TBT INDUCED IMPOSEX IN MOLLUSCS
ORGANIC CHEMISTS PRODUCE UP TO 50,000 CHEMICALS PER YEAR

CHEMICALS AND XENOBIOTICS THAT MIMIC HORMONES THAT ACCUMULATE IN ORGANISMS CAN PERTURB ENDOCRINE FUNCTION

THOSE CHEMICALS ARE TERMED ENDOCRINE DISRUPTORS
What is endocrine disruption?

- Definition of the EU commission (1998):
  *Exogenic compounds which negatively affect the health of an intact organism or its offspring by interference with its endocrine function.*

- Endocrine disruptors (EDCs) are environmental chemicals which directly or indirectly influence the hormonal system and may be active at low concentrations.
Suspected endocrine disrupting compounds

Insecticides
- Carbaryl
- Chlordane
- DDT and DDE
- Dicofol
- Dieldrin
- Endosulfan
- Lindan
- Methoxychlor
- Mirex
- Oxychlordane
- Parathion
- Toxaphen
- Pyrethroid

Herbicides
- Alachlor
- Amitrol
- Atrazine
- 2,4-Dichlorophenoxy-acetic acid
- Metribuzin
- Nitrofen
- 2,4,5-Trichlorophenoxy-acetic acid

Nematicides
- Aldicarb
- DBCP (1,2-Dibrom-3-chloropropan)

Fungicides
- Benomyl
- Fenarimol
- Mancozeb
- Maneb
- Tributyltin compounds (TBT)
- Triphenyltin compounds (TPT)
- Vinclozolin
- Zineb

Industrial chemicals
- Alkylphenols
- Bisphenol A
- Dioxine (2,3,7,8-TCDD)
- Pentachlorophenol
- Phthalate
- Polybromated Biphenyls (PBB)
- Polychlorinated Biphenyls (PCB)

(from Oehlmann & Markert, 1997)
STRUCTURE OF TRIBUTYL TIN

Bis(tri-n-butyltin oxide)
TRIBUTYL TIN (TBT)

USES
- Biocidal agent used as wood preserver, disinfectants etc
- Antifouling agent (1970’s) used in free association or self-polishing copolymer paints

TOXICITY
- 96 HOUR LD-50’S=300ug L⁻¹ TBTO in Ostrea edulis

BIOASSIMILATION
- Highly lipophilic-Cf 100,000 in molluscs
First effects of TBT in oysters

- since 1960: use of TBT-based antifouling paints
  since 1970: also on leisure boats/yachts
- 1979: first observation of deleterious effects caused by TBT in oysters at Arcachon (France) which led to massive economical damage
  - no larvae - reproductive failure
  - shell deformations (formation of chambers and "balling"):
    - normal shell
    - deformed shell
IMPOSEX

- Occurs in Gastropods, especially stenoglossans
- Pseudo-maleness if female individuals
- Penis-like outgrowth
- Vas deferens formation
- Sperm production
- Sterility in affected females
- Prevalent and Ubiquitous
- Known in at least 160 species worldwide
- Threshold concentrations <0.5ng/L TBT
- Used as a “Bioindicator” of TBT exposure
New Dangerous Chemicals in the Environment: Lessons from TBT

DEREK V. ELLIS

Derek Ellis is a Professor of Biology at the U Victoria, Canada. He started biological research related to TBT in 1987, and is now new ways to bring the biology and chemistry

TBT (Tributyltin) was a new chemical in the sea during the early 1960s. It is no longer new, but it is now recognized as dangerous. The case has been well reviewed many times (e.g. Stebbing, 1985; Hall & Pim Thompson et al., 1986; Laughlin & Linden, thern, above, of a viewpoint.

Viewpoint is a column which allows authors to express their own opinions about current

Printed in Great Britain.
Widespread Organotin Pollution in New Zealand Coastal Waters as Indicated by ImPOSEX in Dogwhelks

J. Smith and M. McVeagh
Marine Research Centre, MAF Fisheries Greta Point, P.O. Box 297, Wellington, New Zealand

Three species of Neogastropod snails were assessed as indicators of organotin (TBT) pollution around New Zealand. ImPOSEX, a condition in which females develop a penis, was induced in two dogwhelks, *Lepsiella oblina* and *L. albomarginata*, exposed to low levels of TBT. The relative penis size (RPS) in female dogwhelks was used to survey TBT pollution. ImPOSEX was found at all sites close to permanent mooring areas and in areas with a high seasonal input of pleasure craft. It was present only on open coastal sites or in isolated bays.

Over the past decade, organotin compounds have been used extensively in antifoulants to inhibit the settlement of fouling organisms on vessels and marine structures. Laboratory studies have shown that tributyltin (TBT) in particular is acutely or chronically toxic to a wide range of non-target organisms including algae (Thain, 1983), molluscs (Beaumont & Budd, 1984; Lawler & Aldrich, 1987; Bryan et al., 1988), crustaceans (Laughlin & French, 1980; Laughlin et al., 1983; Uren, 1983) and teleosts (Kakuno & Kimwa, 1987; Thain, 1983). Com-
13. Tributyltin (TBT) antifoulants: a tale of ships, snails and imposex

David Santillo, Paul Johnston and William J. Langston

13.1. Introduction

There is little doubt that, without some form of control, the accumulation of marine fouling communities on vessels and man-made structures at sea increases drag and, in the case of vessels, fuel consumption, with substantial consequences in terms of economics and emissions. There is no doubt, also, that tributyltin (TBT) compounds (and the less widely used triphenyltin)s are extremely effective and relatively economical as antifouling biocides, contributing to the rapid take-up of organotin-based paints by the shipping industry and small boat owners in the 1970s. These two arguments have formed the basis of the defence of TBT antifouling formulations since the first undesirable consequences of their use became apparent in the late 1970s, and in the 1980s, but the strength of the evidence for severe effects on biota, including regional extermination of some species, could hardly be denied.

The prohibition of the use of TBT paints on vessels under 25 metres in length, effective in France from 1982, in the United Kingdom from 1987 and more widely from the end of the 1980s and early 1990s, did much to improve the situation within marinas and sheltered harbours where use on leisure craft had predominated. Some regional recovery of affected mollusc populations has since been recorded. Through the late 1980s and 1990s, however, a picture of more widespread TBT contamination and population-level effects emerged, coincident with improved monitoring and understanding of the properties and environmental distribution of
APPEARANCE OF PALLIAL-GENITAL TRACT

NORMAL FEMALE  NORMAL MALE  FEMALE WITH IMPOSEX

EFFECTS OF TBT ON GENITAL TRACT OF Ocenebra erinacea AFTER GIBBS ET AL., {1990}. 
Spermatogenesis in Female Molluscs

- Ultimately, a sex change (from female to male) can be induced on the level of the gonad:

- spermatogenesis
- ripe oocyte
- disintegrating oocytes
Effects of TBT on Female Gonads

1 ng/l

20 ng/l

20 ng/l
Spermatogenesis in Testes of TBT exposed Female
WHAT IS THE MECHANISM BY WHICH TBT IS INDUCING IMPOSEX?
<table>
<thead>
<tr>
<th><strong>Phylum/Effect</strong></th>
<th><strong>Causal Link</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crustaceans</strong></td>
<td><strong>Hormonal imbalance</strong></td>
</tr>
<tr>
<td>Decapod Metamorphosis</td>
<td>20-hydroxyecdysone</td>
</tr>
<tr>
<td>Sex determination in Mysids</td>
<td>Androgen/estrogen</td>
</tr>
<tr>
<td><strong>Molluscs</strong></td>
<td>1,25(OH)$_2$-D3</td>
</tr>
<tr>
<td>Shell thickening (Oysters)</td>
<td>Androgen/estrogen</td>
</tr>
<tr>
<td>Impossex (Gastropods)</td>
<td>Androgen/estrogen</td>
</tr>
</tbody>
</table>
OBSERVED SYNDROMES RELATED TO STEROID METABOLISM
BIOGENESIS OF STEROID HORMONES

ANDROSTENEDIONE → C17-Oxo-reductase → TESTOSTERONE → 5α-Reductase → DIHYDROTESTOSTERONE

Aromatase → ESTRONE

Aromatase → 17B-ESTRADIOL

C17-Oxo-reductase
BIOGENESIS OF STEROID HORMONES

5α Reductase

DIHYDROTESTOSTERONE

Aromatase

17β-ESTRADIOL
EFFECTS OF TESTOSTERONE ON MALE CHARACTERISTICS
Effects of Testosterone on Imposed Expression

Penis Length (mm)

Penis length in female N. Lapillus 28 days after injection of various quantities of testosterone (Spooner et al., 1991)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.01ug Testosterone</th>
<th>0.1ug Testosterone</th>
<th>10ug Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penis Length</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

- CONTROL
- INJECTED
Sensitivity to TBT of various prosobranch snails:

<table>
<thead>
<tr>
<th>Species</th>
<th>threshold values (ng TBT/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>imposex / intersex</td>
</tr>
<tr>
<td>Ocinebrina aciculata</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td><em>Nucella lapillus</em></td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td><em>Ocenebra erinacea</em></td>
<td>≤ 1.0</td>
</tr>
<tr>
<td><em>Nassarius reticulatus</em></td>
<td>≤ 1.0</td>
</tr>
<tr>
<td><em>Nassarius incrassatus</em></td>
<td>≤ 1.5</td>
</tr>
<tr>
<td><em>Littorina littorea</em></td>
<td>≤ 5.0</td>
</tr>
<tr>
<td><em>Hydrobia ulvae</em></td>
<td>≤ 10</td>
</tr>
<tr>
<td><em>Buccinum undatum</em></td>
<td>≤ 15</td>
</tr>
</tbody>
</table>
Littorina littorea (Mollusca, Gastropoda, Prosobranchia)
STEROID METABOLISM & EXCRETION

PHASE 1
OXIDATIVE METABOLISM

steroid → Sulfur Transferase → Sulfate
steroid → UDP Glucuronyltransferase → Glucuronic Acid
steroid → Glucose Transferase → Glucose

NON-POLAR (ORGANIC PHASE)

PHASE 2
CONJUGATION

UDP Glucuronyltransferase

POLAR (AQUEOUS PHASE)
ANIMALS (n=3)

Inject 7nmoles $^{14}$C Testosterone

Expose to 0, 0.5, 5μM TBT (15°C 42 hours)

Dissect and Homogenize Gills, Kidney, Visceral Complex

Organic Extract of Homogenates and Water

Aqueous Phase

Enzymatic Digestion

Organic Phase

2-Dimensional HPTLC
NADPH DEPENDENT METABOLISM OF TESTOSTERONE IN DIGESTIVE GLAND CELLS

- NADPH

+ NADPH
EFFECTS OF TBT ON TESTOSTERONE METABOLISM IN THE GILL

Phase 1 metabolites (pmol)

0.0 uM TBT  0.5 uM TBT  5.0 uM TBT
EFFECTS OF TBT ON TESTOSTERONE METABOLISM IN THE DIGESTIVE GLAND
EFFECTS OF TBT ON TESTOSTERONE METABOLISM IN THE KIDNEY

[Graph showing the effects of TBT on testosterone metabolism in the kidney with bars for DHA, A, DHT, T, and DHT-diols at 0.0 μM, 0.5 μM, and 5.0 μM TBT concentrations.]
EFFECTS OF TBT ON AROMATASE ACTIVITY
DISTRIBUTION OF RADIOACTIVITY IN ANIMAL & SEAWATER

- **SEAWATER**
  - 0.0 uM TBT
  - 0.5 uM TBT
  - 5.0 uM TBT

- **ANIMAL**
  - 0.0 uM TBT
  - 0.5 uM TBT
  - 5.0 uM TBT

%
DETERMINATION OF CONJUGATE IDENTITY

AQUEOUS EXTRACT

Digestion with Enzymes
- Sulfatase
- β-Glucosidase
- β-Glucuronidase

Organic Extraction II

AQUEOUS PHASE

SEAWATER

TISSUES

ORGANIC PHASE

RADIOACTIVE β-COUNTING

HPTLC
EFFECTS OF TBT ON TESTOSTERONE SULFUR CONJUGATES
MECHANISM OF IMPOSEX INDUCTION BY TBT

• Aromatase Inhibition (17β-estradiol)

• Inhibition of Conjugation and build up of androgens such as DHT
AROMATASE INHIBITION BY TBT

pregnenolone

3β-hydroxysteroid-DH

progesterone

17α-hydroxylase

17α-hydroxyprogesterone

C_{17,20}-lyase

androstenedione

testosterone

CYP19 = aromatase

estrone

17β-estradiol

TBT

TBT
MECHANISM OF AROMATASE INHIBITION?

Cytochrome P-450
Metabolism and dealkylation

Suicide Inhibition

TBT
DBT
MBT
Sn

1-Butene
Dealkylation

1-Butene
## EFFECTS OF TBT ON BENZO(a)PYRENE METABOLISM

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>F.U/min/mg</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO TBT</td>
<td>7.13 ± 0.5</td>
<td>+ CONTROL</td>
</tr>
<tr>
<td>-NADPH</td>
<td>0</td>
<td>- CONTROL</td>
</tr>
<tr>
<td>+ 1uM TBT</td>
<td>4.8 ± 0.4</td>
<td>33</td>
</tr>
<tr>
<td>+ 2.5uM TBT</td>
<td>4.4 ± 0.4</td>
<td>38</td>
</tr>
<tr>
<td>+ 5.0 uM TBT</td>
<td>3.9 ± 0.7</td>
<td>46</td>
</tr>
<tr>
<td>10.0 uM TBT</td>
<td>1.1 ± 0.2</td>
<td>85</td>
</tr>
<tr>
<td>12.5 uM TBT</td>
<td>0.43 ± 0.1</td>
<td>94</td>
</tr>
<tr>
<td>25.0 uM TBT</td>
<td>0.16 ± 0.07</td>
<td>98</td>
</tr>
<tr>
<td>50.0 uM TBT</td>
<td>0.044 ± 0.02</td>
<td>99</td>
</tr>
<tr>
<td>200.0 uM TBT</td>
<td>0.0</td>
<td>100</td>
</tr>
</tbody>
</table>

Reconstituted Rat Microsomal system. Approximately 0.5mg of microsomal protein from 3MC preinduced rats preincubated with TBT and NADPH prior to addition of 5nm substrate. (1 F.U. + florescence of 0.3g/ml quinine sulphate in H₂SO₄.)
MECHANISM OF TRANSFERASE INHIBITION?

UNKNOWN
MECHANISM OF IMPOSEX INDUCTION BY TBT

- Aromatase Inhibition (17β-estradiol)
- Inhibition of Conjugation and build up of androgens such as DHT
- Neuropeptide perturbation
Mechanisms of imposex induction in the mud snail, Ilyanassa obsoleta: TBT as a neurotoxin and aromatase inhibitor.

Oberdorster E, McClellan-Green P.

Southern Methodist University, Dallas, TX 75275-0376, USA. eoberdon@mail.smu.edu

The occurrence of imposex, imposition of male sex characteristics on female snails, has been extensively documented throughout the world. Tributyltin (TBT) and other organotins have been causally linked to imposex induction at levels as low as 2 ng/l. There are several proposed mechanisms of action. First, TBT has been shown to be neurotoxic and to accumulate in snail ganglia. Peptide hormones control sexual differentiation in gastropods, and one hypothesis is that TBT acts as a neurotoxin to abnormally release the peptide hormone Penis Morphogenetic Factor (PMF). However, PMF has not been characterized to date. The neuropeptide APGWamide significantly induces imposex in the mud snail, Ilyanassa obsoleta, at 10(-16) moles sub-cutaneous (SQ) injection over 2 weeks, and could be the PMF in this species. A second hypothesis is that TBT inhibits aromatase activity leading to increased testosterone levels and decreased estradiol. In vitro studies with snail digestive gland microsomes showed that TBT-dosed snails not exhibiting imposex had a 52% reduction in aromatase activity. Although the role of vertebrate sex steroids is not known in gastropods, it is possible that the combination of changes in peptide and steroid hormones may lead to imposex induction at extremely low doses of TBT.
TBT REGULATION

• Ban on boats <25m length

  FRANCE 1982
  UNITED KINGDOM 1987
  USA 1988
  EUROPE 1991

• Levels lowered by slow biodegradation and release from scrapings in sediments means that imposex will still be an acute problem in the future