

論文内容の要旨

論文題目 Assessment of organotin contamination and imposex incidence in tropical gastropods (*Thais* spp.) collected along the Malaysian coastal areas.

(マレーシア沿岸域におけるレイシガイ類 (*Thais* spp.) の有機スズ蓄積とインポセックスについて)

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This study was conducted as a part of monitoring works to evaluate how effective the rules and regulation of global banning was enforced, or how the legislation was enforced among participated parties on the use of organotin (OTs) based antifouling paints, specifically tributyltin (TBT) based paints applied on submerged marine structure and vessels. The global banning was first set in action on year 2003 on vessels less than 25m, and total restriction on all marine structure was started on September 2008. This monitoring works was done using the tropical gastropods collected along the Malaysia coastal areas as the best bioindicator for imposex development in 2007. The selection of Malaysia as the sampling location was due to the important role of Malaysian waterways that has been listed as one of the busiest water ways in the world. Therefore, this study could serve as a good model for environmental conservation of the organotin contamination enforced by legislatures around the world. The selection of imposex development (formation of male sexual organs in female individual) is because this morphological change in the female gonochoric gastropods has been known as the most sensitive to the organotin contamination.

Samples of *Thais gradata*, *T. clavigera*, *T. tuberosa* and *T. bitubercularis* were collected from 41 locations along the coastal areas of Peninsular Malaysia and East Malaysia. Samples were analyzed for imposex incidence with support of the histopathological analysis of the gonad, organotin analysis (butyltins (BTs), including tributyltin, dibutyltin (DBT), and monobutyltin (MBT), and phenyltins (PTs), including triphenyltin (TPT), diphenyltin (DPT), and monophenyltin (MPT) concentrations), and trace elements analysis (copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn) concentrations) in tissue burden. *T. gradata*

was found widely distributed along the Straits of Malacca and the Johor Strait. *T. clavigera* was found abundant in area facing the South China Seas such as Sedili, Batu Layar, Sg. Rengit and along Sarawak and Sabah coast. *T. tuberosa* and *T. bitubercularis* were the rare species in Malaysia and were found only at seven sites on the northern part of Peninsular Malaysia. Data obtained from this study were discussed in comparison with data from literature. Since sample number of *T. tuberosa* and *T. bitubercularis* was limited, the results and discussion of these two species were pooled together by taking into account of the similarity of their shell length and mostly co-existing at the same site.

This study revealed new scheme of imposex gradual expression and the evident of sterile condition in imposex affected snails among *T. gradata*, *T. calvigera*, *T. bitubercularis* and *T. tuberosa*. Morphological expressions of the imposex scheme for *T. gradata* were classified into seven stages (stage 0 to stage 6) based on the development of vas deference sequence (VDS), penis bulk and the gonad. The latest imposex scheme in *T. gradata* clearly presented the process of VDS development, the structural changes from penis bulk to a penis with flagellum, changes of a normal vaginal opening to a swollen vaginal opening. All these morphological development have never been documented in previous studies. Histopathological analysis of the gonad revealed that imposex affected individual had a gonad condition similar to that of normal female; presence of mature oocytes filled with yolk globules. No abnormalities of the gonad in the latest imposex scheme in *T. gradata* suggested that physical condition caused the sterility in the end of the imposex stages was caused by physical condition including prohibition of copulation and / or barren capsule formation, and/or blocking the eggs releasing process.

In the case of *T. clavigera*, the morphological expressions of imposex and histopathological analysis of the gonad managed to classify the imposex development in affected individuals into five stages (Stage 0 to Stage 4). This gradual developmental scheme was based on the VDS development, the penis bulk formation, adhesion of vas deferens with the end of oviduct, presence of aborted egg capsules in the capsule gland and other abnormality such as incomplete ontogenetic closer of the pallial oviduct and gonad alteration. Three types of imposex expression were found from Stage 2 (Type A and Type *), followed by Stage 3 -Type (A and B). The physical evidence leading to sterility was observed in the end of each imposex Type; imposex Type * caused prohibition of copulation and capsule formation. Imposex Type-A prohibited releasing process of eggs. Gonad in imposex Type-B was infertile ovo-testis with huge number oocytes atresia and testicular necrosis. This kind of gonad alteration in imposex affected snails was documented for the first time in this study. However, further analysis is needed to confirm the relationship between the gonad malformations in imposex affected snails and organotin, specifically TBT contamination.

New imposex development scheme was also obtained for *T. tuberosa* and *T. bitubercularis*. For these two species, the gonad condition in imposex affected snails was consistent with that in normal female. Both *T. tuberosa* and *T. bitubercularis* showed imposex development occurred up to four stages (Stage

0-Stage3) with two additional types (Type-A and-B), which were different from the conditions obtained for *T. gradata* and *T. clavigera*. Type-A showed a continuing growth of VDS without the development of penis. Type-B showed continuing growth of penis and the flagellum. The final imposex stages (Stage 3) for both types were classified by blockage of the vulva opening with the development of the VDS. The morphological findings suggested that serious imposex level could disturb the normal function of the snail's reproductive system.

The assessment of OTs concentration in four species of *Thais* collected from the Malaysian coastal areas revealed that concentrations of BTs species were higher than concentrations of PTs species in all samples across the sampling sites. The BTs levels in samples of *T. gradata* collected from Johor and Johor Baru were found to be higher than the levels in other biota as reported in literature. Meanwhile BTs levels of samples from east channel of the Strait of Johor, Penang and Selangor were lower than the levels recorded in literature. For PT compounds, samples collected from Johor and Penang showed lower levels of PT than those reported in other biota. The remaining sites had no reference data for comparison. It was found that locations with high levels of imposex incidences tended to show high BTs levels in the tissue of snail except for several sites located in the east channel of the Strait of Johor. Among six organotin species, correlation analysis showed a significant relationship between the levels of DBT and TBT and the percentages of imposex incidence in the *T. gradata* samples. The DBT and TBT levels were also found to relate with the level of imposex severity.

For organotin analysis in *T. clavigera*, the highest organotin levels of all six organotin species were found in samples from Sarawak (Marina Bay). In site with available data for a comparison such as in Selangor (Tg. Harapan and P. Carey), all six organotin species were found higher than levels reported in literature. In Sabah, level of all three BTs species and MPT were found greater than ever recorded in other biota as reported 10 years ago. The BTs concentration pattern across the sampling sites for *T. clavigera* showed MBT was the dominant compounds in most of the sampling sites, which was followed by DBT and TBT. This indicates the degradation products of TBT occurred at higher level than the source of the pollutant (TBT) suggesting that in-put rate of TBT contamination decreased in recent years. By referring to the correlation analysis between OTs level and the number of boats present in the sampling site, significant positive relationship was found between the number of boats and TBT. The most suitable assumption or conclusion regarding this matter is that TBT was extensively used in antifouling paints applied on boat hulls in previous years, since the degradation rate of TBT in the sediment was slow or not occurred at all.

In the case of *T. tuberosa* and *T. bitubercularis*, OTs tissue burden in *T. tuberosa* and *T. bitubercularis* were examined for samples from three sampling sites. The results showed contradict pattern between BTs and PTs among the sampling sites. The highest MBT and DBT levels were found in sample from Pantai Kok (Langkawi), and TBT was found highest in sample from Lumut (Perak). For PTs, the highest level was found in sample from Pantai Bersih (Penang). Sample from Pt. Kok (Langkawi) showed

greater increment of all three BTs species, MPT and DPT as compared to levels previously reported in other biota from Langkawi. In sample collected from Pt. Bersih (Penang), the levels of all six organotin species were found almost equal with the level reported in literatures.

By taking into account all OTs concentrations from all four species of *Thais* along the Malaysian coastal areas, two possibilities were regarded as the source of the contamination. First, the TBT-based paint still applied on boats and ships hulls even after the 2003 regulation as referred to positive relationship between TBT burden in *Thais* tissue and number of boats across 30 sampling sites that were available for OTs analysis. Source of the contaminations is also believed to be the sediments which is supported by the fact that the concentrations of TBT was lower than the degradation products (MBT and DBT) at all study sites.

For trace element analysis, various concentrations of Cu, Zn, Cd and Pb were found in *Thais* collected along the Malaysian coastal areas. The concentration variation was predicted to be caused by the difference in the metabolic rate of each trace element in gastropods. The concentration of Cu, Zn, Cd and Pb seemed to basically reflect the anthropogenic sources in the vicinity. *Thais* collected from sites with high exposure of biocides and food industrial waste accumulated more Zn and Cu, while lower levels of these two elements were found in samples collected from sites with either of those anthropogenic sources. High level of Cd was found in the tissue of *Thais* collected from sites received industrial waste of textile and petrochemicals manufactures. Very low of Pb concentration in tissue sample was found throughout this study suggested an effective depuration system of this element in snails.

The estimation of daily exposure of TBT in Malaysian people using average consumption rate of *Thais* (3.6g/day), showed all sites having lower than the WHO TDI of 0.1 μ g Sn/kg/day (Equivalent to 0.3 μ g TBTO/kg/day). For potential health risk of trace element, this study managed to propose ideal amount of daily consumption of *Thais* (5g/day). Based on the value, calculated Cu, Zn, Cd and Pb dose rate and daily intake rate were below the EPA dose limit and guideline values. Therefore, there seems to be no possibility of acute toxicities of Cu, Zn, Cd and Pb by consuming *Thais* collected from Malaysian coastal areas.

In this study, it was obvious that different species showed different imposex criteria although *T. tuberosa* and *T. bitubercularis* expressed similar process of imposex. Level of the imposex severity (imposex stages) also differed among species. The concentration pattern of OTs tissue burdens and level of imposex stages obtained in *T. gradata* and *T. clavigera* was also differed. These differences strongly indicate the species specific responses of the gastropods toward the contamination level of responsible pollutant inducing imposex (TBT) in the gastropods. Meanwhile, the insignificant results for the imposex levels of *T. tuberosa* and *T. bitubercularis* and the possible factors influenced the imposex incidence, accompanied with limited distribution of these two species, indicate that not all of the *Thais* species are good bioindicator for the monitoring program. The species specific mechanisms of imposex incidence in snails should be studied in future.