THE ROLE OF EXOGENOUS ANTIOXIDANTS L-ASCORBIC ACID AND α-TOCOPHEROL ON THE DEPURATION OF POLYCHLORINATED BIPHENYL 126 (PCB 126) IN THE MARINE MUSSEL, *Perna viridis*

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Abstract of 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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July 2014

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Retention of persistent organic pollutants (POPs) in organisms is one of the key concerns in environmental safety. The retention time of POPs in organisms is regulated by bioaccumulation and depuration. Depuration of POPs from an organism depends on the homeostatic balance of oxidant and antioxidant at its cellular level. Oxidative stress caused by the bioaccumulated POPs would collapse the depuration process and eventually lead to mortality. Hypothetically, presence of exogenous antioxidants shall be able to enhance the depuration process in an organism by re-instating the oxidant and antioxidant balance. Hence, this study aims to reveal the role of exogenous antioxidants in depuration process of a POP, PCB126 in marine mussel, *Perna viridis*. *P. viridis* was used as the test organism and toxicated with PCB126 under controlled environment. α-Tocopherol and L-ascorbic acid were introduced to the toxicated mussels at different concentrations to determine the efficiency of the antioxidants in PCB126 depuration. The depuration efficiency was then assessed kinetically and the role of the exogenous antioxidants was investigated by measuring biochemical responses of the mussels which are CYP450, Glutahione (GSH), Glutathione-S-Transferase (GST), superoxide dismutase (SOD), catalase

(CAT) and lipid peroxidation (LPO). The α-tocopherol and L-ascorbic acid supplementation had significantly improved PCB126 depuration in *P. viridis*. Both lipophilic and lipophobic antioxidants when applied individually to the toxicated mussels were able to reduce accumulated PCB126 from 108 μg g^{-1} down to 9 μg g^{-1} (α-tocopherol at 200 mg L^{-1}) and 20 μg g^{-1} (L-ascorbic acid at 200 mg L^{-1}). As compared to the control, PCB 126 concentration remained at 55 μg g^{-1} in the mussels. Kinetically, the depuration rate constant, k_2 for α-tocopherol and L-ascorbic acid were 0.1325 ± 0.01 day^{-1} and 0.0753 ± 0.01 day^{-1} respectively which was higher than control (0.0261 ± 0.01 day^{-1}). There was no significant difference between the combination (0.1032 ± 0.02 day^{-1}) and optimum individual α-tocopherol and L-ascorbic acid treatments. The PCB126 depuration increased the CYP450 concentration and decreased in GST. α-Tocopherol was used to neutralize the reactive metabolites and maintained the LPO level as compared to L-ascorbic acid and GSH. No significant changes in SOD and CAT concentration showed that the majority of reactive metabolites produced were electrophiles. The results from the biochemical reactions were used to propose the PCB126 depuration pathway in *P. viridis*. In the case of combining both α-tocopherol and L-ascorbic acid, the antioxidants interacted antagonistically by reducing the damage caused by reactive metabolites on the cell walls of the mussels. This improved the survival of the mussels. Based on the results, the exogenous antioxidants may become a potential solution to decontaminate POPs in seafood and improve their survival in the aquaculture industry.

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PERANAN ANTIOKSIDAN LUARAN ASID L-ASKORBIK DAN α-TOCOPHEROL TERHADAP PENYINGKIRAN POLYCHLORINATED BIPHENYL126 (PCB126) DALAM KUPANG MARIN, *Perna viridis*

ONG PEI THING

July 2014

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Pengekalan bahan pencemar organik dalam organisma adalah salah satu kekhuatiran utama dalam keselamatan persekitaran. Tempoh pengekalan bahan pencemar organik kawalatur oleh proses pengumpulan dan penyingkiran dalam organisma. Penyingkiran bahan pencemar organik daripada organisma adalah bergantung kepada keseimbangan homeostatik bagi oksidan dan antioksidan pada tahap sel. Tekanan oksidatif yang disebabkan oleh pengumpulan bahan pencemar organik boleh melemahkan proses penyingkiran dan akhirnya menyebabkan kematian pada organisma. Secara hipotesis, kehadiran antioksidan luaran boleh meningkatkan proses penyingkiran dalam organisma dengan mengembalikan keseimbangan antara oksidan dan antioksidan. Maka, objektif penyelidikan ini adalah untuk mendedahkan peranan antioksidan luaran dalam proses penyingkiran bahan pencemar organik, PCB126 dalam kupang marin, *Perna viridis*. *P. viridis* digunakan sebagai organisma ujian dan dicemarkan dengan PCB126 dalam keadaan terkawal. Pelbagai kepekatan α-tocopherol dan asid L-askorbik diberikan kepada kupang yang telah dicemari PCB126 untuk mengetahui keupayaan antioksida dalam penyingkiran PCB126. Keupayaan penyingkiran juga dinilai secara kinetik dan peranan antioksidan luaran

ditentukan dengan pengukuran biokimia dalam kupang iaitu CYP450, Glutahione (GSH), Glutathione-S-Transferase (GST), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO). Penambahan α-tocopherol dan asid L-askorbik didapati meningkatkan penyingkiran PCB126 dalam *P. viridis*. Apabila kedua-dua antioksidan luaran larut dan tak larut lemak diberikan secara individu kepada kupang tercemar, kepekatan PCB126 diturunkan daripada 108 μg g^{-1} kepada 9 μg g^{-1} (α-tocopherol pada 200 mg L^{-1}) dan 20 μg g^{-1} (Asid L-askorbik pada 200 mg L^{-1}). Berbanding dengan kupang kawalan, kepekatan PCB126 kekal pada 55 μg g^{-1}. Kadar penyingkiran konstan, k_2 untuk rawatan α-tocopherol dan asid L-askorbik adalah 0.1325 ± 0.01 $hari^{-1}$ dan 0.0753 ± 0.01 $hari^{-1}$ dimana ia adalah lebih tinggi daripada tiada rawatan (0.0261 ± 0.01 $hari^{-1}$). Tiada perbezaan signifikan antara rawatan kombinasi (0.1032 ± 0.02 $hari^{-1}$) dan rawatan α-tocopherol dan asid L-askorbik secara individu. Penyingkiran PCB126 meningkatkan kepekatan CYP450 dan menurunkan GST. α-Tocopherol digunakan untuk meneutalkan metabolit reaktif dan mengekalkan tahap LPO. Tiada perubahan ketara dalam kepekatan SOD and CAT menunjukkan bahawa kebanyakan metabolit reaktif adalah elektrofil. Keputusan daripada tindakbalas biokimia digunakan untuk mencadangkan mekanisma penyingkiran PCB126 dalam *P. viridis*. Dalam rawatan kombinasi α-tocopherol dan asid L-askorbik, kedua-dua antioksidan tersebut berinteraksi secara 'antagonistic' dengan mengurangkan kerosakan disebabkan oleh radikal bebas pada dinding sel kupang. Keadaan ini meningkatkan kadar hidup pada kupang. Berdasarkan kepututsan, antioksidan luaran mempunyai potensi untuk menyingkir bahan pencemar organik dalam makanan laut dan meningkatkan kadar hidup mereka dalam industri akuakultur.