A case study on the effects of TRICLOSAN: personal care products in the aquatic environment

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Dann and Hontela. 2011. J. Applied Toxicology 31: 285
Hontela and Habibi. 2013. Fish Physiology, Chap.3

Masaryk University, April 2013

Outline:

Triclosan

Synopsis
1. Chemical and physical characteristics
2. Sources
3. Exposure
4. Fate in the environment
5. Effects
6. Risk and Remediation

Triclosan – Synopsis (identity, use)

- **Broad spectrum antimicrobial**
- **personal care products: Irgasan, Aquasept, …**

EU Cosmetic Directive

(0.1-0.3%)

- **sport clothing:**

  Ultra-Fresh,

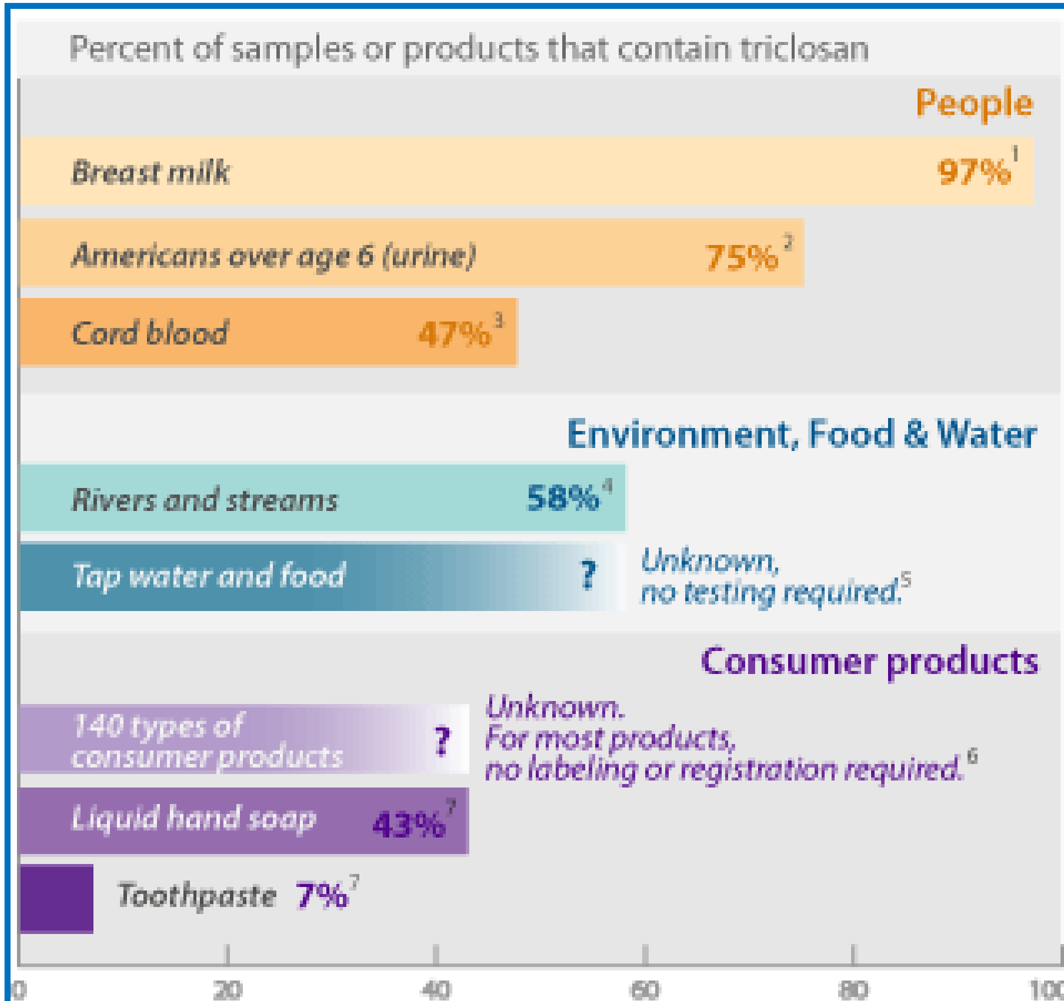
  Microban,

  Sanitized, …

- **food packaging**

Triclosan – key issues

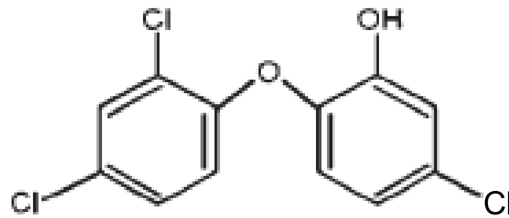
- Detected in water, human plasma, breast milk
- Bioaccumulation
- By-product formation
- Endocrine disruption
  - Thyroid hormones
  - Sex hormones
- Antibiotic resistance, efficacy



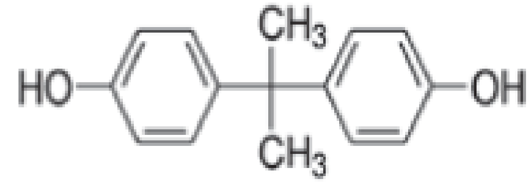
(Adolfsson-Erici et al. 2002; Queckenberg et al. 2010; Dann & Hontela, 2011)

1. Identity and mode of action

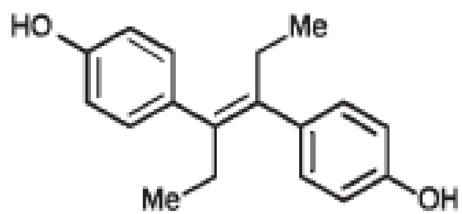
Triclosan



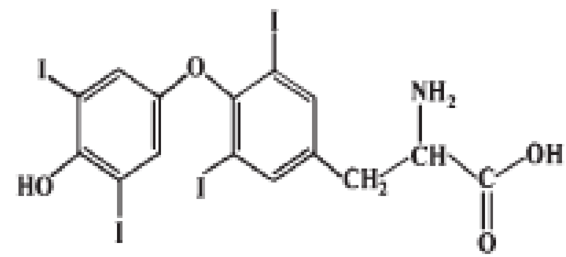
Bisphenol-A



Diethylstilbestrol



Thyroxine



- **Structural similarity of TCS to Bisphenol A,**
  to DES
  to thyroxine
- **Antimicrobial action of TCS :**
  multiple sites in bacteria,
  blocks synthesis of FFA

2. Sources

WWTP influent	In-flowing waste water	USA	2.70–26.80 μg l^{-1}
		Canada	0.01–4.01 μg l^{-1}
		Germany	1.2 μg l^{-1}
		Sweden	0.38 μg l^{-1}
		Japan	2.7–11.9 μg l^{-1}
WWTP effluent	Treated water	Switzerland	0.042–0.213 μg l^{-1}
		Germany	0.01–0.6 μg l^{-1}
		Canada	0.01–0.324 μg l^{-1}
		USA	0.03–2.7 μg l^{-1}
		UK	0.34–3.1 μg l^{-1}
		Australia	0.023–0.434 μg l^{-1}
		Sweden	0.16 μg l^{-1}
		Japan	0.26–0.27 μg l^{-1}

WWTP – Waste Water Treatment Plant

3. Exposure

Table 1. Concentrations of triclosan (TCS) in the aquatic environment

Medium	Sample description	Location	Concentration of TCS
Surface water	Natural streams/rivers	USA	ND[a] to 2.3 µg l^{-1}
		Switzerland	ND to 0.074 µg l^{-1}
		Germany	ND to 0.01 µg l^{-1}
		Sweden	ND
		Australia	0.075 µg l^{-1}
		Japan	<0.0006–0.059 µg l^{-1}
	Streams with inputs of raw wastewater	Switzerland	0.011–0.098 µg l^{-1}
		USA	1.6 µg l^{-1}
	Estuarine waters	USA	0.0075 µg l^{-1}
Sediment	Freshwater	Switzerland	53 µg kg^{-1}
		Spain	ND to 35.7 µg kg^{-1}
	Estuarine	USA	ND to 800 µg kg^{-1}
	Marine	Spain	0.27–130.7 µg kg^{-1}
Sewage sludge	Activated sludge	USA	0.5–15.6 µg g^{-1}
		Spain	0.4–5.4 µg g^{-1}
		Germany	1.2 µg g^{-1}
		Canada	0.62–1.45 µg g^{-1}
	Biosolids	Australia	90–16 790 µg kg^{-1}
		USA	10 500–30 000 µg kg^{-1}
		Spain	1508 µg kg^{-1}
		Canada	680–12 500 µg kg^{-1}

link to experimental studies

3. Concentrations of Triclosan in organisms

Table 2. Concentrations of triclosan (TCS) in aquatic organisms

Organisms	Type of sample	Site description	TCS ($\mu g\ kg^{-1}$)
Algae and invertebrates			
Filamentous algae (*Cladophora* spp.)	Whole organism	Receiving stream for the city of Denton (TX, USA) WWTP	100–150
	Whole organism		50–400
Freshwater snails (*Helisoma trivolvis*)	Muscle		50–300
Vertebrates			
Rainbow trout (*Oncorhynchus mykiss*)	Bile	Upstream from WWTP, Sweden (caged); downstream 2 km from WWTP (caged)	710 17 000
Breams, male (*Abramis brama*)	Bile	River sites (Netherlands)	14 000–80 000
	Muscle	River sites (Germany)	0.25–3.4
Pelagic fish	Plasma	Detroit River (USA)	0.75–10
Atlantic bottlenose dolphins (*Tursiops truncates*)	Plasma	Estuary, South Carolina	0.12–0.27
		Estuary, Florida	0.025–0.11
Killer whale (*Orcinus orca*)	Plasma	Vancouver Aquarium Marine Science Centre	9.0

Bioaccumulation (?)

4. Fate in the environment
Degradation products of TCS

- **Methyltriclosan**

  biological methylation, K_{ow} = 5.2

  (TCS K_{ow} = 4.7)

  used as marker of exposure to WWTP effluent

  more persistent than TCS

- **Dioxins**

  generated during photodegradation of TCS

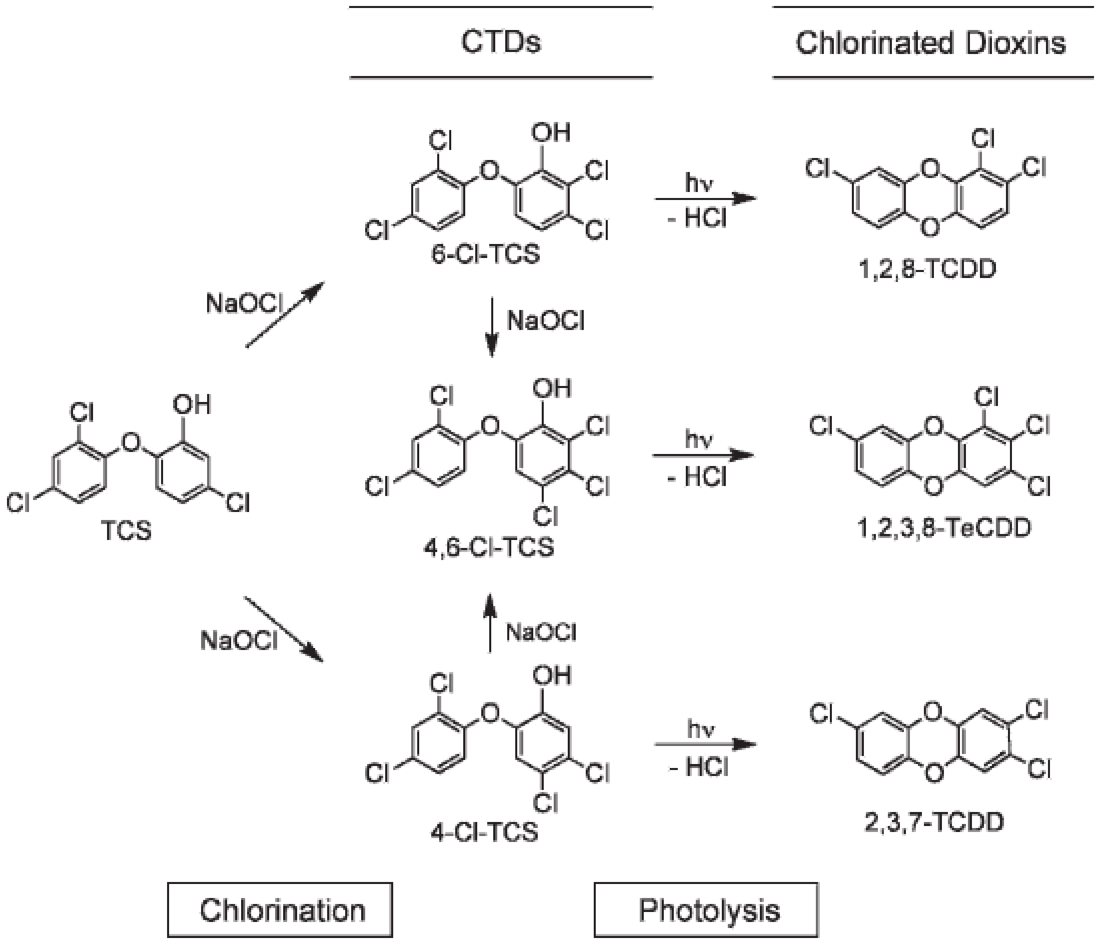
  at pH > 8

- **Chloroform and chlorophenols**

  generated in presence of chlorine or chloramine

fate in the environment …

Buth et al. 2009 Environ Toxicology Chem 28: 2555, 2009



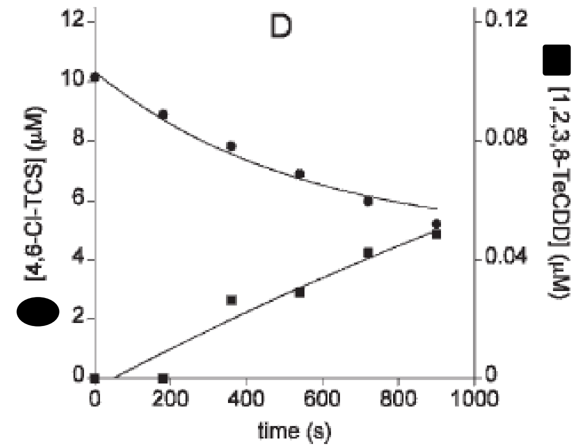
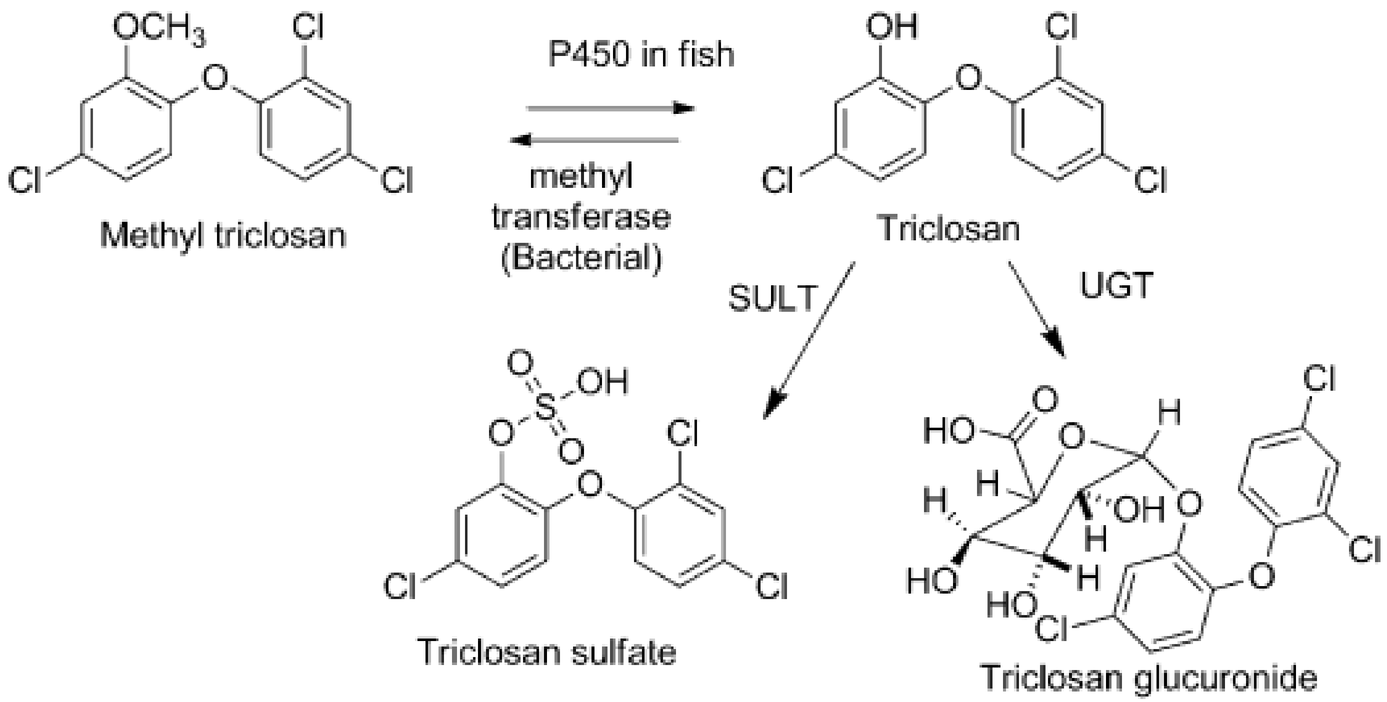


Fig. 4. Solar photolysis of phenoxyphenol substrates

Fig. 1. Hypothesized pathway for the formation of 2,3,7-trichlorodibenzo-*p*-dioxin (2,3,7-TCDD), 1,2,8-trichlorodibenzo-*p*-dioxin (1,2,8-TCDD), and 1,2,3,8-tetrachlorodibenzo-*p*-dioxin (1,2,3,8-TeCDD) from the respective photolysis of 4,5-dichloro-2-(2,4-dichlorophenoxy)phenol (4-Cl-TCS), 5,6-dichloro-2-(2,4-dichlorophenoxy)phenol (6-Cl-TCS), and 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol (4,6-Cl-TCS) formed from the chlorination of triclosan (TCS).

Biotransformation of Triclosan

catfish hepatocytes



James et al. 2012 Aquat Toxicol 124: 72

5. Effects of Triclosan on aquatic organisms

Test species	Life stage	System type	Route of exposure	Test duration	TCS exposure	Endpoint
Algae						
Phytoplankton (*Dunaliella tertiolecta*)		SW	Water (static)	Acute (96 h)	3.5 μg l^{-1}	EC_{50} (population density)
Green alga (*Selenastrum capricornutum*)		FW	Water (static)	Acute (72 h)	4.7 μg l^{-1}	EC_{50} (growth)
Green alga (*Scenedesmus subspicatus*)		FW	Water (static)	Acute (96 h)	1.4 μg l^{-1}	EC_{50} (biomass)
Alga (*Closterium ehrenbergii*)		FW	Water (static)	Acute (48 h)	620 μg l^{-1};250 μg l^{-1}	EC_{50} genotoxicity
Blue-green alga (*Anabaena flos-aquae*)		FW	Water (static)	Acute (96 h)	1.6 μg l^{-1}	EC_{50} (biomass)
Invertebrates						
Daphnia magna		FW	Water (renewal)	Acute (48 h)	390 μg l^{-1}	EC_{50} NOEC reproduction
				21 days	40 μg l^{-1}	
Ceriodaphnia dubia		FW	Water (renewal)	Acute (48 h)	240 μg l^{-1}	EC_{50} NOEC reproduction
				7 days	182 μg l^{-1}	
		FW	Water (renewal)	6–7 days	220 μg l^{-1}	IC_{50} (growth)
Chironomus tentans		FW	Water (renewal)	10 days	400 μg l^{-1}	LC_{50}
Hyalella azteca					200 μg l^{-1}	
Grass shrimp (*Palaemonetes pugio*)	Embryo	SW	Water (renewal)	Acute (96 h)	651 μg l^{-1}	LC_{50}
	Larvae				154 μg l^{-1}	LC_{50}
	Adult				305 μg l^{-1}	LC_{50}
Crustacean (*Thamnocephalus platyurus*)		FW	Water (static)	Acute (24 h)	470 μg l^{-1}	LC_{50}
Bivalve (*Mytilus galloprovincialis*)	Hemocytes	SW	*In vitro*	Acute (30 min)	1 μM	↓ lysosomal membrane stability
	Whole animal	SW	Injection	Acute (24 h)	2.9 ng g^{-1}	Altered hemocyte and digestive gland function
Zebra mussel (*Dreisena polymorpha*)	Hemocytes	FW	*In vitro*	Acute (60 min)	0.1 μM	Genotoxicity
			In vivo	Acute (96 h)	1 M	Genotoxicity

Table 3. Effects of triclosan (TCS) on freshwater (FW) and marine (SW) organisms

Fish	Adult	FW	Water	Acute (96 h)	390 μg l^{-1}	LC_{50}
Rainbow trout (*Oncorhynchus mykiss*)	Embryo	FW	(flow-through)	61 days 35 days	71.3 μg l^{-1}	Delayed swim-up ; ↓ 35 dph survival; erratic swimming, locked jaw
Medaka (*Oryzias latipes*)	Fertilized eggs	FW	Water (renewal)	14 days	313 μg l^{-1}	↓ hatching; delayed hatching
	Larvae (24 h old)			Acute (96 h)	602 μg l^{-1}	LC_{50}
	Male fish			21 days	20 μg l^{-1}	↑ liver Vtg
	Fry	FW		Acute (48 h)	350 μg l^{-1}	LC_{50}
	Eggs	FW		14 days	400 μg l^{-1}	IC_{50} (hatching)
		SW	*In ovo* injection	1 day post-fertilization	4.2 ng egg^{-1}	EC_{50} (survival)
Amphibians						
Bullfrog (*Rana catesbeiana*)	Tadpoles	FW	Water	Acute (96 h)	0.15 μg l^{-1}	↑hindlimb development, ↓ body weight, disruption of thyroid hormone-associated gene expression
Xenopus leavis	XTC-2 cells	FW	*In vitro*	Acute (24 h)	0.03 μg l^{-1}	Altered thyroid hormone receptor mRNA expression
Acris crepitans blanchardii	Larvae	FW	Water	Acute (96 h)	367 μg l^{-1}	LC_{50}
Bufo woodhousii woodhousii	Stage 30				152 μg l^{-1}	

Toxicity of Triclosan

- **Algae are highly sensitive to TCS**
  LC50 96 hr = 1- 4 µg/L

- **Invertebrates and fish**
  average sensitivity

- **Amphibians - highly sensitive**
  effects in tadpoles at 0.15 µg/L
  effects on thyroid status

EFFECTS ...

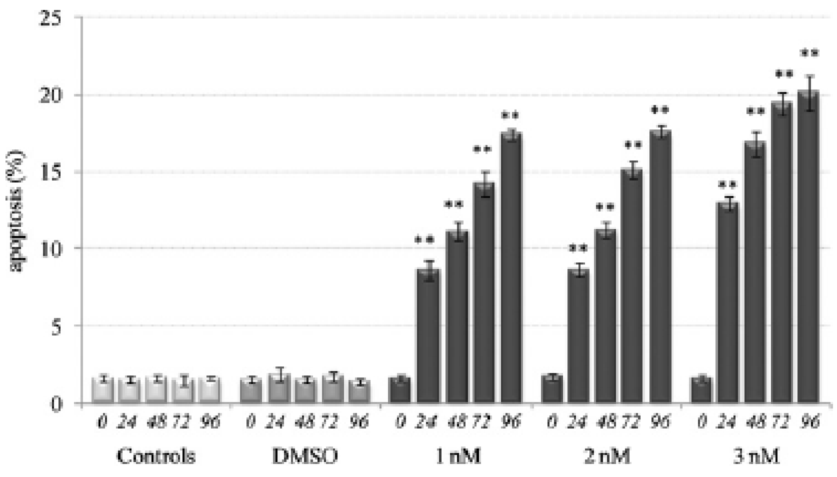


Fig. 2. Temporal trend (h) of the means of apoptosis frequency ± SEM calculated for Zebra mussel hemocytes for controls, solvents and treated samples with TCS. Significant values (two-way ANOVA, Bonferroni *post-hoc* test, $p < 0.05$) were obtained for the comparison between treated samples and controls at the same time.

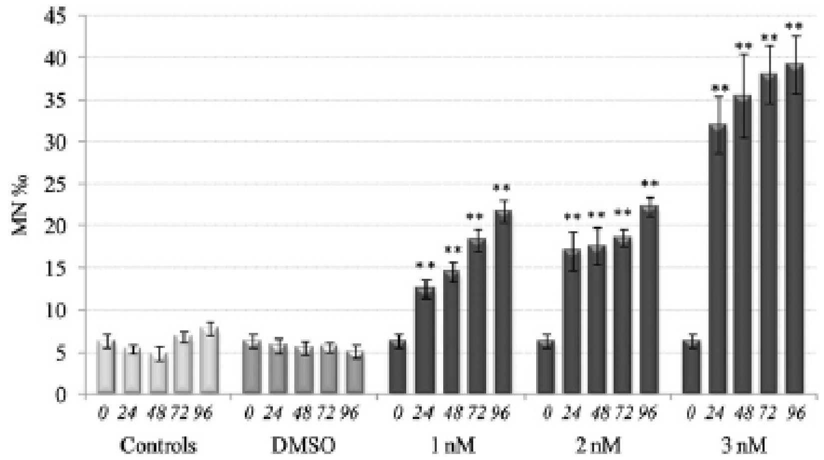


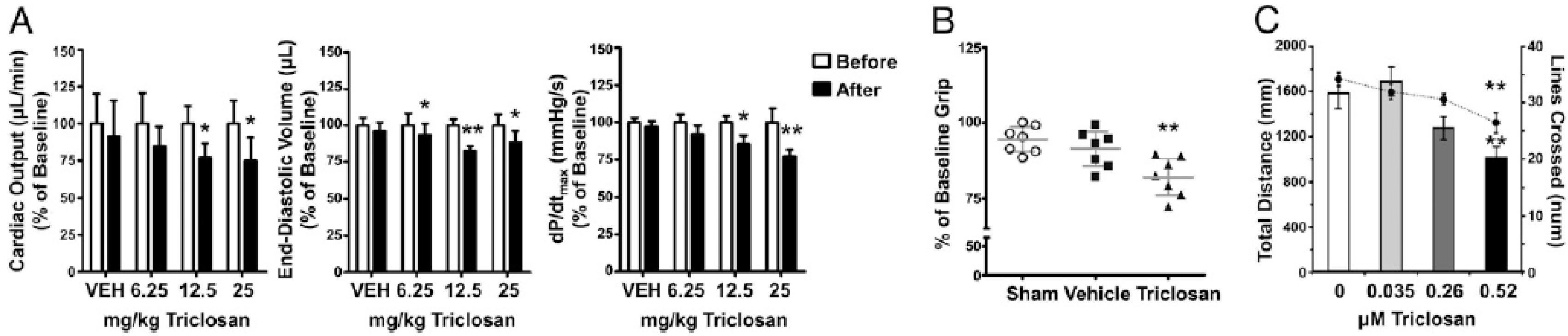
Fig. 3. Temporal trend (h) of the means of micronuclei frequency ± SEM calculated for Zebra mussel hemocytes for controls, solvents and treated samples with TCS. Significant values (two-way ANOVA, Bonferroni *post-hoc* test, $p < 0.05$) were obtained in the comparison between treated samples and controls at the same time.

Zebra mussel hemocytes

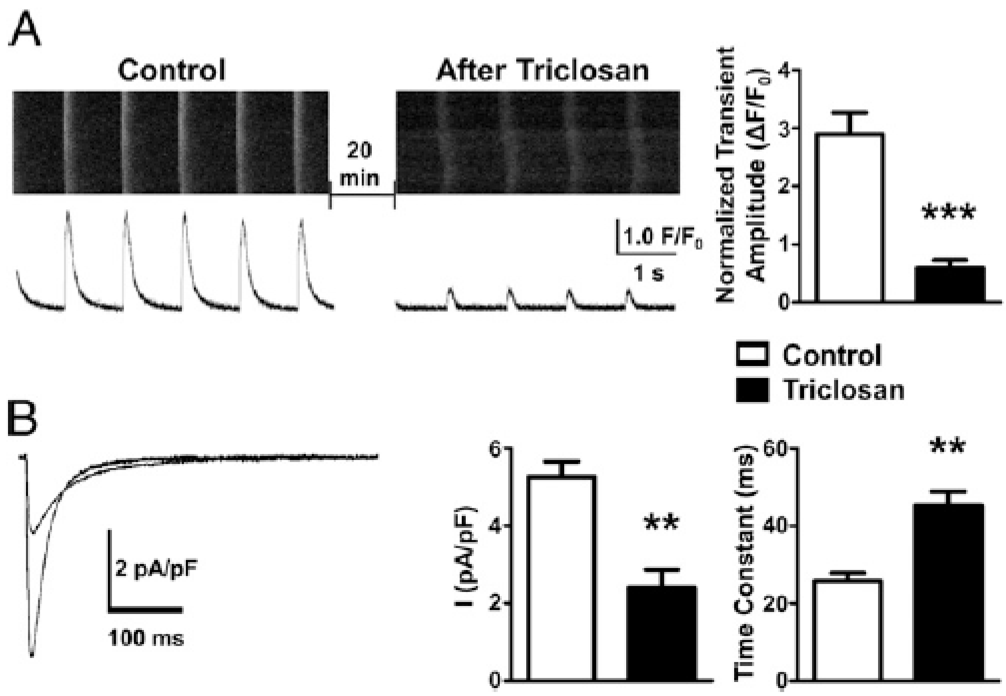
genotoxic effects of Triclosan in vitro and in vivo

- **Micronuclei formation**
- **Appoptosis**

Binelli et al. 2009 Aquatic Toxicology, 2009



- Reduction of cardiac function in mice treated with TCS in vivo



- TCS impairs excitation-contraction coupling in isolated cardiomyocytes
- damage to Ca++ channels

Cherednichenko et al. 2012 PNAS 109:14159

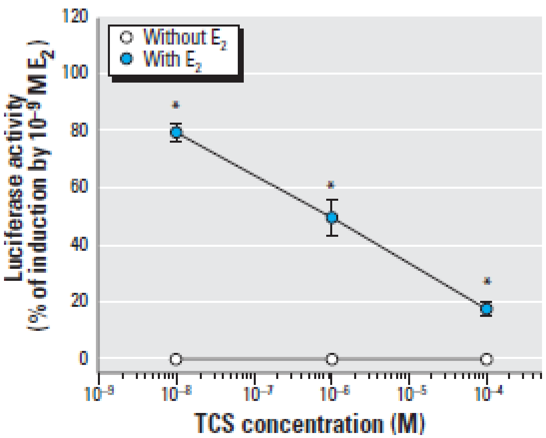


Figure 6. Activity of TCS in the ER-mediated bioassay.
*Significantly different from the control.

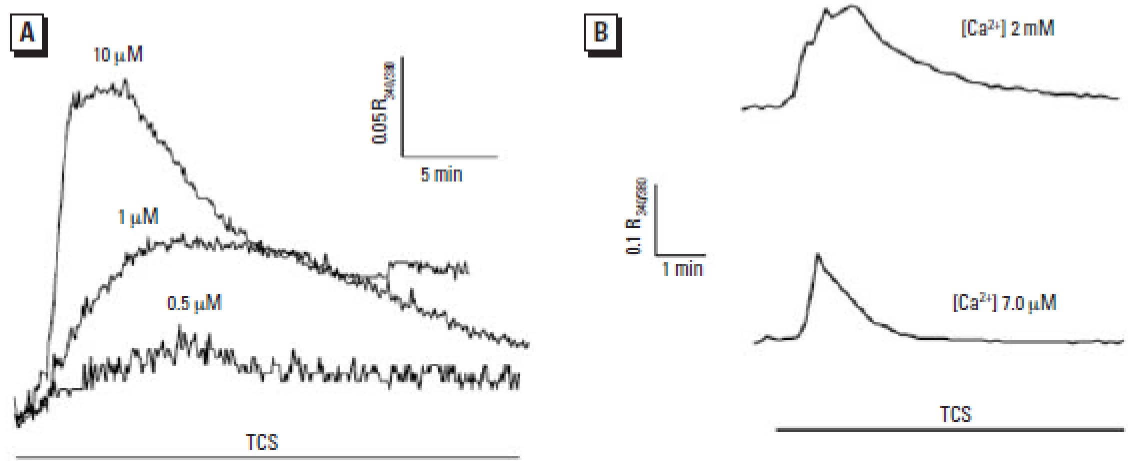
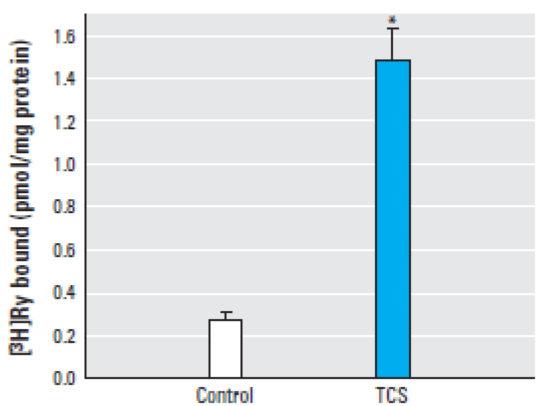


Figure 8. Effect of TCS on cytosolic Ca^{2+} concentration. (*A*) Cytosolic Ca^{2+} concentration in resting myotubes increased in a dose-dependent manner after TCS treatment; each trace is an average of $n \geq 5$ cells in separate cell cultures in Ca^{2+}-replete (1.8 mM) buffer. (*B*) TCS 1 µM triggered an increase in the cytosolic Ca^{2+} concentration even in nominally Ca^{2+}-free (~ 7 µM) extracellular buffer.

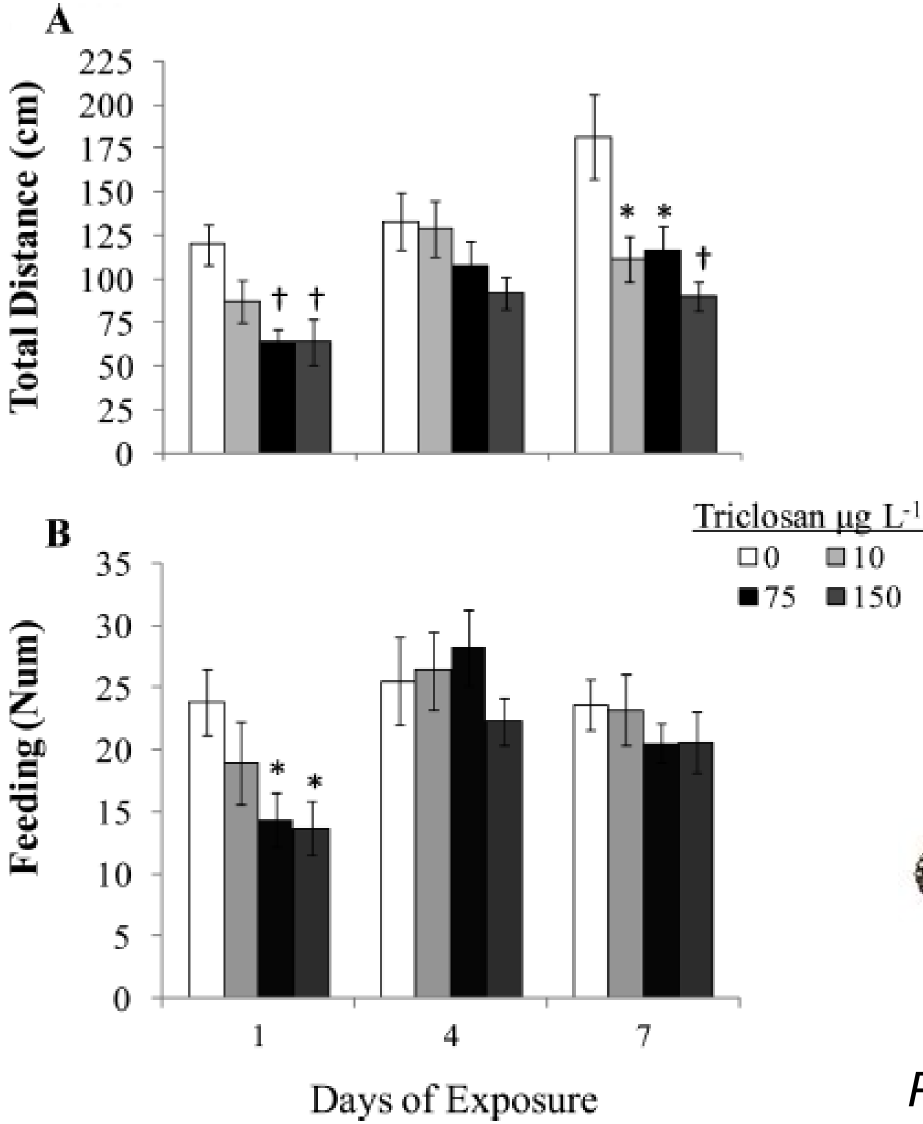


- **Effects of Triclosan on intracellular Ca^{2+} homeostasis in myotubes**
- **Interaction with estrogen**

Figure 7. [^{3}H]Ry binding with or without 1.2 µM TCS in skeletal muscle sarcoplasmic reticulum vesicles.
*Significantly greater than the control at $p < 0.05$.

Ahn et al. Environ Health Perspectives 2008

Triclosan alters DHPR and RyR protein expression in fish larvae



Effect of Triclosan on

- swimming performance
- feeding behaviour

Pimephales promelas, fathead minnow

Fritsch et al 2013 Env Science and Technology

Table 4. Endocrine-disrupting effects of triclosan (TCS)

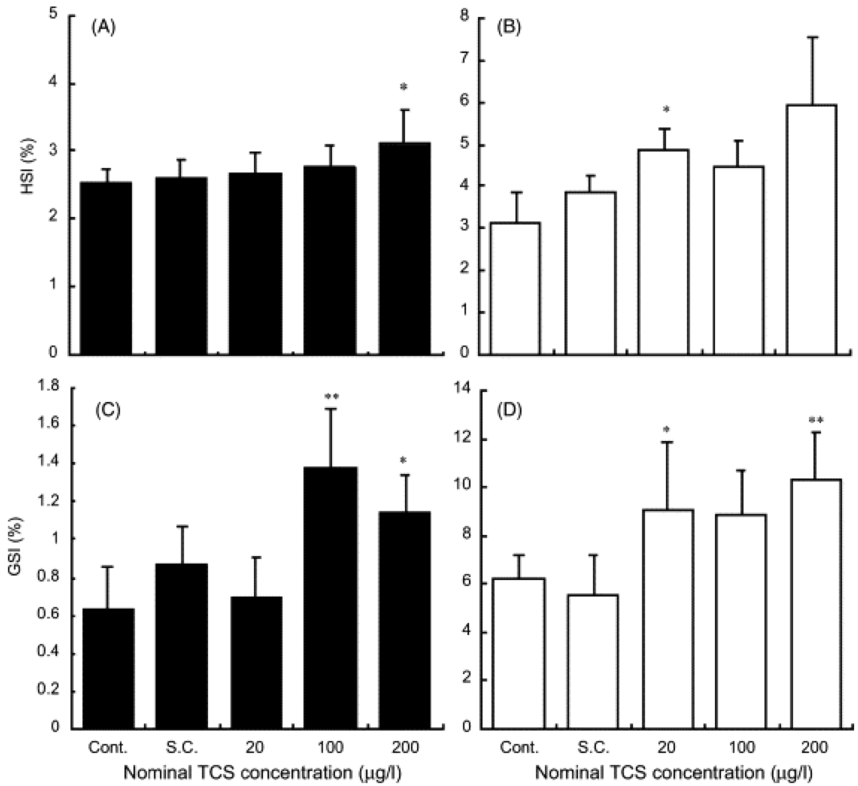
Test species/system	Life stage	Aquatic system	Route of exposure	Test duration	TCS exposure	Effects
Fish Medaka (*Oryzias latipes*)	Embryos	FW	Water	14 days	100 μg l^{-1}	Weak androgenic (or anti-estrogenic) effect (↑male fin size, slight male bias sex ratio)
	Male fish	FW	Water	14 days	20 μg l^{-1}	Weak estrogenic activity; ↑Vtg in male fish; activity in yeast assay
Mosquitofish (*Gambusia affinis*)	Male fish	FW	Water	35 days	101.3 μg l^{-1}	↑vitellogenin, ↓sperm count
Bream (*Abramis brama*)	Bile of male fish	FW	Field sites, Netherlands		No activity up to 0.1 mM	No estrogenic activity detected in ER-CALUX assay
Amphibians						
North American bullfrog (*Rana catesbeiana*)	Tadpoles	FW	*In vivo*	18 days	0.15 μg l^{-1}	Disruption of T_3-dependent developmental metamorphosis processes
South African clawed frog (*Xenopus laevis*)	Tadpoles	FW	*In vivo*	21 days	1.5 μg l^{-1} 0.6–32.4 μg l^{-1}	↓larval growth; no effect on metamorphosis
	Males	FW	Water; i.p. injection	14 days	20–200 μg l^{-1}; inject 4-400 μg g^{-1} body weight	No effect on Vtg in males; no effects on CYP1A and EROD; ↓Vtg in i.p. injected males

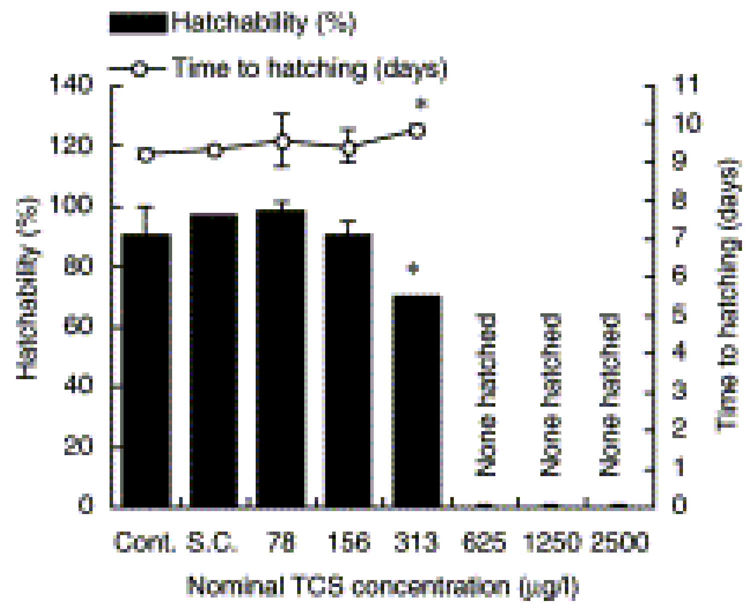
Medaka, *Oryzias latipes*
Japanese killifish

Isihibashi et al. 2004 Chemosphere

Increase in GSI in male Medaka

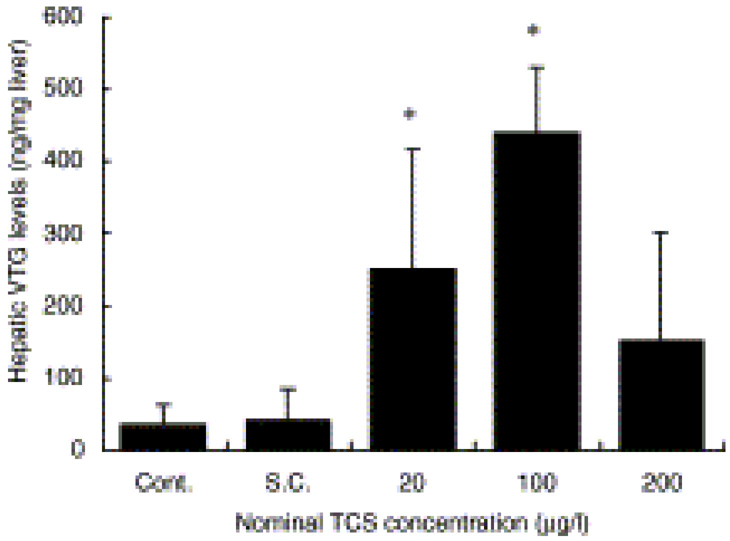
Lower hatchability of eggs in female

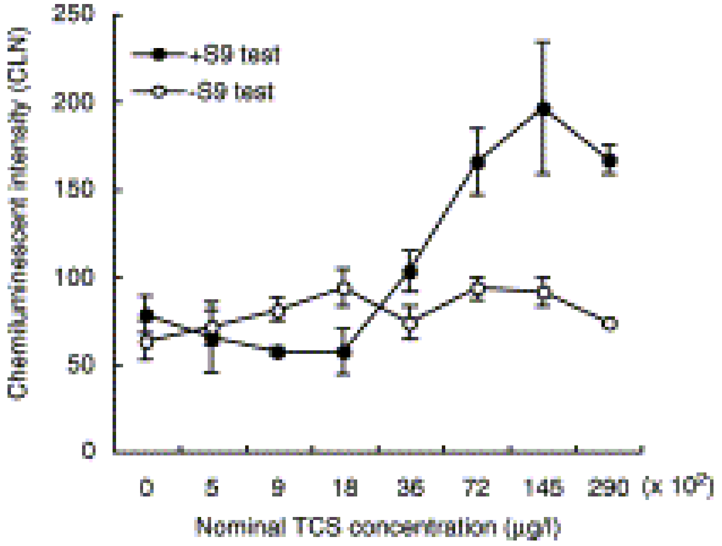




Raut and Angus 2010 Env Toxicol Chem

Decrease in sperm count in Mosquitofish *Gambusia affinis*



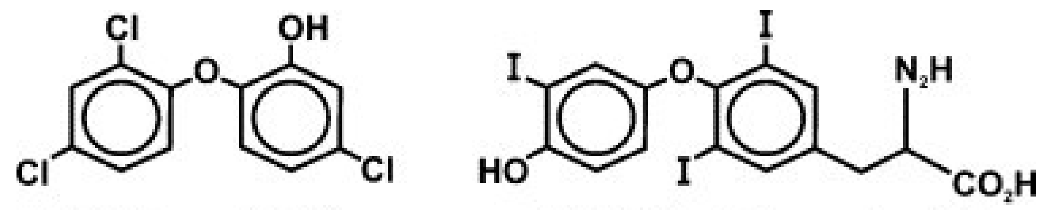


- **Estrogenic effects in male medaka**
  induction of Vtg
- **Estrogenic activity of TCS enhanced by S9 activation**

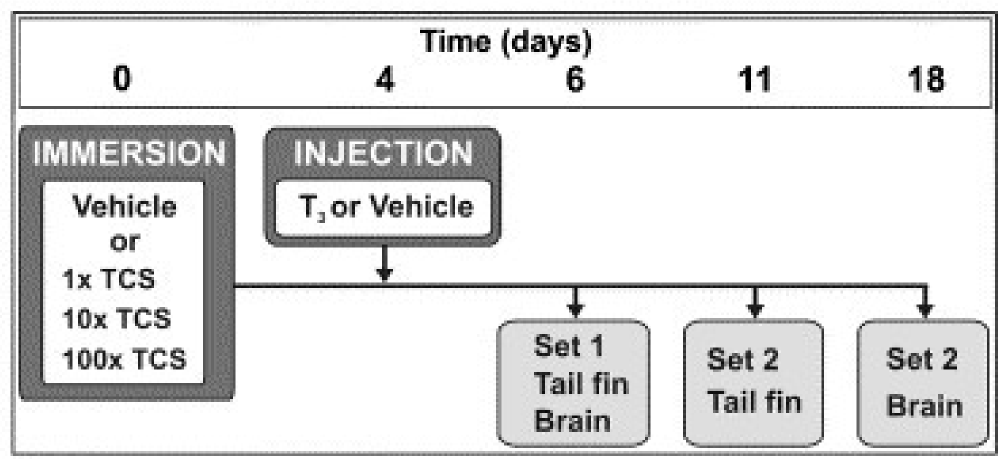
Veldhoen et al. 2006, Aquat Toxicol

Effects of Triclosan on amphibian metamorphosis
Effects of Triclosan on thyroid axis

In vivo
Exposure of tadpoles
(*Rana catesbeiana*)



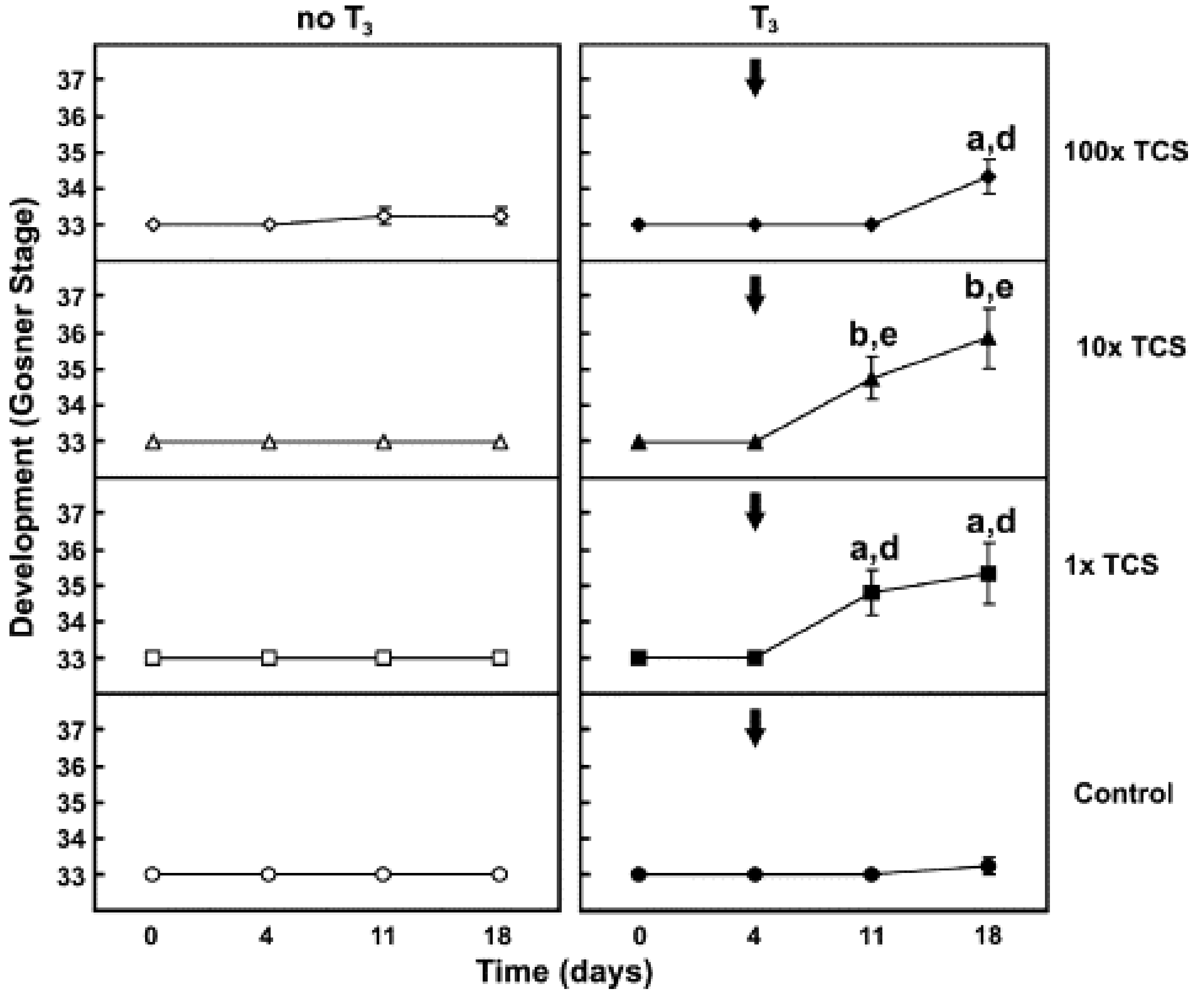
(A) Triclosan (TCS) 3,3',5-triiodothyronine (T_3)



(B)

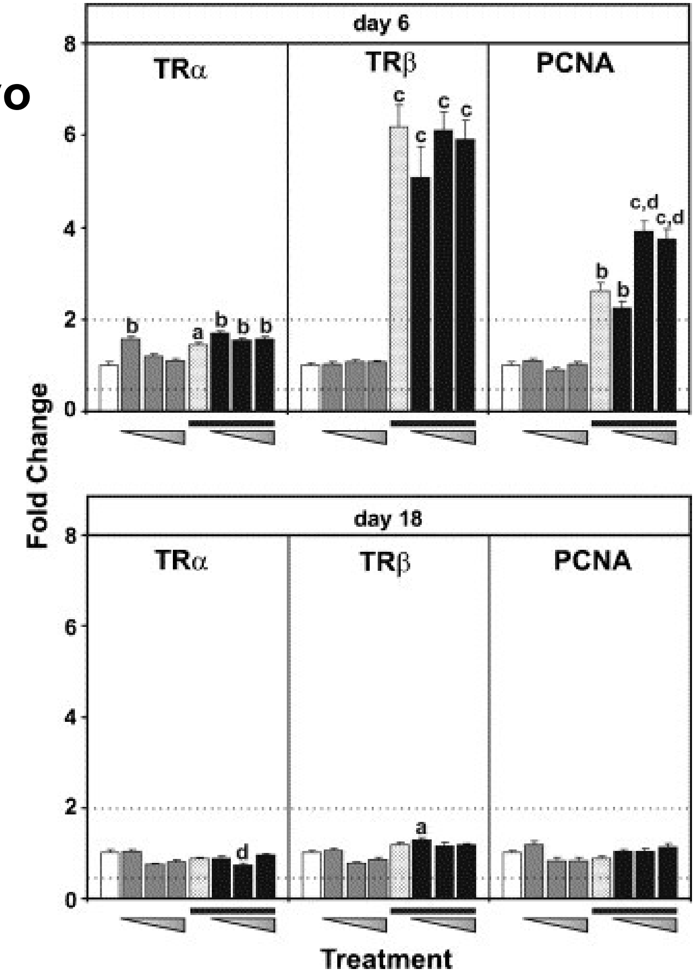
In vitro
Exposure of XTC-2 cells
(*Xenopus laevis* cells)

North American bullfrog
Rana catesbeiana

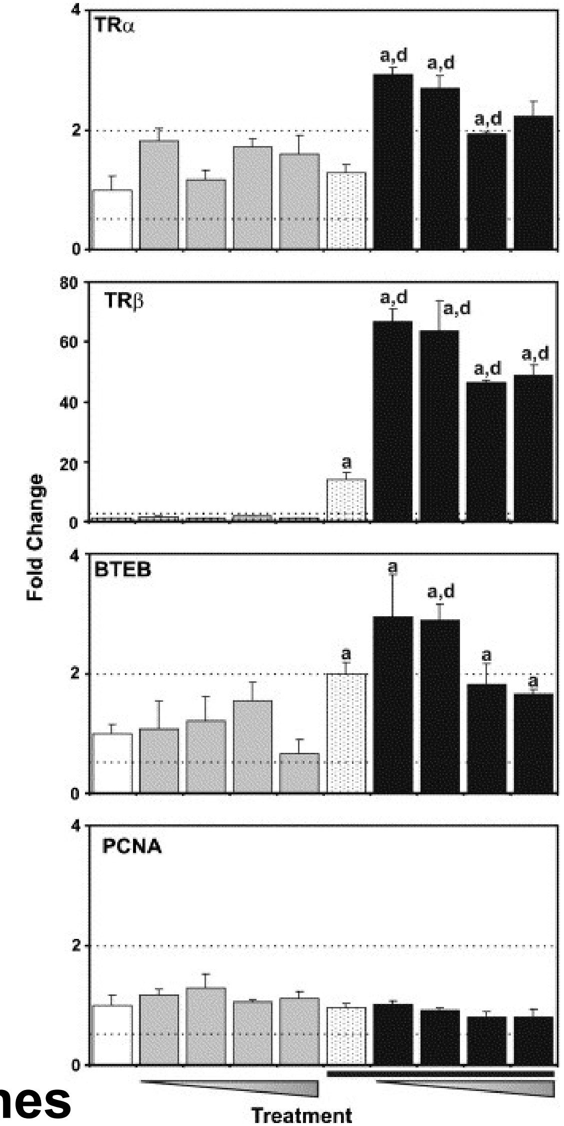


- **Acceleration of development in tadpoles exposed to T3 and TCS**
- **T3 alone has no effect**

In vivo



In vitro



- **Relative mRNA expression of TH-regulated genes**
- **Transcripts levels affected by Triclosan at a sensitive life stage of anuran development**
- **No evidence for estrogenic effects (no VTG induction)**

Effects of Triclosan in human ?

Cell –based assays

- **estrogenic and androgenic effects in MCF37 breast cancer cells**
- **weak AhR activity**

In vivo effects ???

Efficacy of Triclosan

- Use in oral hygiene +++
- Use in clinical setting as soap +++
- Use in personal care ?
- Use in food wrapping ?
- Use in sport clothing ?


Antibacterial Chemical Raises Safety Issues

Fred R. Conrad/The New York Times

Products like soaps and toothpaste contain triclosan, an antibacterial additive.

By ANDREW MARTIN
Published: August 19, 2011

Risk assessment for Triclosan

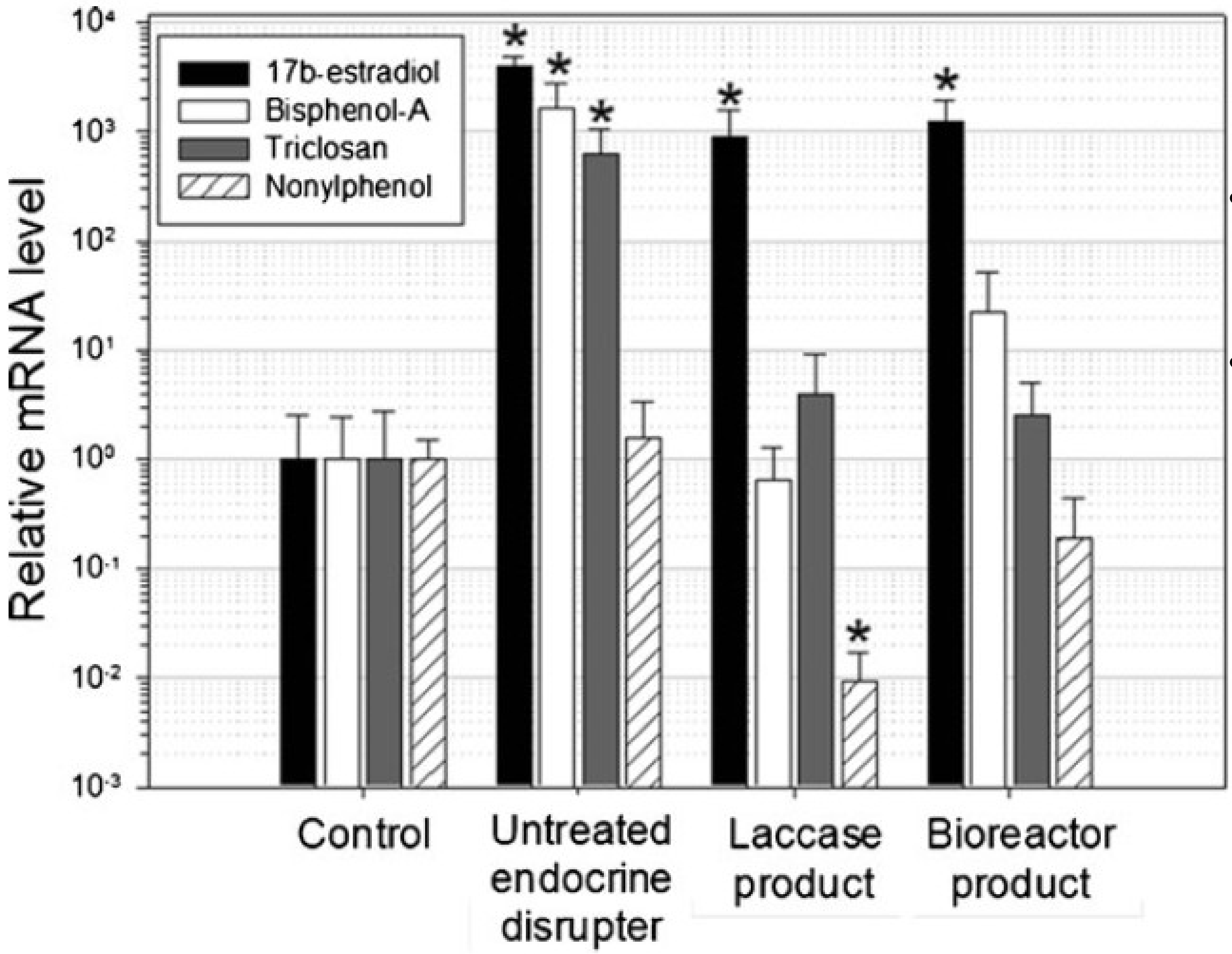
Antibacterial control
- hospitals
- oral care

Ecosystem integrity
- (algal populations)

Endocrine disruption
- amphibians, fish

Antibacterial resistance


Remediation – inactivationl of Triclosan by enzymatic degradation



- Vtg mRNA in zebrafish
- ER activity

Torres-Duarte 2012 Appl Biochem Biotechnol 168: 864