eISSN 2236-5257
10.46551/ruc.v24n1a

EFFECTS OF TRIBUTYLTIN EXPOSURE IN THE MALE MAMMAL'S REPRODUCTIVE SYSTEM: A SYSTEMATIC REVIEW

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Abstract

Objective: To conduct a systematic review of the literature describing male mammals' reproductive and sexual parameters exposed to the biocide TBT. **Method:** A formal computer-assisted search was performed independently by two authors using five online databases and keywords. A manual search of the reference list of the articles found for relevant original articles was also used. We initially identified potentially eligible publications related to the topic of interest through this procedure. The last systematic search resulted in adequate data on TBT toxicity in the mammalian male reproductive tract. **Results:** Decreases in testicular, epididymal, prostate and seminal vesicle weights were observed at higher TBT ranges. Decreases in serum testosterone levels were reported in some studies, with some histological changes in the surveyed tissues and decreased transcriptional expressions of steroidogenic

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Recebido em	Aceito em	Publicado em
18/02/2022	05/03/2022	11/03/2022

enzymes. Notably, there were significant reductions in sperm count and motility and increased abnormalities. **Conclusions:** Further studies are needed to elucidate the precise manner of TBT's deleterious mechanisms of action on the spermatogenesis process. Therefore, a comprehensive survey of TBT levels in food and water sources should also be conducted better to protect susceptible populations from potentially deleterious reproduction effects.

Keywords: Tributyltin; Spermatogenesis; Sperm; Gametogenesis; Male reproductive system.

INTRODUCTION

Tributyltin (TBT) is part of the class of organotin biocide compounds containing a $(n\text{-}C_4H_9)_3Sn\text{-}X$ group. It is mainly used as a defocusing agent in paints to coat structures exposed to the aquatic environment, such as ships, oil platforms, pleasure boats, and water pipes[1,2]. TBT can also be used as impregnation material in prints and textiles, wood preservation, cooling water disinfection, fungicide, and polyvinyl chloride (PVC) heat stabilizer[3,4]. TBT toxicity exceeds its intended target and its bioaccumulation affects the environment through the leaching of paint by wastewater, oil platforms, boats and ships[1].

TBT residue was detected in fish[5] and human blood[6,7], indicating that human contamination may occur through food intake. Absorption through dermal contact and inhalation is also reported among the population occupationally exposed to the biocide[8].

The widespread pollution of seawater and coastal sediment area by TBT in conjunction with its lipophilicity, ionic properties, and persistence have raised concerns about its bioaccumulation and biomagnification in food chains and adverse effects on human health and the environment[9,10]. Data on toxicity levels in mollusks, commonly found in the aquatic environment, led to the banning of TBT use as anti-fouling paint by some agencies[11,12]. Although such usage is now under strict legislation, it remains a widespread environmental contaminant[13,14,15] still being detected in water, sediment, and urban runoff, generating unease about possible impacts on estuaries, freshwater, and coastal areas[16,17,18].

TBT is an endocrine disruptor which compromises reproductive capacity and sexual development in many species[19]. The best known adverse effect caused by TBT is imposex, which occurs in neogastropod females and is characterized by the development of male sex organs, such as penis and vas deferens, overlapping with other female organs[11,20]. Data indicate that this organotin acts on the male reproductive system, decreasing sperm parameters (sperm

density, count, and viability)[21], delaying sexual development[22], reducing testicular size[23] and inhibiting the aromatase enzyme[24].

Aromatase inhibition is a particularly relevant mechanism of action of TBT, given that estrogen hormones are essential for typical male and female reproductive development[25]. The hormonal balance between estrogens and androgens depends on the activity and availability of steroid synthesizing enzymes, mainly cytochrome (CYP) P450, responsible for the production of estrogen through the aromatization of androgens. It is proposed that much of the reproductive toxicity of organotin compounds is due to the inhibition of this aromatase enzyme[22].

Nishikawa *et al.*[26] reports that TBT is a high-affinity ligand of the retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPARɣ) playing an essential role in gastropod-imposex development. In addition, TBT exposure blocks the cellular function of Leydig and Sertoli cells through negative regulation of androgen receptor and estrogen receptor[27].

The toxicity of TBT in the aquatic environment, its bioaccumulation, as well as alterations in the female reproductive system are well documented[28,29,30,31]; however, there are limited data on its effects on relevant environmental concentrations in male vertebrates. In view of these findings, the aim of this systematic review was at evaluating the possible deleterious effects of TBT on the male reproductive system of mammals, such as changes in the weight of reproductive organs, serum concentration of hormones, gene and protein expression, as well as sperm parameters.

METHODS

A systematic review was conducted using the following question: What are the effects of tributyltin on the reproductive tract of male animals belonging to the Mammalia class?

Initially, a formal computer-assisted search was conducted independently by two authors (LSG, TJM), in the period from April to August 2020, using five online databases - Medical Literature Analysis and Retrieval System Online (MEDLINE), Latin American and Caribbean Literature in Health Sciences (LILACS), Web of Science, SCOPUS, and Cochrane - using the keywords: tributyltin; TBT; gametogenesis; spermatogenesis and male reproductive system.

The search for unpublished or unindexed studies in the literature (referred to as "grey literature") included the following websites: Agency for Healthcare Research and Quality (https://www.ahrq.gov/), Google Scholar (http://scholar.google.com/), Grey Literature Report from New York Academy of Medicine (http://www.greylit.org/), and World Health Organization – WHO (http://www.who.int/).

A cross-reference search was performed to locate possible relevant papers that were not eventually found in the electronic databases. Date limits were not applied in the search strategy. In order to trace the publications, Boolean operators, represented by the connecting terms AND and OR were used with the descriptors. The entire strategic flow of the aforementioned survey constituted the final set of original studies selected to proceed to the subsequent steps of this review.

By associating the aforementioned descriptors, we initially identified 862 potentially eligible publications which seemed to be related to the topic of interest. In the first stage of the research, these possible abstracts were classified after a screening using the inclusion criteria: written in English and selected according to the search terms. Exclusion criteria were: abstract in a language other than English, articles that were in duplicate from the searched databases, case reports, other systematic or bibliographic reviews and articles not fully available online. After analyzing the title and abstract of each article, those which met the criteria were considered potentially eligible. (Step 1)

In the second step, the 199 abstracts selected in Step 1 were retrieved for another screening. These articles were fully analyzed in order to shortlist those which should be included in this systematic review. Publications that, although contemplating the descriptors, did not analyze the parameters in the reproductive tract of male animals were excluded. (Step 2)

In the third and final phase, the 93 shortlisted articles were again screened using the inclusion criteria: direct treatment in male mammals *(in vivo)* or in male cell culture *(in vitro)*. Exclusion criteria were: another Animalia class, direct treatment in females, or research during embryonic development (Step 3). The systematic search resulted in 26 publications with adequate data on TBT toxicity in the male reproductive tract of mammals (Figure 1). The complete bibliography of accepted and rejected studies is available upon request from the corresponding author.

Data extraction was applied to studies with methodological quality, conducted by one evaluator (LSG) and subsequently verified by a second evaluator (TJM). The information contained in the selected articles was systematized in a spreadsheet, namely the Excel® for Windows® program, according to the objectives of the review and the aforementioned eligibility criteria. The following criteria were coded: species studied, sexual parameters analyzed, age, identification of the first author and year of publication, as well as the place (country of origin) where it was performed (Table 1).

All the research was based on data from studies published in electronic databases, thus not requiring approval by the CEP-CONEP system. This work did not require any type of funding, and there were no conflicts of interest in its development.

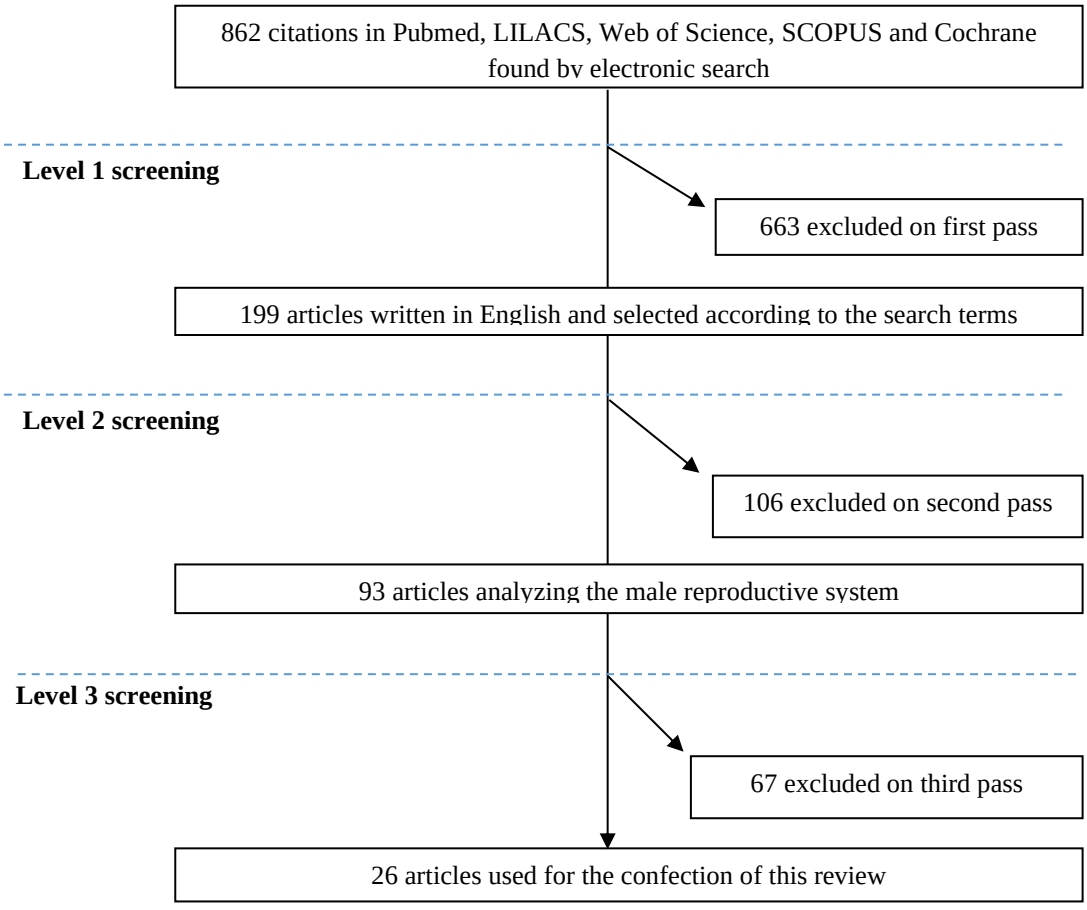

Figure 1. Flowchart of manuscript selection for review.

RESULTS

The 26 selected studies date from 2000 to 2018, including countries located on the coast. Most were conducted in the Asian continent (03 Korea, 05 China, 05 India, and 08 Japan), and one study was conducted simultaneously in two countries (Table 1).

Table 1: Mammalian species and analyzed parameters obtained from screened articles at the level 3.

Species	Parameters	Age	Author	Country
Sprague-Dawley	Seminal vesicle and accessory sex organ weight, detached debris and sloughed cells, seminal vesicle width and androgen level.	45 days	Yu *et al*., 2003	Korea
Sprague-Dawley	Sperm count and motion kinematic parameters	46 days	Yu *et al*., 2003	Korea
Sprague-Dawley	Activity of 3β-HSD, 17-OHase and 17β-HSD	90 days	Mcvey & Cooke, 2003	Canada
Sprague-Dawley	Testis weight and tissue architecture	21, 35 and 50 days	Kuwada *et al*., 2006	Japan
Sprague-Dawley	Serum testosterone levels, LH, FSHR, Leydig cell regeneration, mRNA and protein levels of Leydig and Sertolli cells and Leydig cell proliferation	58 days	Wu *et al.*, 2017	China
Wistar	Organ weight, spermatid and sperm count, histopathology, 17β-estradiol concentration, LH and testosterone levels	119 or 91 days	Omura *et al.*, 2001	Japan
Wistar	Preputial separation completion, weights of reproductive organs, testosterone concentration and LH	53 days	Grote *et al.*, 2004	Germany
Wistar	Organs and body weigh, serum testosterone, luteinizing hormone, follicle-stimulating hormone concentrations, and epididymal sperm count	12 weeks	Makita *et al*., 2005	Japan
Wistar	p38 and JNK phosphorylation, stress proteins (Nrf2, MTand GST) induction and mitochondrial depolarization leading to caspase-3 activation	28 days	Mitra *et al.*, 2013	India
Wistar	Leydig cells viability, activity of 3β-HSD and Star and testosterone production	5 weeks	Mitra *et al.*, 2014	India
Wistar	Ability of to reach testicular tissue, impact on BTB Permeability, effect on testicular biology and tissue architecture	4-5 weeks	Mitra *et al.*, 2017	India
Wistar	Metabolic profile of Sertolli cells	20 days	Cardoso *et al.*, 2017	Portugal
Chinese Kun Ming	Organs and body weight, sperm parameters, histopathology of the testis, hormone levels (Testosterone, 17β-estradiol), estrogen receptors	≈ 60 days	Chen *et al*., 2008	China
Chinese Kun Ming	Sperm parameters, epididymal function activity of acid phosphatase, acrosin and lactate dehydrogenase-x	≈ 70 days	Yan *et al.*, 2009	China
Chinese Kun Ming	Organs and body weight, Serum hormone levels (testosterone, 17β-estradiol, LH), testis hormone extraction and tissue analysis	49 or 84 days	Si *et al.*, 2010	China
ICR mice	Testicular weight, testicular sperm head counts, testicular histology and testicular total Sn concentration	9 weeks	Kumasaka *et al.*, 2002	Japan
ICR mice	Measurement of body and organ weights, determination of serum testosterone and estradiol concentration, leydig cells- and seminiferous tubule apoptosis and testicular gene expressions	24 days	Kim *et al*., 2008	Korea
Syrian Hamster	Body and testis weight, sperm count and morphology, testis histology, ApoE expression, serum lipid profile, testosterone level, FSHR, and steroid hormone receptor expression	6-7 weeks	Kanimozhi *et al.*, 2014	India and USA
Syrian Hamster	Testis morphology, immunohistochemistry of iNOS, 3β-HSD and 17β-HSD, cholesterol transport receptor, nuclear receptors, and transcription factors	6-7 weeks	Kanimozhi *et a*l., 2017	India

Pigs strain LWD	Testosterone production in isolated Leydig cells, cAMP level and hormones levels	2 weeks	Nakajima *et al.*, 2003	Japan
Pigs strain LWD	Testosterone production in isolated Leydig cells, cAMP level, hormones levels and P450cl7 levels	2 weeks	Nakajima *et al.*, 2005	Japan
Pigs strain LWD	Enzymes involved in testosterone biosynthesis and17β-hydroxusteroid dehydrogenase activity	2 weeks	Ohno; Nakajima; Nakajin, 2005	Japan
Human prostate cancer cell line (LNCaP)	Androgenic effects via the activation of AR in mammalian cells	not applicable	Yamabe *et al.*, 2000	Japan
Human prostate tissue	Human 5- reductase isoenzymes	not applicable	Doering *et al.*, 2002	Germany
Human prostate tissue	Human 5- reductase isoenzymes	not applicable	Lo *et al.*, 2006	Germany
Human H295R cell	Production of steroid hormones and expression of steroidogenic genes	not applicable	Yan *et al.*, 2018	China

Tributyltin administration showed no obvious signs of toxicity, since no mortalities and abnormal activities were observed in the animals of any group analyzed. In the study by Grote *et al.* [32], one animal receiving 15 mg TBT/kg body weight died, but no signs of general toxicity were observed. Regarding the male reproductive organs, most studies have shown no changes in testes weight among the groups treated with TBT over a wide range of doses [33,34,35,32,36,24,37]. However, a weight decrease was observed at higher TBT dose ranges [38,36,24,37] (Table 2).

In the work by Omura *et al.*[39] testes weight decreased significantly in the 5 and 25 ppm/kg/d TBT groups. In the epididymis, weight decreased significantly in the 5 ppm TBT group in the F1 generation in a two-generation study. In addition, the weights of the testis, epididymis and ventral prostate of the rats fed in the 125 ppm TBT diet decreased significantly compared to the control rats (Table 2).

TBT chloride treatments caused a dose-dependent decrease in seminal vesicle weights and there was significance at doses of 10 and 20 mg TBT chloride/kg body weight compared to the control[34] (Table 2).

Exposure to 0.5 mg TBT/kg body weight led to a statistically significant increase in the absolute and relative weights of the epididymis and prostate. On the other hand, exposure to 15 mg TBT/kg body weight resulted in a statistically significant decrease in the absolute and relative weights of the epididymis, prostate, and seminal vesicle compared to control[39] (Table 2).

Table 2. Weight changes induced by TBT in the reproductive organs of male mammals obtained from screened articles at the level 3.

Author	Dose/concentration	Treatment duration	Testis	Epididymis	Prostate	Seminal vesicle	Vas deferens	n

Wu *et al.*, 2017	0.1 mg /kg			NS		-		-		-		-		18	
	1.0 mg /kg	10 days		NS		-		-		-		-			
	10.0 mg /kg			NS		-		-		-		-			
Omura *et al.*, 2001	5.0 ppm /kg/d	F1	F2	↓	NS	↓	NS	NS	NS	NS	NS	-		14	08
	25.0 ppm /kg/d	F1	F2	↓	NS	NS	NS	NS	↓	NS	NS	-		16	13
	125.0 ppm /kg/d	F1	F2	↓	↓	↓	↓	↓	↓	NS	NS	-		16	10
Yu *et al.*, 2003b	5.0 mg /kg	10 days		NS		NS		NS		NS		-		10	
	10.0 mg /kg			NS		NS		NS		↓		-			
	20.0 mg /kg			NS		NS		NS		↓		-			
Kuwada *et al.*, 2006	4 µM	20 µM	Single dose	↓	↓	-		-		-		-		10	
Makita *et al.*, 2005	2 mg/kg		6 weeks	NS		NS		NS		NS		-		06	
Grote *et al.*, 2004	0.5 mg /kg		30 days	NS		↑		↑		NS		-		15	
	15.0 mg /kg			NS		↓		↓		↓		-			
Chen *et al.*, 2008	0.5 µg /kg			NS		-		-		-		-			
	5.0 µg /kg		3 days	NS		-		-		-		-		08	
	50.0 µg /kg			↓		-		-		-		-			
Kim *et al.*, 2008	25.0 mg /kg		Single dose	NS		NS		NS		NS		NS			
	50.0 mg /kg			NS		NS		NS		NS		NS		05	
	100.0 mg /kg			↓		NS		NS		↓		NS			
Kanimozhi *et al.*, 2014	50.0 ppm /kg/d			NS		NS		↓		NS		-			
	100.0 ppm /kg/d		65 days	↓		↓		↓		↓		-		08	
	150.0 ppm /kg/d			↓		↓		↓		↓		-			
Kanimozhi *et al.*, 2017	50.0 ppm /kg/d			NS		-		-		-		-			
	100.0 ppm /kg/d		65 days	NS		-		-		-		-		05	
	150.0 ppm /kg/d			↓		-		-		-		-			

NS = not significant; ↑ = increase; ↓ = decrease; n = number of experiments performed.

According to the work of Wu *et al.*[33], in adult rats, testosterone levels decreased in a TBT dose-dependent manner. Additional analysis showed that TBT significantly increased serum LH and FSH levels. After exposure to 15 mgTBT/kg body weight, Grote *et al.*[32] observed a statistically significant decrease in testosterone concentration compared to control, while treatment with 0.5 mg TBT/kg body weight did not lead to a significant change in testosterone levels. No effect was observed on LH concentration after exposure to 15 mg TBT/kg body weight (Table 3). Mitra *et al.*[17] demonstrated that after exposure to TBT for 6h, the amount of testosterone in rats also decreased significantly in a dose-dependent manner. Various TBT concentrations (300, 600, and 1000 nM) evaluated were based on reported human blood values ranging from 50 to 400 nM[6]. According to Kim *et al.*[36], the serum testosterone concentration in mice exposed to 100 mg/kg TBT at the immature stage was reduced to almost 26% of the value observed in vehicle-exposed animals. A significant decrease in testosterone production in pigs was also observed at concentrations between 0.03-0.3 µM TBT[40] (Table 3).

In one study conducted by Omura *et al.*[39], a dose-dependent increase in serum testosterone concentration of the TBT diet-treated rats was demonstrated in the F1 generation, but serum testosterone concentration did not increase in the F2 generation. Serum LH concentrations of the TBT-treated rats did not increase in the F1 generation, but increased dose dependently in the F2 generation. Despite the absence of reductions in LH and testosterone concentrations, serum 17β-estradiol concentration was decreased in the rats fed with the 125 ppm TBT diet (Table 3).

On the other hand, TBT treatments during the pubertal period of rats did not alter serum androgen levels[34]. Makita *et al.*[35] also observed no differences in testosterone, LH and FSH concentrations in any treated group.

Chen *et al.*[38] detected no significant change in testosterone levels in the testes of mice compared to control after TBT treatment, despite there was a slight tendency for a dose-dependent increase. As for 17β-estradiol levels, on the other hand, TBT exposure resulted in a decrease in a dose-dependent manner compared to control[38] (Table 3).

Table 3. Hormones serum concentration changes induced by TBT in male mammals obtained from screened articles at the level 3.

Author	Dose/concentration	Treatment duration		Testosterone		FSH	LH		17β-estradiol		n	
Wu *et al.*, 2017	0.1 mg/kg	10 days		↓		NS	NS		-		18	
	1.0 mg/kg			↓		↑	↑		-			
	10.0 mg/kg			↓		↑	↑		-			
Yu *et al.*, 2003b	5.0 mg/kg	10 days		NS		-	-		-		10	
	10.0 mg/kg			NS		-	-		-			
	20.0 mg /kg			NS		-	-		-			
Makita *et al.*, 2005	2 mg/kg	6 weeks		NS		NS	NS		-		06	
Grote *et al.*, 2004	0.5 mg/kg	30 days		NS		-	NS		-		15	
	15.0 mg/kg			↓		-	NS		-			
Mitra *et al.*, 2014	300 nM	6 hours		NS		-	-		-		1×10^5/ml cells	
	600 nM			↓		-	-		-			
	1000 nM			↓		-	-		-			
	3000 nM			↓		-	-		-			
Omura *et al.*, 2001	5.0 ppm/kg/d	F1	F2	NS	NS	-	NS	NS	NS	NS	14	08
	25.0 ppm/kg/d	F1	F2	NS	NS	-	NS	NS	NS	NS	16	13
	125.0 ppm/kg/d	F1	F2	↑	NS	-	NS	↑	↓	↓	16	10
Si *et al.*, 2010	0.05 mg/kg	PND 49	PND 84	NS	NS	-	NS	↓	↓	NS	25	
	0.5 mg/kg	Seven times		↓	NS	-	NS	↓	NS	↑		
Chen *et al.*, 2008	0.5 µg/kg	3 days		NS		-	-		NS			
	5.0 µg/kg			NS		-	-		NS		08	
	50.0 µg/kg			NS		-	-		↓			
Kim *et al.*, 2008	25.0 mg/kg	Single dose		NS		-	-		NS		05	
	50.0 mg/kg			NS		-	-		NS			

	100.0 mg/kg		↓	-	-	NS	
Kanimozhi *et al.*, 2014	50.0 ppm/kg/d		NS	-	-	-	
	100.0 ppm/kg/d	65 days	↓	-	-	-	08
	150.0 ppm /kg/d		↓	-	-	-	
Nakajima *et al.*, 2003	0.01 µM	3 hours	NS	-	-	-	6×10^4 cells/well/ 200 µl
	0.03 µM		↓	-	-	-	
	0.1 µM		↓	-	-	-	
	0.3 µM		↓	-	-	-	
Yan *et al.*, 2018	10 nM	48 hours	NS	-	-	NS	12-well plates 1×10^6 cells mL^{-1}
	50 nM		NS	-	-	↓	
	100 nM		↑	-	-	↓	

NS = not significant; ↑ = increase; ↓ = decrease; FSH = follicle-stimulating hormone; LH = luteinizing hormone; n = number of experiments performed; F = generation; PND = postnatal days.

Rat Leydig cells were stained by Hsd 3β1 biomarker and Sertoli cells were stained by Sox9 biomarker. When compared to control, TBT negatively regulated the expression levels of Leydig cell genes, Hsd17β3 and Cyp17α1, without affecting StAR levels. TBT at 10 mg/kg also reduced the expression levels of Sox9 genes in Sertoli cells, while lower doses of TBT did not affect the expression levels of these genes[33] (Table 4).

In the study by Mitra *et al.*[41], the expression of steroidogenic markers in rats, such as StAR and Hsd 3β1, was down-regulated at almost all doses, but was only significantly affected in the 30 mg TBT group. In another study, relating time of administration, the same group showed a significant decline in Hsd 3β1 expression starting at 30min, and over time, levels were further suppressed. StAR levels, however, remained unchanged, except at 1h, when there was a surge in expression levels[17] (Table 4).

The mRNA expressions of Cypscc and Cyp17α1 were markedly decreased in the testes of mice exposed to 50 and 100 mg/kg TBT. For Hsd 3β1 and Hsd17β3, animals exposed to 100 mg/kg TBT showed significant reductions in gene expression in the testes compared to the vehicle-exposed control[36] (Table 4).

Steroidogenic acutely regulated proteins (StAR) and other hamster steroidogenic enzymes (expressions of Cypscc, Hsd 3β1 and Cyp17α1) were studied by real-time PCR, which revealed significantly decreased mRNA expression of StAR, Cypscc, Hsd 3β1 and Cyp17α1, all involved in normal testicular function and steroidogenesis. Immunoblotting research showed that the expression of StAR, 3β-HSD, Cypscc, Hsd 3β1 and Cyp17α1 was also significantly down-regulated in hamsters treated with TBT in a dose-dependent manner compared to control[37] (Table 4).

Human H295R cells exposed to TBT at 100 nM concentration also reduced the expressions of StAR and Hsd 3β1. The enzymes transcribed by these genes play key roles in the biosynthesis of steroid hormones in H295R[42] (Table 4).

Table 4. Gene and protein expression changes induced by TBT in male mammals obtained from screened articles at the level 3.

Author	Dose/concentration	Duration	StAR	Cyp scc	Cyp cl7	Cyp 17α1	Hsd 3β1	Hsd 17β3	Sox9	n
Wu et al. (2017)	0,1 mg / kg		NS	-	-	NS	↓	↓	NS	18
	1,0 mg / kg	10 days	NS	-	-	↓	↓	↓	NS	
	10,0 mg / kg		NS	-	-	↓	↓	↓	↓	
Mitra et al. (2014)	600nM	30min	NS	-	-	-	NS	-	-	3×10^6 cells
		1h	↑	-	-	-	↓	-	-	
		3h	NS	-	-	-	↓	-	-	
Mitra et al. (2017)	10,0 mg /kg	Single dose	NS	-	-	-	NS	-	-	05
	20,0 mg /kg		NS	-	-	-	NS	-	-	
	30,0 mg /kg		↓	-	-	-	↓	-	-	
Kim et al. (2008)	25,0 mg /kg	Single dose	-	NS	-	NS	NS	NS	-	05
	50,0 mg /kg		-	↓	-	↓	NS	NS	-	
	100,0 mg /kg		-	↓	-	↓	↓	↓	-	
Kanimozhi et al. (2017)	50,0 ppm /kg/d		NS	↓		↓	↓	-	-	
	100,0 ppm /kg/d	65 days	↓	↓		↓	↓	-	-	05
	150,0 ppm /kg/d		↓	↓		↓	↓	-	-	
Nakajima et al. (2005)	0,1 µM		↓	NS	↓	NS	NS	-	-	03-07
Yan et al. (2018)	10 nM	48 hours	NS	-	-	NS	NS	NS	-	12-well plates 1 × 10^6 cells mL^{-1}
	50 nM		NS	-	-	NS	NS	NS	-	
	100 nM		↓	-	-	NS	↓	NS	-	

NS = not significant; ↑ = increase; ↓ = decrease; Cyp = Cytochrome P450; Hsd = hydroxysteroid dehydrogenase; Sox9 = SRY-Box transcription factor 9; n = number of experiments performed.

In the study by Yu *et al.*[43], no histological changes related to TBT compound were observed in the testes and prostate of rats in all experimental groups. In the epididymis and seminal vesicle, however, microscopic changes were induced by TBT treatments. Increases in detached debris and some desquamated cells were observed in the epididymal tubules in treated rats compared to control rats. (Table 5).

In mice exposed to 100 mg/kg TBT, the formation of the lumen of the seminiferous tubules was delayed and the total number of germ cells in the tubules in the testes was reduced. In addition, the number of cells with pyknotic nuclei and multinucleated bodies increased in the tubules of TBT-exposed animals[36]. Degenerative changes and desquamation of the differentiating cells of the germ layer were also present in the seminiferous tubules of the testes

of TBT-treated mice[38]. The seminiferous tubules of these animals' testes showed a reduction in spermatogenesis. Increases in detached debris and some shredded cells were observed in the animals treated with 50 µg/kg TBT[38] (Table 5).

TBT exposure in rats, performed through the study by Mitra *et al.*[41], showed the development of interstitial edema, along with Leydig cell loss, being evident at all doses. One of the most prominent effects of TBT exposure was cell loss, which was most evident on day 7, indicating disruption in Sertoli cells.

In hamster testes, on the other hand, TBT treatment resulted in abnormalities such as desquamation of epithelial cells, retraction of the outer membrane, presence of vacuoles, remnants of dead cells, binucleated giant cells with enlarged nuclei, and granulation of cells. The presence of these abnormalities was widely found in the groups treated at the highest concentrations (TBT 100 and 150 ppm/kg/d). The maximum number of distorted seminiferous epithelium, vacuoles, multinucleated giant cells, and dead cell debris was also demonstrated at these TBT doses[24] (Table 5).

Table 5. Histopathological changes induced by TBT in the reproductive organs of male mammals obtained from screened articles at the level 3.

Author	Dose/ concentration	Treatment duration	ST	SV	Testis	Prost.	Epid.	CL	CS	n
Yu *et al.*, 2003	5 mg /kg	10 days	-	alteration	NS	NS	alteration	-	-	10
	10.0 mg /kg		-		NS	NS		-	-	
	20.0 mg /kg		-		NS	NS		-	-	
Mitra *et al.*, 2017	10.0 mg /kg	Dose única	↓	-	-	-	-	↓	↓	05
	20.0 mg /kg		↓	-	-	-	-	↓	↓	
	30.0 mg /kg		↓	-	-	-	-	↓	↓	
Chen *et al.*, 2008	0.5 µg /kg	3 days	NS	-	-	-	-	-	-	
	5.0 µg /kg		NS	-	-	-	-	-	-	03
	50.0 µg /kg		alteration	-	-	-	-	-	-	
Kim *et al.*, 2008	25.0 mg /kg	Single dose	NS	-	-	-	-	-	-	05
	50.0 mg /kg		NS	-	-	-	-	-	-	
	100.0 mg /kg		↓	-	-	-	-	-	-	
Kanimozhi *et al.*, 2014	50.0 ppm /kg/d		NS	-	-	NS	-	-	-	
	100.0 ppm /kg/d	65 days	alteration	-	-	alteration	-	-	-	08
	150.0 ppm /kg/d		alteration	-	-	alteration	-	-	-	

NS = not significant; ↑ = increase; ↓ = decrease, ST = seminiferous tubule; SV = seminal vesicle; Prost = Prostate; Epid = Epididymis; CL = Leydig cells number; CS = Sertoli cells number; n = number of experiments performed.

Sperm counts recovered from the testes of TBT-treated rats and hamsters decreased in a dose-dependent manner. There was a change in both daily sperm production and sperm

motility[43,24]. A significant decrease in mouse sperm count and viability compared to control was also observed [38,42,44]. On the other hand, the percentage of abnormal spermatozoa, such as highly bent tail, curled tail, small head, unformed head, headless tail, and tailless head was increased in a dose-dependent manner[38,42,24] (Table 6).

In a two-generation study, sperm counts decreased significantly to approximately 80% of the control value in the F2 generation. However, sperm motility and morphology were not affected by TBT treatment in any experimental group[39] (Table 6).

Table 6. Spermatic parameters changes induced by TBT in male mammals obtained from screened articles at the level 3.

Author	Dose/concentration	Treatmant duration	Sperm count	Spermatid count	Motility	Sperm abnormality	Viability	n
Yu *et al.*, 2003	5 mg /kg		NS	-	NS	-	-	10
	10.0 mg /kg	10 days	↓	-	NS	-	-	
	20.0 mg /kg		↓	-	↓	-	-	
Makita *et al.*, 2005	2 mg/kg	6 weeks	NS	-	-	-	-	06
Omura *et al.*, 2001	0.4 mg / kg	F1 F2	NS NS	NS NS	NS NS	NS NS	-	14 08
	2.0 mg / kg	F1 F2	NS NS	NS ↓	NS NS	NS NS	-	16 13
	10.0 mg / kg	F1 F2	NS ↓	↓ ↓	NS NS	NS NS	-	16 10
Chen *et al.*, 2008	0.5 µg /kg		↓	-	-	NS	↓	
	5.0 µg /kg	3 days	↓	-	-	↑	↓	08
	50.0 µg /kg		↓	-	-	↑	↓	
Yan *et al.*, 2009	0.5 µg /kg		↓	-	-	↑	NS	
	5.0 µg /kg	3 ou 45	↓	-	-	↑	NS	06
	50.0 µg /kg		↓	-	-	↑	↓	
Kumasaka *et al.*, 2002	0.4 mg /kg		NS	-	-	-	-	
	2.0 mg /kg	6 weeks	↓	-	-	-	-	06
	10.0 mg /kg		↓	-	-	-	-	
Kanimozhi *et al.*, 2014	50.0 ppm /kg/d		NS	-	NS	NS	-	
	100.0 ppm /kg/d	65 days	↓	-	↓	↑	-	08
	150.0 ppm /kg/d		↓	-	↓	↑	-	

NS = not significant; ↑ = increase; ↓ = decrease, n = number of experiments performeds.

DISCUSSION

There are some reports that high concentrations of organotin compounds have accumulated in marine organisms due to such possible causes as biological accumulation[45]. For instance, TBT average concentration in oysters in the United Kingdom was found to be 16.7 µg/g, and their biological concentration factor is approximately 10,000[46]. TBT average concentration in fish and shellfish purchased from retail markets in Niigata, Japan was 0.669 µg/g[47]. TBT average concentration in Pacific oysters in Chinhae Bay, Korea was reported to

be 0.095–0.885 µg Sn/g and it was also reported that TBT could be biologically concentrated in oysters up to 25,000 times[48]. TBT average concentration in 11 kinds of fish in the ports of Osaka and Yodo River, Japan was reported to be 0.011–0.082 µg/g wet weight[49].

Most studies have focussed on marine species, yet due to drinking water contamination and consumption of seafoods from TBT contaminated waters, mammals can be exposed to significant quantities of TBT. Based on immunotoxicity studies, the tolerable daily intake of TBT for humans has been set at 0.25ug/kg body weight [50].

Male infertility is one of the challenging problems encountered by human society worldwide. Reports indicate the involvement of several environmental factors in inducing this problem[51]. There are scarcely reports identifying effects of TBT compounds on reproductive system of male mammals, mainly humans[34].

In the literature, it has been reported that high levels of TBT have been detetected in human liver tissue and human blood samples. For example, total TBT concentrations in human livers collected from Poland were in the range of 2.4–11 ng/g wet wt[52], and concentrations of butyltin in the livers of Japanese were in the range of 59–96 ng/g wet wt [53]. Sanocka and Kurpisz (2003)[54] stated that 20% of couples are infertile in Poland, and 40-60% of those couples' cases are due to male factor alone, whereas a more recente study by Bablok *et al.* (2011)[55] states that 56% of infertility cases are due to an involved male factor. Kannan *et al.* (1999)[56] also reported tributyltin levels in the human blood collected from U.S.A. were up to 8.18 ng/ml. North America demonstrates rates of male infertility 4.5-6%[57]. While a calculated percentage reveals 4.5-6% of North American males are infertile, the Centers for Disease Control (CDC) estimates that 9.4% of males in the United States are infertile[58]. In general, among Asian and Oceanian countries, the maximum estimated butyltin intake was 3.8 µg/(person day)[59]. Australia's rates infertility are similar to those in North America and the United States, at 8-9%; additionally, 40% of infertility cases in Australia are due to male factor involvement[60].

There is increasing evidence that normal male reproductive function can be disrupted by exposure to environmental pollutants that mimic or antagonize endogenous sex hormone function[61,62] . In addition to some known endocrine disruptors (such as bisphenol A, phthalates and polychlorinated biphenyls), organotin compounds such as TBT have also been reported to interfere with the endocrine system[63].

The influences experienced by the male and female reproductive organs of sex hormones in relation to endocrine disruptors include changes in the weight of the ovaries[47] and testes[39]. Regarding male reproductive organs, most studies in this review showed no differences in testes weight between groups treated with low doses of TBT[33,34,35,32,36,24,37], but with reduced weight in higher TBT ranges [38,36,24,37].

The weight of the testes varies with the amount of differentiated spermatogenic cells, so a decrease in organ weight may suggest a reduction in sperm production[64]. Furthermore, several studies indicate that inhibition of the aromatase enzyme by TBT, generates a "hyper-androgenic" status in male mammals[65,66,67], which also entails a reduction in testes weight [68].

O'Connor *et al* (1998)[69] elucidated a decrease in seminal vesicle and prostate weights after treatment with finasteride, an inhibitor of the 5α-reductase that converts testosterone to dihydrostostostosterone, and with anstrozol, an aromatase inhibitor that converts testosterone and androstenedione to 17β-estradiol and estrone, respectively, demonstrating that TBT inhibits the activities of human 5α-reductase[70] and aromatase[71], results that cororoborate with the work of Yu *et al.*[34] and Omura *et al.*[39]. Thus, the decreases in prostate and seminal vesicle weights in these studies were likely induced by the inhibition activity of TBT for 5α-reductase and aromatase.

In the study by Grote *et al.*[32] the increase in prostate and epidymis weight observed in the 0.5 mg group and the decrease observed in the 15 mg TBT group are apparently opposite effects. However, within the field of endocrine disruption, such effects often occur and are controversial.

It is well known that the progression of sexual maturation is endocrine mediated and the onset of puberty in the male rat is triggered within the central nervous system with the testes playing a key role[32]. Leydig cells (LCs) and Sertoli cells (SCs) are responsible for testicular development and normal spermatogenesis[72,73]. In the testes, spermatogenesis is under the control of two gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH)[74,75].

FSH acts directly on SCs promoting spermatogenesis while LH induces testosterone production in Leydig cells. Sertoli cells express both the FSH receptor (FSHR) and the androgen receptor (AR), thus integrating androgen and FSH signaling[76]. FSH concentrations increase steadily after birth, which promotes SC proliferation and induces AR expression in

these cells[77,78,79]. In addition, the concentration of FSH and testosterone are increased at puberty and increases the expression of AR, which is essential for the final maturation of SCs[79]. After binding to hormones, AR translocates to the nucleus where it regulates transcription of androgen responsive genes.

Serum LH levels during puberty are controversial, with some studies reporting an increase in LH during puberty[80,81], others have found no change in LH levels throughout [82,83]. While some antiandrogenic substances result in an increase in serum LH and testosterone levels others decreased[84,39] or did not alter testosterone and/or LH concentrations[85].

When it comes to serum hormone concentration, most studies have shown that testosterone levels in mice and pigs decreased in a TBT dose-dependent manner[33,32,17,36,40]. Additional analysis showed that TBT treatment significantly increased serum LH and FSH levels[32,39], while reducing to 17β-estradiol levels compared to control[39,39].

The antiandrogenic properties are mediated by different mechanisms. While some chemicals cause pubertal changes through antagonistic binding to the androgen receptor, others disrupt hypothalamic-pituitary function, steroid hormone synthesis or metabolism[32].

The production of sex steroid hormones in mammals results from a pathway involving both cytochrome P450 enzymes, which are mixed-function oxidases and steroid dehydrogenases. Gonadal androgen production is important in males for brain masculinization [86], androgen target tissue function[87] and spermatogenesis[88].

As reported, TBT causes damage to many organs, including the testes, and the Leydig cell is also a target of TBT. The LCs of the testes has the ability to synthesize testosterone from cholesterol[89]. Testosterone biosynthesis depends on several steroidogenic enzymes. Steroidogenic acute regulatory protein (StAR) is required for the transport of cholesterol to the mitochondrial membrane. Additional conversions of cholesterol to testosterone occurring through mitochondria to the smooth endoplasmic reticulum are catalyzed by the cholesterol side chain cleavage enzyme (Cyp scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and Cyp17α.

Leydig cells also express aromatase P450 (Cyp), which catalyzes the aromatization of testosterone into estradiol[89]. Since differentiation of adult Leydig cells during the immature stage is crucial for testosterone production in adulthood, it is important to clarify the inhibitory effects of TBT exposure on steroidogenesis during this developmental stage[36].

Kim *et al.* (2008)[36] demonstrated association of TBT with induced apoptosis of testicular germ cells in mice and inhibition of steroidogenesis by reduced expression of steroidogenic enzymes in interstitial Leydig cells.

Decreased expression of steroidogenic acute regulatory protein (StAR)[41,37,42] also results in the inhibition of steroidogenesis, as StAR mediates the limiting step in steroidogenesis. Reduced levels of 3β-HSD, on the other hand, may be a causal factor for decreased testosterone production[33,17,41,36,40].

Significant degenerative changes and shedding of differentiating cells of the germ layer were found in the seminiferous tubules of the testes of TBT-treated mice. Increases of detached debris and some shredded cells were observed, whereas these testicular changes were not reported in some TBT treatment experiments[43,34,35]. Ingestion of TBT from adult male rats in a two-generation toxicity study induced mild testicular histological changes that were vacuolization of the seminiferous epithelium, retention of spermatids in the epithelium, delayed spermiation, and germ cell degeneration[39].

The homeostasis of the seminiferous epithelium depends on cell death and proliferative activity of the epithelium, and any imbalance of the two processes can result in histological changes[90]. Proliferating protein nuclear antigen (PCNA) is essential for several cell cycle pathways, such as the initiation of DNA replication[91]. It has been proposed that PCNA is a useful molecular marker to assess germ cell kinetics[92] and the intensity of labeling could assess testes spermatogenic function, including cell proliferation[93]. Kishta *et al.* (2006)[94] demonstrated that TBT is an inhibitor of protein synthesis, and is possibly able to increase the expression of a number of gene stress responses that lead to lower PCNA expression. Organotin compounds have induced significant delay in cell kinetics[95], inhibited DNA synthesis of spleen cells in mice[96] and decreased proliferation of human B lymphocytes[97]. It is reasonable to assume that not only one, but several factors are involved in the mutagen-induced delay in the cell cycle.

Delayed lumen formation in the seminiferous tubules and a reduction in the total number of germ cells were seen in TBT-exposed animals compared to vehicle-exposed animals. Several multinucleated bodies and cells with pyknotic nuclei in the tubules were also observed, and data from *in situ* TUNEL analysis indicated that these testicular germ cells underwent apoptosis after TBT exposure[36]. Jurkiewicz *et al.* (2004)[98] showed the involvement of mitochondrial and

receptor pathways in TBT-induced apoptosis in rat hepatocytes. These pathways are also known to play important roles in spontaneous and heat- or chemical-induced apoptosis in testicular germ cells[99,100].

TBT treatment also produced histological changes in the seminal vesicle of adult rats such as vacuolization of the seminiferous epithelium, retention of spermatids in the epithelium, delayed spermiation, and degeneration of germ cells. The epithelium of the epididymal head showed the normal, but disturbances characterized by increments of detached debris and some desquamated cells thought to originate from seminiferous tubules of the testes were detected[43].

TBT exposures caused a significant reduction in both sperm count and sperm motility compared to control[43,39,38,23,44,24]. Haubruge *et al.* (2000)[61] reported that TBT exposure to adult male guppies caused a significant decline in total sperm count. This decline was not due to endocrine system-mediated alteration, but in vivo interference with normal Sertoli cell function[101].

After exposures of 0.27 to 27.0 µg/L for 24 hours to African catfish spermatozoa a significant decrease in the duration and intensity of sperm motility was observed[86]. The decrease in sperm motility was probably associated with an instantaneous decrease in ATP content and simultaneous increase in AMP content following exposure in catfish semen to TBT. An exposure to 2.7 µg/L for 24 hours in carp also caused a significant reduction in sperm motility, but no change in adenylate concentrations[86].

On the other hand, spermatozoa with morphological abnormalities observed in treated animals suggest that TBT may cause a spermatotoxic effect[38,42,24]. The increased frequency of abnormalities, such as highly bent tail, curled tail, small head, unformed head, headless tail, and tailless head, can impair sperm motility, affecting male fertility[102]. Since the chemical composition of epididymal tissue fluid plays an important role in both sperm maturation and storage, it is possible that chemicals, such as organotin biocidal compounds, could disrupt these processes and produce toxic effects[103].

CONCLUSION

In conclusion, TBT exposure to male animals produces several reproductive disorders. Decreased weight of the testicles, epididymis, prostate and seminal vesicle were observed at higher TBT ranges, according to the articles surveyed. In some studies, decreased serum

testosterone levels were reported, with some histological changes in the surveyed tissues, as well as decreased transcriptional expressions of steroidogenic enzymes. Importantly, there were significant reductions in sperm count and motility and increased abnormalities, perhaps due to its direct effect on accessory sex organs and spermatogenesis.

Further studies are needed to elucidate the precise mode of its deleterious mechanisms of action on the spermatogenesis process. It is theorized that the androgenic effects of TBT are mediated by inhibition of the aromatase activity of cytochrome P450 and 5α-reductase. This is of special interest because high levels of tributyltin have been detected in human blood samples.

Therefore, a comprehensive survey of TBT levels in food and water sources should also be conducted in order to better protect susceptible populations from potentially deleterious reproductive effects.

NO CONFLICT OF INTEREST

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