OCCURRENCE OF BOOSTER BIOCIDES AROUND PENINSULAR MALAYSIA: *Implication of its toxicity on selected coral reef organisms*

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DEDICATION

This work is dedicated to my late father *Sheikh* Rashid bin Ali, Grandfathers *Sheikh* Ali and *Sheikh* Jadi, Grandmother Bi Mariam bit Rashid, Aunt Halima bint Hassan and my beloved brother Suleiman Rashid who laid the foundation for my education. May Almighty Allah rest their souls in Paradise. Again, the work is dedicated to my wonderful mama; Fatma bit Jadi, brothers, sisters and my wife whose unfailing interest as well as supports for my education has kept me going. Thank you and long live all.

ABSTRACT

Abstract of the thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Doctor of Philosophy

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Booster biocides were introduced as alternative antifouling products to replace tributyltin (TBT) since 1987. Irgarol (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) and Diuron (*N*-(3,4-dichlorophenyl)-*N,N*-dimethylurea) are the most commonly used biocides in recent decades. This thesis therefore provides the baseline information of Irgarol and Diuron from various coastal compartments such as ports and coral reef Islands waters of Malaysia Peninsular as well as the eco-toxicology effect data of the biocides to the coral reefs using fatty acid composition as biomarker.

The average concentrations observed in ports (commercial and fisheries) and islands (mean ± SD) were 239 ± 64.2 ng/L (Kemaman), 447 ± 104 ng/L (Johor), 423 ± 230 ng/L (Klang North), 447 ± 270 ng/L (Klang South), 663 ± 693 ng/L (Klang West), 622 ± 432 ng/L (Redang) and 40.8± 27.9 ng/L (Bidong). Individual results were from non-detected (ND) to 2021ng/L at Klang, ND to 1397ng/L at Johor, and 5

to 846ng/L at Kemaman. Results from Coral reef islands, Redang and Bidong ranged from ND to 1370 ng/L. Temporal variation has shown no significant difference ($P > 0.05$) for November and January, while at April there is significant difference between the sampling sites. Temporal variation shows that, only 1% of 28 stations sampled on November, 2011 was above the environmental risk limit of 24 ng/L as restricted by the Dutch Authorities for marine organisms. Contrary to November 2011, of 28 stations sampled at January and April 2012, 46% and 92% were above the limit respectively.

The concentrations of diuron in water ranged from ND to 285ng/L were detected. The detection frequency was higher (100% of all 15 samples) at Klang North port, compared to (80%, 12/15 samples) and (73%, 11/15 samples) at Southern and Western Port of Klang respectively. Detection frequency of diuron at Kemaman was 87% (13/15 samples) whereby Johor Port was 92% for 22/24 samples. Coral reef islands of Terengganu, Redang and Bidong were sampled at March, 2012 during the boats activity period and results showed that (100%, 10/10) sampled sites were contaminated by Diuron. Temporal variation results show relatively high concentrations of diuron but no significant difference ($P>0.05$) during November and January (North-East Monsoon) in Klang ports (North, South and West), while higher levels of diuron were detected during April, 2012 (Inter monsoon) in Kemaman, and Johor port. Although no site shows concentration above the maximum permissible concentration of 430ng/L for Diuron as restricted by the Dutch Authorities for marine organisms, however, long term exposure studies for environmental relevance levels of diuron around coral reefs should be given a priority in future.

The 96 h-LC_{50}'s of Irgarol and Diuron were established for acute exposure and found to be 0.535±0.065 and 1.627±0.181mg/L respectively for *Lates Calcarifer* used in this study. The sublethal exposure (21 days) was done for *Lates calcarifer,* and short term exposure (4 days) for *Galaxea fascicularis* and *Fungia fungites.* The results showed that, fresh and control samples of *Lates calcarifer* and *Galaxea fascicularis* were not significant different (P>0.05) and dominated by Polyunsaturated Fatty Acids (PUFA) followed by Saturated Fatty Acids (SAFA) and then Monounsaturated Fatty Acids (MUFA), while *Fungia fungites* oppose the trend with SAFA dominated more followed by PUFA and MUFA. The trends for other tested groups (50, 30 and 10% of LC_{50} values) *Lates calcarifer* and (20, 100 and 500µg/L) *Galaxea fascicularis* and *Fungia fungites* were significant different (P<0.05) for both Irgarol and Diuron, with species suffered more as the dose of chemicals increased. The results showed that, corals species exposed in Irgarol were suffered more than those exposed in Diuron even if they were exposed at the same concentration ratios.

In general the results of this thesis demonstrate the detection of novel antifouling materials Irgarol and Diuron which advocates the need of proper monitoring and conservation strategies for the coastal resources. Comparing to eco-toxicological data, the present results indicate that at present the level of contamination of Irgarol is much higher in this region while Diuron is not at an alarming stage for the health of corals and other marine organisms. Furthermore the ecotoxicological data proved that both Irgarol and Diuron may affect the fatty acids composition of coral reef organisms even if exposed at the low levels of these biocides.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu bagi memenuhi keperluan untuk mendapatkan Ijazah Doktor Falsafah

KEHADIRAN BAHAN PENGGALAK BIOSIDA DI SEKITAR SEMENANJUNG MALAYSIA: ***Implikasi ketoksikannya kepada organisma terumbu karang terpilih***

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Januari 2013

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Penggalak racun biologi telah diperkenalkan sebagai produk alternatif bagi anti-teritip untuk menggantikan tributyltin (TBT) sejak tahun 1987. Irgarol (2-methylthio-4-tert-butylamino-6-cyclopro-pylamino-s-triazine) dan Diuron (N-(3,4-dichlorophenyl)-N, N-dimethylurea) adalah racun biologi yang paling biasa digunakan sejak beberapa dekad kebelakangan ini. Maka, tesis ini menyediakan maklumat asas mengenai Irgarol dan Diuron dari pelbagai sudut kawasan pantai seperti pelabuhan dan kepulauan terumbu karang di perairan Semenanjung Malaysia. Tesis ini juga membentangkan data kesan eko-toksikologi racun biologi terhadap terumbu karang menggunakan komposisi asid lemak sebagai penanda bio.

Kepekatan purata yang diperhatikan di pelabuhan (komersial dan perikanan) dan pulau-pulau (min ± SD) adalah 239 ± 64.2 ng/L (Kemaman) ,447±104ng/L (Johor), 423 ± 230 ng/L (Klang Utara), 447 ± 270 ng/L (Klang Selatan), 663 ± 693 ng/L

(Klang Barat), 662 ± 432 ng/L (Redang) dan 40.8 ± 27.9 ng/L (Bidong). Keputusan individu adalah daripada tidak dikesan (TD) kepada 2021 ng/L di Klang, TD 1397 ng/L di Johor, dan 5 hingga 846 ng/L di Kemaman. Keputusan dari Kepulauan terumbu karang, Redang dan Bidong antaranya daripada TD kepada 1370ng/L. Variasi masa telah menunjukkan tiada perbezaan yang signifikan ($P>0.05$) bagi bulan November dan Januari, manakala pada April terdapat perbezaan yang signifikan antara tapak persampelan.Variasi temporal menunjukkan bahawa, hanya 1% daripada 28 stesen yang disampel pada bulan November 2011, adalah melebihi had risiko alam sekitar 24 ng/L seperti yang dihadkan oleh Pihak Berkuasa Belanda untuk organisma marin. Bertentangan dengan bulan November 2011, 28 stesen yang disampel pada Januari dan April 2012, 46% dan 92% masing-masing telah melebihi had yang ditetapkan

Kepekatan Diuron di dalam air adalah dari TD kepada 285 ng/L telah dikesan. Kekerapan pengesanan adalah lebih tinggi (100% bagi semua 15 sampel) di pelabuhan Klang Utara, berbanding dengan (80%, 12/15 sampel) dan (73.3%, 11/15 sampel) masing-masing di Pelabuhan Selatan dan Barat Klang. Kekerapan pengesanan Diuron di pelabuhan Kemaman adalah pada 86.7% (13/15 sampel) manakala Pelabuhan Johor pula adalah 91.7% untuk 22/24 sampel. Kepulauan terumbu karang Terengganu, iaitu Redang dan Bidong telah disampel pada bulan Mac 2012, semasa tempoh aktiviti bot dan keputusan menunjukkan bahawa (100%, 10/10) kawasan yang disampel telah dicemari oleh Diuron. Keputusan variasi masa menunjukkan kepekatan yang agak tinggi Diuron tetapi tiada perbezaan yang signifikan ($P>0.05$) pada November dan Januari (Monsun Utara-Timur) di pelabuhan Klang (Utara, Selatan dan Barat), manakala tahap yang lebih tinggi Diuron telah

dikesan pada bulan April, 2012 (antara monsun) di Kemaman, dan Johor port. Walaupun kawasan yang telah disampel tidak menunjukkan kepekatan melebihi kepekatan maksimum yang dibenarkan, iaitu, 430 ng/L untuk Diuron yang telah dihadkan oleh Pihak Berkuasa Belanda bagi organisma marin, walaubagaimanapun, kajian bagi pendedahan jangka panjang untuk tahap relevan Diuron di persekitaran terumbu karang haruslah diberi keutamaan di masa hadapan.

96 h-LC_{50} daripada Irgarol dan Diuron telah dijalankan bagi pendedahan akut dan didapati 0.535 ± 0.065 dan 1.627 ± 0.181 mg/L masing-masing untuk ikan Siakap ‚*Lates calcarifer'* telah digunakan dalam kajian ini. Pendedahan sublethal (21 hari) telah dilakukan untuk *Lates calcarifer*, dan pendedahan jangka pendek (4 hari) untuk *Galaxea fascicularis* dan *Fungia fungites*. Keputusan menunjukkan bahawa, sampel baru serta sampel kawalan bagi *Lates calcarifer* dan *Galaxea fascicularis* tidak berbeza secara ketara iaitu (P> 0.05) telah didominasi oleh Asid lemak-Poli Tidak Tepu (PUFA) diikuti oleh Asid lemak Tepu (SAFA) dan kemudian Asid lemak-Mono Tidak Tepu (MUFA), manakala *Fungia fungites* menentang trend dengan SAFA lebih menguasai diikuti oleh PUFA dan MUFA. Trend untuk kumpulan lain yang diuji (50, 30 dan 10% daripada LC_{50} nilai*) Lates calcarifer* dan (20, 100 dan 500 µg/L) *Galaxea* fascularis dan *Fungia fungites* mempunyai perbezaan yang signifikan (P<0.05) untuk kedua-dua Irgarol dan diuron, dengan spesies mengalami lebih menderita apabila dos bahan kimia meningkat. Keputusan menunjukkan bahawa, spesies karang terdedah dalam Irgarol telah mengalami lebih penderitaan daripada yang terdedah dalam Diuron walaupun mereka terdedah pada nisbah kepekatan yang sama.

Secara umum keputusan tesis ini menunjukkan pengesanan bahan novel antifouling Irgarol dan Diuron yang menyokong keperluan pemantauan yang betul dan strategi pemuliharaan bagi sumber pantai. Perbandingan data eko-toksikologi, keputusan yang hadir menunjukkan bahawa pada masa ini tahap pencemaran Irgarol adalah lebih tinggi di rantau ini manakala Diuron tidak pada peringkat yang membimbangkan untuk kesihatan batu karang dan lain-lain organisma marin. Tambahan pula data eko-toksik membuktikan bahawa kedua-dua Irgarol dan Diuron boleh menjejaskan komposisi asid lemak organisma terumbu karang walaupun terdedah pada peringkat rendah biosid ini.