

NOTE

Increasing levels of Tributyltin-induced Imposex in Muricid Gastropods at Phuket Island, Thailand

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Tributyltin (TBT)-induced imposex, or the masculinization of female gastropods, was used in an investigation of TBT contamination around the major harbours at Phuket Island, Thailand. From 21 stations along the south-east coast of Phuket Island a minimum of 50 specimens of the muricid *Thais distinguenda* were collected and examined for the presence of imposex in February–April 1996 and in January–April 1998. The results showed clearly that both the incidence and the distribution of imposex increased dramatically from 1996 to 1998. In 1996, imposex was recorded at 10 stations within 3.5 km of the nearest port, but in 1998 imposex was present at 18 stations up to 7.5 km from the nearest port. A similar tendency to increase in the incidence and distribution of imposex was found using *Thais bitubercularis* and *Morula musiva* as indicators. Copyright © 1999 John Wiley & Sons, Ltd.

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INTRODUCTION

Imposex, or the masculinization of female gastropods, was first observed in *Nucella lapillus*

(Linnaeus, 1758) (family Muricidae)¹ and a decade later it was linked to the presence of organotin.²

Numerous investigations have shown that there is a significant correlation between incidence of imposex and the concentration of TBT in the snail tissue of gastropods^{3–5} the sediment⁶ or in the water.⁷ It has furthermore been shown in laboratory experiments that imposex in neogastropods was initiated and promoted by exposure of part-per-billion (1:10⁹) concentrations of bis(tributyltin) oxide (TBTO)⁸ or TBT.⁵ Because imposex development is a TBT dependent matter, a scale for six species of gastropods has been developed to calculate TBT concentrations in seawater based solely on biological measurements in terms of imposex.⁹ Little doubt now exists that TBT is the major organotin causing imposex in gastropods.¹⁰ However, triphenyltin (TPT)-induced imposex has been produced in the Japanese rock shell *Thais clavigera*,¹¹ even though *Nucella lapillus* did not produce imposex when exposed to TPT.¹⁰ A few field observations have suggested that the causative agent is not always TBT,^{12,13} but none of them has proved this.¹⁴

Imposex has mainly been observed close to ports, ship yards, marinas and around mariculture facilities where organotin compounds are used in antifouling paints,^{19,20} but it has also been found in *Buccinum undatum* (Linnaeus, 1758) from the open sea, in relation to the deep sea routes in the North Sea.²¹

The dilemma of tributyltin is that its extreme effectiveness as an antifouling agent also makes it a potent toxin of anthropogenic origin that threatens seafood resources in coastal environments.²² Numerous investigations have shown that the presence of TBT is harmful to the culture of commercially important molluscs such as *Crassostrea gigas* (Thunberg, 1819),^{23,24} *C. angulata* (Lamarck, 1819)²⁵ and *Mytilus edulis* (Linnaeus, 1758).^{26–28}

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Figure 1 Sampling sites in 1996 and 1998 along the south-east coast of Phuket Island, Thailand. Notice the location of Phuket Harbour (station 4), the deep-sea port (station 6) and Chalong Marina (station 15).

In Archachon Bay in France it was discovered that TBT caused reduced settling of oyster spat and declining growth rates of adult oysters, resulting in a significant reduction of oyster culture.²⁹ This was the first report of economic consequences caused by the use of TBT, which in 1982 led to the implementation of legislation in France to reduce the use of TBT-based paints to boats larger than 25 m.³⁰

Since then similar legislation has been implemented in Europe, USA, Australia, New Zealand and Japan.^{18,31} Subsequent investigations have documented the positive effects of this legislation in terms of declining concentrations of TBT in water, sediment and mollusc tissue.³² In south-east Asia, however, there is no restriction on the use of TBT-based antifouling paints, despite the fact that these countries derive a substantial part of their income from mollusc culture.³³ Concern about the impact of TBT in south-east Asia has been expressed after high levels of imposex were found in muricids from Singapore,³⁴ Malaysia,³⁵ Thailand^{36,37} and Indonesia.³⁸

The only published investigation on concentrations of butyltin compounds in sediments from

Table 1 Imposex incidence of *T. distinguenda* from 21 sites in 1996 and 1998^a

Station no. and name	Sample size		Imposex (%)	
	1996	1998	1996	1998
1. Laem Nga	58	50	0	0
2. Laem Mai Phai	53	61	0	62
3. Laem Phap Pha	51	50	57.4	83
4. Phuket Harbour	50	50	89.67	100
5. Laem Nam Bor	50	50	84	91
6. Deep-sea port	88	60	65.79	100
7. Tin smelting plant	54	61	34.38	68
8. Taphao Yai (west)	92	59	7.1	74
9. Taphao Yai (south)	95	51	0	62
10. Koh Taphao Noi	50	58	0	32
11. Hotel Cape Panwa	124	59	0	63
12. Laem Panwa	111	63	0	58
13. Laem Yam Yen	52	51	50	71
14. Laem Wing	51	61	22.2	62
15. Chalong Bay (East)	16	50	57	58
16. Koh Lon (East)	52	50	5.56	4.3
17. Koh Thanan	56	50	0	34
18. Koh Lon (West)	58	61	0	14
19. Koh Aew	50	63	0	2
20. Koh Hey	50	50	0	0
21. Koh Maiton	50	50	0	0

^a A significant increase was found between 1996 and 1998 ($P < 0.001$; Wilcoxon signed-rank test).

Table 2 Imposex incidence of *T. bitubercularis* and *M. musiva* from five sites in 1996 and 1998

Station no. and name	Sample size				Imposex (%)			
	<i>T. bitubercularis</i>		<i>M. musiva</i>		<i>T. bitubercularis</i>		<i>M. musiva</i>	
	1996	1998	1996	1998	1996	1998	1996	1998
1. Laem Nga	59	58	50	50	21	79	0	0
3. Laem Phap Pha	52	57	52	48	42	100	0	10
4. Phuket Harbour	32	51	40	41	100	100	19	62.5
5. Laem Nam Bor	50	42	50	23	41.4	100	0	0
15. Chalong Bay	50	25	50	31	100	100	0	5

Thailand showed that the deep-sea port on the south-east coast of Phuket Island had the second-highest level in the country. Furthermore, the high concentrations of TBT in sediments at Phuket were associated mainly with harbours used by large commercial vessels.³⁹

The present investigation focuses on the reported TBT contaminated areas on the south-east coast of Phuket Island, and reports the incidence and change in TBT-induced imposex levels of three muricid gastropods from 1996 to 1998.

MATERIALS AND METHODS

Along the south-east coast of Phuket three different sites of high boating activity were identified in 1996.³⁶ The first site was the deep-sea port of Phuket, which is the only port on the west coast of Thailand where large cruise ships and oil tankers can go alongside the quay. The second site was Chalong Marina, which is the largest marina on the west coast of Thailand. The third site chosen was Phuket Harbour, which is used by a large fleet of fishing boats and ferries. In addition, several shipyards discharge their waste directly into the harbour.

Twenty-one stations, including eight islands, were selected to represent gradients of contamination from the three identified hotspots along the south-east coast of Phuket Island.³⁶ Remote islands were chosen as controls where TBT contamination was presumed to be absent (Fig. 1).

The muricid specimens were collected at the same stations on two occasions in February–April 1996 and two years later in January–March 1998. From each of these 21 stations, a minimum of 50 *Thais distinguenda* (Dunker and Zelebor, 1866) were collected in both 1996 and 1998 (Table 1).

Furthermore, *Thais bitubercularis* (Lamarck, 1822) and *Morula musiva* (Kiener, 1836) were collected at each of five stations in 1996 and in 1998 (Table 2). The samples were preserved by deep-freezing.

The total length of each snail was measured from the apex to the distal end of the siphonal canal to the nearest 0.5 mm using callipers. The snail's shell was crushed with a hammer; the soft parts were removed gently, placed in a Petri dish and examined using a binocular microscope. The gender was determined by identification of the sperm-ingesting gland and the capsule gland in females and the presence of the prostate gland in males. In most specimens ovaries could be distinguished from testes by the colour as an aid in determination of gender.

The lengths of both male penis and female pseudo-penis were measured to the nearest 0.1 mm using an eyepiece graticule mounted on a binocular

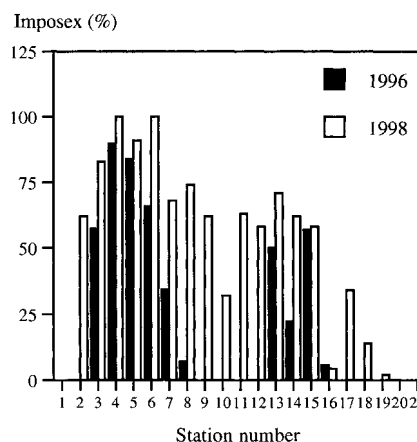


Figure 2 Proportion of female *T. distinguenda* with imposex in each sample in 1996 and 1998. Station numbers correspond to Fig. 1 and Table 1.

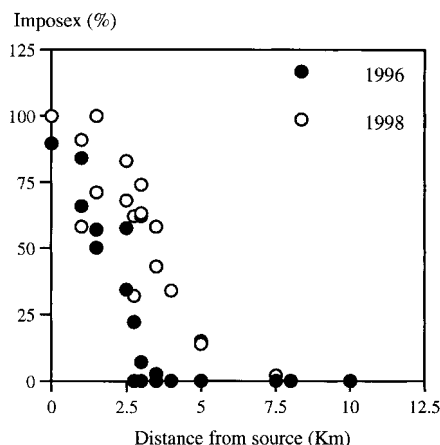


Figure 3 Distribution in 1996 and 1998 of female *T. distinguenda* with imposex in relation to the nearest presumed source of TBT.

microscope. The total length of a penis was measured from the beginning of the penial flagellum to the base of the penis. The imposex frequency was calculated as the proportion of females with imposex compared with the total number of females in the sample. A relative penis size index (RPSI) was calculated as (mean length of female penes \times 100)/(mean length of male penes).⁴⁰ Juvenile snails (*T. distinguenda* less than 20 mm, *T. bitubercularis* less than 25 mm and *M. musiva* less than 15 mm) were not included in the imposex measurements because of difficulties in the determination of gender.

RESULTS

In 1996, two very distinct peaks of imposex levels of *T. distinguenda* were observed: one as a combined effect of the deep-sea port and Phuket Harbour, and the second around Chalong marina (Fig. 2). The deep-sea port and Phuket Harbour are only separated by a shallow bay 4 km wide.

The presence of imposex in *T. distinguenda* in 1996 was clearly localized around these two hot-spots and the distribution was limited to approximately 3.5 km from these sources of TBT (Fig. 3).

The relative penis size index (RPSI) is a useful measurement to distinguish between two stations with 100% imposex, but in this investigation the RPSI values were excluded from the tables because they added no further information to the imposex incidence.

In 1998 the peaks of imposex levels of *T. distinguenda* were located around the same sites but the levels were much higher than those found in 1996 (Fig. 2 and Table 1). Additionally, the distribution range of imposex from the hotspots had been extended considerably—from 3.5 km in 1996 to approximately 7.5 km in 1998 (Fig. 3). In 1996 imposex was recorded at 10 stations along the south-east coast of Phuket whereas in 1998 imposex was recorded at 18 stations. The average level of imposex at the 21 stations was 22.5% in 1996 and 49.4% in 1998. A similar pattern of increased incidence of imposex was also observed for *T. bitubercularis* and *M. musiva* between 1996 to 1998 (Table 2). In 1996 *T. bitubercularis* showed imposex levels of 100% at two of the five stations, but in 1998 100% imposex was found at four stations (Table 2). Although *T. bitubercularis* is a more sensitive indicator of imposex than *T. distinguenda*, its distribution is less widespread at Phuket because it can only be found at stations with muddy water. Of the three muricids tested, *M. musiva* is the least sensitive indicator of imposex. The only incidence of imposex in *M. musiva* in 1996 was recorded at Phuket Harbour (station 4). In 1998, low levels of imposex was furthermore recorded at stations 3 and 15 (Table 2).

DISCUSSION

The results of this investigation show clearly that both the incidence and distribution of TBT-induced imposex have increased dramatically from 1996 to 1998 along the south-east coast of Phuket Island.

In 1996 the incidence of imposex in *T. distinguenda* and *T. bitubercularis* showed a clear localization around the deep-sea port, Phuket Harbour and Chalong Marina. In 1998 both the incidence and the distribution of imposex increased at most stations.

The average incidence of imposex in *T. distinguenda* from all stations doubled from 1996 to 1998. The dramatic increase in imposex levels can hardly be explained solely by increasing shipping activities or current patterns in the area. There may be some other contributing factor involved recently as two years is a relatively short timescale. A threshold level for the development of imposex by the muricids may have been exceeded. From a translocation experiment done in 1996, where 300 tagged *T. distinguenda* were moved from a station without imposex (station 21) to the deep-sea port,

16% imposex ($n = 60$) was found after three months, indicating that the development of imposex is relatively fast.³⁶

The development of imposex is an irreversible process¹⁷ specifically induced by organotin compounds with three alkyl groups attached to the tin atom, such as TBT, TPT and tricyclohexyltin.³³ In the marine environment the main origin of TBT and TPT is considered to be from the use in antifouling paints. It is not likely to be caused by the tin smelting plant (station 7) located 1 km south of the deep-sea port, because the imposex level of *T. distinguenda* at that station was considerably lower than the level at the deep-sea port in both 1996 and 1998 (Table 1).

Because imposex is irreversible and *T. distinguenda* can develop imposex in a relatively short time, the high level of imposex recorded in 1998 is not necessarily a product of a steady increase in TBT; it could be caused by one short exposure of the snails to high concentrations of TBT in the period from 1996 to 1998. One possible source of a sudden discharge of TBT could be from the shipyards located in the narrow channel that constitute Phuket Harbour (station 4), where the highest levels of imposex were recorded for all three species.

Among the muricid gastropods, mainly species belonging to the genus *Thais* have been used as indicators of TBT contamination in tropical areas.^{18,34,38} To cover a large area with different habitats and water quality it is often necessary to use different species. In this investigation *T. distinguenda* was chosen mainly because it was the most abundant muricid and present on all stations. Because of species-specific differences in sensitivity to TBT-induced imposex,³⁴ it is not possible to compare imposex levels from different species unless they were collected from the same station. *T. bitubercularis* has been used in an investigation from Singapore where imposex levels of 100% and sterilization of this species were recorded.³⁴ *M. musiva* is the least sensitive of the species studied and was only included in this investigation because it could be found in high numbers, and because it was previously found reliable as an indicator of TBT in an investigation from Malaysia.³⁵

An optimal indicator of TBT should be sensitive, easy to examine and widely distributed in the area in question, and in that respect *T. distinguenda* and *T. bitubercularis* should be considered as good indicators in future studies in the south-east Asian region because they are sensitive to TBT, easy to

examine, and widely distributed in south-east Asia.⁴¹

Few publications on TBT have been made from the south-east Asian region, and from tropical areas in general.⁴² Approximately 600 km south of the present study area, on the west coast of Malaysia, TBT levels up to 216.5 ng TBT g⁻¹ sediment wet weight were found in 1992.⁴²

The first paper on TBT concentrations in sediments from Thailand showed that among the 20 major ports of Thailand, the deep-sea port of Phuket had the second-highest concentration of TBT in the sediment (3900 ng TBT g⁻¹ sediment dry weight).³⁹ The sediment samples analysed in that investigation were collected from the deep-sea port of Phuket Island in March 1995 (station 6), where the present samples of gastropods were collected (S. Kan-atireklap, Eastern Marine Fisheries Development Center, Rayong 21160, Thailand, personal communication). The TBT level from 1995 in the deep-sea port was a ten fold higher than the concentrations measured in the Archachon Bay in France, where oyster culture was severely reduced from 1977 to 1981.²⁹ In view of the increasing imposex incidence recorded in the present study, it seems clear that the TBT concentrations at Phuket Island are a danger to the molluscan aquaculture industry in the area, and to the environment in general.

The legislation to reduce the use of TBT-based antifouling paints to ships greater than 25 m in length implemented in Europe, the USA, Australia and New Zealand has had significant positive effects in terms of declining imposex levels in several species of gastropods and declining concentrations of TBT in organisms, water and sediment.³⁰ The absence of similar legislation in the countries of south-east Asia could pose a serious threat to the economically important aquaculture industry of molluscs in the near future.

These findings strongly indicate that imposex is an effect of TBT as the highest incidence of imposex in both 1996 and 1998 were found close to the main areas of shipping activities at the deep sea port and fishing port (Table 1). Similarly the highest levels of imposex of both *T. bitubercularis* and *M. musiva* were found at the fishing port (Table 2).

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