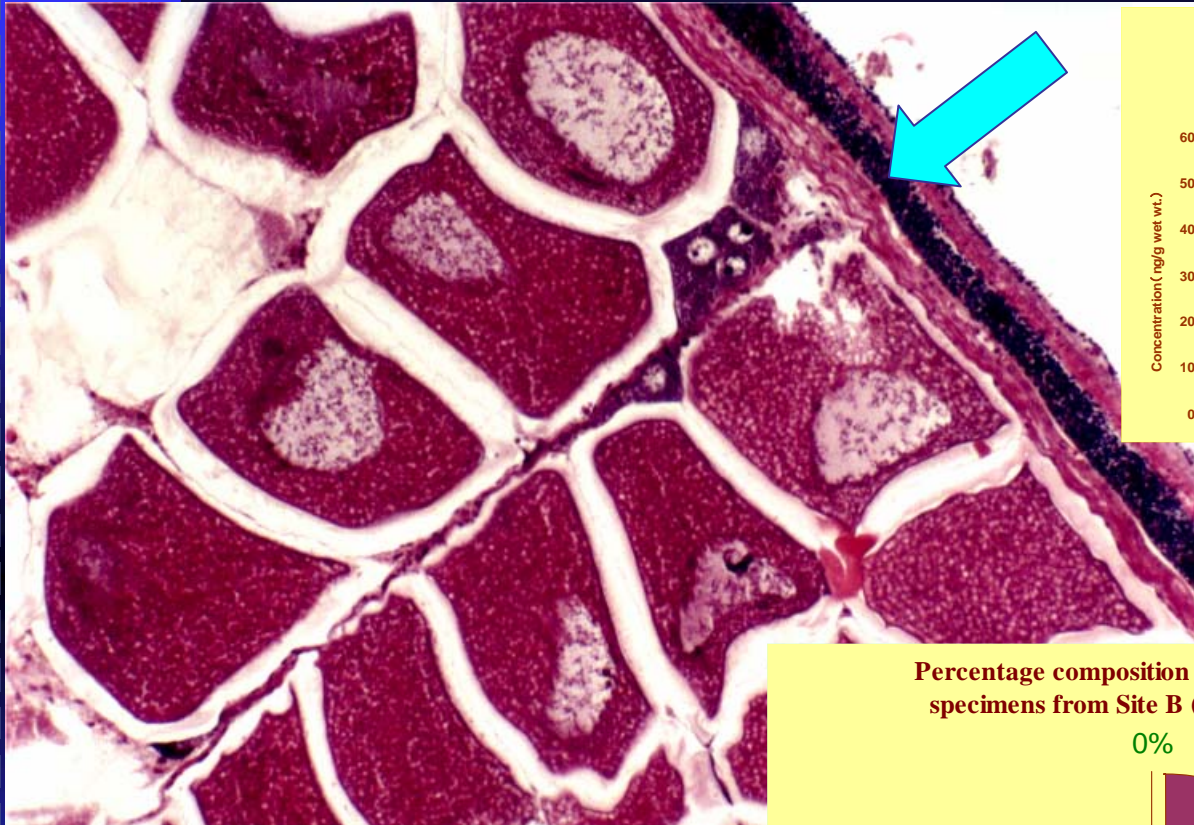
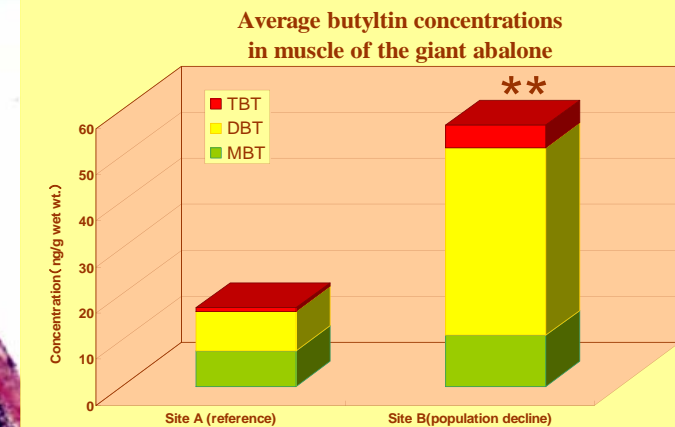


Ovarian spermatogenesis in *Haliotis madaka*



Spermatogenesis in ovary of the giant abalone (*H. madaka*) from Site B, Japan (April 1996)

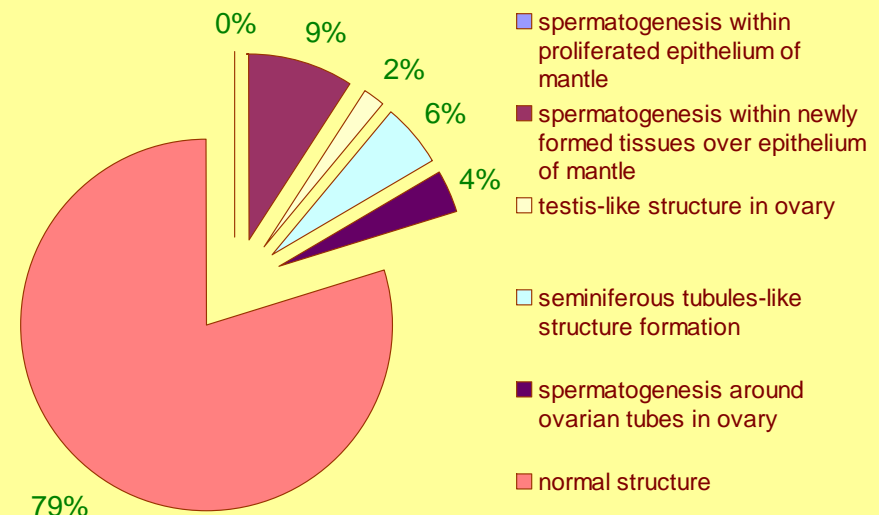
Horiguchi *et al.* (2000)



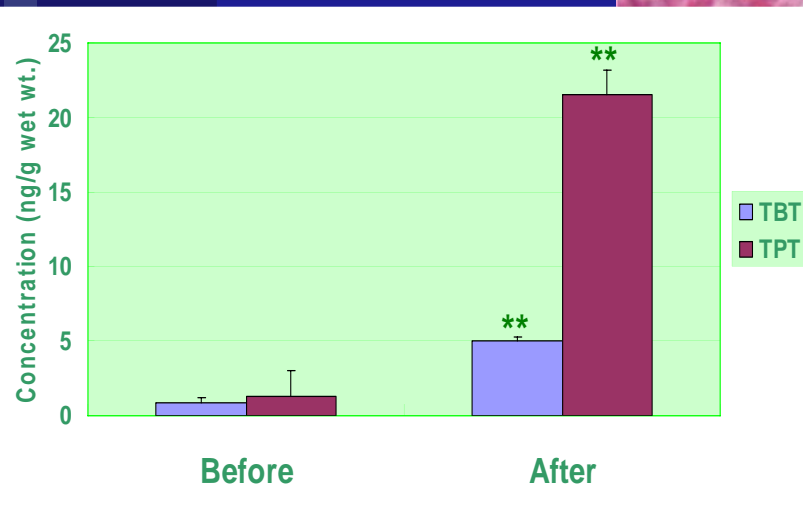
n=125

n=83

Percentage composition of masculinized females of the giant abalone specimens from Site B (a site representative of population decline)



In situ exposure experiment nearby a shipyard in Site B, Japan



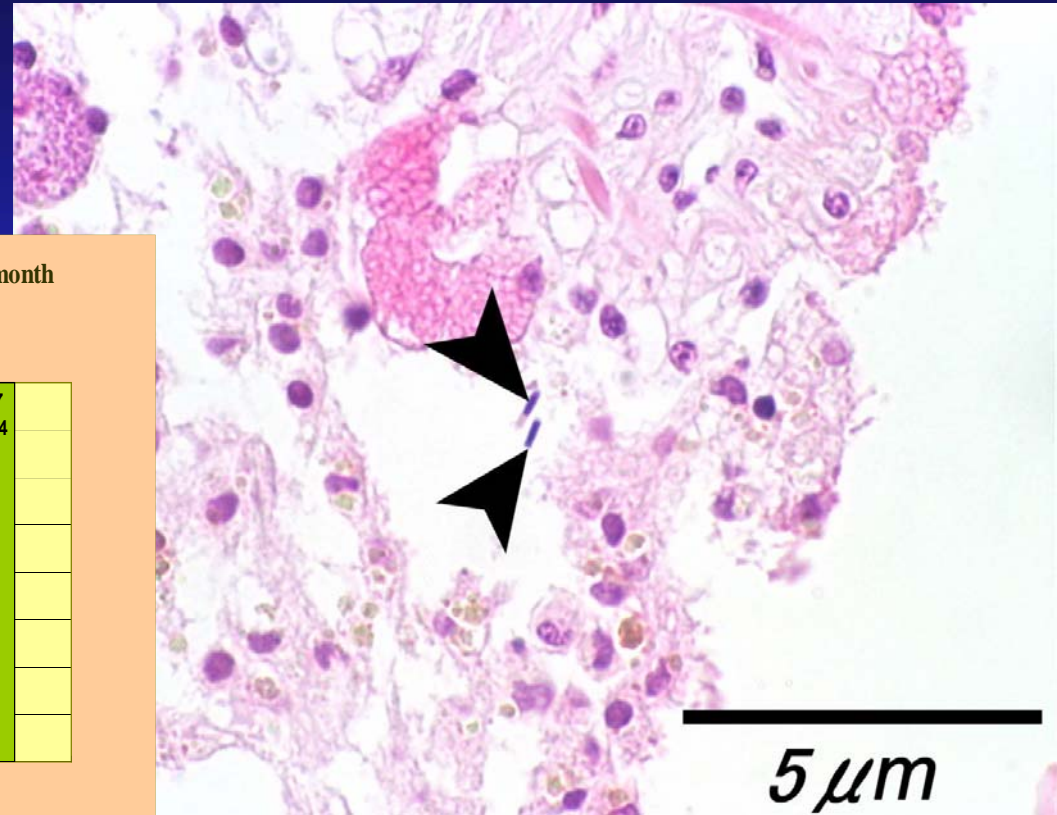
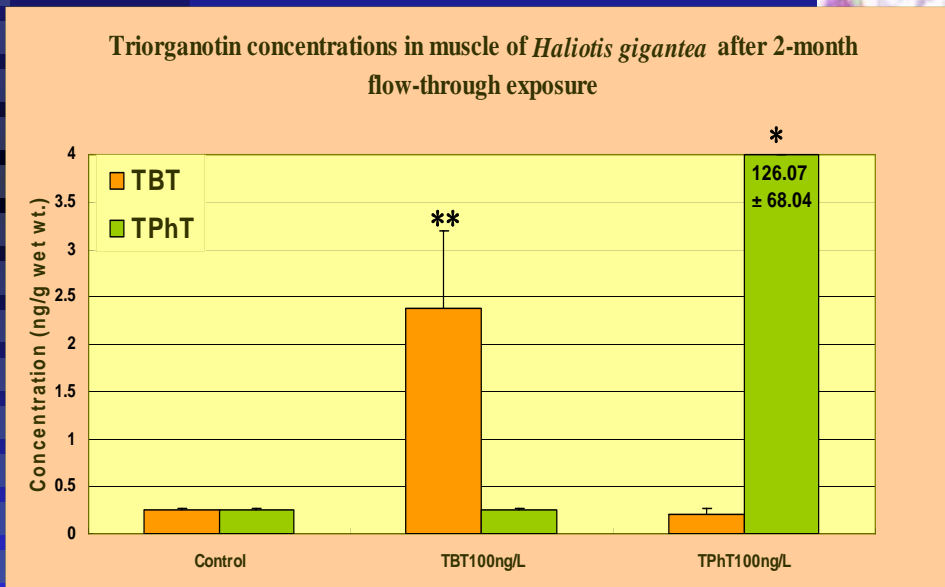
**a Female Abalone
Exposed for 7 Months**

Significant accumulation of TBT and TPT in muscle of abalone from Site A (reference), after 7-month *in situ* exposure experiment (June 1998 – Jan. 1999)

Ovarian spermatogenesis was observed in 90% of females *in situ* exposed nearby a shipyard in Site B

Horiguchi *et al.* (2000)

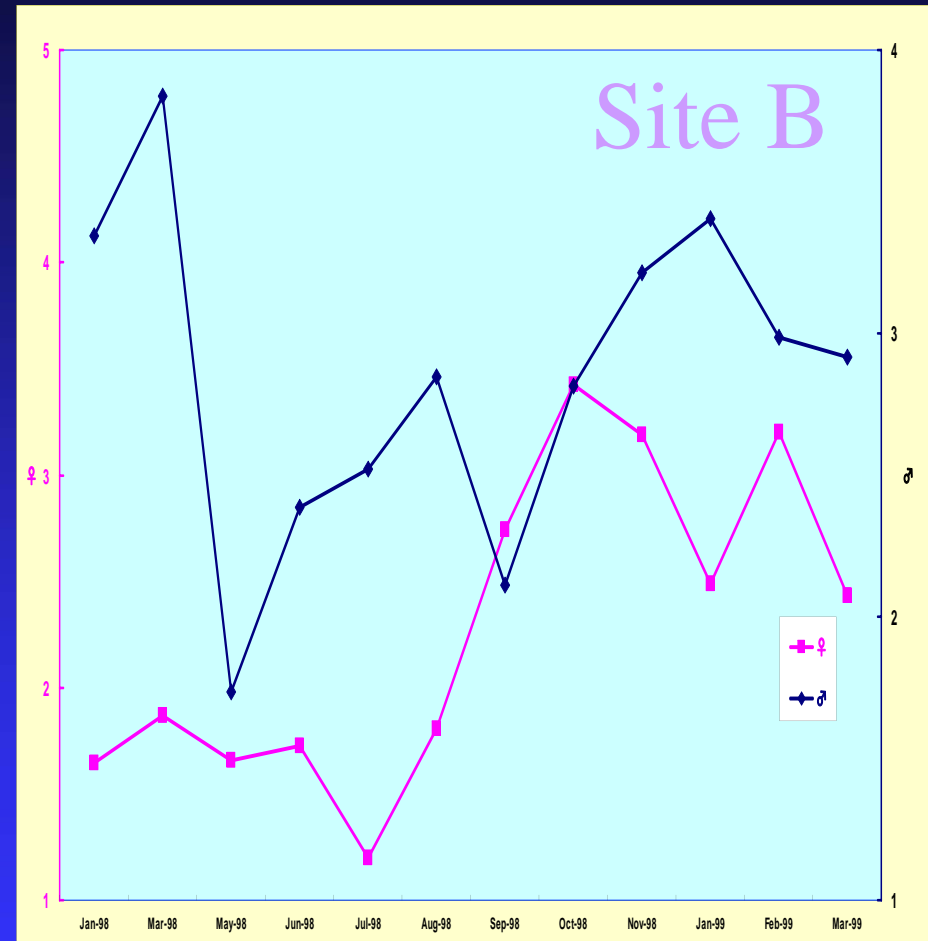
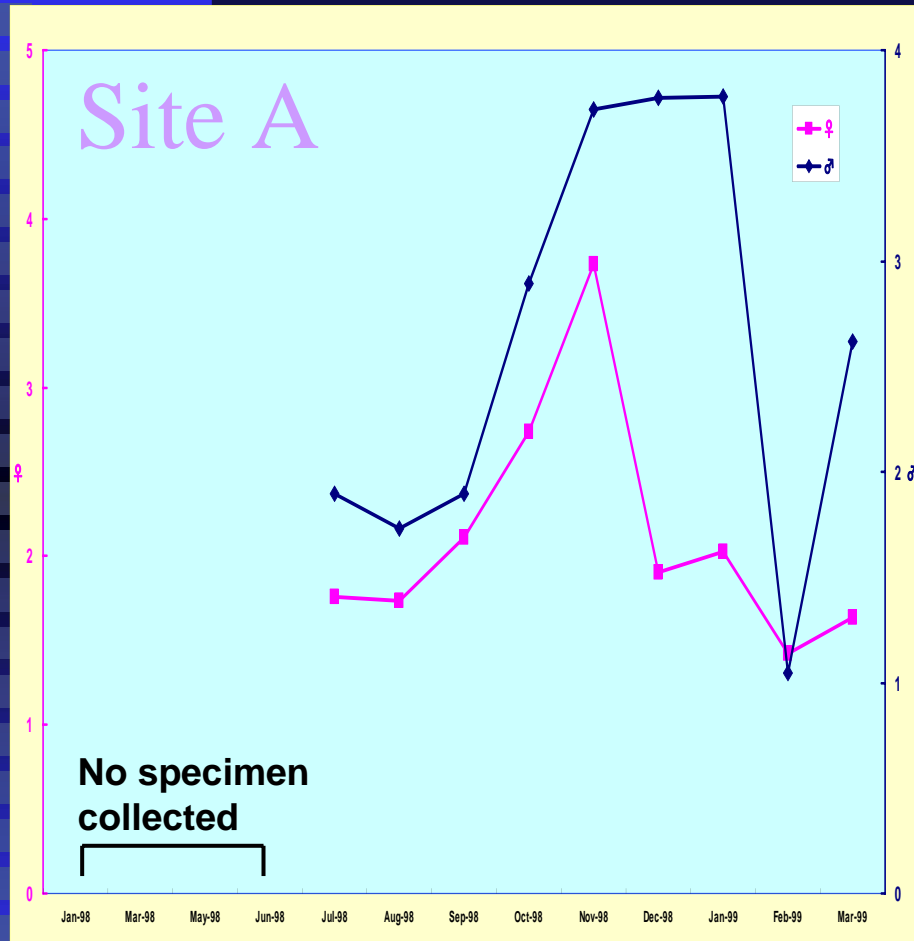
Laboratory flow-through exposure experiments of TBT and TPhT with abalone, *H. gigantea*



Ovarian spermatogenesis in female *H. gigantea* exposed to 100 ng/L of TBT for 2 months

Horiguchi *et al.* (2002)

Reproductive Cycles in the Giant Abalone (January 1998 – March 1999)



Ovarian spermatogenesis: 0 % (Site A)
19 % (Site B)

Horiguchi et al. (2005)

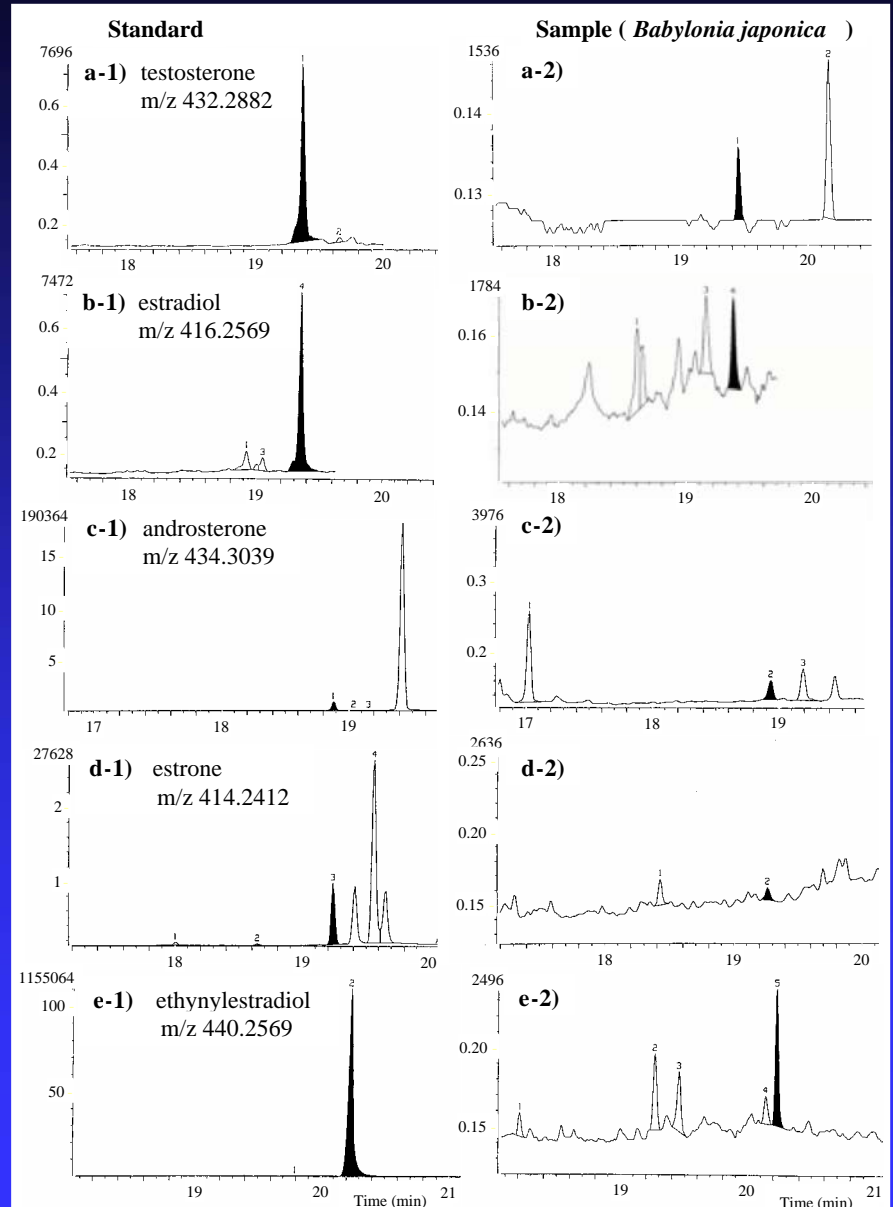
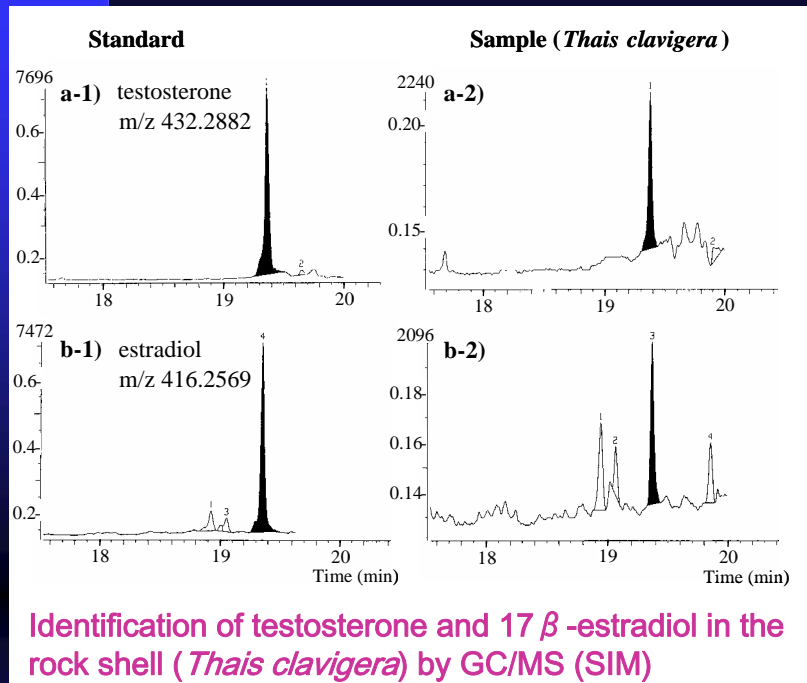
Conclusions

- Imposex has been observed in 39 species of gastropods from Japan.
- Reproductive failure involved by imposex is thought to have led some Japanese gastropod populations to population decline/mass extinction.
- Causal substances of gastropod imposex are TBT and/or TPhT from antifouling paints.
- Similar endocrine disruption to gastropod imposex was also observed in abalone from Japan.
- Ovarian spermatogenesis was also induced by TBT and TPhT in abalone.
- TBT is still used worldwide in antifouling paints despite of the AFS Convention, which may bring about delayed recovery of gastropods from imposex.

Hypotheses on Induction Mechanism of Imposex Caused by TBT in Gastropods

- Aromatase inhibition (Bettin *et al.*, 1996)
- Testosterone excretion-inhibition (Ronis & Mason, 1996)
- Interfering with the release by female cerebropleural ganglia of a “retrogressive” factor for penis growth (Féral & Le Gall, 1983)
- Promotion of APGWamide as a penis morphogenetic factor (Oberdörster & McClellan-Green, 2000)

Identification of Steroid Hormones & Metabolism



E2 transformed from T in gonad/digestive gland of *T. clavigera* from Iguchi et al. (2007)

Structure of Estrogen Receptor

	C	D	E	F
rock shell				
human	89	35	39	34
mouse	89	33	40	26
alligator	89	34	39	30
chicken	89	34	40	29
zebrafish	84	33	39	24

Iguchi et al. (2007)

Comparison of ER Homology among Species



Iguchi et al. (2007)

Aromatase Inhibitor and Testosterone: Less Effective on the Development of Imposex

★ Injection experiments

	Control	Fadrozole	Fadrozole + Testosterone	TPTCI
Incidence (%)	15	35	20	100**
Penis Length (mm)	0.02 ± 0.10	0.12 ± 0.19*	0	3.10 ± 3.57***
VDS Index	0.20 ± 0.52	0.70 ± 1.03	0.20 ± 0.41	2.80 ± 1.51***

* p<0.05 ; ** p<0.01 ; *** p<0.001

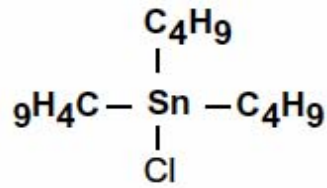
Iguchi et al. (2007)

★ 72-day flow through exposure experiments of only Fadrozole (0.3 mg/L) and Fadrozole (0.3 mg/L) in combination with 3 conc. of Testosterone (0.1, 1 and 10 μ g/L) did NOT result in significant promotion of imposex symptoms (Sugimoto, Horiguchi, Shiraishi, Morita, Takahashi & Miura, unpublished data), either.

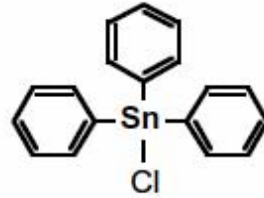
Hypotheses on Induction Mechanism of Imposex Caused by TBT in Gastropods

- Aromatase inhibition (Bettin *et al.*, 1996) **X**
- Testosterone excretion-inhibition (Ronis & Mason, 1996) **X**
- Interfering with the release by female cerebropleural ganglia of a “retrogressive” factor for penis growth (Féral & Le Gall, 1983) **X?**
- Promotion of APGWamide as a penis morphogenetic factor (Oberdörster & McClellan-Green, 2000) **X**

**NOT supported in the experiments
with *Thais clavigera***

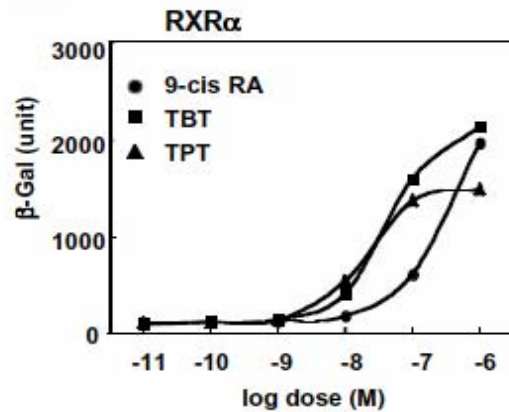
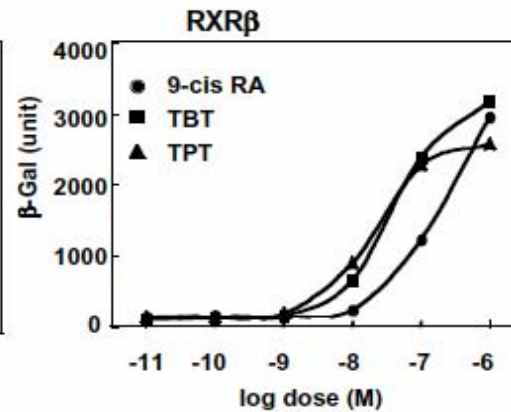
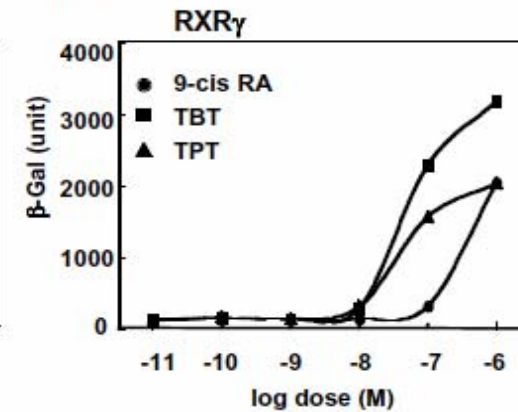
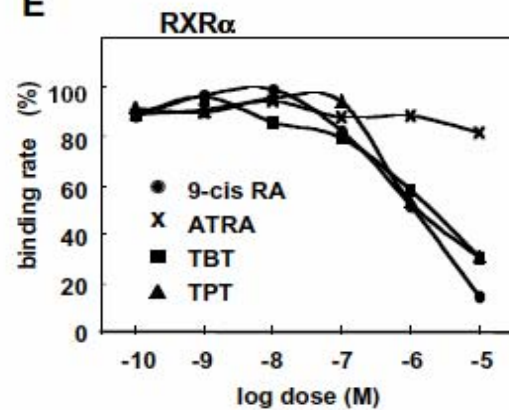
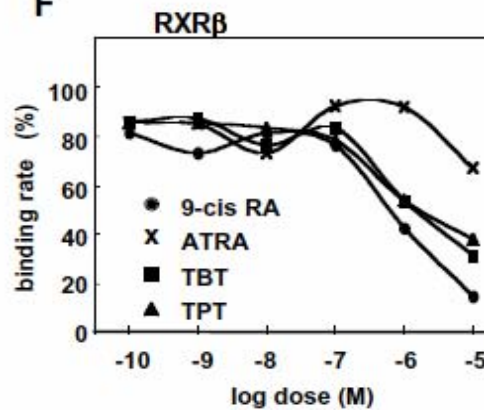
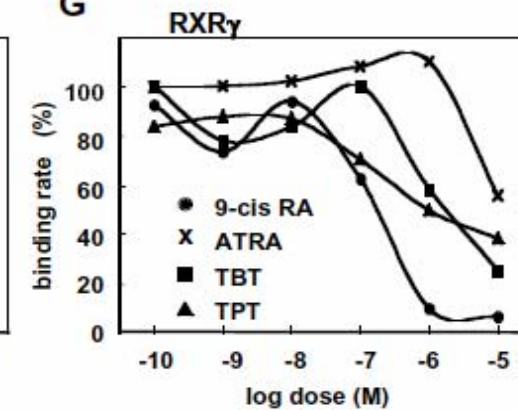
A

Tributyltin chloride



Triphenyltin chloride

From Nishikawa, J., Mamiya, S., Kanayama, T., Nishikawa, T., Shiraishi, F., Horiguchi, T.: *Environ. Sci & Technol.* **38**: 6271-6276, 2004.

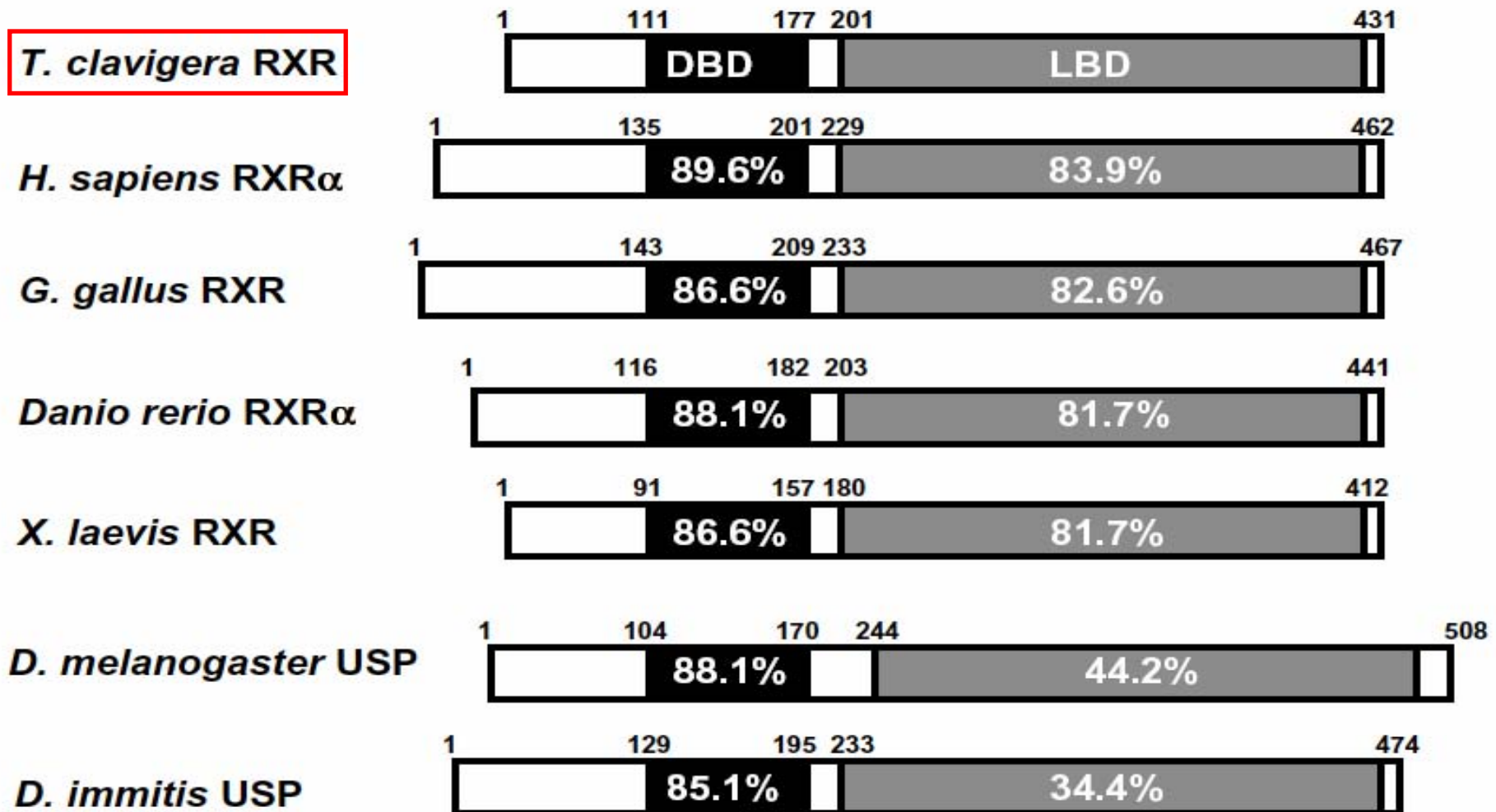
B**C****D****E****F****G**

We know TBT and TPhT cause imposex in *T. clavigera*.

Retinoid X Receptor (RXR)

- One of nuclear receptors
- Ligand-dependent transcriptional activation
- 9-*cis* retinoic acid (9CRA): the natural ligand to human RXR (hRXR)
- Heterodimer coupled with non-steroid nuclear receptors
- Three subtypes: hRXR α , hRXR β and hRXR γ
- TBT and TPhT are agonists for hRXR, having strong affinity, comparable to 9CRA
- Phylogenetically well conserved

Homology of RXR among Species



Nishikawa, J., Mamiya, S., Kanayama, T., Nishikawa, T., Shiraishi, F., Horiguchi, T.: *Environ. Sci & Technol.* **38**: 6271-6276, 2004.