TRICLOSAN : environmental exposure, toxicity and mechanisms of action

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Innovation Lectures (INNOLEC)
Masaryk University, May 2012

Triclosan – identity, use

Broad spectrum antimicrobial

- **personal care products – Irgasan, Aquasept, Sapoderm, …**

EU Cosmetic Directive (0.1-0.3%)

- **sport clothing – Ultra-Fresh, Microban, Sanitized, …**
- **food packaging**

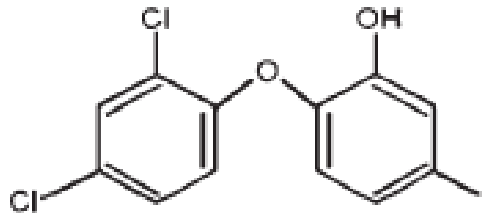
Economy – Life style

Triclosan – key issues

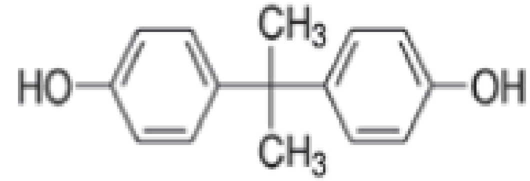
- Detected in surface water, human plasma, breastmilk
- Bioaccumulation
- By-product formation
- Endocrine disruption
  - Thyroid hormones
  - Sex hormones
- Antibiotic resistance, efficacy

1. Identity and mode of action

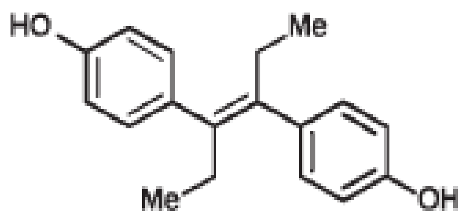
Triclosan



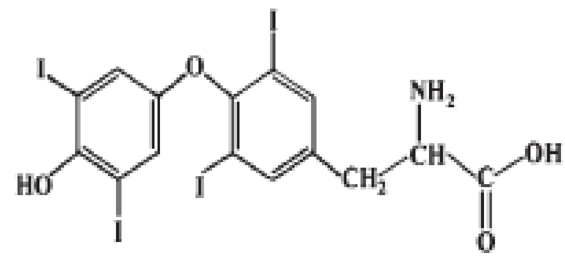
Bisphenol-A



Diethylstilbestrol



Thyroxine



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

2. 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 $\mu g\ l^{-1}$
		Switzerland	ND to 0.074 $\mu g\ l^{-1}$
		Germany	ND to 0.01 $\mu g\ l^{-1}$
		Sweden	ND
		Australia	0.075 $\mu g\ l^{-1}$
		Japan	<0.0006–0.059 $\mu g\ l^{-1}$
	Streams with inputs of raw wastewater	Switzerland	0.011–0.098 $\mu g\ l^{-1}$
		USA	1.6 $\mu g\ l^{-1}$
	Estuarine waters	USA	0.0075 $\mu g\ l^{-1}$
Sediment	Freshwater	Switzerland	53 $\mu g\ kg^{-1}$
		Spain	ND to 35.7 $\mu g\ kg^{-1}$
	Estuarine	USA	ND to 800 $\mu g\ kg^{-1}$
	Marine	Spain	0.27–130.7 $\mu g\ kg^{-1}$
Sewage sludge	Activated sludge	USA	0.5–15.6 $\mu g\ g^{-1}$
		Spain	0.4–5.4 $\mu g\ g^{-1}$
		Germany	1.2 $\mu g\ g^{-1}$
		Canada	0.62–1.45 $\mu g\ g^{-1}$
	Biosolids	Australia	90–16 790 $\mu g\ kg^{-1}$
		USA	10 500–30 000 $\mu g\ kg^{-1}$
		Spain	1508 $\mu g\ kg^{-1}$
		Canada	680–12 500 $\mu g\ kg^{-1}$

link to experimental studies

Concentrations of triclosan (TCS) in the aquatic environment

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

WWTP – Waste Water Treatment Plant

3. 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**

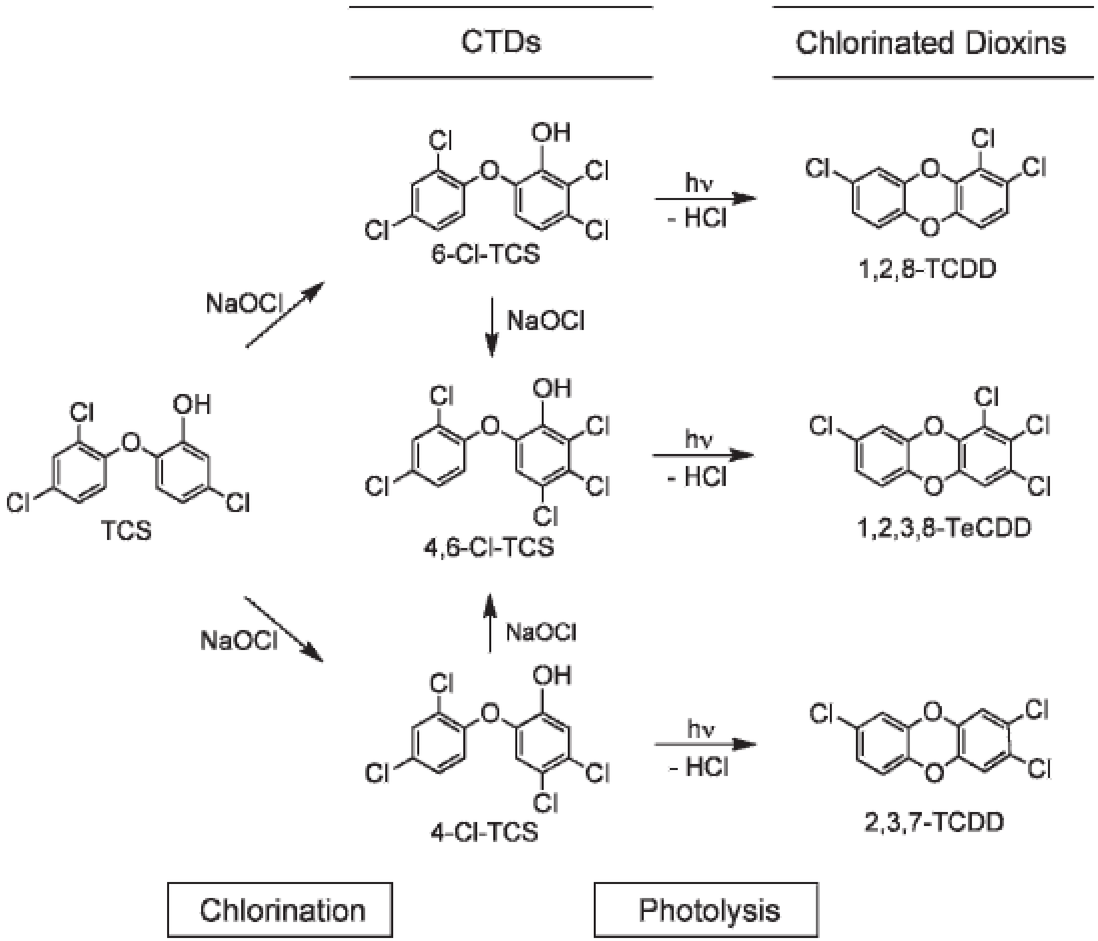
  generated during photodegradation of TCS at pH > 8

- **Chloroform and chlorophenols**

  generated in presence of chlorine or chloramine

Environmental chemistry, Environmental fate

Buth et al. 2009 Environ Toxicology Chem 28: 2555-2563, 2009



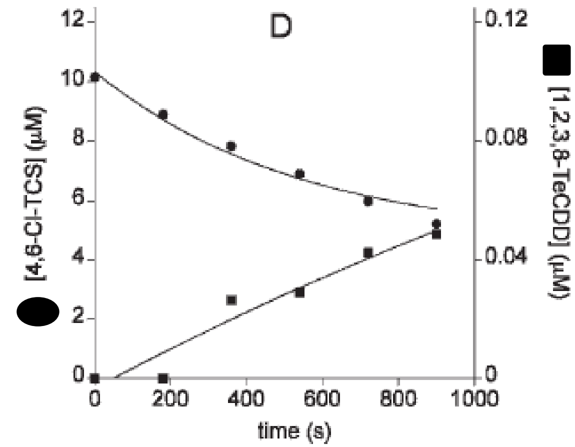
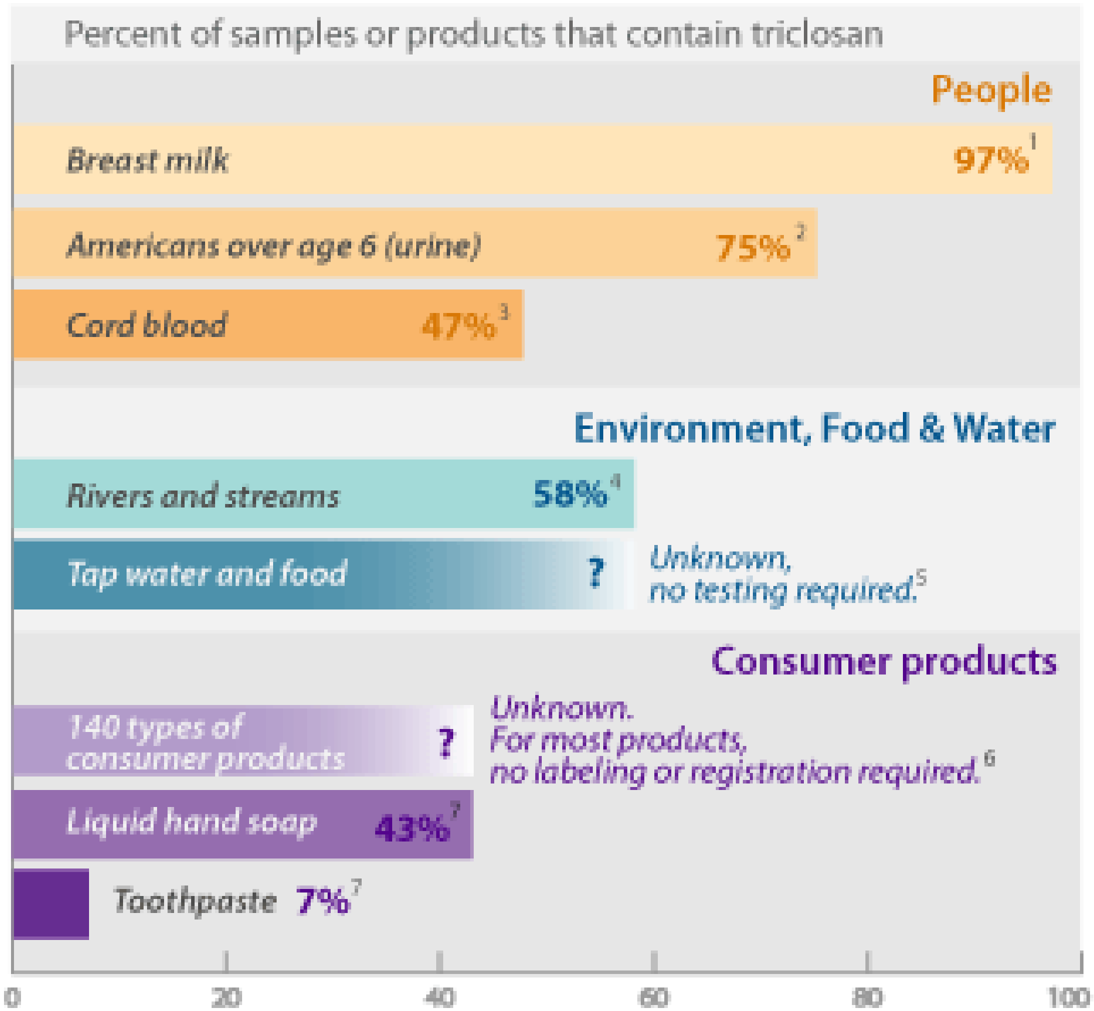


Fig. 4. Solar photolysis of phenoxyphenol substrates

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).

4. Concentrations of Triclosan in organisms



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

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

Organisms	Type of sample	Site description	TCS (μg 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 (?)

Table 3. Effects of triclosan (TCS) on freshwater (FW) and marine (SW) 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	71.3 µg l^{-1}	Delayed swim-up ;
				35 days		↓ 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)	$\boxed{0.15\ \mu g\ l^{-1}}$	↑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

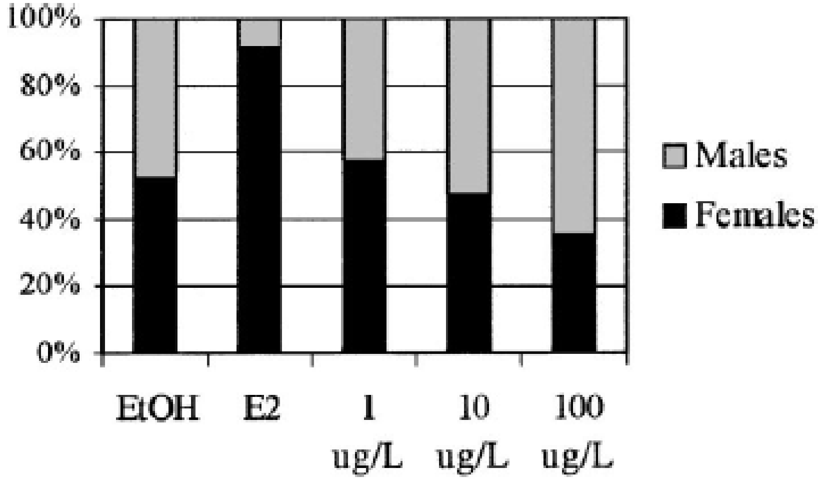


Fig. 1. Resultant sex ratio from exposed medaka fry.

Foran et al. 2000
Mar Environ Res

Triclosan
weak androgenic action
in medaka, *Oryzias latipes*

Table 1
Fin lengths (mean±S.D.) from adult animals

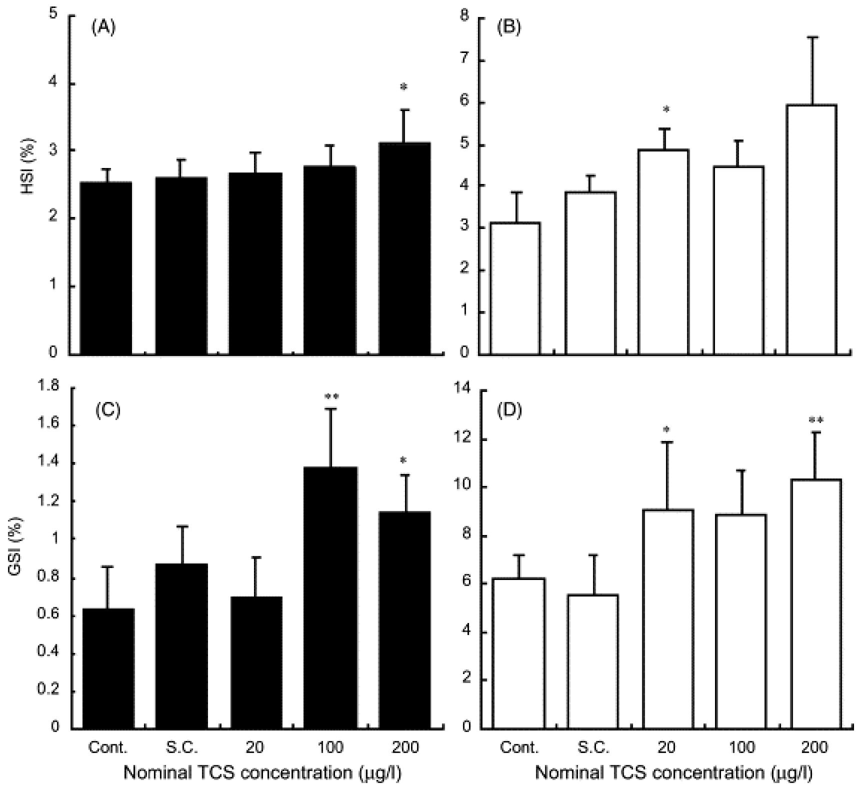
	EtOH	1 μg/l	10 μg/l	100 μg/l	E2
Dorsal fin					
Males	3.87±0.33	4.00±0.33	3.51±0.63	4.15±0.41	3.30±0.25
Females	2.63±0.09	2.73±0.20	2.78±0.18	2.83±0.18	2.62±0.25
Anal fin					
Males	4.20±0.57	4.06±0.37	3.48±0.85	4.48±0.86	3.09±0.45
Females	2.90±0.32	2.63±0.29	2.70±0.31	2.62±0.38	2.55±0.22

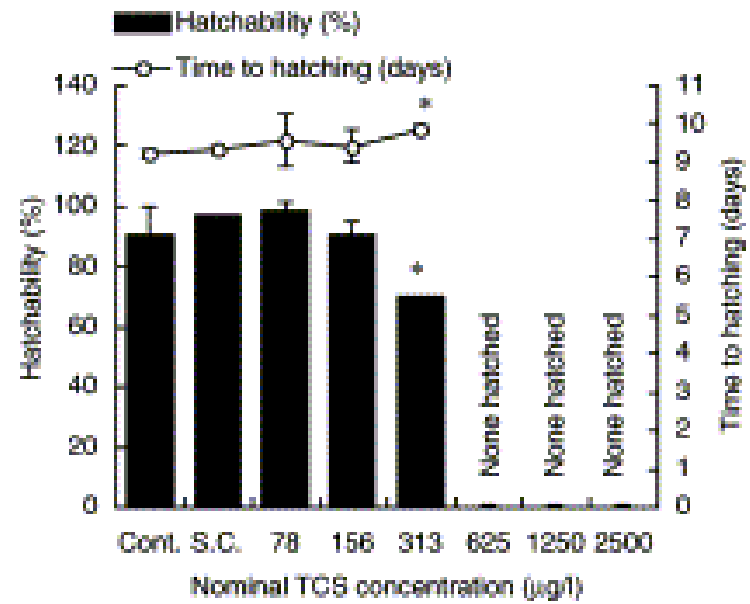
Medaka, *Oryzias latipes*
Japanese killifish

Isihibashi et al. 2004 Chemosphere

Increase in GSI in male Medaka

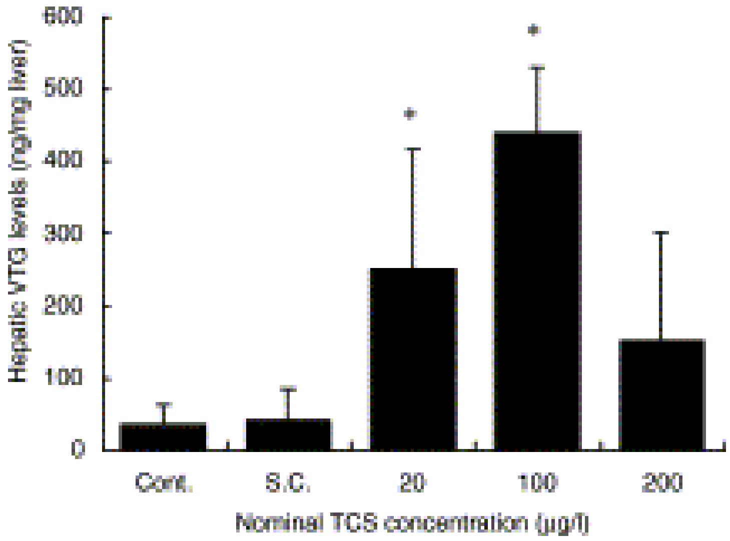
Lower hatchability of eggs in female

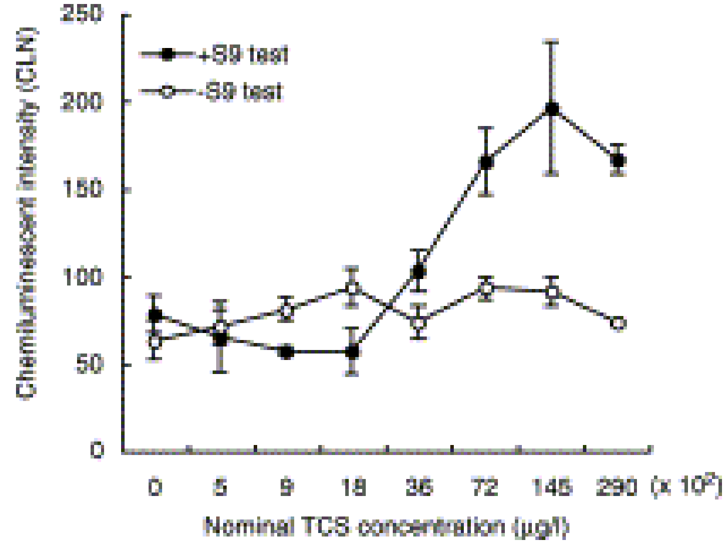




Raut and Angus 2010 Env Toxicol Chem

Decrease in sperm count in Mosquitofish *Gambusia affinis*





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

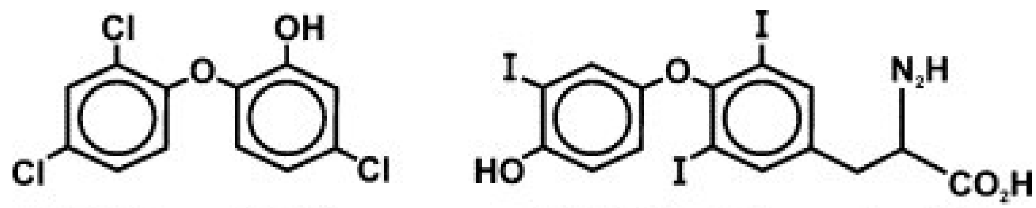
Table 4. Endocrine-disrupting effects of triclosan (TCS)

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

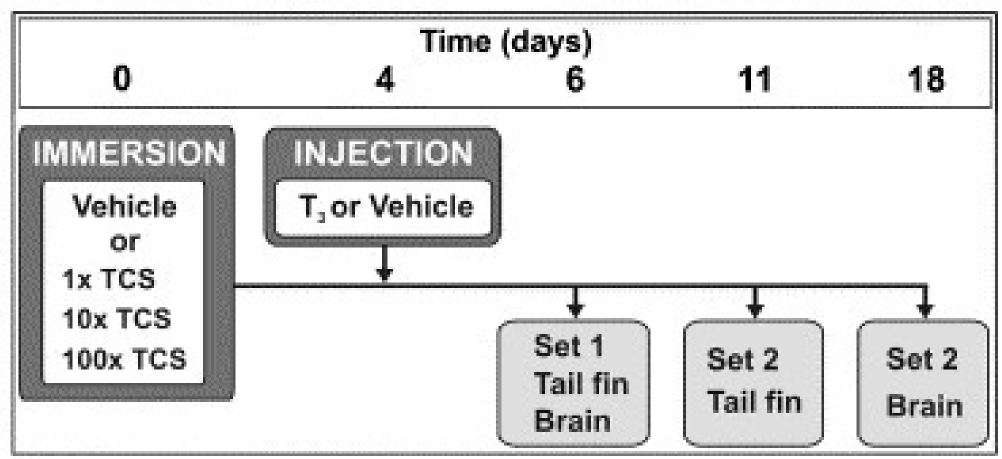
Veldhoen et al. 2006, Aquat Toxicol

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

In vivo
Exposure of tadpoles
(*Rana catesbeiana*)



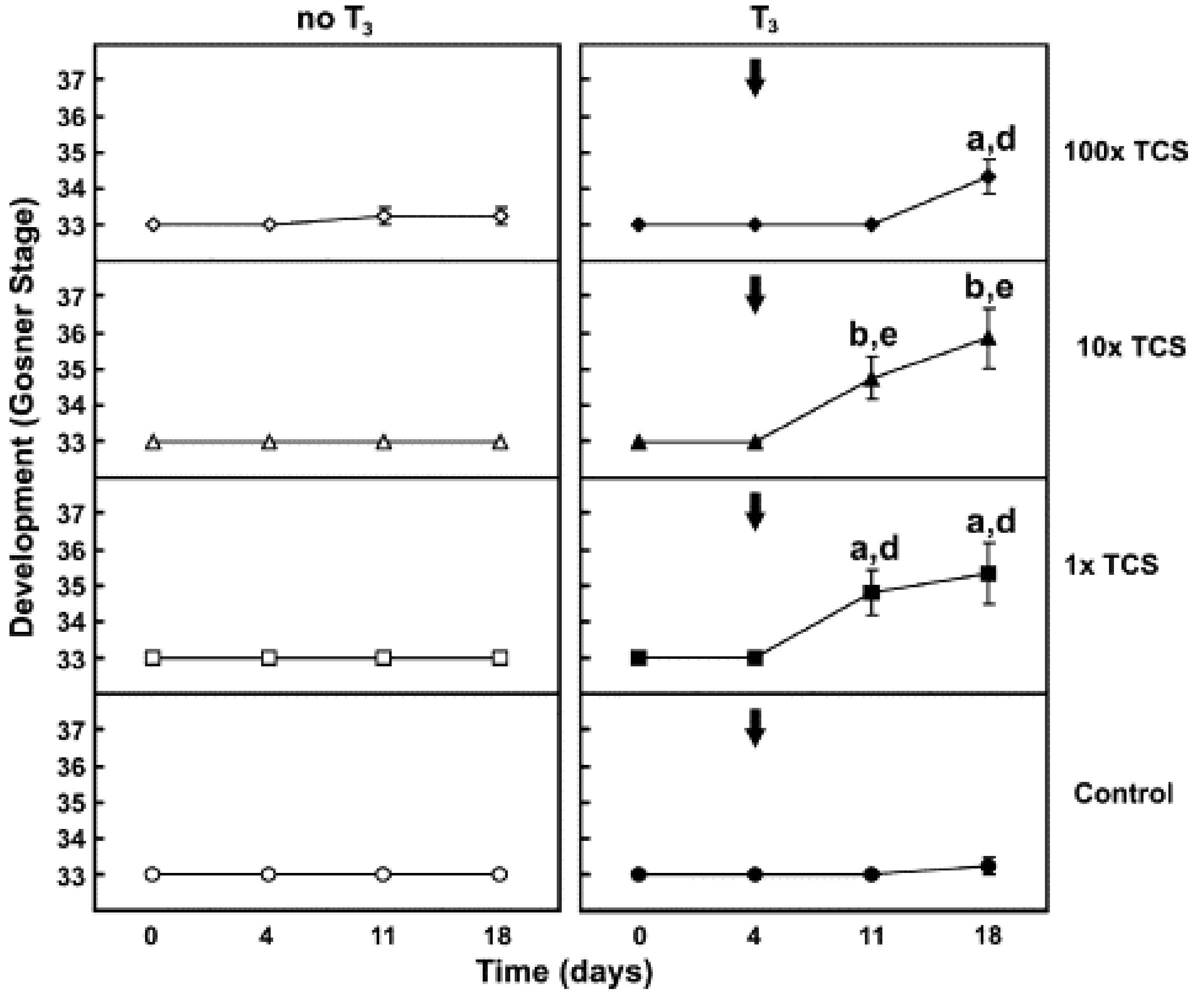
(A) Triclosan (TCS) 3,3',5-triiodothyronine (T_3)



(B)

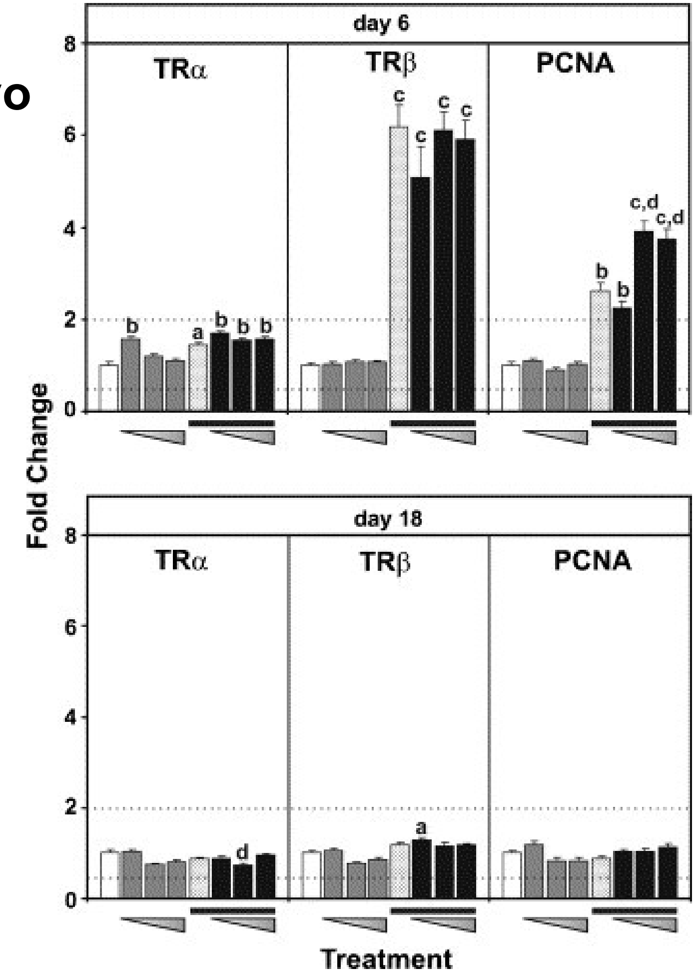
In vitro
Exposure of XTC-2 cells
(*Xenopus laevis* cells)

North American bullfrog
Rana catesbeiana

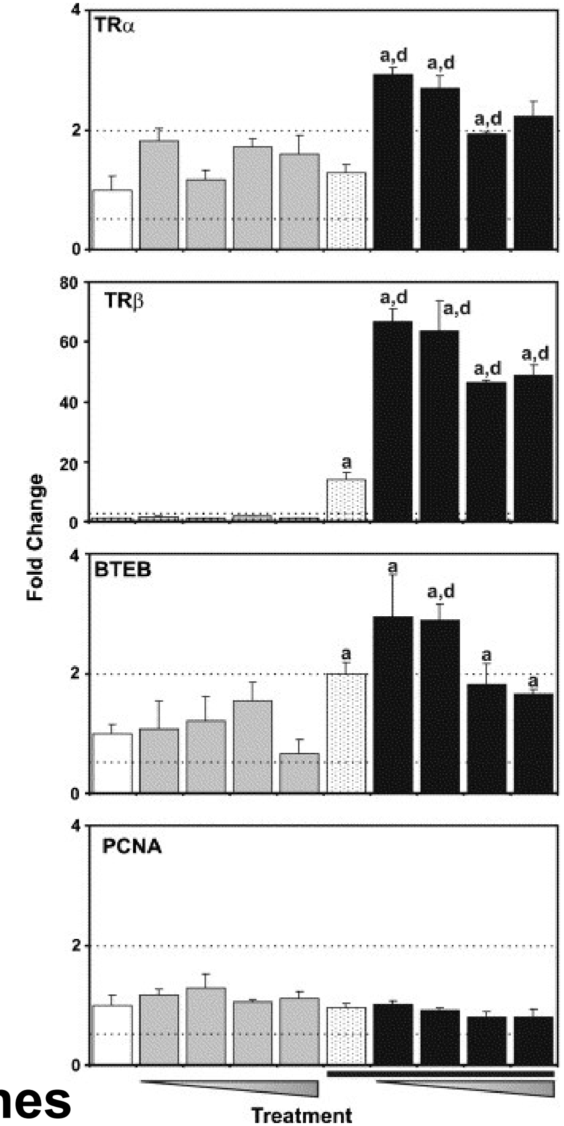


- **Acceleration of development in tadpoles exposed to T3 and TCS**
- **T3 alone has no effect**

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)**

South African clawed frog
Xenopus laevis

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

Mammals					
Sheep	Placenta	*In vitro*		0.6 nM	↓Estrogen sulfonation (IC_{50})
Rats (Wistar)	Pre-pubescent males	Oral (gavage)	31 days	200 mg kg^{-1} 30 mg kg^{-1}	No effect on timing of puberty; ↓levels of plasma testosterone and T_4
	Males; isolated	*In vivo* (daily intubation)	60 days	5–20 mg kg^{-1}	Disruption of LH, FSH, pregnenolone and testosterone synthesis; ↓mRNA expression of StAR and steroidogenic enzymes
	Leydig cells	*In vitro*	2 h	0.01–10 μM	
Rats (Long–Evans)	Adult female	Oral (gavage)	4 days	100 mg kg^{-1} day^{-1}	↓Plasma T_4
	Female weanlings	Oral (gavage)	4 days	300 mg kg^{-1} day^{-1}	↓Plasma T_4 and T_3
Human	Adults	brush 2/day with TCS tootpaste	14 days	0.3% w/w TCS	No effect on thyroid status

Crofton et al. 2007
Environ Toxicol Pharmacol

Paul et al 2010
Toxicological Sciences

Inhibition of thyroid axis in rats exposed to Triclosan

Effect on Phase II enzymes (metabolism of thyroid hormones)

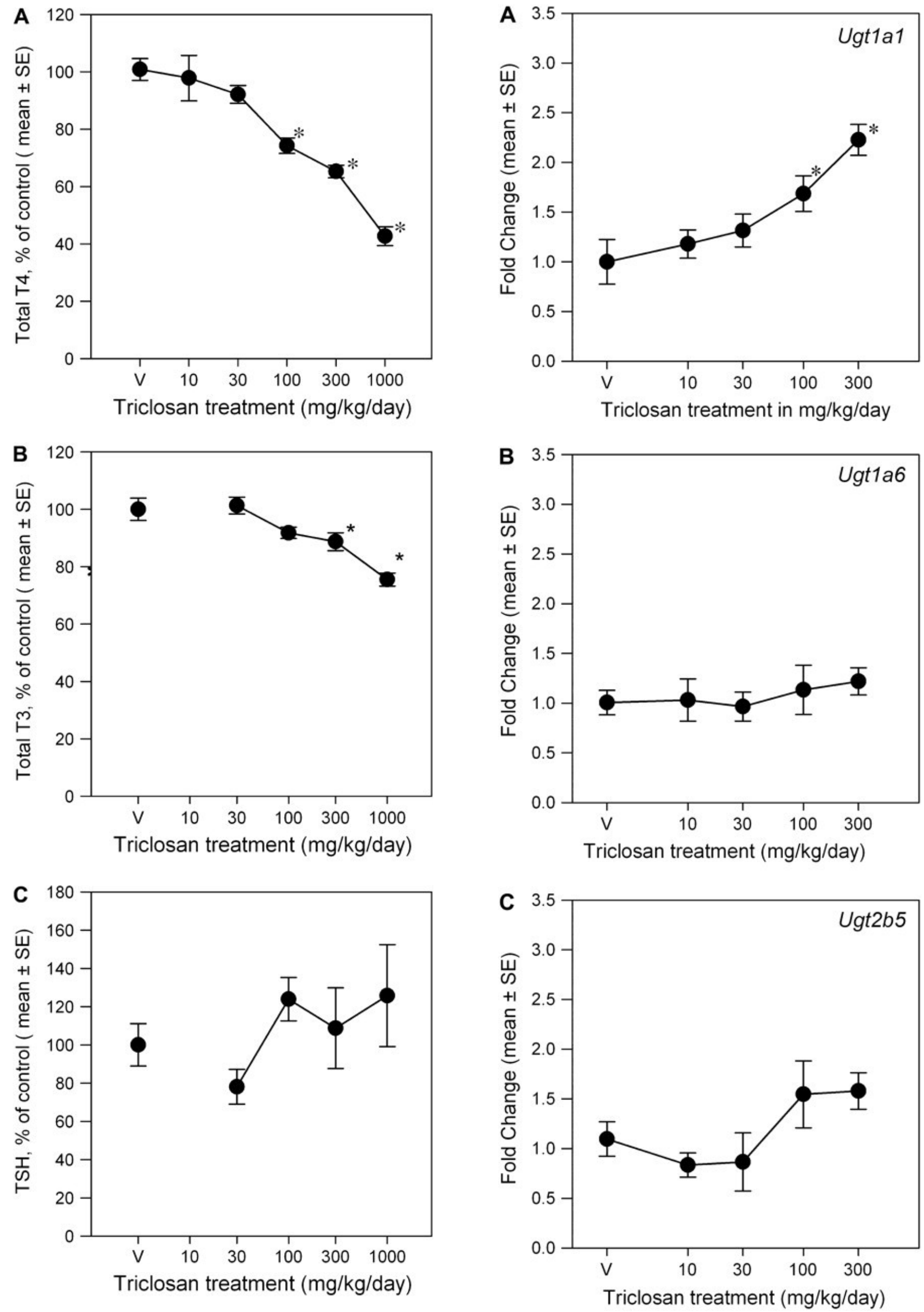


Table 4. Endocrine-disrupting effects of triclosan (TCS)

Cell-based assays			
MCF37 breast cancer cells	*In vitro*	10 μM	Estrogenic and androgenic effects
2933Y cells (human)	*In vitro*	1.0 and 10 μM	↓Testosterone-induced transcriptional activity
Cell-based nuclear-receptor-responsive and calcium signaling bioassays (AhR, ER, AR, RyR)	*In vitro*	1–10 μM (for ER- and AR-responsive gene expression; 0.1–10 μM (for RyR response)	Weak AhR activity; antagonistic activity in ER- and AR-dependent gene expression; interaction with RyR1, ↑Ca^{2+} mobilization in skeletal myotubes
HuH7 cells (human hepatoma cell line) transfected with human pregnane X receptor (hPXR)	*In vitro*	>10 μM	Activation of hPXR
Induced rat liver microsomes	*In vitro*	3.1 μM (IC_{50})	↓Diiodothyronine (T_2) sulfotransferase activity

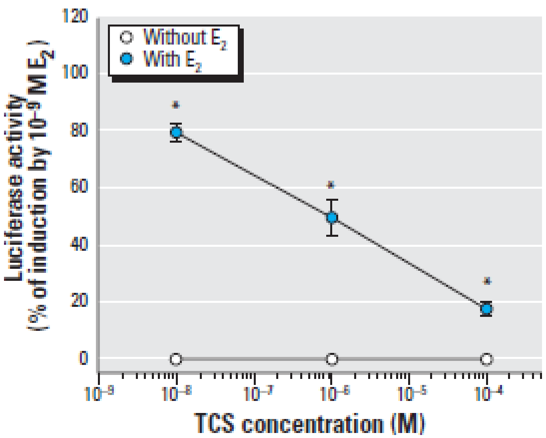


Figure 6. Activity of TCS in the ER-mediated bioassay.
*Significantly different from the control.

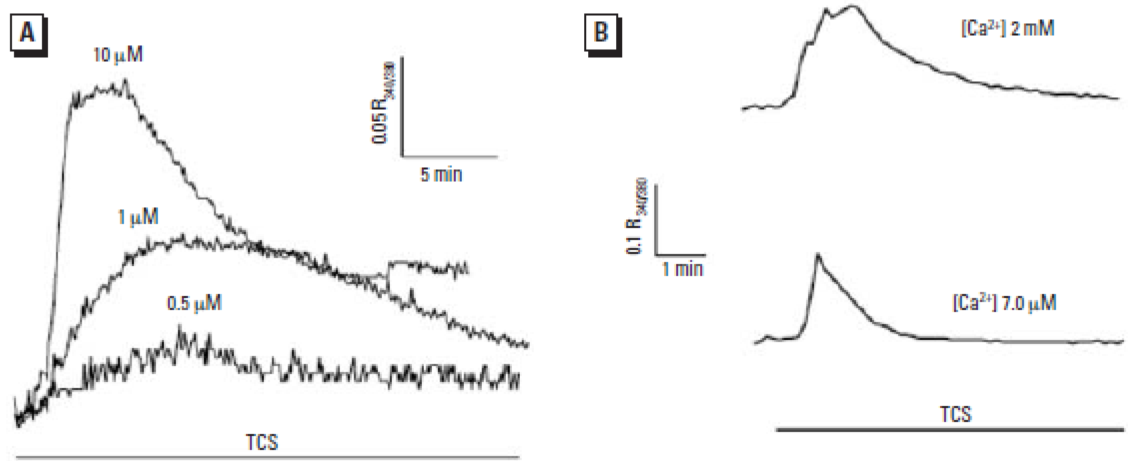


Figure 8. Effect of TCS on cytosolic Ca^{2+} concentration. (*A*) Cytosolic Ca^{2+} concentration in resting myotubes increased in a dose-dependent manner after TCS treatment; each trace is an average of $n \geq 5$ cells in separate cell cultures in Ca^{2+}-replete (1.8 mM) buffer. (*B*) TCS 1 µM triggered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

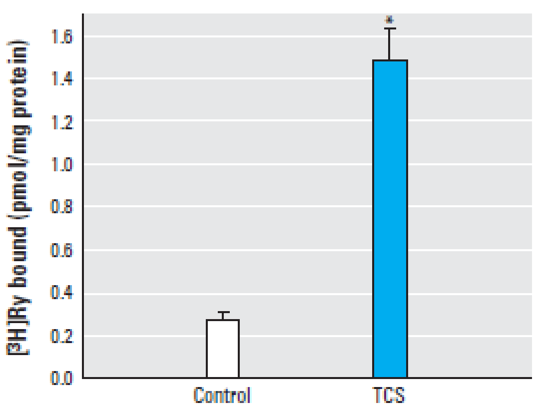


Figure 7. $[^3H]$Ry binding with or without 1.2 µM TCS in skeletal muscle sarcoplasmic reticulum vesicles.
*Significantly greater than the control at $p < 0.05$.

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

Ahn et al. Environ Health Perspectives 2008

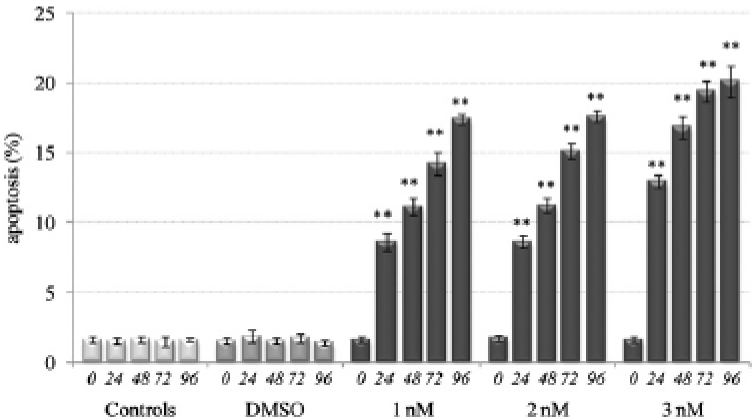


Fig. 2. Temporal trend (h) of the means of apoptosis frequency ± SEM calculated for Zebra mussel hemocytes for controls, solvents and treated samples with TCS. Significant values (two-way ANOVA, Bonferroni *post-hoc* test, $p < 0.05$) were obtained for the comparison between treated samples and controls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

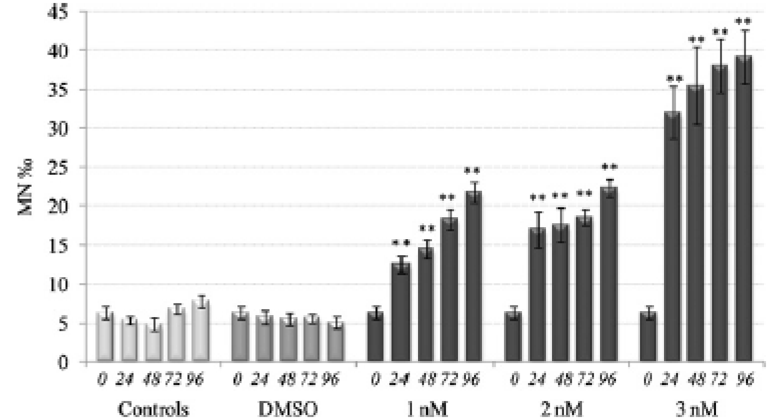


Fig. 3. Temporal trend (h) of the means of micronuclei frequency ± SEM calculated for Zebra mussel hemocytes for controls, solvents and treated samples with TCS. Significant values (two-way ANOVA, Bonferroni *post-hoc* test, $p < 0.05$) were obtained in the comparison between treated samples and controls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

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