                                       |
| Sungai Buloh, Selangor| KA1       | Industrial, fisheries, palm oil plantation |
|                       | KA2       |                                       |
|                       | KA3       |                                       |
|                       | KA4       |                                       |
|                       | KA5       |                                       |
|                       | KA6       |                                       |
| Kapar, Selangor       | SA1       | Fisheries, palm oil plantation        |
|                       | SA2       |                                       |
|                       | SA3       |                                       |
|                       | SA4       |                                       |
|                       | SA5       |                                       |
2.2. Determination of Booster Biocide Concentration

2.2.1. Sample Collection

Blood cockles, *Tegillarca granosa*, were collected by using a wire collecting basket, while sediment samples were taken by using a core sampler at 20 sampling sites along the west coast of Peninsular Malaysia. The lengths of the blood cockles collected were within 40 to 50 mm. All the samples were kept in a zip-lock bag prior to being brought back to the Ecotoxicology Laboratory at University of Putra Malaysia in a cold box. Later, the samples were stored in a freezer at −20 °C until analysis.

2.2.2. Analytical Procedures

Booster biocides including Irgarol 1051, diuron, 3,4-DCA and chlorothalonil were analyzed using the method described by Harino et al. [14] and Mukhtar et al. [15] with some modifications. Approximately 3 g of sediment and homogenized blood cockles were extracted by 10 mL of acetone for 10 min using a mechanical shaker at 300 rpm. Later, the samples were continuously extracted by centrifuge for 5 min at 2500 rpm at a temperature of 5 °C. The supernatant was transferred into a conical flask while the remaining pellet was re-extracted with 10 mL acetone. The combined supernatant was ethylated by 50 mL distilled water, 0.5 g celite and 1 g zinc acetate dehydrate to remove the lipid. Then, the analytes were rested for 30 min before being filtered into the separatory funnel. The analytes were added to 10 mL dichloromethane (DCM) and shaken for 10 min using a vertical shaker at 300 rpm. The organic layer was then transferred into an anhydrous sodium sulfate set, and the remaining water base was supplemented with another 10 mL DCM followed by repeated shaking. About 20 mL of methanol was added into the combined analytes prior to concentrating to 2 mL. The concentrated analytes were injected with 100 µl of internal standard solution (atrazine-d5, 1 µg/L) and the volume was adjusted to 1 mL of nitrogen gas (N₂).

2.2.3. Quantification of Booster Biocides by High-Performance Liquid Chromatography (HPLC)

The prepared samples were analyzed using an HPLC system equipped with a pump (LC-20AD, Shimadzu, Kyoto, Japan), a sample injector (SIL-20A, Shimadzu, Kyoto, Japan) and a UV/VIS detector (SPD-20AV, Shimadzu, Kyoto, Japan). The oven temperature was 25 °C (CTO-20AC, Shimadzu, Kyoto, Japan) and the system used a Hewlett-Packard 1050 system fitted with a quaternary pump and a Bakerbond ENV reversed-phase column (150 × 4.6 mm ID) fitted with a guard (Mallinckrodt Baker, Milton Keynes, UK). A mixture of methanol and Milli-Q water (1:2) was used as the mobile phase with isocratic elution. Ten microliters were injected into the column. The flow rate of eluent was maintained at 1 mL/m, and the UV detector wavelengths for Irgarol 1051, diuron, DCA and chlorothalonil were 223, 254, 247 and 230 nm, respectively. The retention times of Irgarol 1051, diuron, DCA and chlorothalonil were 9.1, 5.8, 6.1 and 9.8 min, respectively.

2.2.4. Quality Assurance

All the glassware used in the liquid–liquid extraction was washed with detergent, Decon-90, followed by rinsing with distilled water to remove external contamination. Later, the glassware was dried and covered using aluminum foil.

Blanks were performed to identify the presence of any contamination during the extraction. Blanks were prepared while conducting the extraction sample procedure. The prepared blanks were analyzed together with each batch sample, and the results found that no interference was detected in the measurement. The actual concentration of the booster biocides detected in the sample was obtained by subtracting the blank concentration. The value of the blanks was mostly negligible because of the detection limits. The known concentration standard was run through every 5 to 10 samples to ensure the sensitivity and recovery of the instrument. The percentages of recoveries found were 108.2% for Irgarol 1051, 90.2% for diuron, 95.0% for DCA and 91.4% for chlorothalonil. The instrument was also flushed every 10 samples to remove the impurities in the HPLC process.
To ensure the stability of the HPLC, the instrument was warmed up before use to analyze the samples. The instrument was flushed for at least 2 h using acetonitrile (ACN) and Milli-Q water as the mobile phase. Next, the instrument was flushed with ACN and Milli-Q water/methanol (2:1) for another hour before continuing to analyze the samples.

Five standard solutions (0.025, 0.05, 0.1, 0.5 and 1.0 ppm) were used to create the calibration curve. The calibration curve was used to determine unknown sample concentrations by comparing them to the several standard samples with a known concentration.

Atrazine-d5 was used as an internal standard (IS) in this study. The known concentration of IS was injected into each blank, sample and standard solution. The purpose of the internal standard was to improve the precision of quantitative analysis by plotting the ratios of analyte signal to the IS signal. Thus, the presence of IS has the potential to correct the loss of the analyte during sample preparation.

2.2.5. Statistical Analysis

Statistical analysis was conducted using SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). Tukey and Duncan’s post-hoc method with one-way ANOVA was applied to obtain the differences in biocides concentration in the cockle and sediment between locations of the cultivation areas. Levene’s test was performed to identify the variance of biocide concentrations from different locations. Pearson’s correlation was conducted to test the relationship between the blood cockle and sediment concentration of biocides.

3. Results

3.1. Booster Biocides Concentration in the Blood Cockle, Tegillarca granosa

Table 2 and Figure 2 present the concentration of Irgarol 1051, diuron, 3,4-DCA, and chlorothalonil for the analyzed blood cockles, Tegillarca granosa. Samples from Kapar, Selangor (98.92 ± 13.65 µg/kg, w/w) have the highest mean concentration of Irgarol 1051 in the blood cockles, followed by Sungai Buloh, Selangor > Sungai Ayam, Johor > Bagan Pasir, Perak. The Irgarol 1051 concentration detected in Kapar, Selangor is significantly different from those in Sungai Ayam and Bagan Pasir (Tukey’s HSD, \( p < 0.05 \)) but not Sungai Buloh. The mean concentration of diuron in blood cockle is highest in Kapar, Selangor (40.31 ± 7.62 µg/kg, w/w) while 3,4-DCA and chlorothalonil show the highest means in Sungai Ayam, Johor with values of 41.42 ± 21.58 and 29.76 ± 8.80 µg/kg, w/w, respectively. However, the means are not significantly different from other locations (Tukey’s HSD, \( p > 0.05 \)).
Table 2. The concentration of booster biocides in blood cockles, *Tegillarca granosa* (µg/kg ± SD).

| Location       | Site Code | Irgarol 1051 | Diuron 3,4-DCA | Chlorothalonil |
|----------------|-----------|--------------|----------------|----------------|
| Bagan Pasir, Perak | BP1       | 6.05 ± 0.11  | 35.32 ± 1.21   | 9.62 ± 0.63    | 18.17 ± 1.98  |
|                | BP2       | 17.77 ± 6.57 | 7.80 ± 0.24    | 10.10 ± 1.54   | 11.74 ± 0.76  |
|                | BP3       | 13.58 ± 5.47 | 2.41 ± 0.05    | 0.62 ± 0.13    | 13.11 ± 1.52  |
|                | BP4       | 28.06 ± 7.20 | 1.99 ± 0.25    | 0.67 ± 0.05    | 9.35 ± 1.50   |
| Sungai Buloh, Selangor | SB1     | NS          | NS             | NS             |
|                | SB2       | 69.91 ± 14.54 | 33.75 ± 4.32  | 36.19 ± 3.34   | 11.75 ± 0.41  |
|                | SB3       | NS          | NS             | NS             |
|                | SB4       | 84.43 ± 0.97 | 55.59 ± 3.31   | 20.85 ± 3.34   | 19.37 ± 1.26  |
|                | SB5       | 20.96 ± 6.04 | 9.56 ± 0.41    | 3.09 ± 0.12    | 18.90 ± 0.42  |
| Kapar, Selangor | KA1       | 94.39 ± 1.98 | 37.32 ± 1.05   | 28.65 ± 0.91   | 10.02 ± 2.19  |
|                | KA2       | 97.20 ± 17.39 | 34.63 ± 0.40   | 20.22 ± 4.22   | 26.51 ± 1.00  |
|                | KA3       | 68.24 ± 1.13 | 26.17 ± 2.11   | 13.27 ± 2.74   | 9.95 ± 1.47   |
|                | KA4       | 163.97 ± 16.25 | 72.80 ± 1.97  | 17.08 ± 1.83   | 19.25 ± 1.33  |
|                | KA5       | 83.97 ± 1.03 | 49.57 ± 1.11   | 10.50 ± 1.25   | 14.67 ± 1.03  |
|                | KA6       | 85.77 ± 3.61 | 21.40 ± 0.08   | 11.54 ± 4.01   | 15.11 ± 0.24  |
| Sungai Ayam, Johor | SA1       | 11.22 ± 1.06 | 13.30 ± 3.67   | 10.24 ± 0.58   | 41.69 ± 3.50  |
|                | SA2       | 10.0 ± 0.62  | 16.59 ± 0.14   | 5.97 ± 1.20    | 58.85 ± 2.66  |
|                | SA3       | 9.86 ± 0.15  | 3.30 ± 0.64    | 8.38 ± 0.88    | 16.23 ± 3.13  |
|                | SA4       | 31.93 ± 0.46 | 77.51 ± 6.09   | 68.59 ± 7.5    | 16.41 ± 2.70  |
|                | SA5       | 49.48 ± 0.45 | 76.41 ± 5.10   | 113.90 ± 9.66  | 15.62 ± 2.66  |

SD: standard deviation, NS: no sample.

Figure 2. Mean concentration (±SE) in µg/kg wet weight of booster biocides in the blood cockle. Abbreviations: 3,4-DCA: 3,4-dichloroaniline.

3.2. Booster Biocides Concentration in the Sediment

The identified concentrations of booster biocides in sediment are shown in Table 3 and Figure 3. The mean concentration of Irgarol 1051 in sediment decreases according to the following order: Kapar, Selangor > Bagan Pasir, Perak > Sungai Buloh, Selangor > Sungai Ayam, Johor. Kapar has a mean that is significantly different from other locations (Tukey’s HSD, p < 0.05). The sediment also differed in diuron level for Sungai Buloh, Selangor with the mean value of 139.69 ± 29.06 µg/kg, w/w. The mean is significantly different from other locations (Tukey’s HSD, p < 0.05). The mean of 3,4-DCA is highest in sediment from Sungai Buloh, Selangor (25.73 ± 6.40 µg/kg, w/w) but is not significantly different from Sungai Ayam and Kapar; the opposite is true of Bagan Pasir (Tukey’s HSD, p > 0.05). For chlorothalonil, the highest mean is detected in Bagan Pasir, Perak (23.87 ± 13.87 µg/kg, w/w);
however, there is no significant difference for chlorothalonil in sediment collected from blood cockle cultivation areas (Tukey’s HSD, \( p > 0.05 \)).

**Table 3.** The mean concentration of booster biocides in the sediment (\( \mu g/kg \pm SD \)).

| Location       | Site Code | Irgarol 1051          | Diuron          | DCA            | Chlorothalonil |
|----------------|-----------|-----------------------|-----------------|----------------|----------------|
| Bagan Pasir, Perak | BP1       | 12.08 ± 0.60          | 57.23 ± 1.60    | 1.16 ± 0.05    | 10.00 ± 1.09   |
|                | BP2       | NS                    | NS              | NS             | NS             |
|                | BP3       | NS                    | NS              | NS             | NS             |
|                | BP4       | 12.50 ± 0.63          | 28.42 ± 0.07    | 12.96 ± 0.26   | 37.73 ± 2.29   |
| Sungai Buloh, Selangor | SB1       | 6.66 ± 0.33           | 172.39 ± 9.11   | 32.10 ± 2.80   | 17.51 ± 2.00   |
|                | SB2       | 10.07 ± 0.50          | 134.65 ± 24.29  | 24.33 ± 1.93   | 2.96 ± 0.20    |
|                | SB3       | 19.95 ± 0.10          | 181.03 ± 15.33  | 36.90 ± 2.69   | 15.34 ± 0.38   |
|                | SB4       | 15.19 ± 1.55          | 181.67 ± 11.43  | 33.80 ± 4.00   | 10.17 ± 1.50   |
|                | SB5       | 5.44 ± 0.27           | 28.69 ± 13.92   | 1.54 ± 0.29    | 8.44 ± 0.77    |
| Kapar, Selangor | KA1       | ND                    | 72.76 ± 10.17   | 14.53 ± 1.47   | 3.61 ± 1.03    |
|                | KA2       | 83.22 ± 4.16          | 82.06 ± 6.17    | 15.70 ± 4.34   | 1.85 ± 0.81    |
|                | KA3       | ND                    | 45.35 ± 3.88    | 8.19 ± 0.86    | 11.30 ± 1.15   |
|                | KA4       | ND                    | 48.13 ± 1.04    | 11.36 ± 1.64   | 13.65 ± 0.89   |
|                | KA5       | ND                    | 58.29 ± 1.20    | 15.41 ± 0.13   | 11.30 ± 2.29   |
|                | KA6       | 52.70 ± 3.65          | 39.09 ± 1.37    | 8.54 ± 0.96    | 3.44 ± 1.34    |
| Sungai Ayam, Johor | SA1       | 13.29 ± 0.66          | 61.87 ± 1.56    | 17.87 ± 1.11   | 2.12 ± 0.19    |
|                | SA2       | 9.75 ± 0.48           | 48.83 ± 6.63    | 9.37 ± 0.91    | 2.68 ± 0.26    |
|                | SA3       | 15.68 ± 0.78          | 88.93 ± 1.81    | 18.40 ± 3.08   | 4.34 ± 0.50    |
|                | SA4       | 12.65 ± 0.83          | 12.72 ± 1.66    | 25.77 ± 1.50   | 25.30 ± 0.51   |
|                | SA5       | 0.93 ± 0.05           | 79.47 ± 2.04    | 15.92 ± 0.80   | 22.16 ± 0.67   |

SD: standard deviation, NS: no sample, ND: not detected.

**Figure 3.** Mean concentration (±SE) in \( \mu g/kg \) wet weight of booster biocides in the sediment.

In this study, the variances of biocide concentrations from all locations for both blood cockle and sediment were equal since \( p > 0.05 \). A significant correlation is found between sediment and blood cockle for Irgarol 1051 (\( r = 0.70, p < 0.05 \)). The insignificant correlation (\( p > 0.05 \)) found in the field of diuron, 3,4-DCA, and chlorothalonil is probably due to environmental factors such as the physical-chemical parameters of the study areas.
4. Discussion

4.1. Booster Biocide Distribution

4.1.1. Irgarol 1051

Irgarol 1051 is an algaecide which was specifically designed as a protective antifouling coating. It is a highly specific and effective inhibitor of photosynthesis by blocking electron binding sites at photosystem II [16,17]. Irgarol 1051 has low solubility in water and contributes to very slow leaching rates and extended antifouling action. As an antifouling paint, Irgarol 1051 is often combined with copper or copper thiocyanate [18]; while Irgarol 1051 is effective in controlling algae, copper is effective in controlling animals.

Since Irgarol 1051 was detected as a marine contaminant in Côte d’Azur, France [19], many researchers have performed studies on the occurrence of this compound in the marine ecosystem. Irgarol 1051 concentrations were found to be lowest in *Tegillarca granosa* from Bagan Pasir, Perak. In contrast, the concentrations of Irgarol 1051 were found to be high in the rest of the sampling sites—mainly in Kapar, Selangor—suggesting that Irgarol 1051 is being used widely in this particular area. The sampling site of Kapar, Selangor is located near to Port Klang, with the busiest shipping activities and a resident jetty for fishing activities that contribute to the high concentrations of Irgarol 1051. The same is true for Sungai Buloh, Selangor, which also recorded a high concentration of Irgarol 1051 in the blood cockles. The concentrations are believed to arise from tourism and residential jetties. The differences of Irgarol 1051 levels depend upon the density of boating use in these particular areas.

The concentrations of Irgarol 1051 were compared with previous studies on biological samples (Table 4). The Irgarol 1051 value found in clam (*Meretrix* spp.) was under the detection limit, with a value of 0.05 µg/kg in samples collected from Vietnamese marinas [20]. The same was true for the mussels (*Perna viridis*) collected from the coastal areas of Thailand, which showed values under the detection limit [21]. Meanwhile, a study in the coastal area of Hong Kong detected an Irgarol 1051 concentration in the range of 0.4 to 1.2 µg/kg in green mussel [22]. The study also detected the metabolites of Irgarol 1051 known as M1 (2-methylthio-4-tert-butylamino-s-triazine), M2 (3-[4-tert-butylamino-6-methylthiol-s-triazen-2-ylamino]propanol) and M3 (N,N’-di-tert-butyl-2,4-diamino-6-methylthiol-s-triazine) in the green mussels’ tissue at concentrations of 1.0 to 5.2, 1.6 to 2.8 and 8.9 to 44.4 ng/g, respectively. The data indicated that metabolites in green mussels have a higher concentration than the parent compound.

To date, many studies have been conducted to monitor the occurrence of Irgarol 1051 in the sediment. Table 3 shows the concentration of Irgarol 1051 in the sediment samples. A high concentration of Irgarol 1051 was detected in Kapar, Selangor, which is located near Port Klang; large ships from around the world are moored at and sail in and out of the port. It was reported that Irgarol 1051 concentrations in the sediment of the Malacca Strait and Sungai Pulai, Johor were much lower, with concentrations of <0.1 to 14 and <0.1 to 1.4 µg/kg, respectively [15,23]. In the present study, the concentrations of Irgarol 1051 on the west coast of Peninsular Malaysia are comparable to those results in other parts of the world. For example, the Irgarol 1051 concentrations in the sediment from Indonesia coastal water and Seto Inland Sea, Japan were in the range 0.1 to 76, 1.79 to 73.5 and 11 to 69 µg/kg, respectively [14,24,25]. The marinas with heavy traffic in terms of shipping activities and poor water exchange rates presented higher Irgarol 1051 concentrations than those with less boating activity. Although Irgarol 1051 has a low affinity to particulate matters (organic carbon partition coefficient (log Koc) 3.0, octanol-water partition coefficient (log Kow) 3.9), the biocide is able to bind into the sediment when the water marinas are sump, such as is found in enclosed marinas [26]. However, lower Irgarol 1051 concentrations were detected in sediment from Californian marinas, shipping and shipbuilding in Korea, Korean coastal marinas and Panamanian marinas at levels of <0.3 to 8.9, n.d. to 11.5, n.d. to 3.4 and <0.08 to 2.8 µg/kg, respectively [11,27–29].
Table 4. Concentration of Irgarol 1051 in various locations around the world.

| Location                                      | Water Concentration (µg/L) | Sediment Concentration (µg/kg) | Biological Sample Concentration (µg/kg) | References |
|-----------------------------------------------|----------------------------|--------------------------------|-----------------------------------------|------------|
| Malaysia coastal area                         | <0.02–14                   |                                |                                         | [23]       |
| Sungai Pulai, Johor                           | <0.1–1.4                   |                                |                                         | [15]       |
| Thailand coastal area                         | 0.03–3.2                   | <0.76 (green mussel)           |                                         | [21]       |
| Indonesia coastal area                        | 0.1–76                     |                                |                                         | [14]       |
| Vietnam coastal area                          | 0.05–4                     | 0.05 (clam)                    |                                         | [20]       |
| Peninsular Malaysia                           | 5–2121                     |                                |                                         | [30]       |
| Major bays and fishing ports, South Korea     |                            | 1.0–11.5                       |                                         | [27]       |
| Major harbors, South Korea                    |                            | 1.1–3.5                        |                                         | [27]       |
| Jinhae Bay and harbors, Korea                 | 0.2–14.1                   |                                |                                         | [10]       |
| Korean special management sea areas           | <0.12–2.05                 | <0.02–7.79                     |                                         | [31]       |
| Busan Bay, Korea                              | 1.79–73.5                  |                                |                                         | [28]       |
| Ulsan Bay, Korea                              | ND–38.8                    |                                |                                         | [28]       |
| Southern England                              | <1.7–45                    |                                |                                         | [32]       |
| Hong Kong waters                              | 0.22–21.26                 | 0.4–1.2 (green mussel)         |                                         | [22]       |
| California marinas                            | 2–254                      | <0.3–8.9                       |                                         | [11]       |
| Iran, Bushehr                                 | <1.0–63.4                  |                                |                                         | [33]       |
| French Mediterranean coast                    | 43–689                     |                                |                                         | [34]       |
| Panama coastal area                           | <0.08–2.8                  |                                |                                         | [29]       |
| San Diego area, California, USA               | 1–304                      |                                |                                         | [35]       |
4.1.2. Diuron

Diuron is a herbicide that has been used since the 1950s. It is used in paints and stains, acting as an algaecide in commercial fish production [36]. Various studies have indicated that diuron has a half-life ranging from 43 to 2180 days and that it is stable in seawater [37].

The study conducted by Harino et al. [21] detected a diuron concentration in green mussel (Perna viridis) from Thailand marinas of between <0.69 to 9.6 µg/kg. Another study on clams from Vietnam reported there was no diuron detected in the samples [20]. A study on plankton collected from the Seto Inland Sea, Japan showed a diuron concentration between 75 to 455 µg/kg, which was the highest concentration detected in Harimanada and Osaka bay [24]. The study indicated that diuron has been used as a pesticide in these areas and accumulates in the marine environment through regular discharge from the rivers.

Previous studies have reported diuron concentration for various areas. For example, a study conducted by Harino et al. [14] reported a concentration of diuron in sediment from Indonesian coastal areas of between 0.04 to 740 µg/kg, and the concentration recorded was the highest in the Asian coastal area. Another study conducted in sediment from Panama and Port Klang, Malaysia showed concentrations within <0.75 to 14.1 µg/kg and 2.24 to 19.25 µg/kg [29,38]. These values were much lower compared to the studies conducted in Korean coastal areas. Kim et al. [27] reported diuron concentrations in Busan Bay and Ulsan Bay, Korea of 6.89 to 29.9 and 15.3 to 39.2 µg/kg.

4.1.3. 3,4-DCA

3,4-dichlorothalonil (3,4-DCA) is the main degradation product of diuron [37] and endangers the growth and development of aquatic organisms [39–41]. 3,4-DCA has a high water solubility of 580 mg/L at 20 °C and an estimated half-life of 18 days. Compared to the parent material, 3,4-DCA is highly toxic and is classified as a secondary poisonous substance (Chemical Abstracts Service (CAS) Registry Number: 95-76-1) [42]. Besides the leaching route from ship and boat hulls, 3,4-DCA is also released into the environment through application in plant protection agents—for example, linuron, diuron, and propanil—in agricultural activities.

The result in Tables 2 and 3 suggest that the concentrations of 3,4-DCA in both blood cockle and sediment were lower compared to the parent material of diuron. 3,4-DCA has high solubility in various discharge paths, mainly in the water and soil, and passes through the biodegradation process either by bacteria or fungi [43–47]. Another study revealed that algae has the potential to remove 3,4-DCA in the aquatic environment. For example, the green algae Chlorella pyrenoidosa reduced 3,4-DCA about 78.4% over a period of 7 days [48].

Several studies have been conducted on the assessment of 3,4-DCA in the marine environment. For example, Saleh et al. [33] reported 3,4-DCA concentrations in ports and marinas of Bushehr, Iran of between the limit of detection (LOD) to 390 ng/L. The study on Arabian Killifish, Aphanius dispar, embryo exposed to 3,4-DCA at a concentration above 2.5 mg/L showed the effects of severe pericardial edema and tail shortage [49]. 3,4-DCA is also believed to have endocrine effects on fish. Testing 3,4-DCA concentrations of 200 and 400 µg/L decreased androgen synthesis in breeding male stickleback, Gasterosteus aculeatus. At a concentration of 100, 200 and 400 µg/L, it is possible to change the secondary sex character and the color of the breeding males to become regressive as well as causing the disappearance of courtship behavior [42].

4.1.4. Chlorothalonil

Chlorothalonil is among the common compounds which are widely used in antifouling paint and fungicide in agricultural activities [50]. Chlorothalonil (2,4,5,6–tetrachloroisophthalonitrile) is less toxic and less persistent in the environment than other compounds.
Chlorothalonil is very effective against marine organisms, including crustaceans and fish. In fact, several studies have been conducted on chlorothalonil in the marine environment, including on its distribution and toxicity effect on aquatic organisms [51]. Chlorothalonil is the most toxic compound for the early growth stages (embryotoxicity, larva inhibition and mortality) of invertebrate species including *Paracentrotus lividus*, *Ciona intestinalis*, and *Mytilus edulis*. A study on crustaceans indicated that many of them were sensitive toward chlorothalonil; for example, *Amphiascus tenuiremis* and *Daphnia magna* have LC50 concentrations of 27 to 53 and 130 to 200 µg/L, respectively. The toxicity concentration of fish varied depending on their sensitivity. *Pseudaphritis urvillii* was very sensitive toward chlorothalonil, with an LC50 concentration of 8.2 µg/L [52], while *Oryzias melastigma* and *Anguilla japonica* have an LC50 concentration of 110 µg/L [6,53], showing the least sensitivity toward chlorothalonil.

A study in Sungai Pulai, Johor recorded that chlorothalonil in the sediment was within the range of <0.1 to 6.2 µg/kg [15]. A study in the Salton Sea, USA showed a similar pattern to the study in Sungai Pulai, Johor with a recorded concentration of <0.12 to 8.9 µg/kg [54]. However, high chlorothalonil was detected in Korean marinas as recorded by Kim et al. [28]. The chlorothalonil was detected in ranges from 1.2 to 99, 22 to 1065 and 1.3 to 422 µg/kg in Korean coastal waters, Busan Bay and Ulsan Bay, respectively. Chlorothalonil might be the main ingredient of the antifouling paint used widely in these areas and be subsequently released into the marine environment.

### 4.2. Potential Adverse Effect

Malaysia does not currently have guidelines regarding the quality of sediment to protect aquatic organisms from biocides. The potential risks of booster biocide level in sediment for Irgarol 1051 (1.4 µg/kg) were assessed by referring to the environmental risk limits (ERLs) derived by the equilibrium partition (EqP) method in the Netherlands [55]. ERLs are the lowest compound concentration values which begin to cause a risk for the ecosystem. About 72% of Irgarol 1051 concentrations detected in sediment from blood cockle cultivation areas of Peninsular Malaysia (range values, 5.44 to 83.22 µg/kg, w/w) exceed the ERLs. The sediment quality criteria (SQC) for both Irgarol 1051 and diuron were revised by referring to the Norwegian guidelines [56]. More than 70% of Irgarol 1051 concentrations exceed 2.5 µg/kg, which is suggested to be “very bad” (Figure 4). Thus, the level of Irgarol 1051 in sediment possibly causes toxic effects toward marine organisms, including marine plants, benthic biota, and fishes.

Diuron in the sediment was evaluated by referring to the maximum permissible concentration (MPC) derived by applying the equilibrium partition (EqP) method [57]. The diuron concentrations in the sediment from all blood cockle cultivation areas exceed the MPC (9 µg/kg). Based on the Norwegian guidelines, only SA4 can be classified as moderately contaminated by diuron, which suggests that toxic effects can occur following short-term exposure (Figure 5). However, more than 90% of the sediment samples are higher than the Norwegian guidelines values of 13 ng/g, which is classified as “very bad”, suggesting that severe acute toxic effects are likely in marine organisms.
4.3. Human Risk Assessment

The purpose of risk assessment is to predict the environmental concentration exposure towards organisms and humans. The assessment is based on formulae proposed by the European Uniform System for the Evaluation of Substances (EUSES) [58]. The predicted human dose (PHD) via the consumption of blood cockle (mg/kg/d) is calculated using the following equation:

$$\text{PHD} = \frac{(C_{\text{cockle}} \times \text{IH}_{\text{cockle}})}{\text{BW}}$$
where $C_{\text{cockle}}$ is the concentration of booster biocide in blood cockle (in mg/kgwwt), $I_{\text{Hcockle}}$ is the blood cockle intake (kg/person/day) and BW is the body weight, assumed to be 62 kg [59]. The average mollusk consumption (including blood cockle) in Malaysia is 0.0044 kg person [60].

The amount of blood cockle consumed varies from one individual to another. Referring to the Food and Agriculture Organization (FAO) [60], the average Malaysian consumption of mollusk, including blood cockle, is 1.6 kg/yr [60]. The intake of the compound from seafood consumption is dependent on the booster biocide concentration in the seafood and the total amount of seafood consumed. Blood cockle is categorized as a sessile organism that has high potential to accumulate pollutants in the soft tissue; thus, an assessment of whether booster biocides pose a risk to the consumer was conducted. The predicted human dose (PHD) of booster biocides was compared based on the blood cockle collected from different cultivation areas. The results show that the PHD for daily intake in all blood cockle samples is below the human reference dose (HRD) for both Irgarol 1051 (NOEL: 0.097 mg/kg/d) and diuron (ADI: 0.007 mg/kg/d) (Tables 5 and 6). The highest PHD levels of Irgarol 1051 and diuron are detected in KA4 with values of $1.16 \times 10^{-5}$ and $4.7 \times 10^{-6}$ mg/kg/d, respectively. Not many studies have been conducted on the PHD of booster biocides towards humans. The PHD of Irgarol 1051 and diuron through fish consumption in Spanish marines was much lower, with recorded values of $5.8 \times 10^{-8}$ and $9.6 \times 10^{-10}$ mg/kg/d, respectively [61].

| Location             | Site Code | Irgarol 1051 | Diuron       |
|----------------------|-----------|--------------|--------------|
| Bagan Pasir, Perak   | BP1       | $4.29 \times 10^{-7}$ | $2.28 \times 10^{-6}$ |
|                      | BP2       | $1.26 \times 10^{-6}$  | $5.03 \times 10^{-7}$  |
|                      | BP3       | $9.64 \times 10^{-7}$  | $1.55 \times 10^{-7}$  |
|                      | BP4       | $1.99 \times 10^{-6}$  | $1.28 \times 10^{-7}$  |
| Sungai Buloh, Selangor | SB2    | $4.96 \times 10^{-6}$  | $2.18 \times 10^{-6}$  |
|                      | SB4       | $5.99 \times 10^{-6}$  | $3.59 \times 10^{-6}$  |
|                      | SB5       | $1.49 \times 10^{-6}$  | $6.17 \times 10^{-7}$  |
| Kapar, Selangor      | KA1       | $6.70 \times 10^{-6}$  | $2.41 \times 10^{-6}$  |
|                      | KA2       | $6.90 \times 10^{-6}$  | $2.23 \times 10^{-6}$  |
|                      | KA3       | $4.84 \times 10^{-6}$  | $1.69 \times 10^{-6}$  |
|                      | KA4       | $1.16 \times 10^{-5}$  | $4.70 \times 10^{-6}$  |
|                      | KA5       | $5.96 \times 10^{-6}$  | $3.20 \times 10^{-6}$  |
|                      | KA6       | $6.09 \times 10^{-6}$  | $1.38 \times 10^{-6}$  |
| Sungai Ayam, Johor   | SA1       | $7.96 \times 10^{-7}$  | $8.58 \times 10^{-7}$  |
|                      | SA2       | $7.10 \times 10^{-7}$  | $1.07 \times 10^{-6}$  |
|                      | SA3       | $4.23 \times 10^{-7}$  | $2.13 \times 10^{-7}$  |
|                      | SA4       | $2.27 \times 10^{-6}$  | $5.00 \times 10^{-6}$  |
|                      | SA5       | $3.51 \times 10^{-6}$  | $4.93 \times 10^{-6}$  |
Table 6. Toxicity data for predators and humans.

| Compound | PNEC<sub>oral</sub> (mg/kg<sub>oral</sub>) | Source | Human Reference Dose (HRD) (mg/kg/d) | Source |
|----------|-----------------------------------------|--------|-------------------------------------|--------|
| Irgarol 1051 | 2.2 | Based on NOEL in rat (90 days feeding study) of 9.7 mg/kgbw/d (JECFA, [62]), a conversion factor to NOEC of 20 kgbw/d for rat (TGDRA) and an assessment factor to PNEC<sub>oral</sub> of 90 (TGDRA) | 0.097 | Based on a NOEL of 9.7 mg/kgbw from a 90-days subchronic oral toxicity in Rats (Ciba Specialty Chemicals Corporation, [63]) and assessment factor of 100 [64] |
| Diuron | 0.83 | Base on a NOEC from a 2-years dog feeding study (USEPA, [65]) and an assessment factor of 30 (TGDRA) | 0.007 | ADI (EC, [66]) |

PNEC: Predicted no effect concentration, NOEL: No observed adverse effect level, NOEC: No observed effect concentration, TGDRA: Technical Guidance Document on Risk Assessment, ADI: Acceptable daily intake.

The risk characterization ratios (RCR) compare the exposure concentration to suitable no-effect levels [61]. For the environmental assessment, the RCR is calculated as

$$ RCR_{pred} = \frac{C_{cockle}}{PNEC_{oral}} $$

while for the human toxicity ratio, RCR is calculated with the equation below:

$$ RCR_{human} = \frac{PHD}{HRD} $$

An RCR value of below 1 indicates no adverse effects toward a particular subject, which corresponds to the compound. However, a value above 1 indicates that adverse effects have the potential to occur.

[Figure 6. Risk characterization of Irgarol 1051 for predators.]
The assessment of the risk characterization for Irgarol 1051 and diuron on predators and humans covers a wide range. This can be seen for the blood cockles collected from all cultivation areas of the west coast of Peninsular Malaysia. The graph indicates that none of the locations pose a risk to either predators or humans in terms of either of the biocides—Irgarol 1051 or diuron (Figures 6–8). The highest $RCR_{\text{pred}}$ for Irgarol 1051 and diuron is detected in KA4 (0.075) (Figure 6) and SA4 (0.093) (Figure 7). However, all the identified values are below the risk threshold of 1. The risk toward humans is also detected to be lower than found in predators, as the highest $RCR_{\text{human}}$ value for Irgarol 1051 is $1.2 \times 10^{-4}$ which is detected in KA4, while SA4 shows the highest $RCR_{\text{human}}$ for diuron with a value of $7.1 \times 10^{-4}$ (Figure 8).

5. Conclusions

The presence of booster biocides along the west coast of Peninsular Malaysia showed that shipping, fisheries and agriculture, together with domestic activities, are the sources of these
compounds. More than 66% and more than 90% of the Irgarol 1051 and diuron concentrations in sediment clearly exceed the ERLs and can be classified as “very bad” based on the Norwegian guidelines, probably having toxic effects for marine organisms, especially the benthic organisms. However, the RCR values of blood cockle, *Tegillarca granosa* (RCR < 1), indicated that there is no potential for adverse effects to occur for humans. An assessment of the impact of booster biocides in the marine environment must be conducted to obtain proof to formulate regulations in Malaysia in order to guarantee that the aquatic ecosystem is protected.

**Author Contributions:** Conceptualization, A.M., S.Z.Z., F.M.Y., M.N.A.A., H.H., A.I.; data curation, A.M.; formal analysis, A.M., H.H.; funding acquisition, S.Z.Z., H.H., A.H.; investigation, A.M., S.Z.Z., H.H.; methodology, A.M., H.H.; project administration, S.Z.Z., A.I.; resources, A.M., H.H.; supervision, S.Z.Z.; validation, A.M., F.M.Y., H.H.; visualization, A.M., S.Z.Z.; writing-original draft, A.M.; writing-review & editing, S.Z.Z, F.M.Y. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by The Fundamental Research Grant Scheme (FRGS) (Reference No.: FRGS/1/2017/WAB05/UPM/02/1) from the Ministry of Higher Education, Malaysia and was performed in relation to the JSPS Core-to-Core Program, B. Asia-Africa Science Platforms.

**Acknowledgments:** We are grateful to the members of the Faculty of Science, University Putra Malaysia, in particular to Abdullah Talib and Azizul Aziz for their involvement in sampling and laboratory activities.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Konstantinou, I.K.; Albanis, T.A. Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: A review. *Environ. Int.* 2004, 30, 235–248.
2. Thomas, K.V.; Brooks, S. The environmental fate and effects of antifouling paint biocides. *Biofouling* 2010, 26, 73–88.
3. Mohammat-Yusuff, F.; Zulkifli, S.Z.; Ismail, A.; Harino, H.; Yusoff, M.K.; Arai, T. Imposing in *Thais gradata* as a biomarker for TBT contamination on the southern coast of Peninsular Malaysia. *Water Air Soil Pollut.* 2010, 211, 443–457.
4. Sousa, A.C.; Pastorinho, M.R.; Takahashi, S.; Tanabe, S. History on organotin compounds, from snails to humans. *Environ. Chem. Lett.* 2014, 12, 117–137.
5. Kamarudin, N.A.; Zulkifli, S.Z.; Azmai, M.N.A.; Aziz, F.Z.A.; Ismail, A. Herbicide diuron as endocrine disrupting chemicals (EDCs) through histopathological analysis in gonads of Javanese medaka (*Oryzias javanicus*, Bleecker 1854). *Animals* 2020, 10, 525.
6. Bao, V.W.; Leung, K.M.; Qiu, J.W.; Lam, M.H. Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species. *Mar. Pollut. Bull.* 2011, 62, 1147–1151.
7. Department of Fisheries. *Annual Fisheries Statistics 2017*; Department of Fisheries Malaysia, Ministry of Agriculture and Agro-based Industries: Putrajaya, Malaysia, 2018; Available online: www.dof.gov.my (accessed on 12 June 2019).
8. Khalil, M.; Yasin, Z.; Hwai, T.S. Reproductive biology of blood cockle *Anadara granosa* (Bivalvia: Arcidae) in the northern region of the Strait of Malacca. *Ocean Sci. J.* 2017, 52, 75–89.
9. Mochida, K.; Hano, T.; Onduka, T.; Ichihashi, H.; Amano, H.; Ito, M.; Tanaka, H.; Fujii, K. Spatial analysis of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (Sea-Nine 211) concentrations and probabilistic risk to marine organisms in Hiroshima Bay, Japan. *Environ. Pollut.* 2015, 204, 233–240.
10. Kim, N.S.; Shim, W.J.; Yim, U.H.; Hong, S.H.; Ha, S.Y.; Han, G.M.; Shin, K.H. Assessment of TBT and organic booster biocide contamination in seawater from coastal areas of South Korea. *Mar. Pollut. Bull.* 2014, 78, 201–208.
11. Sapozhnikova, Y.; Wirth, E.; Schiff, K.; Fulton, M. Antifouling biocides in water and sediments from California marinas. *Mar. Pollut. Bull.* 2013, 69, 189–194.
12. Sánchez-Rodríguez, A.; Sosa-Ferrera, Z.; Santana-del Pino, Á.; Santana-Rodríguez, J.J. Probabilistic risk assessment of common booster biocides in surface waters of the harbours of Gran Canaria (Spain). *Mar. Pollut. Bull.* 2011, 62, 985–991.
13. Jusoff, K. Malaysian mangrove forests and their significance to the coastal marine environment. *Pol. J. Environ. Stud.* 2013, 22, 979–1005.
14. Harino, H.; Arifin, Z.; Rumengan, I.F.; Arai, T.; Ohji, M.; Miyazaki, N. Distribution of antifouling biocides and perfluoroalkyl compounds in sediments from selected locations in Indonesian coastal waters. *Arch. Environ. Contam. Toxicol.* 2012, 63, 13–21.

15. Mukhtar, A.; Mohamad-Yusuff, F.; Zulkifli, S.Z.; Harino, H.; Ismail, A.; Inoue, K. Concentration of Organotin and Booster Biocides in Sediments of Seagrass Area from Sungai Pulai Estuary, South of Johor, Malaysia. *Environments* 2019, 6, 26.

16. Hall, L.W., Jr.; Giddings, J.M.; Solomon, K.R.; Balcomb, R. An ecological risk assessment for the use of Irgarol 1051 as an algaecide for antifoulant paints. *Crit. Rev. Toxicol.* 1999, 29, 367–437.

17. Thomas, K.V.; Fileman, T.W.; Readman, J.W.; Waldock, M.J. Antifouling paint booster biocides in the UK coastal environment and potential risks of biological effects. *Mar. Pollut. Bull.* 2001, 42, 677–688.

18. Koutsasitis, A.; Aoyama, I. The interactive effects of binary mixtures of three antifouling biocides and three heavy metals against the marine algae *Chaetoceros gracilis*. *Environ. Toxicol. An Int. J.* 2006, 21, 432–439.

19. Readman, J.W.; Kwong, L.L.W.; Grondin, D.; Bartocci, J.; Villeneuve, J.P.; Mee, L.D. Coastal water contamination from a triazine herbicide used in antifouling paints. *Environ. Sci. Technol.* 1993, 27, 1940–1942.

20. Harino, H.; Midorikawa, S.; Arai, T.; Ohji, M.; Cu, N.D.; Miyazaki, N. Concentrations of booster biocides in sediment and clams from Vietnam. *J. Mar. Biol. Assoc. UK* 2006, 86, 1163–1170.

21. Harino, H.; Ohji, M.; Watahayakorn, G.; Arai, T.; Rungsupa, S.; Miyazaki, N. Occurrence of antifouling biocides in sediment and green mussels from Thailand. *Arch. Environ. Contam. Toxicol.* 2006, 51, 400–407.

22. Tsang, V.W.H.; Lei, N.Y.; Lam, M.H.W. Determination of Irgarol-1051 and its related s-triazine species in coastal sediments and mussel tissues by HPLC–ESI-MS/MS. *Mar. Pollut. Bull.* 2009, 58, 1462–1471.

23. Harino, H.; Arai, T.; Ohji, M.; Ismail, A.; Miyazaki, N. Contamination profiles of antifouling biocides in selected coastal regions of Malaysia. *Arch. Environ. Contam. Toxicol.* 2009, 56, 468–478.

24. Balakrishnan, S.; Takeda, K.; Sakugawa, H. Occurrence of Diuron and Irgarol in seawater, sediments and planktons of Seto Inland Sea, Japan. *Geochem. J.* 2012, 46, 169–177.

25. Koangga, C.C.; Takeda, K.; Sakugawa, H. Antifouling agents and Fenitrothion contamination in seawater, sediment, plankton, fish and selected marine animals from the Seto Inland Sea, Japan. *Geochem. J.* 2015, 49, 23–37.

26. Tolosa, I.; Readman, J.W.; Blaevoet, A.; Ghilini, S.; Bartocci, J.; Horvat, M. Contamination of Mediterranean (Côte d’Azur) coastal waters by organotins and Irgarol 1051 used in antifouling paints. *Mar. Pollut. Bull.* 1996, 32, 335–341.

27. Kim, N.S.; Hong, S.H.; An, J.G.; Shin, K.H.; Shim, W.J. Distribution of butyltins and alternative antifouling biocides in sediments from shipping and shipbuilding areas in South Korea. *Mar. Pollut. Bull.* 2015, 95, 484–490.

28. Kim, U.J.; Lee, I.S.; Choi, M.; Oh, J.E. Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. *Mar. Pollut. Bull.* 2015, 99, 157–165.

29. Batista-Andrade, J.A.; Caldas, S.S.; Batista, R.M.; Castro, I.B.; Fillmann, G.; Primel, E.G. From TBT to booster biocides: Levels and impacts of antifouling along coastal areas of Panama. *Environ. Pollut.* 2018, 234, 243–252.

30. Ali, H.R.; Arifin, M.M.; Sheikh, M.A.; Shazili, N.A.M.; Bachok, Z. Occurrence and distribution of antifouling biocide Irgarol-1051 in coastal waters of Peninsular Malaysia. *Mar. Pollut. Bull.* 2013, 70, 253–257.

31. Lam, N.H.; Jeong, H.H.; Kang, S.D.; Kim, D.J.; Ju, M.J.; Horiguchi, T.; Cho, H.S. Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation: Contamination profiles and risk assessment. *Mar. Pollut. Bull.* 2017, 121, 302–312.

32. Zhou, J.L. Occurrence and persistence of antifouling biocide Irgarol 1051 and its main metabolite in the coastal waters of Southern England. *Sci. Total Environ.* 2008, 406, 239–246.

33. Saleh, A.; Molaei, S.; Fumani, N.S.; Abedi, E. Antifouling paint booster biocides (Irgarol 1051 and diuron) in marinas and ports of Bushehr, Persian Gulf. *Mar. Pollut. Bull.* 2016, 105, 367–372.

34. Cassi, R.; Tolosa, I.; de Mora, S. A survey of antifoulants in sediments from Ports and Marinas along the French Mediterranean coast. *Mar. Pollut. Bull.* 2008, 56, 1943–1948.

35. Sapozhnikova, Y.; Wirth, E.; Schiff, K.; Brown, J.; Fulton, M. Antifouling pesticides in the coastal waters of Southern California. *Mar. Pollut. Bull.* 2007, 54, 1972–1978.
36. USEPA. United States Environmental Protection Agency. Reregistration Eligibility Decision for Diuron. Federal Register: Washington, DC, USA, 2003.
37. Moncada, A. DPR Report: Environmental Fate of Diuron. DPR Pesticide Chemistry Database. Environmental Monitoring Branch, Department of Pesticide Regulation. Available online: http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/diuron.pdf (accessed on 20 January 2020).
38. Hanapiah, M.; Zulkifli, S.Z.; Shahrun, M.S.; Mohamad-Yusuff, F.; Ismail, A.; Harino, H.; Inoe, K. Concentration of antifungal herbicides, Diuron in the vicinity of Port Klang, Malaysia. Isu-isu Terkini Pendid. St. Sains Mar. Malays. 2017, 1, 35–38.
39. Zhu, B.; Liu, T.; Hu, X.; Wang, G. Developmental toxicity of 3,4-dichloroaniline on rare minnow (Gobiocypris rarus) embryos and larvae. Chemosphere 2013, 90, 1132–1139.
40. Xiao, H.; Kuckelkorn, J.; Nüser, L.K.; Floehr, T.; Hennig, M.P.; Roß-Nickoll, M.; Schäffer, A.; Hollert, H. The metabolite 3,4,3',4'-tetrachloroazobenzene (TCAB) exerts a higher ecotoxicity than the parent compounds 3,4-dichloroaniline (3,4-DCA) and propanil. Sci. Total Environ. 2016, 551, 304–316.
41. Carbajal-Hernández, A.L.; Valero-García, R.C.; Martínez-Ruiz, E.B.; Jarquín-Díaz, V.H.; Martínez-Jerónimo, F. Maternal-embryonic metabolic and antioxidant response of Chalpichthys pardalis (Teleostei: Goodeidae) induced by exposure to 3,4-dichloroaniline. Environ. Sci. Pollut. Res. 2017, 24, 17534–17546.
42. European Commission. Summary Risk Assessment Report 3,4-Dichloroaniline (3,4-DCA); European Commission: Brussels, Belgium, 2006.
43. Yao, X.F.; Khan, F.; Pandey, R.; Pandey, J.; Mourant, R.G.; Jain, R.K.; Russell, R.J.; Oaksheott, J.G.; Pandey, G. Degradation of dichloroaniline isomers by a newly isolated strain, Bacillus megaterium IMT21. Microbiology 2011, 157, 721–726.
44. Roehrs, R.; Roehrs, M.; Machado, S.L.D.O.; Zanella, R. Biodegradation of herbicide propanil and its subproduct 3,4-dichloroaniline in water. Clean–Soil Air Water 2012, 40, 958–964.
45. Li, T.; Deng, X.P.; Wang, J.J.; Zhao, H.; Wang, L.; Qian, K. Biodegradation of 3,4-dichloroaniline by a novel Myriophyllum odoratimimus strain LWD09 with moderate salinity tolerance. Water Air Soil Pollut. 2012, 223, 3271–3279.
46. Elleegaard-Jensen, L.; Knudsen, B.E.; Johansen, A.; Albers, C.N.; Aamand, J.; Rosendahl, S. Fungal–bacterial consortia increase diuron degradation in water-unsaturated systems. Sci. Total Environ. 2014, 466, 699–705.
47. Castillo, J.M.; Nogales, R.; Romero, E. Biodegradation of 3,4 dichloroaniline by fungal isolated from the preconditioning phase of winery wastes subjected to vermicomposting. J. Hazard. Mater. 2014, 267, 119–127.
48. Wang, S.; Poon, K.; Cai, Z. Biodegradation and removal of 3,4-dichloroaniline by Chlorella pyrenoidosa based on liquid chromatography-electrospars ionization-mass spectrometry. Environ. Sci. Pollut. Res. 2013, 20, 552–557.
49. Saeed, S.; Al-Naema, N.; Butler, J.D.; Febbo, E.J. Arabian killifish (Aphanius dispar) embryos: A model organism for the risk assessment of the Arabian Gulf coastal waters. Environ. Toxicol. Chem. 2015, 34, 2898–2905.
50. DeLorenzo, M.E.; Fulton, M.H. Comparative risk assessment of permethrin, chlorothalonil, and diuron to coastal aquatic species. Mar. Pollut. Bull. 2012, 64, 1291–1299.
51. Caux, P.Y.; Kent, R.A.; Fan, G.T.; Stephenson, G.L. Environmental fate and effects of chlorothalonil: A Canadian perspective. Crit. Rev. Env. Sci. Technol. 1996, 26, 45–93.
52. Davies, P.E.; Cook, L.S.J.; Goenarso, D. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. Environ. Toxicol. Chem. Int. J. 1994, 13, 1341–1354.
53. Mayer, F.L. Acute Toxicity Handbook of Chemicals to Estuarine Organisms; (No. PB-87-186866/XAB; EPA-600/8-87/017); Environmental Protection Agency: Gulf Breeze, FL, USA; Environmental Research Lab.: Gulf Breeze, FL, USA, 1987.
54. Sapozhnikova, Y.; Bavardi, O.; Schlenk, D. Pesticides and PCBs in sediments and fish from the Salton Sea, California, USA. Chemosphere 2004, 55, 797–809.
55. Van Wezel, A.P.; Van Vlaardingen, P. Environmental risk limits for antifouling substances. Aquat. Toxicol. 2004, 66, 427–444.
56. Bakke, T.; Kålqvist, T.; Ruus, A.; Breedveld, G.D.; Hylland, K. Development of sediment quality criteria in Norway. J. Soils Sediments 2010, 10, 172–178.
57. Crommentuijn, T.; Sijm, D.; De Bruijn, J.; Van Leeuwen, K.; Van de Plassche, E. Maximum permissible and negligible concentrations for some organic substances and pesticides. J. Environ. Manag. 2000, 58, 297–312.
58. Lijzen, J.P.A.; Rikken, M.G.J. European Union System for the Evaluation of Substances 2.0 (EUSES 2.0); Background Report; RIVM Rapport 601900005. Available online: https://www.pbl.nl/sites/default/files/downloads/601900005.pdf (accessed on 20 May 202).

59. Azmi, M.Y.; Junidah, R.; Siti Mariam, A.; Safiah, M.Y.; Fatimah, S.; Norimah, A.K.; Poh, B.K.; Kandiah, M.; Zalilah, M.S.; Wan Abdul Manan, W.M.; et al. Body Mass Index (BMI) of adults: Findings of the Malaysian Adult Nutrition Survey (MANS). *Malays. J. Nutr.* **2009**, *15*, 97–119.

60. FAO, Food and Agriculture Organization of the United Nations. Food Balance Sheets by Main Groups of Fish Species and Fish Nutritional Factors—By Selected Countries. 2012. Available online: ftp://ftp.fao.org/FI/CDrom/CD_yearbook_2010/root/food_balance/section3.pdf (accessed on 5 September 2019).

61. Muñoz, I.; Bueno, M.J.M.; Agüera, A.; Fernández-Alba, A.R. Environmental and human health risk assessment of organic micro-pollutants occurring in a Spanish marine fish farm. *Environ. Pollut.* **2010**, *158*, 1809–1816.

62. JECFA-Joint FAO WHO Expert Committee on Food Additives. WHO Food Additives Series: 53, Flumequine. 2004. Available online: http://www.inchem.org/documents/jeccam/jeccmonov53je06.htm (accessed on 21 April 2009).

63. Ciba Specialty Chemicals Corporation. *Ciba IRGAROL 1051 Material Safety Data Sheet*; Ciba Additives: Tarrytown, NY, USA, 2005.

64. Falk-Filipsson, A.; Hanberg, A.; Victorin, K.; Warholm, M.; Wallén, M. Assessment factors—Applications in health risk assessment of chemicals. *Environ. Res.* **2007**, *104*, 108–127.

65. USEPA. Integrated Risk Information System. Available online: http://www.epa.gov/IRIS/ (accessed on 8 March 2009).

66. Directive, W.F. Environmental Quality Standards (EQS) substance data sheet: Tributyltin compounds (TBT-ion). Available online: https://circabc.europa.eu/sd/d/899759c1-af89-4de4-81bf-488c949887c8/30_Tributyltin_EQS (accessed on 5 May 2020).

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).