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Review

Systematic Review of Exposure to Bisphenol A Alternatives and Its Effects on Reproduction and Thyroid Endocrine System in Zebrafish

Jiyun Lee [1,2], Kyong Whan Moon [1,2] and Kyunghee Ji [3,*]

[1] Department of Health and Safety Convergence Science, Graduate School at Korea University, Seoul 02841, Korea; jiyun9607@gmail.com (J.L.); kwmoon@korea.ac.kr (K.-W.M.)
[2] BK21 FOUR R&E Center for Learning Health System, Graduate School at Korea University, Seoul 02841, Korea
[3] Department of Occupational and Environmental Health, Yongin University, Yongin 17092, Korea
* Correspondence: kyungheeji@yongin.ac.kr; Tel.: +82-31-8020-2747

Abstract: Bisphenol A (BPA), which is widely used for manufacturing polycarbonate plastics and epoxy resins, has been banned from use in plastic baby bottles because of concerns regarding endocrine disruption. Substances with similar chemical structures have been used as BPA alternatives; however, limited information is available on their toxic effects. In the present study, we reviewed the endocrine disrupting potential in the gonad and thyroid endocrine system in zebrafish after exposure to BPA and its alternatives (i.e., bisphenol AF, bisphenol C, bisphenol F, bisphenol S, bisphenol SIP, and bisphenol Z). Most BPA alternatives disturbed the endocrine system by altering the levels of genes and hormones involved in reproduction, development, and growth in zebrafish. Changes in gene expression related to steroidogenesis and sex hormone production were more prevalent in males than in females. Vitellogenin, an egg yolk precursor produced in females, was also detected in males, confirming that it could induce estrogenicity. Exposure to bisphenols in the parental generation induced a decrease in the hatchability associated with offspring generation. In zebrafish exposed to bisphenols, significant decreases in thyroxine concentrations and increases in thyroid-stimulating hormone concentrations were commonly observed. Alternative compounds used to replace a chemical of concern are believed to be less toxic than the original compound; however, several BPA alternatives appear to have similar or greater effects on the endocrine system in zebrafish. Since endocrine systems interact with each other, further studies are needed to assess the primary target of BPA alternatives among the endocrine axes.

Keywords: bisphenol A alternatives; endocrine disruption; reproduction; thyroid; zebrafish

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1. Introduction

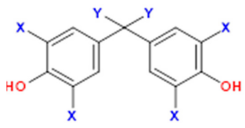
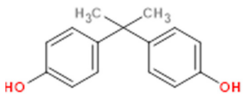
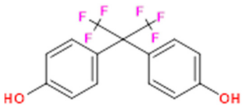
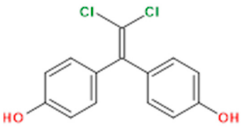
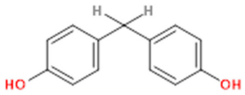
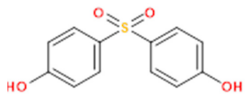
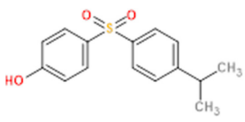
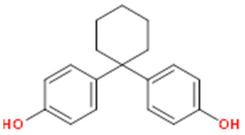
The endocrine system comprises endocrine glands, hormones, and target cells involved in reproduction, development, growth, metabolism, and stress response, thereby maintaining the living organism's homeostasis [1]. Hormones produced by the endocrine glands throughout the blood flow circulate in the whole organism and bind to receptors on target cells thus transmitting chemical signals or inducing hormone action. The endocrine system is present in all vertebrates ranging from fish to mammals and secretes similar hormones [2]. Synthetic or natural chemicals that have an abnormal effect on normal endocrine system function are called endocrine disruptors. The exact definition of endocrine disruptors is different for various organizations, such as the United States Environmental Protection Agency [3] or the Organization for Economic Co-operation and Development [4]. Therefore, a globally established list of endocrine disruptors is non-existent, and chemical substances with potential risks are estimated through prior research [5]. Unlike biological hormones, these endocrine disruptors are stable and not easily degraded; therefore, they

remain in the environment or inside a living organism [6]. Additionally, due to their strong lipid affinity, endocrine disruptors can accumulate in fats and tissues of organisms along the food chain [7]. Chemicals that are estimated to disturb the endocrine system are very diverse, such as plastic plasticizers [8], pesticides [9], heavy metals [10], and persistent organic compounds [11]. Bisphenol A (BPA) is suspected to be a representative endocrine disruptor [12].

BPA is a compound synthesized from phenol and acetone in 1891 [13] and has been used since the 1950s to produce resilient and transparent polycarbonate plastics and epoxy resins [14]. This compound has been used for various purposes, including electronic equipment, medical devices, reusable bottles, food storage containers, canned food, and thermal paper receipts [13,15–17]. The worldwide demand for BPA increases by 5% annually and is classified as a high production volume chemical in the United States [18]. As the use of BPA increases, it is frequently detected in human samples [19–21], as well as in environmental media [22,23].

As BPA causes estrogenic activity [24,25] and is detected at a high frequency in human, environmental, and product samples, concerns regarding its use have increased. According to the precautionary principle, Canada, the European Union, and the United States have banned the use of BPA in baby bottles [26]. Substances similar in structure to BPA are now used as an alternative [27]. As shown in Table 1, bisphenol analogs have two phenolic rings, and the types and positions of functional groups are slightly different. Since the key structure of bisphenol analogs is similar to the original compound, they can replace the role of BPA. However, there is a possibility that the toxicity resulting from the structure remains. BPA analogs are used in many consumer products and are frequently detected in urine samples of the general population [28], surface water [23], and indoor dust [22].

Table 1. Structural resemblance of bisphenol A analogs.

Classification	Bisphenol	Bisphenol A (BPA)	Bisphenol AF (BPAF)	Bisphenol C (BPC)
Structure [a]				
CAS number	X, Y: regulating factor for estrogenic andanti-and rogenic effects	80-05-7	1478-61-1	14868-03-2
Molecular formula		$C_{15}H_{16}O_2$	$C_{15}H_{10}F_6O_2$	$C_{14}H_{10}Cl_2O_2$
Molecular weight (g/mol)		228.29	336.24	281.14
Classification	**Bisphenol F (BPF)**	**Bisphenol S (BPS)**	**Bisphenol SIP (BPSIP)**	**Bisphenol Z (BPZ)**
Structure [a]				
CAS number	620-92-8	80-09-1	95235-30-6	843-55-0
Molecular formula	$C_{13}H_{12}O_2$	$C_{12}H_{10}O_4S_1$	$C_{15}H_{16}O_4S_1$	$C_{18}H_{20}O_2$
Molecular weight (g/mol)	200.24	250.27	292.35	268.36

[a]: chemical structure drawn by CkemSketch program.

The zebrafish has become a powerful model for testing endocrine disruptors based on their higher fecundity, fast embryonic development, and a conserved neuroendocrine system, which are also observed in humans [2]. This small fish has a hypothalamus-pituitary-endocrine gland axis, which connects the central nervous system and the endocrine system. Depending on the prominent glands, the axis can be divided into the hypothalamic-pituitary-gonad (HPG) axis, hypothalamic-pituitary-thyroid (HPT) axis, and hypothalamic-pituitary-adrenal (HPA) axis. All axes cooperate with other neuroendocrine systems to control body physiology [2]. Both the technical and practical advantages have

made zebrafish an ideal organism for the identification of endocrine-disrupting chemicals [29].

As their production and discharge into the environment are estimated to increase worldwide, the health risk potentials of BPA and its alternatives are of increasing concern. Unlike BPA, whose endocrine disruption has been investigated thoroughly, limited information is available regarding the toxicity of BPA alternatives. This review focuses on the endocrine disruption of BPA alternatives in zebrafish that have been reported in previous studies and presents the current status of related knowledge to identify gaps for future research. The first step involves a systematic review to define the protocol through a population-based comparator-outcome (PECO) statement (Table 2). Our review included the literature in which zebrafish were exposed to BPA and its alternatives, with control groups or vehicle-treated groups included for comparison. The effects on the reproduction (fertility, fecundity, vitellogenesis, sex hormones, and genes related to the HPG axis) and development (hatchability, time-to-hatch, spontaneous movement, body length, thyroid hormones, and genes related to hypothalamic-pituitary-thyroid (HPT) axis) were considered as the potential adverse outcomes. The PECO statement serves as a guide for the entire review process, including the literature search strategy, criteria for the inclusion/exclusion of studies, type of data extracted from studies, and strategy for reporting results [25].

Table 2. Elements of a population-based comparator-outcome (PECO) statement in the present study.

Element	Explanation	Inclusion Criteria	Exclusion Criteria
(P) Population	What are the characteristics of the receptors?	• Experimental zebrafish studies	• All humans and rodents studies
(E) Exposures	What are the types of chemicals and the timing of exposure?	• Exposure to BPA, BPAF, BPC, BPF, BPS, BPSIP, and BPZ in adult stages or early life stages	• Exposure to other chemicals
(C) Comparator	Which exposure groups will be compared to each other?	• Exposed groups versus vehicle-treated or • negative controls	• No controls in experimental studies
(O) Outcome	Which outcomes will be included or covered?	• Fertility, fecundity, sex ratio, gonad weight, sex hormones, and genes related to the hypothalamic-pituitary-gonad (HPG) axis, vitellogenesis • Length, weight, thyroid hormones, and genes related to the hypothalamic-pituitary-thyroid (HPT) axis	• All other endocrine disruption effects
Publication parameters	-	• Peer-reviewed • Original data • Available in English	• Non-peer reviewed • Not an original data (e.g., reviews, editorials) • Unavailable in English

2. Methods

2.1. Search and Selection of Studies for Inclusion

Relevant studies were selected using two screening phases. The first selection was based on title and abstract screening, and the second selection was based on full-text screening. Studies were selected for full-text screening when they met the inclusion criteria. Articles published before April 1, 2020, were identified in PubMed. A comprehensive search strategy was developed and included the search components "bisphenol A alternatives", "zebrafish", "HPG axis", and "HPT axis". The reference lists of the included articles and relevant reviews were screened manually for potentially relevant new articles. In case of doubt, articles were also analyzed based on their full text. Studies were included in this systematic review when they met all of the following criteria (Table 2): (a) an original full paper that presented unique data; (b) a study where exposure to BPA and its alternatives was elucidated; and (c) an article related to HPG or HPT axes. Studies were excluded if they met one of the following criteria: (a) unoriginal paper, (b) studies involving the exposure to a chemical other than the BPA alternatives, (c) no outcome of interest, and (d) not a zebrafish study. Moreover, selection was restricted to English-language articles.

2.2. Reliability Assessment of Individual Studies

The quality of evidence of the systematic review outcomes was rated using the criteria for reporting and evaluating ecotoxicity data (CRED) [24]. A total of 20 criteria were divided into 6 categories for evaluation, including general information, test design, test substance, test organism, exposure conditions, and statistical method. Data above the standard score were judged as high-quality toxicity data. Five points were assigned for each criterion, and the reliability level of the toxicity data was determined based on the satisfaction of 12 essential items and the total score. The CRED evaluation method uses 4 reliability categories, similar to the Klimisch scores [26]: reliable without restrictions (R1), reliable with restrictions (R2), not reliable (R3), and not assignable (R4). R1 grade is given when all 12 essential items are satisfied, and the total score is 80 points or more. The R2 grade is a case where the total score is 60–80 points, and one of the 12 required items is not satisfied. We selected the data corresponding to R1 and R2 grades.

3. Results and Discussion

3.1. Study Selection and Characteristics

Study selection is summarized in a flow chart (Figure 1). Literature searches in PubMed identified 40,262 studies related to zebrafish and 24,133 studies related to bisphenols. After removing duplicate records, 241 articles were identified as relevant. After title and abstract screening, 58 articles were excluded based on the following criteria: (a) unavailability in English, (b) unoriginal data, and (c) irrelevant research. Of these 183 publications, 49 were included in this review based on full text screening.

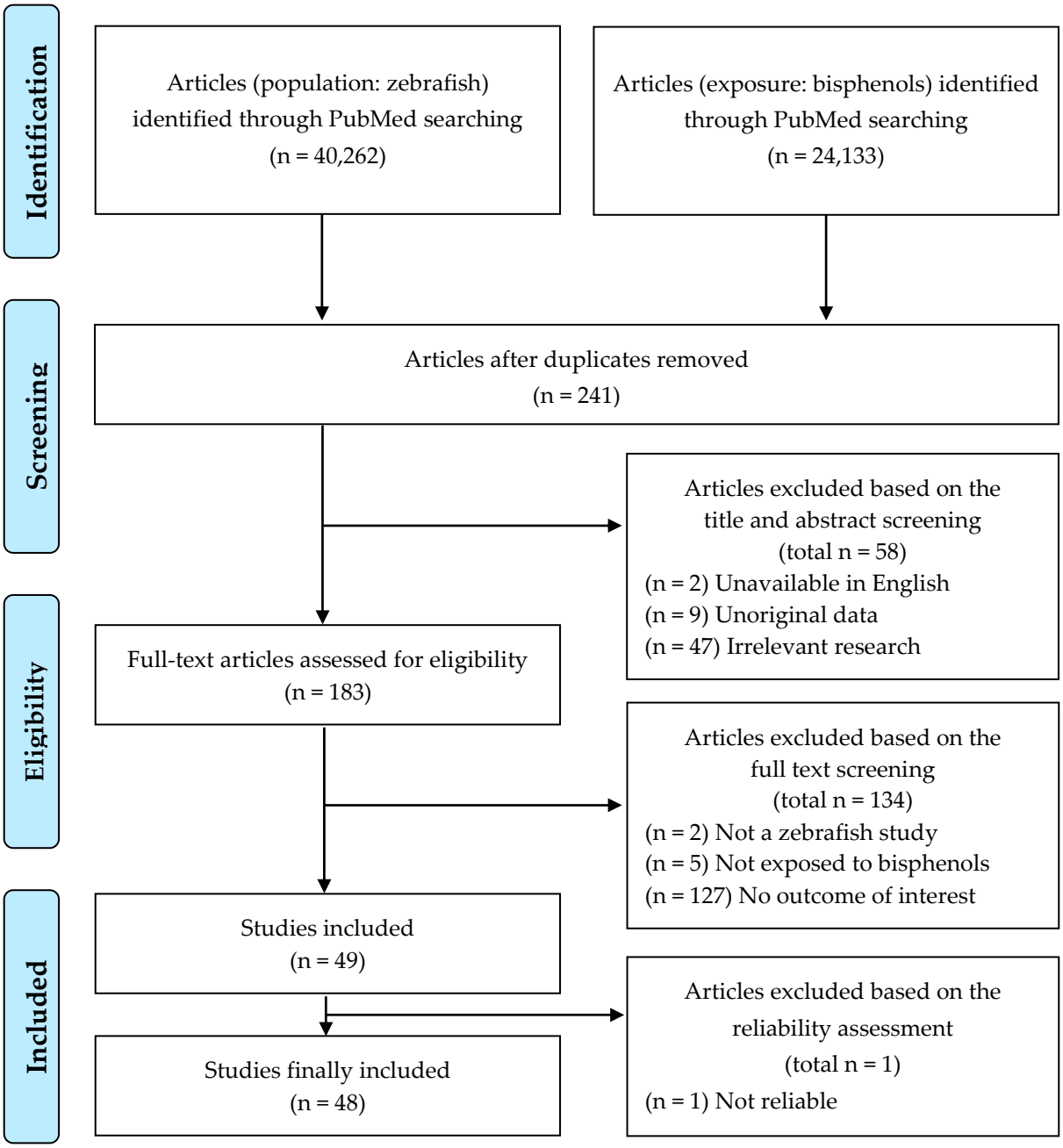


Figure 1. Flow chart of study selection process.

Among 49 individual studies, 9 cases were R1 grade, 39 cases were R2 grade, and 1 case was R3 grade. Therefore, 48 high-quality studies (38 studies related to the HPG axis and 10 studies related to the HPT axis) are summarized in the following section.

3.2. Effects of BPA and Its Alternatives on HPG Axis

It has been reported that functions of the reproductive system are susceptible to disruption by endocrine-disrupting chemicals. Thirty-eight studies showed that BPA [27,28,30–56], BPAF [42,45,51,57,58], BPC [45], BPF [42,46–57,59–61], BPS [42,46–48,62–64], and BPSIP [65] disturbed fecundity, fertility, relative organ weight, vitellogenin protein production, sex hormone production, and mRNA expression related to the HPG axis (Table 3).

Table 3. Summary of studies about hypothalamic-pituitary-gonad (HPG) axis in zebrafish exposed to bisphenol A and its alternatives.

Chemical	Stage (Type)	Exposure Concentration (μg/L)	Exposure Duration	No Observed Adverse Effect Level (μg/L)	Lowest Observed Adverse Effect Level (μg/L)	Toxicity Effect					Ref
						Gene	Hormone	Protein	Organ	Organism	
BPA	Embryo	0, 0.5, 5, 50, 500	96 h	-	0.5	↑*esr1*, *esr2a*, *vtg1*	-	-	-	-	[27]
				0.5	5	↑*cyp19a1b*	-	-	-	-	
				50	500	↑*ar*	-	-	-	-	
BPA	Adult	0, 1, 10, 100, 1000	14 days	-	1	↓*cyp19a* (♀)	-	↑VTG (♀)	-	-	[28]
BPA	Adult	0, 100, 300, 600, 800, 1000	96 h	>1000	-	-	-	VTG (♂, ♀)	-	-	[30]
			168 h	>1000	-	-	-	VTG (♂, ♀)	-	-	
BPA	Adult	0, 2, 20, 200	21 days	-	2	-	-	↑VTG (♀)	-	-	[36]
				2	20	-	-	↑VTG (♂)	-	-	
				20	200	-	↓FSH, LH, T, E2 (♂, ♀)	-	-	-	
BPA	Embryo	0, 0.02, 0.2, 2, 22, 228, 2282, 6848 (=0, 0.0001, 0.001, 0.01, 0.1, 1, 10, 30 μM)	5 days	0.2	2	↓*esr2*	-	-	-	-	[44]
				22	228	↓*ar*	-	-	-	-	
				228	2282	↓*esr1*	-	-	-	-	
BPA	Adult	0, 5	21 days	-	5	↑*vtg*, *erα*, *cyp19a* (♂)	↑E2 (♂), ↓E2 (♀)	-	↓GSI (♂)	↓Fecundity	[37]
BPA	Adult	0, 0.01, 0.1, 1, 10, 100	96 h	1	10	-	-	↑VTG (♂)	-	-	[54]
BPA	Adult	0, 10, 200, 400	180 days	-	10	-	-	↑VTG (♂)	-	-	[39]
BPA	Embryo	0, 0.1, 1, 10, 100, 1000	120 h	1	10	↑*gnrh3*, *lhβ*, *kiss1r*	-	-	-	-	[48]
				10	100	↑*erα*, *kiss1*	-	-	-	-	
				100	1000	↑*fshβ*	-	-	-	-	
BPA	Adult	0, 20	4 min	-	20	↑F1 *vtg2*	↓E2 (♂),↓T (♀)	↑F1 VTG2	-	↓Fecundity	[32]
BPA	Adult	0, 5, 10, 20	21 days	10	20	↑*star*, *esr2b*, *fshr* (♀)	-	-	-	-	[50]
BPA	Adult (F2)	0, 20	28 days	-	20	↑*star*, *fshr* (♀)	-	-	↓GSI (♀)	↓Fertility	[49]
BPA	Embryo	0, 100	120 h	-	100	↑*erα*	-	-	-	-	[47]
BPA	Juvenile	0, 100	60 days	-	100	↑*kiss1*, *kiss2*, *gnrh3*, *erα*, *cyp19a*, *cyp19b*	-	-	-	-	[46]
BPA	Adult	0, 10, 100, 1000	15 days	10	100	↓*vtg1* (♀)	-	-	-	-	[40]
				100	1000	↑*vtg1* (♂)	-	-	-	-	
BPA	Adult	0, 100, 2000	42 days	-	100	↑*vtg1*(lai♂)	-	-	↓GSI (♂)	-	[52]
				100	2000	↑*esr1*, *vtg2* (♂)	-	-	-	-	

Table 3. *Cont.*

Chemical	Stage (Type)	Exposure Concentration (μg/L)	Exposure Duration	No Observed Adverse Effect Level (μg/L)	Lowest Observed Adverse Effect Level (μg/L)	Toxicity Effect					Ref
						Gene	Hormone	Protein	Organ	Organism	
BPA	Embryo	0, 100, 1000, 5000	96 h	-	100	↑*vtg1, cyp17a1*	-	-	-	-	[42]
				100	1000	↑*esr1, esr2a, esr2b, hsd17b1*	-	-	-	-	
				1000	5000	↑*cyp19a1*	-	-	-	-	
BPA	Adult	0, 25, 50, 100, 250, 500	15 days	100	250	↑*vtg1* (♂)	-	-	-	-	[31]
BPA	Adult	0, 500, 1000, 1500	21 days	-	500	-	-	↑VTG (♂)	-	-	[51]
BPA	Adult	0, 500, 1000, 1500	21 days	-	500	↑*vtg1, esr2b, cyp19a1a* (♂)	↑E2 (♂)	↑VTG (♂)	-	-	[56]
				500	1000	↑*esr1* (♂), ↓*star, cyp17a1* (♂)	↓T (♂)	-	-	-	
BPA	Embryo	0, 10, 100, 500, 750, 1000, 2500, 5000	7 days	500	750	↑*cyp19b*	-	-	-	-	[55]
				1000	2500	↑*vtg*	-	-	-	-	
BPA	Adult	0, 40, 200, 1000	21 days	200	1000	-	-	↑VTG (♂)	-	-	[53]
BPA	Adult	0, 2000	35 days	-	2000	↑*vtg* (♂)	-	-	-	-	[41]
BPA	Embryo	0, 1141, 2282, 3424 (=0, 5, 10, 15 μM)	120 h	1141	2282	↑*vtg1*	-	-	-	-	[43]
BPA	Embryo(GFP)	0, 11, 22, 114, 228, 1141, 2282 (=0, 0.05, 0.1, 0.5, 1, 5, 10 μM)	2 days	1141	2282	↑*vtg1, cyp19a1b*	-	-	-	-	[45]
BPA	Juvenile(albino)	0, 2282 (=10 μM)	20 days	-	2282	↓*fshβ*	-	-	↓Ovary growth	↑♀/♂ratio	[34]
BPA	Embryo	0, 114, 228, 570, 1141, 2282 (=0, 0.5, 1, 2.5, 5, 10 μM)	7 days	>2282	-	*vtg1*	-	-	-	-	[33]
BPA	Embryo	0, 804, 2010, 4020, 6030	96 h	4020	6030	↑*vtg1*	-	-	-	-	[35]
BPA	Adult	0, 0.1, 2, 20, 200, 400, 1000, 2000	11 days	1000	2000	↑*vtg1* (♂)	-	-	-	-	[38]
BPAF	Embryo	0, 20, 200, 1000	96 h	-	20	↑*vtg1, esr2b*	-	-	-	-	[42]
				20	200	↑*esr1, esr2a, cyp19a1, hsd17b1*	-	-	-	-	
				200	1000	↑*cyp17a1*	-	-	-	-	
BPAF	Adult	0, 5, 25, 125	120 days	5	25	↑*vtg1* (♂)	↑E2 (♂) ↓T (♂)	-	-	-	[57]
				25	125	↑*gnrh2, fshβ, lhβ, fshr, star, cyp17, cyp19a, cyp19b* (♂) ↑*fshr* (♀) ↓*star* (♀)	↑E2 (♀)	-	-	↓F1 fertility	

Table 3. *Cont.*

Chemical	Stage (Type)	Exposure Concentration (μg/L)	Exposure Duration	No Observed Adverse Effect Level (μg/L)	Lowest Observed Adverse Effect Level (μg/L)	Toxicity Effect					Ref
						Gene	Hormone	Protein	Organ	Organism	
BPAF	Embryo(GFP)	0, 16, 33, 168, 336, 1681 (= 0, 0.05, 0.1, 0.5, 1, 5 μM)	2 days	33	168	↑*cyp19a1b*	-	-	-	-	[45]
				168	336	↑*vtg1*	-	-	-	-	
BPAF	Adult	0, 500, 1000, 1500	21 days	-	500	-	-	↑VTG (♂)	-	-	[51]
BPAF	Adult	0, 50, 250, 1000	28 days	250	1000	↑*vtg* (♂)	↑E2 (♂)	-	-	-	[58]
BPC	Embryo(GFP)	0, 14, 28, 140, 281, 1405 (= 0, 0.05, 0.1, 0.5, 1, 5 μM)	2 days	-	14	↑*vtg1*	-	-	-	-	[45]
				140	281	↑*cyp19a1b*	-	-	-	-	
BPF	Juvenile	0, 0.1, 1, 10, 100, 1000	60 days	-	0.1	-	-	↑VTG	-	-	[46]
				1	10	↑*kiss1r*, *fshr*, *vtg*, *erα*, *cyp19a*	↑LH, FSH, GnRH	-	-	-	
				10	100	↑*kiss1*, *lhr* (♀), *erβ*, *cyp19b*	-	-	-	-	
				100	1000	↑*gnrh3*, *lhr* (♂)	-	-	-	-	
				>1000	-	*kiss2*, *kiss2r*, *sv2c*	-	-	GSI (♀)	-	
BPF	Adult	0, 1, 10, 100, 1000	21 days	-	1	↑*fshβ* (♂)	-	-	-	-	[61]
				1	10	↑*lhβ*, *gnrh3*, *vtg* (♂)	↓T (♂)	-	-	-	
				10	100	↑*gnrh2*, *gnrhr1*, *gnrhr2*, *cyp19a*, *fshr*, *lhr* (♂)	↑E2 (♂)	-	-	-	
						↑*fshr* (♀)					
						↓*fshβ*, *17βhsd*, *star* (♀)					
				100	1000	↑*cyp11a* (♂, ♀)	↓T (♀),↑E2 (♀)	-	↓GSI (♂, ♀)	↓Fecundity, ↓F1 survival	
						↓*17βhsd*, *cyp17*, *star* (♂)					
						↓*lhr* (♀)					
BPF	Juvenile	0, 1, 10, 100, 1000	60 days	-	1	↑*cyp19a1a*, *vtg*	-	-	-	-	[60]
				1	10	↓*amh*, *foxl2*	↑E2 (♂, ♀),↓T (♂, ♀)	-	-	-	
				10	100	↑*dmrt1*	-	-	-	↑Intersex	
				100	1000	↓*ff1d*	-	-	-	-	
BPF	Adult(GFP)	0, 2.28, 22.8, 228, 2282(=0, 0.01, 0.1, 1, 10 μM)	7 days	2.28	22.8	-	-	↑VTG (♂)	-	-	[59]
BPF	Embryo	0, 0.1, 1, 10, 100, 1000	120 h	10	100	↑*erα*	-	-	-	-	[47]
BPF	Embryo	0, 200, 2000, 10,000	96 h	200	2000	↑*vtg1*	-	-	-	-	[42]
				2000	10,000	↑*esr1*, *esr2a*, *esr2b*, *cyp19a1*, *cyp17a1*, *hsd17b1*	-	-	-	-	

Table 3. *Cont.*

Chemical	Stage (Type)	Exposure Concentration (μg/L)	Exposure Duration	No Observed Adverse Effect Level (μg/L)	Lowest Observed Adverse Effect Level (μg/L)	Toxicity Effect					Ref
						Gene	Hormone	Protein	Organ	Organism	
BPS	Adult	0, 0.5, 5, 50	21 days	-	0.5	-	↑E2 (♂)	-	↓GSI (♀)	↓Fecundity	[62]
				5	50	↑*gnrh3, gnrhr1, gnrhr2, fshβ, lhβ, fshr, lhr, cyp19b, hmgra, hmgrb, cyp11a, 3βhsd, cyp17, 17βhsd, cyp19a* (♂)	↓T (♂)	-	↓GSI (♂)	-	
						↑*gnrh3, fshβ, hmgra, hmgrb* (♀)	↑E2 (♀)				
BPS	Adult	0, 1, 10, 30	120 days	-	1	↑*esr2a, esr2b* (♀)	-	-	-	-	[63]
BPS	Adult	0, 0.1, 1, 10, 100	75 days	0.1	1	-	↑E2 (♂)	-	-	-	[64]
				1	10	-	↓T (♂),↑E2 (♀)	↑VTG (♀)	↓GSI (♂)	↓Fecundity	
				10	100	-		↑VTG (♂)	↓GSI (♀)	-	
BPS	Embryo	0, 100	120 h	10	100	↑*gnrh3, kiss1, erα*	-	-	-	-	[48]
BPS	Embryo	0, 0.1, 1, 10, 100, 1000	120 h	10	100	↑*erα*	-	-	-	-	[47]
BPS	Juvenile	0, 100	60 days	-	100	↑*kiss1, kiss2, kiss1r, gnrh3, erα, erβ, cyp19a, cyp19b*	-	-	-	-	[46]
BPS	Embryo	0, 500, 5000, 25,000	96 h	>25,000	-	*vtg1, esr1, esr2a, esr2b, cyp19a1, cyp17a1, hsd17b1*	-	-	-	-	[42]
BPSIP	Adult	0, 0.5, 5, 50	21 days	0.5	5	↓*cyp19a, cyp19b, fshr* (♂)	↓T (♂)	-	↓GSI (♂)	-	[65]
				5	50	↑*gnrh2, gnrhr2, gnrhr4, erα* (♂)	↓E2 (♂)	-	-	-	
						↓*fshβ, cyp17, 17βhsd* (♂)					
						↑*gnrh2, gnrhr2, gnrhr4, fshβ, cyp19b, erα, er2β, cyp17, cyp19a* (♀)	↑E2, T (♀)				

3.2.1. Effects on Reproduction

The reproductive process in fish is regulated by steroidogenesis and coordinated interactions between steroid hormones on the HPG axis [66]. Therefore, chemicals that alter the concentration of sex hormones and the expression of steroidogenesis-related genes could affect the endocrine system's functionality, eventually influencing the reproduction of fish [67]. Exposure to bisphenols in the parental generation induced a significant decrease in fecundity [32,37,61,62,64]. Parental exposure to bisphenols is essential because no excretion mechanism exists in the eggs [62]. One explanation for the reproductive effect of parental generation and the developmental effect of offspring generation is the change in hormones and genes in the HPG axis.

3.2.2. Effects on Relative Gonad Weight

The gonadosomatic index (GSI), which is the ratio of the gonad weight to body weight, has been used as a biomarker for endocrine disruption. GSI indicates the effects on the development of reproductive organs along with a decrease in fecundity and a change in sex ratio [68]. Male zebrafish were more sensitive to exposure to bisphenols than females. Male zebrafish exposed to BPA, BPF, BPS, and BPSIP significantly decreased GSI at 5–100 µg/L [37,52], 1000 µg/L [61], 10–50 µg/L [62,64], and 5 µg/L [65], respectively (Figure 2). Several studies have reported that estrogenic chemicals reduce GSI by altering the number and size of germ cells in zebrafish [69,70]. These observations indicate that alternatives such as BPS and BPSIP can affect gonadal development, which is similar to BPA.

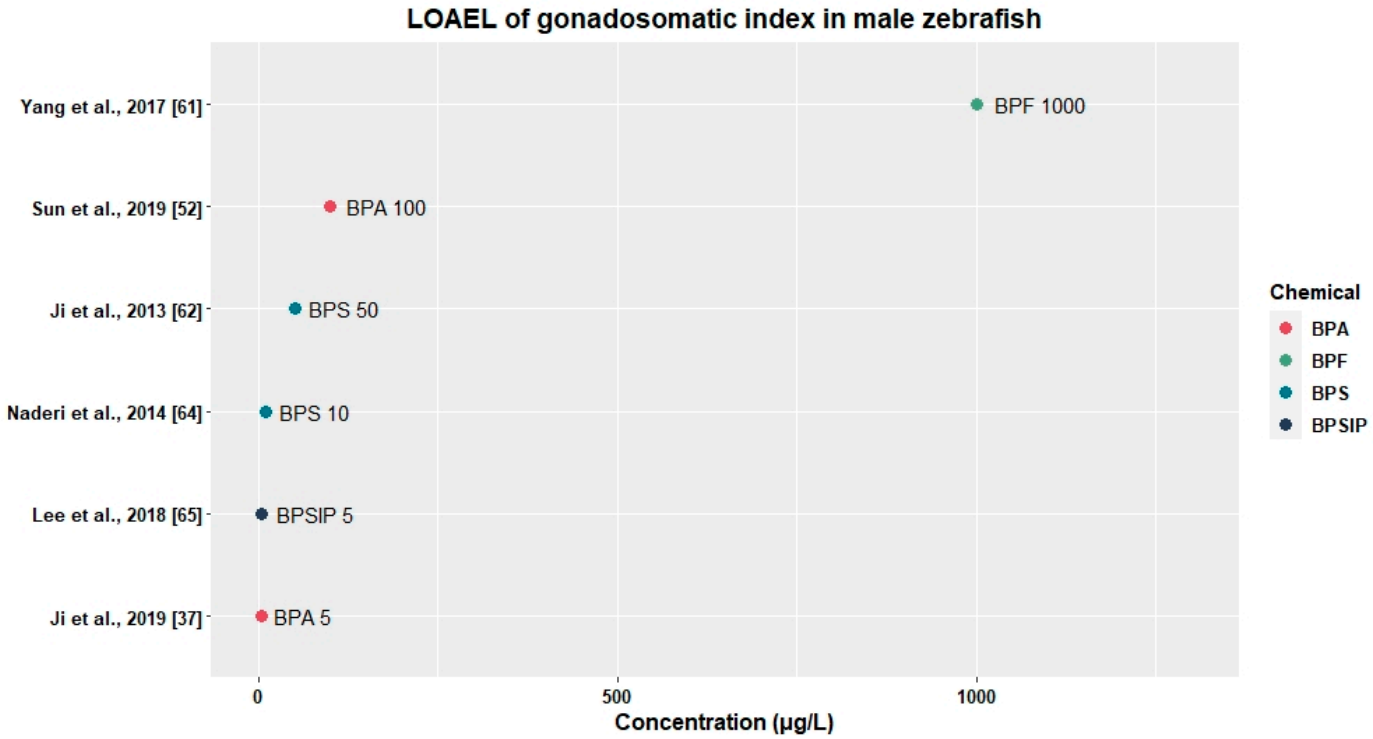


Figure 2. Lowest observed adverse effect level (LOAEL) of gonadosomatic index (GSI) in male zebrafish exposed to BPA, BPF, BPS, and BPSIP.

3.2.3. Effects on Vitellogenesis

Vitellogenin is a yolk precursor expressed in females and is used as a biomarker for endocrine disruptors. Figure 3 shows the studies on vitellogenin protein production and vitellogenin gene transcription in male zebrafish exposed to BPA [31,37,38,40,41,52,56], BPAF [57,58], and BPF [61]. Male zebrafish exposed to BPA, BPAF, BPF, and BPS signifi-

cantly increased vitellogenin production at 10–1000 µg/L [36,39,51,53,54,56], 500 µg/L [51], 22.8 µg/L [59], and 100 µg/L [64], respectively. In male zebrafish exposed to BPA, BPAF, and BPF, vitellogenin mRNA expression was significantly increased at 5–2000 µg/L [31,37,38,40,41,52,56], 25–1000 µg/L [57,58], and 10 µg/L [61], respectively. These studies demonstrate that it is possible to induce endocrine disruption at a detectable concentration in aquatic environments [71].

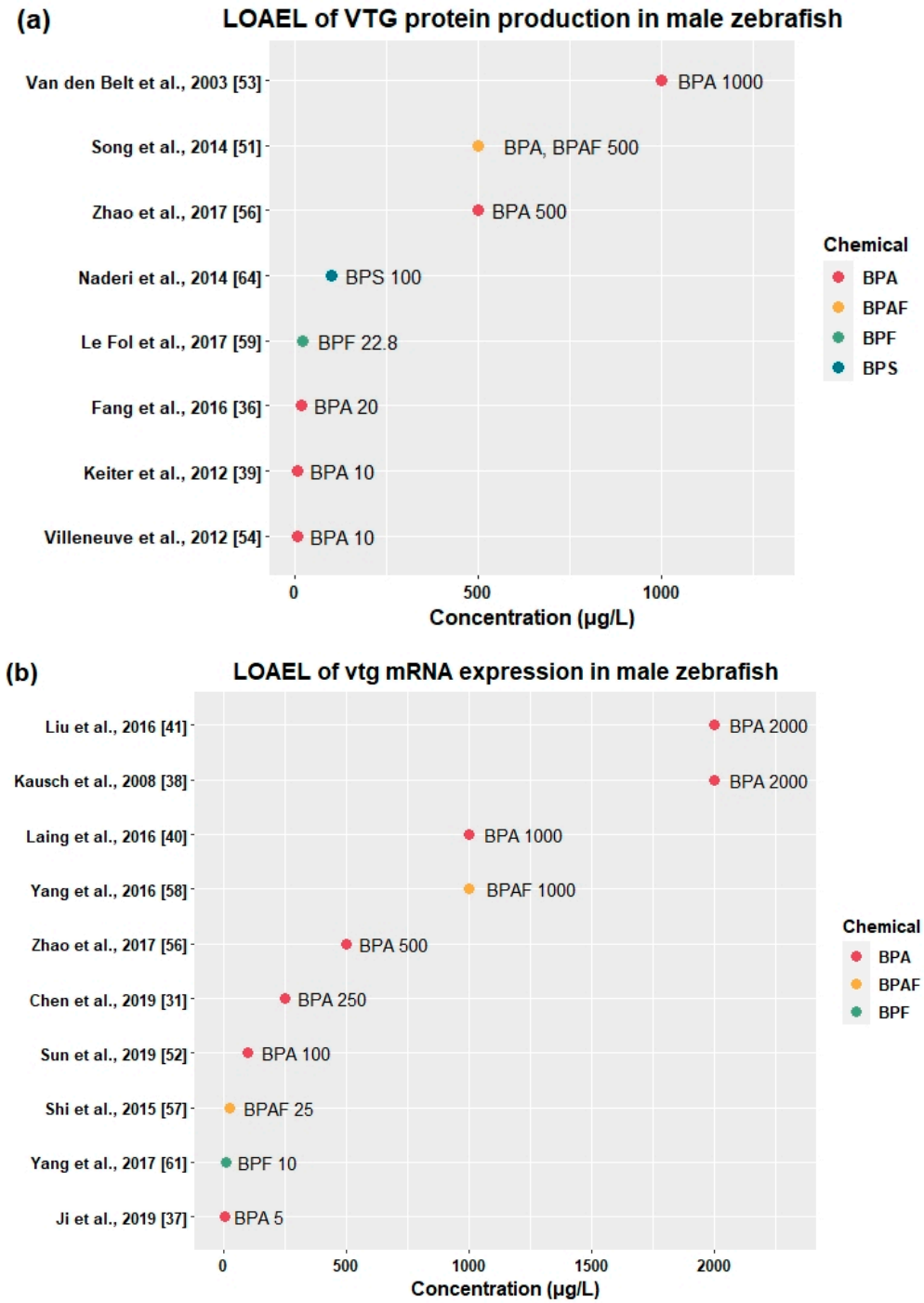


Figure 3. Lowest observed adverse effect level (LOAEL) of vitellogenin protein production (**a**) and *vitellogenin* mRNA expression (**b**) in male zebrafish exposed to BPA, BPAF, BPF, and BPS.

3.2.4. Effects on Sex Hormones and Genes Related to HPG Axis

The concentration and ratio of 17β-estradiol (E2) and testosterone (T) hormones have been widely used as integrated biomarkers for reproduction. In the studies summarized here, the adverse effects of BPA and its alternatives on hormone levels were sex-dependent, and a significant increase in E2 and decrease in T concentrations were observed in male zebrafish exposed to BPA, BPAF, BPF, BPS, and BPSIP (Figure 4). Interestingly, changes in sex hormones in male zebrafish were more sensitive to the toxicity of its alternatives than BPA. For example, male zebrafish exposed to BPS significantly increased E2 compared to the control group even at 0.5–1 μg/L [62,64]. The decrease in T hormone was up to 200 times more sensitive than BPA for most alternatives (e.g., BPAF, BPF, BPS, and BPSIP) [57,60–62,64,65]. These results suggest that the endocrine disruption of BPA alternatives is no less than that of BPA and may significantly affect sex hormones in males.

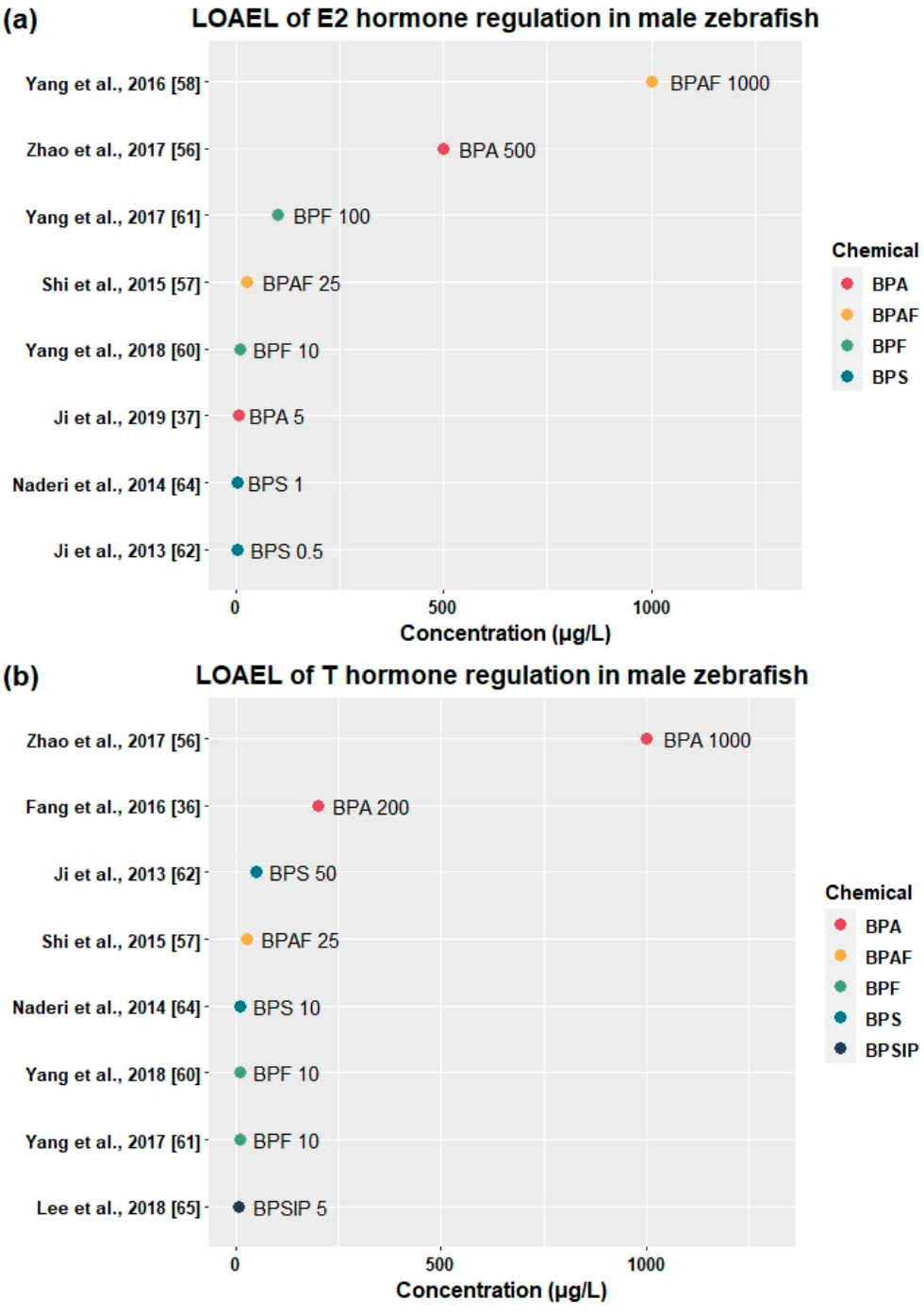


Figure 4. *Cont.*

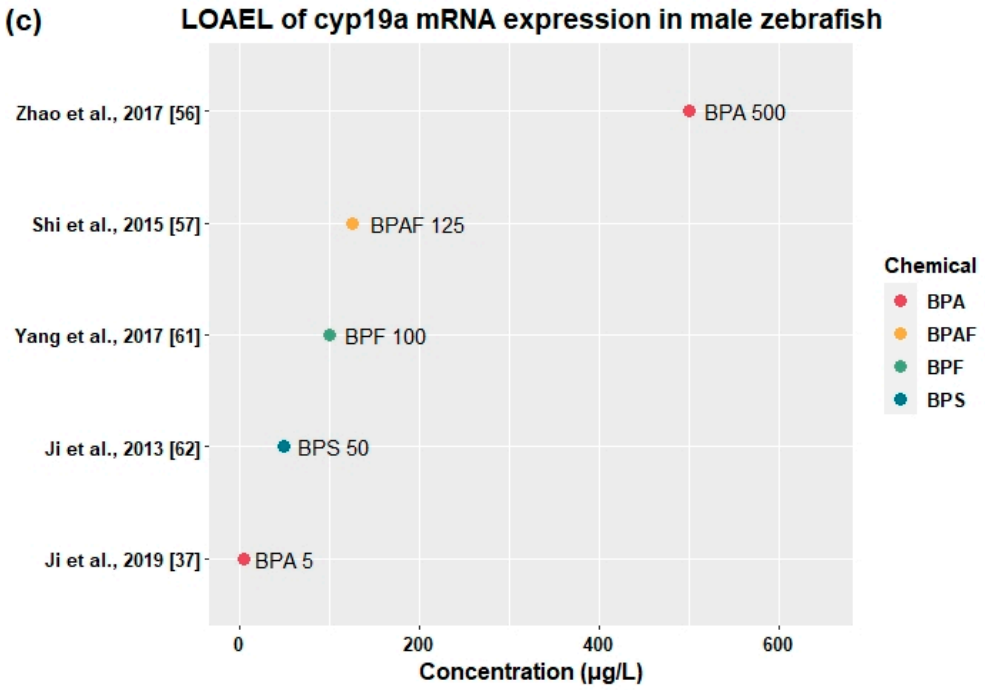


Figure 4. Lowest observed adverse effect level (LOAEL) of 17β-estradiol (E2) hormone (**a**), testosterone (T) hormone (**b**), and *cyp19a* mRNA expression (**c**) in male zebrafish exposed to BPA, BPAF, BPF, BPS, and BPSIP.

Significant increases in aromatase (*cyp19*) genes are well supported by changes in the two sex hormones. CYP19 enzyme is involved in the final step in converting of T to E2 [72], and enzyme activities are generally well correlated with their mRNA levels [73]. *Cyp19a* mRNA expression increased significantly in male zebrafish exposed to BPA [37,56], BPAF [57], BPF [61], and BPS [62] (Figure 4). Interestingly, the adverse effects of BPA alternatives on gene transcription were sex-dependent, with males being more sensitive than females.

In the HPG axis, gonadotropin-releasing hormone (GnRH) in the hypothalamus stimulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in teleosts [74]. Gonadotropin hormones in the pituitary regulate the synthesis of sex hormones, E2 and T. In terms of homeostasis, it is meaningful to examine the effects of BPA and its alternatives on the GnRH, LH, and FSH of zebrafish. Zebrafish possess the hormones GnRH2 and GnRH3, as well as four different GnRH receptors [75]. While there are few studies are measuring the GnRH hormone [46], it was frequently confirmed that the expression of *gnrh2* or *gnrh3* genes in zebrafish exposed to BPA, BPAF, BPF, BPS, and BPSIP was significantly increased (Table 3) [46,48,57,61,62,65]. These results suggest that BPA analogs can directly or indirectly (e.g., a negative feedback action in the hypothalamus compensates for the reduced E2 production) affect GnRH.

LH and FSH, which bind to specific receptors and induce gametogenesis, were also confirmed by measuring hormone or gene expression in zebrafish exposed to BPA and its alternatives. The β-subunit mRNA encoding LH and FSH were generally upregulated in male zebrafish exposed to BPAF [57], BPF [61], and BPS [62]. Two possibilities can be responsible for the decrease in the T hormone, despite a significant increase in gonadotropin hormone-related mRNA expression in male zebrafish exposed to BPA alternatives. The first option suggests that the rate of E2 production with the aromatase enzyme is higher than that observed with the T hormone. In contrast, the second option proposes the possibility of decreased expression of several genes involved in steroidogenesis (e.g., *star*, *17βhsd*, and *cyp17*).

The commonalities and differences in toxicity of BPA analogs have been explained based on their chemical structure [65,76]. Bisphenols have a phenolic group in common.

The hydrophobic group of the propane moiety and the 4-hydroxyl group on the A-phenyl ring are suggested regulatory factors that can cause differences in the BPA analog toxicity [76,77]. If the estrogenicity and anti-androgenicity of BPA are due to the phenolic ring, the endocrine disruption would be possessed even if BPA analogs are used as substitutes.

3.3. Effects of BPA and Its Alternatives on HPT Axis

For elucidating endocrine disruption, bisphenol analogs have primarily been studied focusing on reproductive toxicity, whereas developmental toxicity due to thyroid endocrine disruption has been studied less relatively. Ten studies showed that BPA, BPAF, BPF, BPS, and BPZ influenced hatchability, time-to-hatch, spontaneous movement, thyroid hormone production, and mRNA expression related to the HPT axis (Table 4) [78–87].

Table 4. Summary of studies about hypothalamic-pituitary-thyroid (HPT) axis in zebrafish exposed to bisphenol A and its alternatives.

Chemical	Stage (Type)	Exposure Concentration (μg/L)	Exposure Duration	No Observed Adverse Effect Level (μg/L)	Lowest Observed Adverse Effect Level (μg/L)	Toxicity Effect				Ref
						Gene	Hormone	Organ	Organism	
BPA	Embryo	0, 2, 22, 228 (=0, 0.01, 0.1, 1 μM)	24 h	-	2	↑*tg*, *pax8*	-	-	-	[79]
				2	22	↑*tsh*, *pax2a*	-	-	-	
BPA	Adult	0, 2, 20	4 min	2	20	-	↓T4(♀)	-	↓F1 survival	[80]
BPA	Embryo	0, 80, 400, 2000, 10,000	120 h	-	80	↑*hhex*	-	-	-	[83]
				80	400	↑*ttr*, *dio1*, *ugt1ab*	↑T3	-	-	
				400	2000	↑*tg*, *trα*	-	-	↑Time-to-hatch	
				2000	10,000	-	-	-	↓Hatchability	
				>10,000	-	-	-	-	Length	
BPA	Embryo	0, 804, 2010, 4020, 6030	96 h	-	804	↑*tshβ*	-	-	-	[78]
BPA	Embryo (GFP)	0, 2282 (=0, 10 μM)	168 h	>2282	-	*trα*, *trβ*	-	-	-	[85]
BPAF	Embryo	0, 5, 50, 500	168 h	-	5	↑*ttr*	↓FT3	-	-	[84]
				5	50	↑*tshβ*, *slc5a5*, *tg*, *dio1*, *dio2* ↓*trα*, *trβ*	↓TT4, FT4, TT3	-	-	
BPAF	Adult	0, 24.7	21 days	-	24.7	↑*trh*, *trhr1*, *tshβ*, *dio2* (♂) ↓*tpo* (♂)	-	-	-	[82]
BPF	Embryo	0, 0.2, 2, 20, 200	144 h	0.2	2	↑*crh*, *tg*	-	-	-	[81]
				2	20	↑*nis*, *dio2*, *ugt1ab* ↓*ttr*	↑TSH	-	-	
				20	200	-	↑T3, ↓T4	-	-	
BPF	Embryo	0, 80, 400, 2000, 10,000	120 h	-	80	-	-	-	↑Time-to-hatch	[83]
				400	2000	↑*hhex*, *ugt1ab*	↑T4	-	-	
				>10,000	-	-	-	-	Length	
BPS	Adult	0, 1, 10, 100	120 days	-	1	↑*dio2*, *dio3*, *ugt1ab* (♀), *dio2* (♂)	↑T3(♀), F1 T3	-	↓F1 spontaneous movement	[86]
						↓*crh*, *tshβ* (♂)	↓T4(♂, ♀), F1 T4	-	-	
				1	10	↑*crh*, *dio1* (♂, ♀)	-	-	-	
				10	100	↑*tshβ* (♀), *dio3* (♂)	-	-	-	
BPS	Embryo	0, 1, 3, 10, 30	168 h	1	3	↓*ttr*	-	-	-	[87]
				3	10	↑*crh*, *tg*, *dio1*, *ugt1ab*	↓T4,↑TSH	-	-	

Table 4. *Cont.*

Chemical	Stage (Type)	Exposure Concentration (μg/L)	Exposure Duration	No Observed Adverse Effect Level (μg/L)	Lowest Observed Adverse Effect Level (μg/L)	Toxicity Effect				Ref
						Gene	Hormone	Organ	Organism	
BPS	Embryo	0, 400, 2000, 10,000, 50,000	120 h	-	400	-	-	-	↑Time-to-hatch	[83]
				400	2000	↑*crh*, *tshβ*, *tshr*, *hhex*, *tpo*, *ttr*, *ugt1ab*	-	-	-	
				10,000	50,000	-	↑T3	-	-	
				>50,000	-	-	-	-	Length	
BPZ	Embryo	0, 40, 180, 680, 2900	120 h	180	680	↑*tshβ*	-	-	-	[83]
				680	2900	-	-	-	↑Time-to-hatch	
				>2900	-	-	-	-	Length	

3.3.1. Effects on Development

Experimental evidence showed that hatchability, time-to-hatch, eyeball size normalized to the body length, and spontaneous movement were affected by exposure to BPA [80,83], BPF [83], BPS [83,86], and BPZ [83]. Spontaneous movement, any distinguishable movement inside the chorion of embryos, was significantly decreased in fish exposed to environmentally relevant concentration (1 μg/L) of BPS [86]. Especially, significant delay of the time-to-hatch were observed after the exposure to BPA, BPF, BPS, and BPZ [83]. Longer hatching duration of embryos can make them more susceptible to predators and mortality via environmental factors [88]. The effective concentration of BPA was up to 5~25-fold greater than those of BPF or BPS [83]. These results suggest that the endocrine disruption potential of BPF and BPS is no less than that of BPA.

3.3.2. Effects on Thyroid Hormones and Genes Related to HPT Axis

Similar to the HPG axis, the thyrotropin-releasing hormone (TRH) with the corticotrophic-releasing hormone (CRH) of the HPT axis secrete thyroid-stimulating hormone (TSH) from the hypothalamus [89]. TSH secreted by the pituitary then regulates the synthesis of thyroid hormone (TH), that is thyroxine (T4) and triiodothyronine (T3). Measurement of thyroid hormones is commonly used in zebrafish, as it is the most integrated endpoint in assessing thyroid endocrine disruption [90]. Zebrafish exposed to BPA [80,83], BPAF [84], BPF [81,83], and BPS [83,86,87] were affected by either activating or suppressing hormone production. Interestingly, significant decreases in T4 concentrations along with increases in TSH concentration were commonly observed in fish exposed to BPF and BPS [81,87]. Moreover, changes in thyroid hormones were more sensitive to the toxicity of BPS than BPA (Figure 5). These results suggest that the thyroid endocrine disruption of BPA alternatives is no less than that of BPA and may significantly affect on thyroid hormone homeostasis. Decreases in T4 could reduce the TH availability in target tissues and subsequently influence metabolic pathways [78,84].

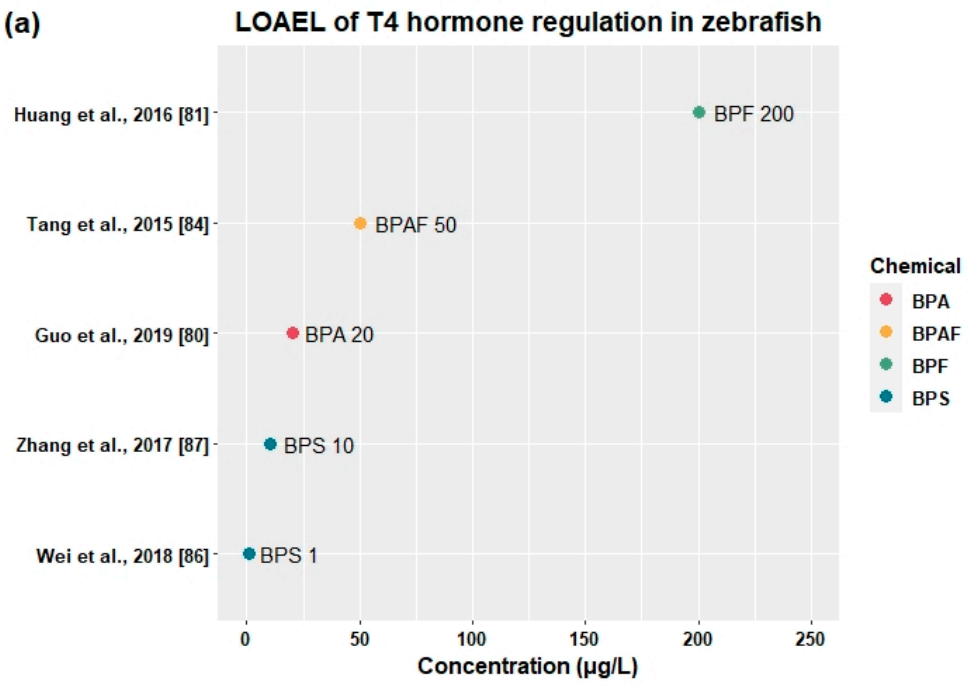


Figure 5. *Cont.*

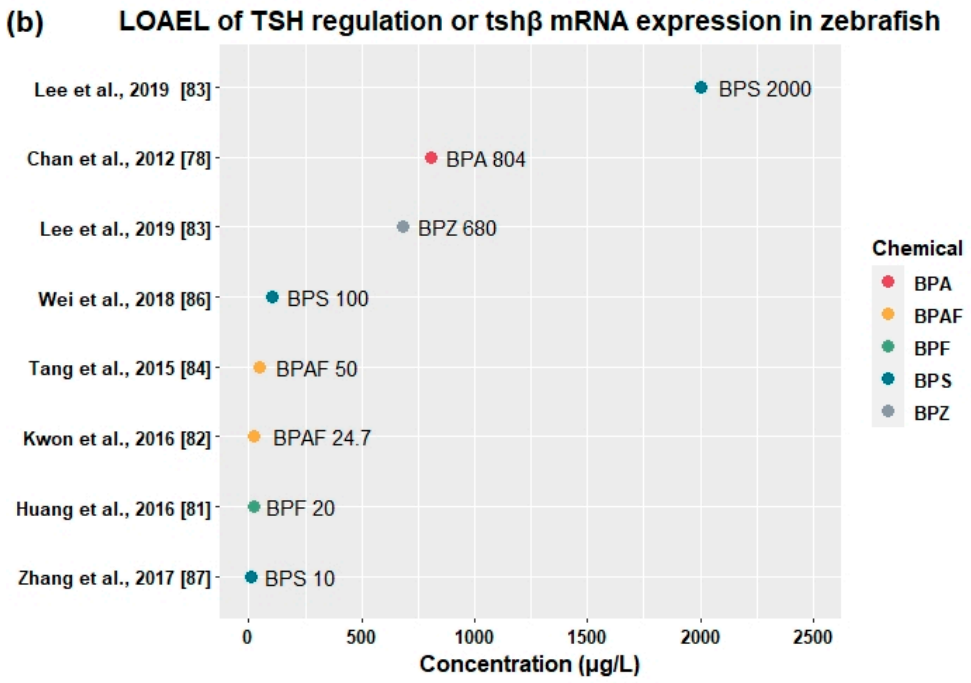


Figure 5. Lowest observed adverse effect level (LOAEL) of thyroxine (T4) hormone (**a**), thyroid stimulating hormone (TSH) or *tshβ* mRNA expression (**b**) in zebrafish exposed to BPA, BPAF, BPF, BPS, and BPZ.

TSH has been measured to assess the potential mechanism of thyroid dysfunction. TSH levels increased significantly after BPA analog exposure [81,87], which might be involved in the negative feedback mechanism. Significant up-regulation of tshβ genes is also well supported by changes in TSH levels. TRH and CRH stimulate TSH secretion, and transcription of trh and crh genes were significantly upregulated after exposure to BPAF, BPF, and BPS [81–83,86,87]. The results of these studies suggest that the increase in TSH concentrations and upregulation of trh and crh caused by bisphenol exposure may be attributed to the promotion of thyroid hormone synthesis and its subsequent release to compensate for the decreased T4 levels in zebrafish.

The transcription of various genes in the HPT axis has been measured in zebrafish exposed to BPA, BPAF, BPF, BPS, and BPZ. Iodothyronine deiodinases (Dio) are the key regulators of T4 and T3 bioavailability [91], and three types of dio enzymes (Dio1, Dio2, and Dio3) and genes (dio1, dio2, and dio3) have been found in zebrafish [92]. Dio2 enzyme plays a vital role in catalyzing the conversion of T4 into biologically active T3 [91]. Significant increases in dio2 mRNA expression in zebrafish exposed to BPAF [82,84], BPF [81], and BPS [86] have been reported, and an increase in T3 and a decrease in T4 were observed together in some studies [81,86]. These results suggest that BPA analogs potentially affect dio2 gene transcription and thyroid hormone production.

Overall, the results demonstrated that BPA analogs significantly changed thyroid hormone concentrations and modified the mRNA expression of key genes involved in the HPT axis, suggesting thyroid endocrine disruption in zebrafish. BPA analogs and thyroid hormones' chemical structures have similarities; therefore, these chemicals exhibit the property of binding to thyroid receptors and competing with the thyroid hormone [93]. Since endocrine systems interact with each other, further studies are needed to assess BPA alternatives' primary target among the endocrine axes.

4. Conclusions

The present study summarized the endocrine-disrupting potential of BPA and its alternatives in zebrafish and identified knowledge gaps. The summary highlights of this study are as follows:

- BPA alternatives have a similar or more significant toxic potential than that of BPA.
- Several BPA alternatives may cause reproductive dysfunction by interfering in the regulatory mechanisms of the HPG axis or inducing vitellogenin in males.
- Males were more sensitive to the adverse effects on sex hormone levels, as well as gene transcriptions, than females.
- Environmentally relevant concentration of BPA alternatives has the potential to inhibit the normal development of embryo/larvae by disrupting thyroid hormone endocrine system.

The modifications of phenolic rings and bridging carbon or the longer length of the alkyl substitutes seem to influence endocrine-disrupting activity. However, the apparent relationship between their structure and endocrine-disrupting activity was not clarified. Further toxicological information on BPA alternatives is required to understand the environmental health implications of these alternatives and to develop proper management strategies.

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Conflicts of Interest: The authors declare that there is no conflict of interest associated with this work.

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