

Effect of Tributyltin on the Sex Ratio in Guppy (*Poecilia reticulata*)

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Cite this article as: Karayücel, İ., Kırkoğlu, O., & Dernekbaşı, S. (2019). Effect of Tributyltin on the Sex Ratio in Guppy (*Poecilia reticulata*). *Aquatic Sciences and Engineering*, 34(1), 1–6.

ABSTRACT

In this study, the effect of tributyltin (TBT) on the sex ratio in guppy (*Poecilia reticulata*) during the labile period covering both embryonic and post hatching periods was investigated. The gravid females was fed an artificial diet containing TBT chloride at an environmental levels of 25, 50 and 150ng/g feed from 16th day after the first parturition until next parturition. The newly hatched larvae from untreated females were also fed by the same diet for 11 days. TBT caused various abnormalities like body shape and fin deformations in gravid females and no parturition was seen. The male ratio significantly increased to 70.74, 87.50 and 87.18 % in the experimental groups of guppy larvae fed by 25, 50 and 150ng TBT/g diet, respectively. Survival and growth of the larvae were negatively affected by TBT treatment. These results clearly showed the masculinization of guppy exposed to TBT for the first time.

Keywords: Guppy, endocrine disrupters, tributyltin, sex reversal

INTRODUCTION

Endocrine disrupting chemicals (EDCs), a range of pollutants in the environment, are both natural and mostly man-made, found in many everyday products including plastic bottles, metals, detergents, flame retardants, additives or contaminants in food, toys, personal care products and pesticides that interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife and have received increasing interest in the possible health threat posed by them. Amongst them organotin compounds (OTs), with a worldwide annual production of 50,000 tons in 1996, particularly tributyltin (TBT) have been widely used for various industrial purposes such as production of PVC, textile, slime control in paper mills, disinfection of circulating industrial cooling water, agricultural fungicides, preservation of woods and rodent repellents. The use of TBT as biocides in antifouling

boat paints is the most important contribution of organotin compounds and leads to the contamination of aquatic environment (Tian et al. 2015). Aquatic pollution resulting from its usage has been of great concerns due to its bioaccumulation potential, persistence in sediment up to several years and highly toxic effects on non-target organisms (Antizar-Ladislao 2008; Anastasiou et al. 2016). After the first negative effects were reported in several studies, the use of organotin-based antifouling from paints was prohibited in the European Union since 2003 and imposed their complete removal from the ship hulls since 2008 (Anastasiou et al. 2016). Because of the relatively long half-life of OTs in sediments (0.9-5.2 years; Dowson et al. 1993), they are priority substances posing a serious threat to aquatic environment for a long time after their deposition (Anastasiou et al. 2016). TBT, currently a cause of great concern, is still found at high levels in water ecosystem and tissues of aquatic organisms in China (Jiang et al. 2001; Zhang et al. 2013),

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Submitted:
10.05.2018

Accepted:
23.10.2018

Online published:
07.01.2019

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Available online at
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Japan (Harino et al. 2000), Great Britain (Harino et al. 2003), Taiwan (Shue et al. 2014), India (Garg et al. 2011), Croatia (Furdek et al. 2012), South Korea (Kim et al. 2014), Spain (Jiang et al. 2001), Italy, Tunisia and Portugal (Anastasiou et al. 2016).

After the demonstration of masculinization effect of TBT on approximately 260 gastropods species worldwide (Tittley-O'Neil et al. 2011), studies of the effects of TBT especially on fish reproduction has also received particular attention. In fish, TBT disrupted steroidogenesis in zebrafish (*Danio rerio*) and brown trout (*Salmo trutta fario*) (McGinnis and Crivello 2011; Marca Pereira et al. 2014) and reproductive behaviour in male medaka (*Oryzias latipes*) (Nakayama et al. 2004) and male guppies (*Poecilia reticulata*) (Tian et al. 2015), decreased fertility and fecundity in Japanese whiting (*Sillago japonica*) (Shimasaki et al. 2006), inhibited gonad development in rockfish (*Sebastes marmoratus*) (Zhang et al. 2007; 2013) and mummichog (*Fundulus heteroclitus*) (Mochida et al. 2007). Although there are many studies dealing with the negative effects of TBT on fish reproduction, only three studies showed that TBT altered the sex ratio towards males at extremely low levels in Japanese flounder (*Paralichthys olivaceus*) (Shimasaki et al. 2003) and zebrafish (McAllister and Kime 2003; Santos et al. 2006). Interestingly, no studies are available dealing with the effects of TBT on sex differentiation in guppy.

In the present study, the guppy, native to fresh and brackish waters of north eastern South America and adjacent islands of the Caribbean and one of the famous tropical ornamental fish in the world was used as an experimental model. They are ovoviviparous fish with a short reproductive period, readily available, easily handled fish and therefore suitable for studying EDCs on the reproductive system since they have also strong visible secondary sex characteristics. They also tolerate comparatively wide ranges of temperature, salinity, and crowding (Tian et al. 2015). Therefore, the aim of this study was to investigate the effect of TBT on the sex ratio of guppy.

MATERIAL AND METHOD

Experimental Fish Stock and their Maintenance

The experiment was conducted at an indoor facility of the Faculty of Fisheries, University of Sinop. Experimental *P. reticulata* with an average weight of 0.50g were obtained from a commercial aquarium fish production facility. Fish were kept at $26 \pm 0.5^\circ\text{C}$ ($\pm\text{SE}$) under a 14/10 h light/dark cycle. They were fed with a commercial diet (JPL Novo Beas Flakes with 45.2% protein and 8% fat) twice a day (at approximately 9:00 am and 3:00 pm) *ad libitum*. O_2 and pH were monitored weekly while the water temperature was checked daily during the study. Throughout the experiments, O_2 and pH were $5.57 \pm 0.03 \text{mgL}^{-1}$ and 8.64 ± 0.02 , respectively ($\pm\text{SE}$). A total of 36 aquarium of 40cmX 20cmX 20cm were used for TBT treatment of gravid females and newly born fry at three different concentrations (25, 50 and 150 ng/g feed) with triplicates. Thermostatically controlled submerged heaters were used to maintain the water temperature at desired level. Aeration was provided to each aquarium through an air pump. All aquariums were cleaned weekly by siphoning the uneaten food and excreta and water were then topped up with clean aerated tap water at the desired temperature.

Preparation of Experimental Feed

TBT chloride (97% purity) was obtained from Merck. TBT incorporated in the diet at nominal concentrations of 25, 50 and 150ng/g feed. The selection of TBT doses was based on the previous studies (McAllister and Kime 2003; Shimasaki et al. 2003; Santos et al., 2006). These doses of TBT incorporated in the diet are also environmentally relevant (Santos et al. 2006). TBT dissolved in acetone was poured onto the food in a fume cupboard and mixed frequently by a spatula to ensure even distribution of the acetone/TBT solution. The food was left in a fume cupboard to dry, then stored in airtight containers at 4°C in a refrigerator. The control diet was prepared in a same manner without using acetone/TBT solution.

Determination of TBT Treatment Timing and Duration

Determination of TBT treatment timing and duration were based on the previous hormonal sex reversal studies indicate that the labile period for successful hormonal sex reversal covers both the embryonic i.e. treating the brooding female and post hatching periods (Takahashi 1975; Kavumpurath and Pandian 1993). Therefore, the gravid females were treated by the experimental diet from 16th day after the previous parturition until the termination of gestation while the experimental diet was also given to the newly born fry from the untreated female for 11 days.

Experimental Design

TBT treatment to gravid female guppy; 9 gravid females which are distinguishable by their black sacs, denoting their pregnancy were chosen, maintained individually and fed with the control diet until the succeeding parturition. The resulting broods were served as control group (first brood). The females were reared for further 15 days and fed by control diet. From 16th day after the first parturition, the females were fed with the experimental diets until next parturition.

TBT treatment to fry guppy; 9 gravid females were chosen, maintained individually and fed with the control diet until the succeeding parturition. After parturition, the females were moved individually to stock tanks and the resulting broods were kept in same unit and fed by the experimental diets at concentrations of 25, 50 and 150ng TBT/g feed for 11 days and the experimental groups were coded with T25 (for replicates T25-1, T25-2, T25-3), T50 (for replicates T50-1, T50-2 and T50-3) and T150 (for replicates T150-1, T150-2 and T150-3), respectively. Fish were reared by feeding with the control diet for further 49 day and sexing of the fish was done at 60th day by external examination based on the presence of the male secondary sex characters using caudal, dorsal and anal fins as markers. The fish lacking these characters were classified as female. At the end of the experiment, the fry were also weighted nearest to 0.01 g to determine the effect of TBT treatments on the average weight of guppy. The second broods from the same females would be used as control group but unfortunately the females died.

Survival rate was calculated as: (Number of fish at the end of the treatment / Number of fish at the beginning of the treatment) x 100. Survival rates were calculated at 11th day and 60th day for experiment.

Statistical Analysis

Survival and average weight were expressed as the mean±SE. Survival (arcsine transformed) and average weight of groups were analysed for significant differences using analyses of variance (ANOVA) with a Tukey posthoc test. A P-value <0.05 was considered as significant. Sex ratios of the experimental groups were tested against one male: one female sex ratio using χ^2 test since the respective control groups could not be obtained because of the dead of the mothers. On the other hand, it should be considered that the sex ratios of progeny of the females from the same stock did not differ the expected sex ratio of 1:1.

Heterogeneity χ^2 tests were also used for the experimental groups to pool the data. Whenever more than 20% of the expected frequencies were less than five or any expected frequency was less than one, expected frequencies increased by combining adjacent categories into a single-pooled category (Armitage and Berry, 1994). Pooled sex ratios of the experimental groups were also tested against one male: one female sex ratio using χ^2 test. Minitab 17 was used for all statistical analyses.

RESULTS AND DISCUSSION

In the present study, we examined the effects of TBT on sex ratio of both gravid and larvae guppy by oral administration of TBT. The chosen concentration of TBT (25, 50 and 150 ng TBT/g feed) were environmental levels according to the previous studies (McAllister and Kime 2003; Shimasaki et al. 2003; Santos et al. 2006).

Nine gravid females were planned to use in order to determine the effect of TBT on the sex ratios of broods by feeding with the diet including different concentration of TBT but females showed various abnormalities like body shape and fin deformations (Fig 1). No parturition was seen and therefore no broods were obtained. Only one female in T25 group gave dead broods and died after parturition. On the other hand, no deformation was observed after TBT application to the guppy fry in this study. Although there is no report on abnormality caused by TBT in any adult ovoviviparous fish species, similar malformations (skeletal and neuromuscular) were reported in medaka embryos exposed to TBT at $\geq 12.5\mu\text{g/l}$ during embryogenesis with concentration and time dependent manner (Bentivegna and Piatkowski 1998). Fent and Meirer (1992) reported that TBT exposure (concentrations ranging from 0.82 to 19.51 $\mu\text{g/l}$) led to mortality, behavioral, gross morphological and histopathological effects in minnows (*Phoxinus phoxinus*) eggs and yolk sac fry. Japanese medaka (*Oryzias latipes*) embryos exposed to TBT by nano-injection at concentrations of 0.16, 0.80, 3.96, 19.2 and 82.1 ng/egg caused impairment of development like abnormal eye development, haemorrhage and deformity of tail (Hano et al. 2007). This study clearly showed that fish species that are livebearing and give birth to free swimming fry like guppy were much more susceptible to TBT than fry and TBT caused severe abnormal development in gravid guppies. Especially effect of TBT on malformations in female livebearing fish species should be studied more detailed.

Survival rates of the experimental groups of TBT treated guppy fry did not differ significantly on 11th and 60th day of the experiment (Table 1). On the other hand, significant differences were found in T25 and T150 groups between 11th and 60th day of the experiment showing that survival was reduced after 11th day of the experiment. Average weight of the experimental groups was significantly reduced by increasing level of TBT. Although this



Figure 1. Body shape and fin deformations in female guppy (*Poecilia reticulata*) after treatment with the diet containing TBT.

Table 1. Survival rates (%) and average weight (g) of guppy (*Poecilia reticulata*) fry fed by diet containing different concentration of TBT.

Experimental groups	Survival rate on 11 th day	Survival rate on 60 th day	Average weight on 60 th day
T25	100±0.00 ^{ax}	53.67±6.06 ^{ay}	0.30±0.04 ^a
T50	83.33±16.67 ^{ax}	54.33±15.07 ^{ax}	0.16±0.01 ^{ab}
T150	100±0.00 ^{ax}	48.66±5.70 ^{ay}	0.15±0.05 ^b

Different superscripts (a, b) within the same column denote significant differences.
 Different superscripts (x, y) within the same row denote significant differences.

Table 2. Sex ratios of guppy (*Poecilia reticulata*) fry treated by diet containing different concentration of TBT at 60th day of the experiment

Experimental groups	Number of female (%)	Number of male (%)	P (χ^2) Test (vs 1:1)	Average female (%)	Average male (%)	P (χ^2) of pooled data of the replicates in the same experimental group (vs 1:1)
T25-1	8 (42.10)	11 (57.89)	0.491 ^{NS}	29.26	70.74	0.008 ^{**}
T25-2	1 (5.55)	17 (94.44)	0.000 ^{***}			
T25-3	3 (75.00)	1 (25.00)	0.317 ^{NS}			
T50-1	3 (100.00)	0 (0.00)	0.083 ^{NS}	12.50	87.50	0.000 ^{***}
T50-2	0 (0.00)	30 (100.00)	0.000 ^{***}			
T50-3	3 (20.00)	12 (80.00)	0.020 ^{**}			
T150-1	2 (20.00)	8 (80.00)	0.058 ^{NS}	12.82	87.18	0.000 ^{***}
T150-2	0 (0.00)	17 (100.00)	0.000 ^{***}			
T150-3	3 (25.00)	9 (75.00)	0.083 ^{NS}			

^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001, NS: not significant (P>0.05)

study did not design for investigation of TBT effect on growth rate in guppy, our result still provide strong support for the fact that TBT do have an considerably suppression in growth of guppy fry. Similar results were also reported in rainbow trout (*Oncorhynchus mykiss*) (Seinen et al. 1981), silverside (*Menidia beryllina*) (Hall et. al. 1988) and Japanese flounder (*Paralichthys olivaceus*) (Shimasaki et al. 2003). Rainbow trout in the yolk sac fry stage exposed to tri-n-butyltin-chloride (TBTC) at concentrations of 0.2 and 1µg/l for 110 days showed a significant and a dose-related growth retardation resulting in a 44% decrease of the body weight in the 1 µg/l group at the end of the experimental period (Seinen et al. 1981). Reduced growth in larval inland silverside (*Menidia beryllina*) was reported by Hall et al. (1988). TBT concentrations of 93 and 490 ng/l significantly reduced growth by 20 to 22% in this species. In Japanese flounder fed an diet containing tributyltin oxide (TBTO) at concentration of 0.1 and 1.0 µg/g diet from 35 to 100 d after hatching showed suppressed growth (Shimasaki et al. 2003). This study was agreed with the previously mentioned researchers indicating that TBT may suppressed the feeding or metabolic rates which causing to a lower growth rate in TBT-treated groups than in the control and the inhibitory effect of triorganotin compounds on mitochondrial energy conserva-

tion. Higher male ratios were obtained in the experimental groups (Table 2) showing male biased sex ratio. Significant deviations from one female: one male sex ratio were observed in some replicates of the experimental groups. No heterogeneity was observed with respect to sex ratios in replicates of the groups and data were pooled. Pooled sex ratios of the replicates in the same experimental group significantly differed from the balanced sex ratio of 1:1. In vertebrates, the genetic sex of the individual determined by the combination of male and female genetic factors at fertilization. However, sexual differentiation which refers to gonadal development after primary sex determination in fish is highly plastic, labile and susceptible to the presence of exogenous steroids. Even after primary sex determination in fish, sexual development can be triggered by external factors in contrast to the genotype. Treatments of embryos or larvae with exogenous androgens and estrogens lead to functional male and female phenotype and/ or vice versa and also called sex reversal which is a valuable tool in the elucidation of sex determining mechanisms and production of monosex populations in aquaculture industry (Pandian and Sheela 1995; Baroiller and D' Cotta 2001; Strüssmann and Nakamura 2002). The first report about the effect of TBT on sexual differentiation of fish was published by McAllister and Kime (2003). In that study, water expo-

sure to zebrafish larvae from hatch to 70 days, to environmentally realistic level of 1 and 10 ng TBT/l resulted 80 and 90% male. 35 days post-hatched Japanese flounder larvae fed an artificial diet containing tributyltin oxide (TBTO) at concentrations of 0.1 and 1.0 µg/g diet from 35 to 100 days after hatching resulted male bias sex ratio (25.7% and 31.1% male, respectively) compared with the control (2.2%) (Shimasaki et al. 2003). Santos et al. (2006) demonstrated that fish exposed to TBT showed a bias sex ratio toward males (62.5% males in control tanks and 86% and 82% in TBT 25 and TBT 100 ng TBT/g, respectively) in zebrafish. Bias sex ratio towards male (62.5% males in control tanks and 86% and 82% in TBT 25 and 100 ng/g diet, respectively) was also reported in five days post-fertilization zebrafish larvae fed a diet containing TBT for a 4-month period (Santos et al. 2006). Our data for guppy are consistent with these results showing male biased sex ratio in the experimental groups (between 70.74-87.50%) with significantly differentiation from the balanced sex ratio of 1:1. Although the respective control groups could not be obtained, the sex ratios of progeny of the females from the same stock did not differ the expected sex ratio of 1:1. The result suggest that either the TBT treatment was lethal to genetic females or the TBT treatment caused to masculinization of genetic females. On the other hand aforementioned literatures support the latter hypothesis. Endocrine disruptors interfere with hormone-signaling by competitively binding to the androgen or estrogen receptor, thus activating the transcription of sex-hormone-dependent genes (e.g., xenoestrogens or xenoandrogens), binding but not activating the androgen or estrogen receptor (e.g., anti-androgens or anti-estrogens), modifying enzymatic pathways involved in biosynthesis of sex hormones and other unclear mechanisms (Liao et al. 2014). It is well known that E2 stimulates the hepatic production of yolk that is necessary for oocyte growth. In the ovary, E2 is produced by conversion of T catalyzed by the steroid synthesising enzymes, in particular the P450 A1. TBT is a well known P450 A1. The present and the earlier studies clearly showed increased levels of T and decreased levels of E2 in the ovaries of the fish exposed to TBT which could be related with an inhibition of P450 aromatase activity resulting male bias sex ratio.

CONCLUSION

This is the first report of TBT causing masculinization in guppy. In summary, the data presented here strongly suggest that TBT exposure to guppy resulted in abnormality in gravid females, biased sex ratio towards male and suppression of growth in guppy larvae.

Ethics Committee Approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

Acknowledgements: This paper is a part of Olcay KIRIKOGLU's Master thesis. Thank you to Sinop University of Aquaculture and Fisheries Faculty for allowing us to use the Research and Application Center.

Conflict of Interest: The authors have no conflicts of interest to declare

REFERENCES

- Anastasiou, T. I., Chatzinikolaou, E., Manolis, M., & Arvanitidis, C. (2016). Imposex and organotin compounds in ports of the Mediterranean and the Atlantic: Is the story over? *Science of the Total Environment*, 569-570, 1315–1329. [CrossRef]
- Antizar-Ladislao, B. (2008). Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. *Environment International*, 34, 292–308. [CrossRef]
- Armitage, P., & Berry, G. (1994). *Statistical Methods in Medical Research* (3rd edition). Blackwell.
- Baroiller, J. F., & D'Cotta, H. (2001). Environmental and sex determination in farmed fish. *Comparative Biochemistry and Physiology, Part C*, 130, 399–409.
- Bentivegna, C. S., & Piatkowski, T. (1998). Effects of tributyltin on medaka (*Oryzias latipes*) embryos at different stages of development. *Aquatic Toxicology*, 44, 117–128. [CrossRef]
- Dowson, P. H., Bubbs, J. M., & Lester, J. N. (1993). Depositional profiles and relationship between organotin compounds in freshwater and estuarine sediment cores. *Environmental Monitoring and Assessment*, 28, 145–160. [CrossRef]
- Fent, K., & Meirer, W. (1992). Tributyltin-induced effects on early life stage of minnows *Phoxinus phoxinus*. *Archives of Environmental Contamination and Toxicology*, 22, 423–428. [CrossRef]
- Furdek, M., Vahčić, M., Ščančar, J., Milačić, R., Kniewald, G., & Mikac, N. (2012). Organotin compounds in seawater and *Mytilus galloprovincialis* mussels along the Croatian Adriatic Coast. *Marine Pollution Bulletin*, 64, 189–199. [CrossRef]
- Garg, A., Meena, R. M., Jadhav, S., & Bhosle, N. B. (2011). Distribution of butyltins in the waters and sediments along the coast of India. *Marine Pollution Bulletin*, 62, 423–431. [CrossRef]
- Hall, L. W. Jr., Bushong, S. J., Ziegenfuss, M. C., Hohnson, R. L., & Wright, D. A. (1988). Chronic toxicity of tributyltin to Chesapeake Bay biota. *Water Air Soil Pollut*, 39, 365–376.
- Hano, T., Oshima, Y., Kim, S. G., Satone, H., Oba, Y., Kitano, T., ... Honjo, T. (2007). Tributyltin causes abnormal development in embryos of medaka, *Oryzias latipes*. *Chemosphere*, 69(6), 927–933. [CrossRef]
- Harino, H., Fukushima, M., & Kawai, S. (2000). Accumulation of butyltin and phenyltin compounds in various fish species. *Archives of Environmental Contamination and Toxicology*, 39, 13–19. [CrossRef]
- Harino, H., Langston, W. J., Burt, G. R., Pope, N. D., & O'Hara, S. C. M. (2003). Occurrence of organotin compounds in Mersey and Thames Estuaries a decade after UK TBT legislation. *Journal of the Marine Biological Association of the United Kingdom*, 83, 11–22. [CrossRef]
- Jiang, G. B., Zhou, Q. F., Liu, J. Y., & Wu, D. J. (2001). Occurrence of butyltin compounds in the waters of selected lakes, rivers and coastal environments from China. *Environmental Pollution*, 115, 81–87. [CrossRef]
- Kavumpurath, S., & Pandian, T. J. (1993). Production of a YY female guppy, *Poecilia reticulata*, by endocrine sex reversal and progeny testing. *Aquaculture*, 118, 183–189. [CrossRef]
- Kim, N. S., Shim, W. J., Yim, U. H., Hong, S. H., Ha, S. Y., Han, G. M. ... Shin, K. H. (2014). Assessment of TBT and organic booster biocide contamination in seawater from coastal areas of South Korea. *Marine Pollution Bulletin*, 78, 201–208. [CrossRef]
- Liao P. H., Chu S. H., Tu T. Y., Wang X. H., Lin A. Y., & Chen P. J. (2014). Persistent endocrine disruption effects in medaka fish with early life-stage exposure to a triazole-containing aromatase inhibitor (letrozole). *Journal of Hazardous Materials*, 277, 141–149. [CrossRef]
- Marca Pereira, M., Eppler, E., Thorpe, K., Wheeler, J. R., & Burkhardt-Holm, P. (2014). Molecular and cellular effects of chemicals disrupting steroidogenesis during early ovarian development of brown trout (*Salmo trutta fario*). *Environmental Toxicology*, 29, 199–206. [CrossRef]

- McAllister, B. G., & Kime, D. E. (2003). Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish (*Danio rerio*). *Aquatic Toxicology*, 65, 309–316. [\[CrossRef\]](#)
- McGinnis, C. L., & Crivello, J. F. (2011). Elucidating the mechanism of action of tributyltin (TBT) in zebrafish. *Aquatic Toxicology*, 103, 25–31. [\[CrossRef\]](#)
- Mochida, K., Ito, K., Kono, K., Onduka, T., Kakuno, A., & Fujii, K. (2007). Molecular and histological evaluation of tributyltin toxicity on spermatogenesis in a marine fish, the mummichog (*Fundulus heteroclitus*). *Aquatic Toxicology*, 83, 73–83. [\[CrossRef\]](#)
- Nakayama, K., Oshima, Y., Yamaguchi, T., Tsuruda, Y., Kang, I. J., Kobayashi, M. ... Honjo, T. (2004). Fertilization success and sexual behavior in male medaka *Oryzias latipes*, exposed to tributyltin. *Chemosphere*, 55, 1331–1337. [\[CrossRef\]](#)
- Pandian, T. J., & Sheela, S. G. (1995). Hormonal induction of sex reversal in fish. *Aquaculture*, 138, 1–22. [\[CrossRef\]](#)
- Santos, M. M., Micael, J., Carvalho, A. P., Morabito, R., Booy, P., Massanisso, P., ... M., Reis-Henriques, M. A. (2006). Estrogens counteract the masculinizing effect of tributyltin in zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 142, 151–155. [\[CrossRef\]](#)
- Seinen, W., Helder, T., Vernij, H., Penninks, A., & Leeuwangh, P. (1981). Short term toxicity of tri-*n* butyltin chloride in rainbow trout (*Salmo gairdneri* Richardson) yolk sac fry. *Science of The Total Environment*, 19, 155–166. [\[CrossRef\]](#)
- Shimasaki, Y., Kitano, T., Oshima, Y., Inoue, S., Imada, N., & Honjo, T. (2003). Tributyltin causes masculinization in fish. *Environmental Toxicology and Chemistry*, 22, 141–144. [\[CrossRef\]](#)
- Shimasaki, Y., Oshima, Y., Inoue, S., Inoue, Y., Kang, I.J., Nakayama, K., ... Honjo, T. (2006). Effect of tributyltin on reproduction in Japanese whiting, *Sillago japonica*. *Marine Environmental Research*, 62(Supplement 1), 245–248. [\[CrossRef\]](#)
- Shue, M. F., Chen, T. C., Bellotindos, L. M., & Lu, M. C. (2014). Tributyltin distribution and producing androgenic activity in water, sediment, and fish muscle. *Journal of Environmental Science and Health, Part B*, 49, 432–438. [\[CrossRef\]](#)
- Strüssmann, C. A., & Nakamura, M. (2002). Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes. *Fish Physiology and Biochemistry*, 26, 13–29. [\[CrossRef\]](#)
- Takahashi, H. (1975). Functional feminisation of genetic males of the guppy, *Poecilia reticulata*, treated with estrogen after birth. *Bulletin of the Faculty of Fisheries Hokkaido University*, 26, 223–234.
- Tian, H., Wu, P., Wang, W., & Ru, S. (2015). Disruptions in aromatase expression in the brain, reproductive behavior, and secondary sexual characteristics in male guppies (*Poecilia reticulata*) induced by tributyltin. *Aquatic Toxicology*, 162, 117–125. [\[CrossRef\]](#)
- Titley-O'Neal, C., MacDonald, B. A., Pelletier, E., SaintLouis, R., & Phillip, O. S. (2011). The relationship between imposex and tributyltin (TBT) concentration in *Strombus gigas* from the British Virgin Islands. *Bulletin of Marine Science*, 87(3), 421–435. [\[CrossRef\]](#)
- Zhang, K. G., Jiang, G. B., & Liu, J. Y. (2013). Organotin compounds in surface sediments from selected fishing ports along Chinese coast. *Chinese Science Bulletin*, 58, 231–237. [\[CrossRef\]](#)
- Zhang, J., Zuo, Z., Chen, Y., Zhao, Y., Hu, S., & Wang, C. (2007). Effect of tributyltin on the development of ovary in female cuvier (*Sebastiscus marmoratus*). *Aquatic Toxicology*, 83, 174–179. [\[CrossRef\]](#)