ORGANOTINS AS A COMPLETE PHYSIOLOGIC AND ENDOCRINE DISRUPTOR: ROLE OF DISEASE DEVELOPMENT

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ORGANOTINS AS A COMPLETE PHYSIOLOGIC AND ENDOCRINE DISRUPTOR: ROLE OF DISEASE DEVELOPMENT

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EDITORIAL
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Editorial: Organotins as a Complete Physiologic and Endocrine Disruptor: Role of Disease Development

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Keywords: organotins, endocrine disrupting chemicals, physiology, dysfunction, disease

Editorial on the Research Topic

Organotins as a Complete Physiologic and Endocrine Disruptor: Role of Disease Development

The metal tin and its alloys have a historically important role for humanity. Studies have reported the existence of organotin (OT) since 1853, but it did not become important in industrial use until the 1940–1960s (Marques et al.). OTs are synthetic chemical tetravalent derivatives of tin (IV) with a general formula of R(4-n) SnXn, where R represents organic substituents and X can be a halide, anion, or an organic group linked covalently through a heteroatom (O, N, S, Cl, etc.) (Nunes-Silva et al.). Mono-, di-, tri-, and tetra-OT have many industrial applications, including as PVC catalysts and broad-spectrum biocides, as well as in antifouling paints for marine ships (Nunes-Silva et al.). As a consequence, the main inputs of tri-OT tributyltin (TBT) into the environment are through contamination of water and sediments by improper disposal of antifouling products. TBT has a degradation half-life of days to months in water and up to several years in sediment. TBT is accumulative in different organisms along of the food chain (Fernandez). Thus, aquatic organisms can be exposed by a contaminated habitat (water and sediment) and/or ingestion of contaminated food. Terrestrial organisms may also be exposed via OT- and TBT-contaminated sediments and by the intake of contaminated food or water. Thus, for the majority people, the main route of OT exposure is intake by consumption of contaminated water and foods, as for example, marine foods (Marques et al.; Nunes-Silva et al.; Fernandez).

Through previous studies, we have learned that the use of TBT as the active component in the marine antifouling ship paints has increased because of its particularly potent algicide/molluscicide effects (Fernandez; Vogt et al.). For example, OT exposure (mainly as TBT) can lead to imposex development, the abnormal induction of male sex features in female gastropod mollusks, representing one the clearest examples of environmental endocrine disruption chemical (EDC) action (Marques et al.; Nunes-Silva et al.; Fernandez; Vogt et al.). In addition, another study has shown that TBT exposure can also induce masculinization in fish species (Berto-Júnior et al.). Widespread environmental contamination of marine ecosystems with TBT began in the 1960s, leading to several adverse effects in numerous organisms. Therefore, for this reason, its use in antifouling ship paints was prohibited by the International Marine Organization (IMO) in 2008 (Fernandez; Vogt et al.). However, beyond its continued utilization in industrial and other processes, it is possible that TBT is still employed in some parts of the world, particularly in countries that are not included in the Antifouling Systems (AFS) convention and/or have poor environmental monitoring/supervision (Fernandez; Barbosa et al.). Unfortunately, previous investigation has confirmed that recreational vessels sampled from north European countries contain high TBT levels in their paints and may be a source of it into the environment (Fernandez; Barbosa et al.). Other important recent studies have shown a higher level of OT pollution in commercial and wild oysters from Asia (Fernandez; Barbosa et al.).

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OTs are a diverse organometallic group of widely distributed environmental xenobiotics, and, today, more than 800 OTs are known (Marques et al.; Nunes-Silva et al.; Fernandez; Vogt et al.; Berto-Júnior et al.; Barbosa et al.). OTs have complex toxicological effects on both invertebrate and vertebrate endocrine systems (Fernandez; Vogt et al.; Barbosa et al.; de Araújo et al.). The story of OTs, as well as that of TBT, is far from reaching an end; in fact, the discovery of its new potential EDC actions has placed it again at the forefront of scientific research. In the USA, more than 80,000 chemicals are registered with the Environmental Protection Agency (EPA), some of which are known or potential EDCs. About 1,000 synthesized chemicals are considered to be EDCs, defined as "exogenous chemical[s], or mixtures of chemicals, that interfere with any aspect of hormone action, as organotin chemicals" (de Araújo et al.). A number of issues have proven to be key to a full understanding of the toxicological mechanisms of action and consequences of exposure to OT as an EDC, for example, age at exposure, latency from exposure, the importance of mixtures with other EDCs, non-traditional dose-response dynamics, and transgenerational, epigenetic effects (Marques et al.; Nunes-Silva et al.; Fernandez; Vogt et al.; Berto-Júnior et al.; Barbosa et al.; de Araújo et al.).

Several *in vitro* and *in vivo* studies on gastropods, crustaceans, amphibians, fish, rodents, and humans demonstrated that TBT is able to interfere with many physiologic processes, thereby inducing complex toxic effects (Marques et al.; Nunes-Silva et al.; Fernandez; Vogt et al.; Berto-Júnior et al.; Barbosa et al.; de Araújo et al.). A wide range of detrimental responses are observed in mollusks, crustaceous, cephalopods, fish, and amphibians exposed to low levels of TBT (0.1–100 ng/L), such as imposex, apoptosis, irregular metamorphosis, and other important abnormalities (Fernandez; Berto-Júnior et al.; de Araújo et al.). Additionally, OT may accumulate in birds and sea mammals, leading to reproductive and metabolic dysfunctions (Fernandez; de Araújo et al.). In rodent models, toxicological studies have shown reproductive, cardiovascular, renal, respiratory, neural, and other abnormalities with different TBT doses (100 ng−100 mg/Kg) (Marques et al.; Nunes-Silva et al.; Barbosa et al.; de Araújo et al.; Ronconi et al.; Ferraz da Silva et al.). Therefore, data in different animal models demonstrate the deleterious effects of TBT exposure on multiple organ and species systems.

TBT is an obesogenic chemical (EDC-subclass) thought to induce obesity and other metabolic abnormalities by increasing the number and/or size of fat cells and/or altering the mechanisms through which the body regulates appetite and satiety (Berto-Júnior et al.). Therefore, obesogen chemicals display the potential to disrupt multiple metabolic pathways in the developing organism, which might result in permanent changes in adult physiology, in various experimental species models (Berto-Júnior et al.; de Araújo et al.). Several TBT obesogenic effects are mediated by PPAR-γ signaling, which acts as a key regulator of adipocyte differentiation and as a transcriptional regulator and/or effector of target genes, such as C/EBP (CCAAT/enhancer-binding proteins), AFABP (adipocyte-specific fatty acid-binding protein), and FATP (fatty acid transport protein) (Berto-Júnior et al.; de Araújo et al.).

TBT is able to impair different physiology functions as result of an increase in oxidative stress processes (Marques et al.; Ronconi et al.). Rodent studies reported that TBT exposure (100 ng/kg/day) led to reproductive tract, neuronal, renal, and cardiovascular oxidative stress (Marques et al.; de Araújo et al.; Ronconi et al.; Ferraz da Silva et al.). Additionally, TBT exposure (0, 1, 10, and 100 ng L^{-1}) played a key role as an inducer of oxidative stress and a positive modulator of pro-inflammatory cytokines in a zebrafish model (Berto-Júnior et al.).

This Research Topic brings together nine review papers on the different and complex toxicological role of organotin in the environment, in wild species, such as crustaceans, gastropods, amphibians, and fish, and in rodent and human experimental models. Understanding the interplay between organotin, as well as TBT exposure from different sources, and physiological abnormalities is highly relevant for wildlife and human health. Evidently, investigation in this field is advancing at a rapid pace. The articles in this Research Topic highlight novel findings and unanswered questions for future investigation.

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The author confirms being the sole contributor of this work and has approved it for publication.

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MINI REVIEW
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Organotins in Neuronal Damage, Brain Function, and Behavior: A Short Review

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The consequences of exposure to environmental contaminants have shown significant effects on brain function and behavior in different experimental models. The endocrine-disrupting chemicals (EDC) present various classes of pollutants with potential neurotoxic actions, such as organotins (OTs). OTs have received special attention due to their toxic effects on the central nervous system, leading to abnormal mammalian neuroendocrine axis function. OTs are organometallic pollutants with a tin atom bound to one or more carbon atoms. OT exposure may occur through the food chain and/or contaminated water, since they have multiple applications in industry and agriculture. In addition, OTs have been used with few legal restrictions in the last decades, despite being highly toxic. In addition to their action as EDC, OTs can also cross the blood–brain barrier and show relevant neurotoxic effects, as observed in several animal model studies specifically involving the development of neurodegenerative processes, neuroinflammation, and oxidative stress. Thus, the aim of this short review is to summarize the toxic effects of the most common OT compounds, such as trimethyltin, tributyltin, triethyltin, and triphenyltin, on the brain with a focus on neuronal damage as a result of oxidative stress and neuroinflammation. We also aim to present evidence for the disruption of behavioral functions, neurotransmitters, and neuroendocrine pathways caused by OTs.

Keywords: behavioral impairments, brain function, environmental contaminant, endocrine disruptor, neurotoxicity, neurodegeneration, neuroinflammation, oxidative stress

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INTRODUCTION

In recent neurotoxicology studies, there is a growing interest in chemical pollutants with endocrine disruptor properties (1). Endocrine-disrupting chemicals (EDCs) are compounds capable of altering and modulating the normal functioning of the endocrine system, either increasing or blocking the synthesis, release, and action of a natural hormone, or acting like a xenohormone and mimicking the physiological effects of a particular endogenous hormone (1, 2). EDCs with neurological and behavioral effects include bisphenol A, phthalates, pesticides, and organometallic compounds, such as methylmercury and organotins (OTs) (3–5). OTs are organometallic compounds with one or more bonds between a carbon atom and a tin atom. They interfere with the metabolism of the gonadal and metabolic hormones (6, 7) and present cytotoxic and genotoxic effects, notoriously

trespassing the blood–brain barrier and presenting neurotoxic effects that lead to nervous system abnormalities (8–11). The outspread use of OTs in the agriculture and industry led to environmental and occupational incidents, as well as their banishment in several countries (12–15). This mini-review aims to summarize the toxicity of the main OTs in the brain and briefly presents what is known about their impact on behavior and on the central nervous system function of experimental animal models and humans.

TRIMETHYLTIN (TMT) NEUROTOXICITY

Trimethyltin is one of the most commonly used OTs in industry and agriculture, known for its role as a fungicide and plastic stabilizer (16). The symptoms of TMT intoxication in humans have been documented after the report of two cases, as described by Fortemps et al. (17). TMT exposure could be associated with neurological disorders, such as headaches, vigilance loss, disorientation, memory deficits, and tonic–clonic seizures. TMT also leads to developmental abnormalities in animal models, as TMT exposure causes morphological changes in the rodent hippocampus, leading to reduced expression levels of reelin (18), which is an important glycoprotein in the extracellular matrix that is involved with the migration of postmitotic neurons in the cerebral cortex and the synaptic plasticity in the developing brain (19).

Increased levels of reactive oxygen species (ROS), protein carbonyl, and malondialdehyde—biomarkers of protein and lipid peroxidation—were found in the rat hippocampus after TMT exposure (20). Those markers for oxidative stress were followed by behavioral abnormalities. The homeostasis of several antioxidant mechanisms can be altered by TMT in the hippocampus, with a decrease in the expression levels of enzymes, such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), and an increase in glutathione *S*-transferase (GST), a detoxifying enzyme of which high levels are considered a signal of tissue damage (21, 22). Neuroinflammation is another outcome of TMT intoxication, and several biomarkers, such as activated glial cells and the expression of inflammation-related genes, appear in the brain of TMT-treated rodents. Both astrocytes and microglia release inflammatory cytokines during brain injury process, microglia being mainly responsible for the inflammatory response (23). TMT induces the activation of microglia and astrocytes and leads to the increase of the inflammatory mediators released by them. The accentuated increase of interleukin IL-1β, IL-6, and tumor necrosis factor TNF-α levels in mice hippocampus, as well as iNOS, arginase-1, IL-1β, TNF-α, and IL-6 levels in cultured astrocytes, follows TMT exposure (23). It is also reported that the TMT exposure is capable of impairing the late stages of autophagic flux in primary cultured astrocytes, leading the accumulation of protein aggregates in the cell (24). Genes involved in neuronal differentiation and astrocyte activity, inflammatory response, and apoptosis are overexpressed in the dentate gyrus region of the hippocampus, while such upregulation is not found in the *cornu ammonis* regions (25), adding evidence to the suggestion that TMT intoxication causes specific neuronal damage (26). We can conclude that the hippocampus is an especially TMT-vulnerable structure in the mammalian brain.

Other important studies have been proposed to explain the physiological mechanism of TMT intoxication. Neuropeptide Y and somatostatin are both upregulated in the rat hippocampus in the first 4 days after TMT exposure, correlating to the occurrence of seizures. Treatment with the anticonvulsant phenobarbital blocked the seizures and the upregulation of those hormones (27). Ogita et al. (28) presented some very interesting data that showed an influence of adrenal hormones after TMT exposure. Whereas aldosterone, an important mineralocorticoid hormone, increased TMT cytotoxicity in the mouse hippocampus, the mineralocorticoid receptor antagonist, spironolactone, protected neurons against the TMT effects. The blocking of the glucocorticoid receptor through administration of the antagonist, mifepristone, had effects similar to those caused by aldosterone (28). Similar studies linking both endogenous and exogenous glucocorticoids with the attenuation of TMT brain damage were found in the literature and suggested that the modulation of glucocorticoid receptors interferes with the oxidative stress and cytokine expression related to TMT rodent neurodegeneration models (29–31).

TRIBUTYLTIN (TBT) NEUROTOXICITY

Tributyltin is a highly toxic OT, used initially as a plastic stabilizer and a molluscicide in agriculture, but due to its higher biocide potential, TBT was subsequently extensively used as an antifouling agent in the ships and other marine structures to prevent the adhesion of plankton and other organisms (32). Several studies have reported TBT as a reproductive toxic compound, hepatotoxic, nephrotoxic, and obesogen, and it exerts its toxicity in several groups of organisms (33–36). Neurotoxicity is also described in various experimental organisms and in cultured brain cells after TBT exposure, with oxidative stress and neuroinflammation being a common feature after TBT exposure, which leads to subsequent brain impairments. Mitra et al. observed that TBT-chloride *in vivo* exposure in different concentrations of 10, 20, and 30 mg/kg is capable of disrupting the blood–brain barrier and metal metabolism in the rat brain, followed by protein carbonylation and lipid peroxidation, 4 days after administration. TBT *in vivo* also caused astrocyte activation and overexpression of inflammatory molecules, such as IL-6, Cox-2, and NF-κB. In the same study, TBT *in vitro* led to neurodegeneration and apoptosis by the activation of caspase-3 and -8 (37). Evidence also suggests that TBT induces neuronal damage by suppressing the effects of GST, an important cellular antioxidant mechanism, and subsequently generating ROS (38). The endocrine disruption caused by TBT leads to reduced levels of estrogens by the competitive inhibition of aromatase (39). As the antioxidant role of estrogens in the central nervous system has been reported (40), it is possible to assume that TBT also influences the ROS generation in the brain by suppressing the circulating levels of ovarian estrogen. Ishihara et al. demonstrated that pretreatment with 17β-estradiol is capable to suppress the neuronal injury *via* oxidative stress in cultured hippocampus slices by the activation of the Akt signaling (41). It is interesting to note that, as we cited above, it is suggested that TBT inhibits GST activity, and the

decreased activity of Akt in the cardiac tissue and subsequent oxidative stress can be caused by other GST inhibitors (42), suggesting that TBT-induced oxidative stress occurs through GST inhibition *via* Akt deactivation induced by low levels of estrogen in the brain, as summarized in **Figure 1**.

Rat neurons cultured with astrocytes are less vulnerable to TBT toxicity than neurons cultured purely in primary neuron cultures, indicating that astrocytes play an important role in neuroprotection against TBT toxicity effects (43). Although the hippocampus is one of the main targets of TBT toxicity, considering the deleterious effects following TBT exposure, the striatum seems to be more vulnerable than other brain regions, as ROS generation, protein carbonylation, and lipid peroxidation are more prominent in this region (44, 45). The damage in the hippocampus and the striatum caused by TBT may have cognitive implications, considering that those areas are involved in mammalian memory and learning (46, 47), but further studies in this specific subject are necessary to evaluate this possibility. Based on the presented evidence, we can infer that, like TMT toxicity that targets specific regions of the hippocampal formation, TBT also has differential effects in different brain regions and cell types.

On a cognitive point of view, estrogens are modulators of different types of memory, and their low serum levels due to aromatase inhibition or ovarian failure may be associated with behavioral abnormalities (48). In fact, the brain itself produces estrogens *via* brain aromatase, and this *de novo* synthesis in the hippocampus is fundamental to proper functioning of memory systems (49). In certain animal models, TBT can inhibit not only gonadal aromatase but also brain aromatase, as is observed in the teleost fish. In the Atlantic salmon (*Salmo salar*), a 7-day TBT exposure impaired neurosteroidogenesis, decreasing both the expression of the cytochrome P450 aromatase gene and activity of the expressed aromatase (50). The same gene-suppressive effects of TBT were found in male guppies (*Poecilia reticulata*), where two isoforms of brain aromatase were under-expressed with subsequent alterations in reproductive behavior, after TBT treatment (51). Therefore, the impact of TBT exposure on endocrine systems and its subsequent influence on the nervous system can be diverse.

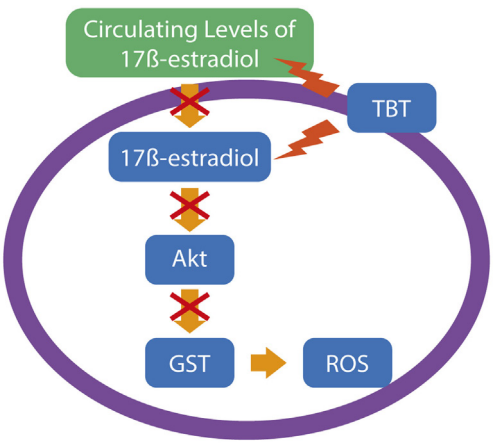

FIGURE 1 | A possible pathway for tributyltin (TBT)-generated oxidative stress in neuronal cells. Considering the data of Ishihara and colleagues (41), supported by data of other groups, TBT leads to neuronal reactive oxygen species (ROS) production by a reduction in the estrogen levels, leading to impairments in the Akt signaling and subsequent downregulation of glutathione *S*-transferase (GST), an important antioxidant mechanism to protect neuronal normal function.

Evidence suggests that the TBT obesogenic effects are not only due to influences on energy metabolism but also on food intake, as follows. Neuropeptide Y is overexpressed in female rats treated for 54 days with TBT (0.5 μg/kg), and their food intake was also increased (52). TBT exerts its toxicity in other regions of the hypothalamus, as it is shown through the disruption of the rat hypothalamus–pituitary–adrenal axis (53), although there is no current knowledge about the influence of the adrenal hormonal imbalance on brain injury caused by TBT, as reported in TMT intoxication cases. We can conclude that, besides causing obvious anomalies in the reproductive behaviors through alterations in the sexual hormone balance, TBT is also capable of interfere with behavior centers in the hypothalamus.

NEUROTOXICITY OF OTHER OTs

Here, we aim to present briefly what is currently known about the neurotoxicity of dibutyltin (DBT), triethyltin (TET), and triphenyltin (TPT), as well as other OTs used in industrial activities (54, 55).

Dibutyltin is a TBT metabolite, and related compounds are used as catalysts in the fabrication of polymers, such as silicone (54). The use of DBT in the polymerization of polyvinyl chloride (PVC) is a focus of special attention, considering that PVC is largely used in water containers and tubes (56). A study with pregnant female rats exposed to DBT (10, 25 ppm, from gestational Day 6 to postnatal Day 21) demonstrated that tin can accumulate in the brain and placenta, is able to cross the placental barrier and is transferred to the offspring (57). TBT is mainly metabolized in the liver, considering the high hepatic concentrations of its metabolites, including DBT, but evidence shows that part of the TBT is also converted into DBT in the brain (58). The brain is highly susceptible to DBT toxicity, and Jenkins et al. showed that DBT causes neuronal death in concentrations 40-fold lower than TMT (59). A sub-chronic exposure to DBT (5, 10, and 20 mg/kg) increased the levels of malondialdehyde, a product from lipid peroxidation, while it decreased the activity of two major antioxidant enzymatic pathways, SOD and GPx, in the rat brain. It was also found to cause DNA damage in the cerebral cortex, probably as a result of oxidative stress. We can assume that, similar to TBT, DBT induces oxidative stress in rat cortical cells (60). The physiological mechanism underlying the DBT-induced oxidative stress is yet to be fully determined. Neurotransmitter systems are influenced by DBT intoxication, as DBT exposure decreased the levels of dopamine and serotonin in the striatum and frontal cortex, respectively, causing impairments in learning and decreased spontaneous locomotion (61). Cholinergic neurotransmission is also affected, as DBT is capable of decreasing the activity of choline acetyltransferase, the uptake of choline into synaptosomes and the myelin content of cholinergic neurons of rodents (62, 63). As the data were obtained through *in vitro*

experiments, the behavioral outcomes of the impaired acetylcholine neurotransmission have yet to be evaluated.

Triethyltin is an environmental contaminant that comes from industrial activities, similar to TMT and TBT (55). The main neurotoxic effects of TET, including demyelination of neurons and edema, are already well-described (64–66), although the molecular mechanisms underlying such effects have yet to be discovered. TET-exposed oligodendrocyte cultures presented disruption in the mitochondrial membrane potential, disturbances in the cytoskeleton, and signs of oxidative stress and apoptosis (67), concluding that, besides causing vacuolization of the myelin sheath, TET is also cytotoxic to oligodendrocytes, the glial cells responsible for forming the myelin in the mammalian central nervous system. In addition to oligodendrocytes, astrocytes are also susceptible to TET neurotoxicity (68). The TET influence on cytoskeleton is also described in primary neuron cultures, where it interferes in actin polymerization through imbalances in calcium metabolism, leading to abnormal neurotransmitter release in different neural cell lines (69). Evidence indicates that calcium homeostasis in the brain is sensible to TET exposure, as it is also capable of inducing noradrenaline spontaneous release in rat cultured hippocampal slices by altering the functioning of calcium channels (70).

Triphenyltin was used as a biocide in antifouling marine paints and acts like an endocrine disruptor, impairing the synthesis of estrogens (55). With this in mind, it is safe to assume that TPT exposure is very similar with TBT exposure. TPT is capable of impairing the expression of brain aromatase when administered in certain periods of developments in rats (71). What is remarkable about TPT neurotoxicity is its effects as an excitotoxic compound. In an isolated cell experiment, TPT increased neuronal excitability through alterations in the voltage-dependent Na^+ current of a hippocampal pyramid cell (72). Another mechanism proposed to explain TPT-induced excitotoxicity is through modifications in glutamatergic transmission by modulation of calcium homeostasis in the pre-synaptic terminal (73). It is not determined yet if calcium modulation in hippocampal neurons by TPT is similar to that observed in TET exposure.

CONCLUDING REMARKS

Although most of the OTs with commercial and environmental relevance share many similarities in their chemical properties, the physiological mechanisms underlying their neurotoxicity are vast and sometimes not fully understood. Each compound has various neuroendocrine and behavioral outcomes in different groups of vertebrates, and a close examination of their biological influence is very important to increase our current knowledge about occupational and environmental health and safety. In summary, OTs are potent neurotoxicants, leading to behavioral impairments due to brain damage in various levels caused, mainly, by oxidative stress and neuroinflammation.

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The Pollutant Organotins Leads to Respiratory Disease by Inflammation: A Mini-Review

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Organotins (OTs) are organometallic pollutants. The OTs are organometallic pollutants that are used in many industrial, agricultural, and domestic products, and it works as powerful biocidal compound against large types of microorganisms such as fungi and bacteria. In addition, OTs are well known to be endocrine-disrupting chemicals, leading abnormalities an "imposex" phenomenon in the female mollusks. There are some studies showing that OTs' exposure is responsible for neural, endocrine, and reproductive dysfunctions *in vitro* and *in vivo* models. However, OTs' effects over the mammalian immune system are poorly understood, particularly in respiratory diseases. The immune system, as well as their cellular components, performs a pivotal role in the control of the several physiologic functions, and in the maintenance and recovery of homeostasis. Thus, it is becoming important to better understand the association between environmental contaminants, as OTs, and the physiological function of immune system. There are no many scientific works studying the relationship between OTs and respiratory disease, especially about immune system activation. Herein, we reported studies in animal, humans, and *in vitro* models. We searched studies in PUBMED, LILACS, and Scielo platforms. Studies have reported that OTs exposure was able to suppress T helper 1 (Th1) and exacerbate T helper 2 (Th2) response in the immune system. In addition, OTs' contact could elevate in the airway inflammatory response, throughout a mechanism associated with the apoptosis of T-regulatory cells and increased oxidative stress response. In addition, OTs induce macrophage recruitment to the tissue, leading to the increased necrosis, which stimulates an inflammatory cytokines secretion exacerbating the local inflammation and tissue function loss. Thus, the main intention of this mini-review is to up to date the main findings involving the inflammatory profile (especially Th1 and Th2 response) in the respiratory tract as a result of OTs' exposure.

Keywords: airway disease, endocrine-disrupting chemicals, inflammation, organotin compounds, reactive oxygen species

INTRODUCTION

The high levels of toxic compounds in the biosphere are elevating the human needs of better comprehension on their impact on earth life and modern society. The utilization of agricultural chemicals products is raising in the world. Concomitantly, the scientific community can observe the incidence of many diseases, such as diabetes, neurodegenerative diseases, asthma, and fertility alterations (1). According to World Health Organization, some toxic compounds are capable of inducing alterations in the endocrine system *via* inflammatory commitment and have been named as endocrine-disrupting chemicals (EDCs). Nowadays, is well known that EDCs is able to modulate important hormone-signaling pathways activities and have received great attention as an inductor of reproductive abnormality and also a chronic obstructive pulmonary disease (2).

Organotins (OTs) is well known as a EDCs. The OTs are organometallic pollutants used in various domestic, industrial, and agricultural products, as a powerful biocidal compound that works against large types of microorganisms, such as fungi and bacteria (3). Summarizing, OTs are frequently used as a chemical part of commercial products because they have a strong biocidal activity against a large spectrum of microorganisms as mentioned before. Tributyltin (TBT), that is a class of OTs, contains (C4H9)3Sn group and are used in many industry products: an example, in wood preservation, antifouling paints for boats and ships, disinfection of circulating industrial cooling water, and in a slime control in paper factory (3). There is some scientific evidence that TBT is able to masculinize the sex organs of the female of several species of meso- and *neogastropods* resulting in a development of a penis and a vast deference along with the female sex organs in these mollusks (4–6). In addition, it has also been also reported that OTs is able to modulate the immune system behavior in mammalian (2, 7). In an animal model, Ohtaki et al. (8), short-term feeding studies using OTs compounds in rats, observed atrophy of the thymus, decreased numbers of lymphocytes in spleen and lymph nodes, as well as increased serum imunoglobulin M level and decreased serum in imunoglobulin G levels (8). This study also shows that mice exposed to TBT compounds were able to reduce spleen weight and induced the reduction in the number of leukocytes and T helper 2 (Th2) polarization (8). Once, OTs are found in various products, human blood and urine have been used to monitor the human exposure to these compounds. However, there are only a few papers involving these quantifications in human. In 1999, Whalen and colleagues (9) detected OTs in human blood at 64–155 ng/mL levels. This OTs' presence in human blood was able to compromise the natural killer (NK) cells activity *in vitro* (9). Also, Brown and colleague, in 2017 (10), reported that OTs alters the interleukin 6 (IL-6) secretion from human immune cells and consequently, affect the immune competence (10). In 2014, Valenzuela and colleagues (11) developed an efficient methodology capable to identify 11 OT compounds in urine from harbor workers exposed to antifouling paints. This methodology confirms the possible human contamination by exposure (11).

The complex immune system is able to develop two different kinds of responses, innate and adaptive to fight against foreign pathogens. These two immune responses involve different immunological effector functions; however, these effectors functions work together through compensatory mechanisms with each other to coordinate optimal immune responses (12). Between these two types of immunity response, innate immunity plays important roles in both detecting invading pathogens and developing a specific adaptive immunological response. Therefore, as discussed until now, the adequate innate immune responses are necessary to prevent several infectious diseases (13) and among cells that comprise the immune system, macrophages play a critical role in the innate immune response to pathogens. However, there is no many information about OTs' (such as TBT) contamination and immune cells in the airway inflammatory disease scenario (**Table 1**).

These cells are sensible to many molecules, including pathogen-associated molecular patterns (PAMPs) and after recognizing by these PAMPs, macrophages are activated, and they induce an inflammatory response by producing pro-inflammatory mediators (13). Kim et al., *in vitro* study, showed that TBT was able to decrease the nitric oxide production in murine macrophage cell line culture (RAW 264.7) and induce significant cell death through mechanisms that varied with the individual chemical (13). The macrophage is an immune cell that is present, as a resident cells, in almost every organ in the body, and they represent the first type of cell that phagocytoses (engulfs a solid particle)

TABLE 1 | Summary of effects of organotins detected using animal experiment and human cell exposure.

Reference	Human/ animal/*vitro*	Experimental designed	Inflammatory mediator	Conclusion
Whalen et al. (9)	Human/*vitro*	Culture	Natural killer cell function	Tributyltin (TBT) affected cell viability
Stridh et al. (43)	Human/*vitro*	Exposed	Lymphocytes apoptosis	TBT induced non-apoptotic morphology
Muller et al. (44)	Human/*vitro*	Exposed	Lymphocytes apoptosis	Lymphocytes more resistant to apoptosis
Ohtaki et al. (8)	C57BL/6 mice	Diet for 2 weeks	Lymphocytes polarization	T helper 2 (Th2) polarization
Kato et al. (2)	C57BL/6 mice	Oral administration of TBT	Modulation of interleukin 10 and IL-12	TBT promotes Th2 polarization
Isomura et al. (18)	*Vitro*	Culture	Neuroblastoma cells (SH-SY5Y)	TBT causes ER stress in human neuroblastoma
Kim et al. (13)	*Vitro*	Culture	Macrophages cells (RAW 264.7 cells)	TBT exerts pathological effects in allergic disease
Kato et al. (19)	C57BL/6 mice	1 μmol/kg TBT (oral gavage)	Modulation of interferon gamma, interleukin 4, and IL-17	TBT induced Treg cells apoptosis
McPherson et al. (14)	Mice (pathogen-free CD-1 male)	i.p. (2.3 mg/kg TMT)	Microglia polarization	TMT elevates M2 anti-inflammatory marker
Brown et al. (10)	Human	Exposed	Interleukin-6	Decrease the secretion of interleukin 6

foreign materials. Thus, it is possible to assume that most EDCs are phagocytosed by macrophages. However, it is no possible to assume whether these cells are influenced by these chemicals through phagocytosis or through receptor-mediated signaling. McPherson et al., *in vivo study*, reported that the androgen hormone receptor is expressed on macrophages surface and that their signaling through this receptor can modify the function of macrophage (13, 14).

In addition, TBT has been known to reduce the cytotoxic activity of NK cells (15). It has been suggested that many pollutant environmental factors are involved in an aging process, elevation of inflammation and oxidative damage to brain tissue. Reactive oxygen species (ROS), produced from many sources, can react with cellular macromolecules such as proteins, lipids, and DNA. Chemicals products that increase ROS production and inflammation may certainly aggravate the situation and may act as a predisposing factor for neurodegenerative diseases (7, 16). Furthermore, studies have reported that OTs exposure was able to suppress T helper 1 (Th1) response, induce exacerbated Th2 immunity and increase airway inflammation, through a mechanism associated with the apoptosis by T-regulatory cells and increased oxidative stress response (2). In addition, OTs induce macrophage recruitment, leading to necrosis increased, stimulation of an inflammatory cytokines secretion in the local inflammatory site (15). Thus, the main intention of this review is to summarize the late findings involving the inflammatory profile as result of OTs exposure directly and/or indirectly associated with developing the abnormal endocrine function.

THE ROLE AND MECHANISM OF Th1/Th2 CELLS ACTIVATION DURING INFLAMMATORY RESPONSE TO RESPIRATORY DISEASE

Tributyltin chloride (TBTC) is an OT compound containing TBT groups that has been used as the heat stabilizer for polyvinyl chloride and catalysts for esterification (3). TBTC is found in industries biocides, wood preservatives, agricultural fungicides, and disinfecting agents in circulating industrial cooling waters, as well as in antifouling paints for marine vessels (3, 17). However, these OTs compounds exhibit various toxicities in mammal organs and organic systems (18) such as adipose tissue, kidney, liver, and lung (19–21), as well as toxicity in the reproductive systems in mammals (3). In pathological features of air pathway inflammatory response, including inflammation induced by the toxicity of TBTC, there is denudation of airway epithelium, collagen deposition, edema, mast cell activation, and inflammatory cell infiltration. Furthermore, the classical inflammatory response induces the elevation of the expression and content of multiple inflammatory mediators in the respiratory tract, including cytokines, chemokines, adhesion molecules, PAMPs, damage-associated molecular pattern, and ROS production (22). This inflammatory scenario results in the activation of immune system and contributes to the recovery of body homeostasis.

The immune system develops both innate (immediate and non-specific) and adaptive (gradual build-up, highly specific, and long-lasting) immune responses to recover the homeostasis in the tissue and also to fight against infection induced by pathogens (23). The first one is orchestrated by neutrophils, monocytes and NK cells that destroy the viruses, bacteria, and fungus, and the second one is orchestrated by lymphocytes (B-cells and T-cells). Lymphocytes B-cells produce antibodies (immunoglobulin), and lymphocytes T-cells are involved primarily in the cell-mediated immune response (23). T-cells and their mediators are involved in inflammation in many diseases scenarios such as diabetes, infectious disease, rheumatoid arthritis, and these cells are likely involved in the pathophysiology of some types of allergy diseases (24). There is two main subpopulations of T-lymphocytes (T-cells). They are differentiated by the presence of cell surface proteins, called cluster of differentiation (CD), and they are classified as lymphocytes CD4 and lymphocytes CD8. T-cell lymphocytes that express CD4 are also known as helper T-cells, and these are regarded as being the most prolific cytokine producers (25).

Cytokines, such as IL-6, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), are small proteins that are involved in autocrine, paracrine, and endocrine signaling as immunomodulating agents, and work as the hormonal messengers responsible for most of the biological effects in the immune system, such as cell-mediated immunity and allergic-type responses (25). Thus, activated lymphocytes (T-cell) are important effector cells in the maintenance of health and controlling diseases, such as inflammatory diseases. Lymphocytes T-cells CD4 can be differentiated into two subgroups: lymphocyte helper type 1 (Th1-cell), and lymphocyte helper type 2 (Th2-cell). Th1 and Th2 are distinguished by the types of cytokines they produce, for example: lymphocyte helper type 1 cells (Th1) produce interleukin 2, TNF-α, and IFN-γ; that is, clearly a pro-inflammatory response and lymphocyte helper type 2 cells (Th2) produce interleukin 4 (IL-4), interleukin 10 (IL-10), and interleukin 13 that induces an anti-inflammatory response (23, 26).

There are no many data highlighting the relationship between OTs and activation of lymphocytes T-cells in a Th1 and Th2 response in respiratory diseases. In this sense, most of analyses of the behavior of the immune system in this scenario are in allergic diseases, such as asthma studies. In an allergic inflammation, there is substantial evidence showing a relevant infiltration of lymphocyte helper type 2 cells bronchoalveolar lung tissue. This subpopulation of lymphocytes is increased in the lungs of allergic asthmatics, as well as increased levels of IL-4, interleukin 5, and IL-10 cytokines, and interestingly the level of Th2 cytokines appears to correlate with the severity of disease (23, 27).

Even some authors have shown that polarization of lung lymphocyte profiles clearly correlates with the sequential development of acute allergic (2). Lloyd and Hessel in a nice revision paper, recently published in Nature, discuss findings from many studies. The authors discuss the results of many studies and defend the idea that there are many potential new lymphocytes T-cell lineages, which suggests that the fate of lymphocyte CD4 subsets may be wider than previously thought. Immunological dogma dictates that, following antigen stimulation and several rounds of division, Th1 and Th2 cells become irreversibly committed to these lineages (28). However, the finding that transforming growth factor beta can subvert Th2 cells to the Th9 cell

lineage has led to the understanding that effector $CD4^+$ T-cell populations might be more plastic than originally thought. In addition, some authors have also shown that Treg lymphocytes and T helper 17 lymphocytes (Th17) cells are not stable populations, and instead have the capacity for dedifferentiation (28). So, the possible therapy in the control of the stimulation of immune system induced by TBT should consider the different types of lymphocytes subpopulations.

EFFECTS OF OT COMPOUNDS IN PULMONARY SYSTEM

Organotin compounds show a high toxicity profile in mammals found in reproductive tracts, liver, and immune system (29, 30). In the pulmonary system, although it has been an important route of exposure, the effects of OT compounds are poorly described and have conflicting findings among organisms.

Tributyltin is the most studied group of OT compounds for all toxicological aspects, including its effects on the pulmonary system. Van Loveren et al. (31) showed that rats exposed to a diet containing up to 80 mg/kg of tributyltin oxide (TBTO) have suppressed NK cells activity in the lungs, which have an important role in surveillance, evident against neoplastic and virus-infected targets (31). This potential immunotoxicity in the lungs could favor respiratory viral infections and neoplasms. Contrasting these findings, Carthew et al. (32) examined whether the exposure of rats to TBTO exacerbated the type of pneumonia caused by pneumonia virus of mice and *Mycoplasma* (32). Animals were exposed for 6 weeks to TBTO in the diet, in a similar protocol employed by Van Loveren et al. (31). Although other signs of TBTO toxicity were found, such as a reduction as a reduction in body and thymus weights, as well as the development of cholangitis, there was no evidence that this OTs favored the occurrence of pneumonia in the animals.

Shelton et al. (33) described a case study about a 52-year-old man, who developed asthma after being, exposed to a carpet deodorizer containing TBTO. Acute symptoms such as retrosternal chest pain, nausea and lethargy appeared a few hours of arriving to work and cleared over 2 days at home (33). On returning to work, exacerbations of symptoms (chest tightness and soreness, dry cough, and wheeze) appeared on at least four occasions within 14 weeks. Although this patient had a smoking history, aeroallergens tests, pulmonary function and the temporal relationship with exposure allowed concluding that TBTO was likely the etiologic factor for this patient's asthma. TBTO also was related to a sore throat, burning nose, and wheezing 24 h after a room had been painted with a paint containing this OT (34). According to Schweinfurth and Gunzel (35), a single 4-h exposure of rats to aerosols of TBTO produced signs of irritation such as nasal discharge, lung edema, and congestion (35).

Although TBT compounds are known to cause irritation of the respiratory tract, eyes, and skin, toxicological data are poorly available. Rats were treated with TBTC, 1 or 5 mg/kg, *via* oral, for 6 weeks. There was observed an increase in lung weight while cell density was reduced 0.3-folds. Indeed, TBTC leads to oxidative stress in the lung, evidenced by ROS production enhancement as well as a bronchi damage with loss of mucosal epithelial lining and fibrocartilaginous shell (36). In a man, cough and difficulty in breathing, characterized by inspiratory discomfort, were observed a few hours after inhaling an unspecified amount of powdered TBTC (37). Shortness of breath and chest discomfort was still present 20 days after the exposure.

In addition, OTs are capable of producing changes in the respiratory system of different organisms. Exposure of man and animals to tricyclohexyltin compounds (tricyclohexyltin hydroxide and tricyclohexyltriazolyltin) employed in agriculture as acaricides lead to severe irritation of airways and pulmonary tissue. In animals, these compounds caused pulmonary lesions and worsening to lung edema after oral or intravenous administration (38).

Triphenyltin (TPT) administered intraperitoneally in rabbits at 16 mg/kg (LD_{50}) immediately lead to hyperpnea. However, beagle dogs dosed with up to 0.62 mg/kg/day of TPT for up to 52 weeks did not show any gross or microscopic alterations in the respiratory tract (39). According to Olushola Sunday et al. (30), tetraorganotins compounds produce respiratory failure as acute effects in mice and dogs, similar to those seen in triorganotins poisoning (30). In a man, cough and difficulty in breathing, characterized by inspiratory discomfort, were observed a few hours after inhaling an unspecified amount of powdered trimethyltin chloride (37). Shortness of breath and chest discomfort was still present 20 days after the exposure.

Diethyltin dichloride produces nasal irritation in mammals after topical administration (40). In rabbits, this compound increased the respiratory rate at lower doses (3–5 mg/kg) (41). Diphenyltin caused generalized weakness and difficulty with respiration in rats that received 100 mg/kg intraperitoneally (42).

CONCLUSION

Although the OTs are widely used in the agro-industry with extreme contact for both human respiratory tracts and skin, the consequence of this exposure remains poorly investigated. Furthermore, the most recent data involving airway inflammation by OTs are from the early 2000s, nearly 20 years ago (2). Considering the context of exposure to stannous compounds, the respiratory system is considered one of the main forms of contact with these toxicants, reinforcing the importance of establishing—or not—a causal relationship between exposure and respiratory diseases.

AUTHOR CONTRIBUTIONS

AN-S, DD, HS, RF, KF, and CC helped to draft the manuscript. LR and IS participated in the study's design. JG and LL contributed to the conception, design, and supervision of the study. LMA answered the reviewer's comments point by point and reviewed all manuscript before submission the final version.

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MINI REVIEW
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Effects of Organotins on Crustaceans: Update and Perspectives

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Organotins (OTs) are considered some of the most toxic chemicals introduced into aquatic environments by anthropogenic activities. They are widely used for agricultural and industrial purposes and as antifouling additives on boat hull's paints. Even though the use of OTs was banned in 2008, elevated levels of OTs can still be detected in aquatic environments. OTs' deleterious effects upon wildlife and experimental animals are well documented and include endocrine disruption, immunotoxicity, neurotoxicity, genotoxicity, and metabolic dysfunction. Crustaceans are key members of zooplankton and benthic communities and have vital roles in food chains, so the endocrine-disrupting effects of tributyltin (TBT) on crustaceans can affect other organisms. TBT can disrupt carbohydrate and lipid homeostasis of crustaceans by interacting with retinoid X receptor (RXR) and crustacean hyperglycemic hormone (CHH) signaling. Moreover, it can also interact with other nuclear receptors, disrupting methyl farnesoate and ecdysteroid signaling, thereby altering growth and sexual maturity, respectively. This compound also interferes in cytochrome P450 system disrupting steroid synthesis and reproduction. Crustaceans are also important fisheries worldwide, and its consumption can pose risks to human health. However, some questions remain unanswered. This mini review aims to update information about the effects of OTs on the metabolism, growth, and reproduction of crustaceans; to compare with known effects in mammals; and to point aspects that still needs to be addressed in future studies. Since both macrocrustaceans and microcrustaceans are good models to study the effects of sublethal TBT contamination, novel studies should be developed using multibiomarkers and omics technology.

Keywords: crustaceans, organotins, endocrine disruption, growth, metabolism, reproduction

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INTRODUCTION

Organotins (OTs) are organometallic compounds in which an atom of tin (Sn) is covalently bounded to one or more organic chains (1). They are considered some of the most toxic chemicals introduced into aquatic environments by anthropogenic activities (1–3). OT's deleterious effects upon wildlife and experimental animals are well documented and include endocrine disruption, immunotoxicity, neurotoxicity, genotoxicity, and metabolic dysfunction including obesity (2, 4). Butyltins (BTs) and phenyltins, the major species of OTs, are widely used for agricultural purposes (insecticides, fungicides), in PVC industry, as industrial catalysts, and as additives on boat hull's paints to avoid encrustations by barnacles, mussels, algae, and other aquatic invertebrates (1–3, 5, 6). Therefore, large quantities of OTs have been released into aquatic ecosystems, either directly as wastewater

treatment plants or indirectly as hull's residues, posing serious environmental risks to non-target species (5, 6). Even though the use of OTs was banned in 2008, as determined by the International Marine Organization in 2001 (7), high levels of OTs can still be detected in different matrices such as surface water, clays, quartz, amorphous silica, natural soils, sediments, and organisms (5, 6, 8–10). OT levels vary in the different matrices and in different geographical regions, since environmental factors (e.g., pH, salinity, temperature) as well as the properties of the matrices can affect their adsorption (5). Recent studies in Europe revealed that OTs are still being released into the environment as outgoing water from boat wash pads, historic paint layers of hulls, and abandoned boats (11).

Marine sediment invertebrates, such as mollusks, ascidians, and crustaceans, can accumulate OTs (6, 8, 12–15). Since mollusks and crustaceans are important fisheries worldwide, many studies on OT accumulation and toxicity were developed in these animals (16, 17). Marine bivalves (mussels, clams, and oysters) tend to accumulate higher OT levels than fishes or crustaceans (13, 14, 16). Tributyltin (TBT) and triphenyltin, the most toxic forms of OTs, are well-recognized endocrine-disrupting chemicals of mollusks causing imposex or masculinization of females in more than 200 species (4, 13, 18, 19). Fishes and marine mammals can be contaminated either by drinking or by ingesting OTs-contaminated invertebrates. Therefore, the consumption of contaminated seafood (fishes, clams, mussels, oysters, crabs, and shrimps) can pose risks to human health (4, 6, 12, 20–22).

Crustaceans form a large and diverse clade of arthropods, whose members are usually free-living aquatic animals, with some terrestrial (isopods), parasitic (fish lice, tongue worms), and sessile (barnacles) species (17, 23, 24). Small crustacean species or microcrustaceans (water flees, brine shrimps, and copepods) and larval forms of larger species of decapods (crabs, lobsters) are major constituents of the zooplankton and have a vital role in the trophic transfer of nutrients and xenobiotics (17, 22, 25, 26). Decapod crustaceans, important worldwide fisheries, are usually marine, with few freshwater (crayfishes) and terrestrial (land crabs) species (17). Since decapods live on the sea floor, they can accumulate OTs dissolved in the water, in their food, or on the sediment (8, 27, 28). However, there is still little information about the mechanisms of OTs' effects in crustaceans. This mini review aims to update information about the effects of OTs on the metabolism, growth, and reproduction of crustaceans; to compare with known effects in mammals, and to point aspects that still needs to be addressed in future studies.

OTs EFFECTS ON THE METABOLISM

The main neuroendocrine center of crustaceans is the X organ–sinus gland system, located inside decapods' eyestalk (**Figure 1**) (29, 30). This system is the functional counterpart of the vertebrate hypothalamus–pituitary axis, controlling many processes such as metabolism, growth, color, and reproduction (17, 29, 31, 32). It secretes neuropeptides, amines (serotonin, melatonin, and catecholamines), and opioids (enkephalins) (29, 32, 33). The most abundant neuropeptide is crustacean hyperglycemic hormone (CHH), which forms a protein family with gonad-inhibiting hormone (GIH), molt-inhibiting hormone (MIH), and mandibular organ-inhibiting hormone (MOIH). As vertebrate pituitary trophic hormones, these neuropeptides regulate other endocrine glands: gonads, androgenic gland, mandibular organ (MO), and Y organ, controlling the synthesis and secretion of other hormones (29, 32, 34).

Both macrocrustaceans and microcrustaceans are considered good animal models to study xenobiotics' ecological and toxicological effects (16, 25, 26, 35–37). Acute toxicity assays of

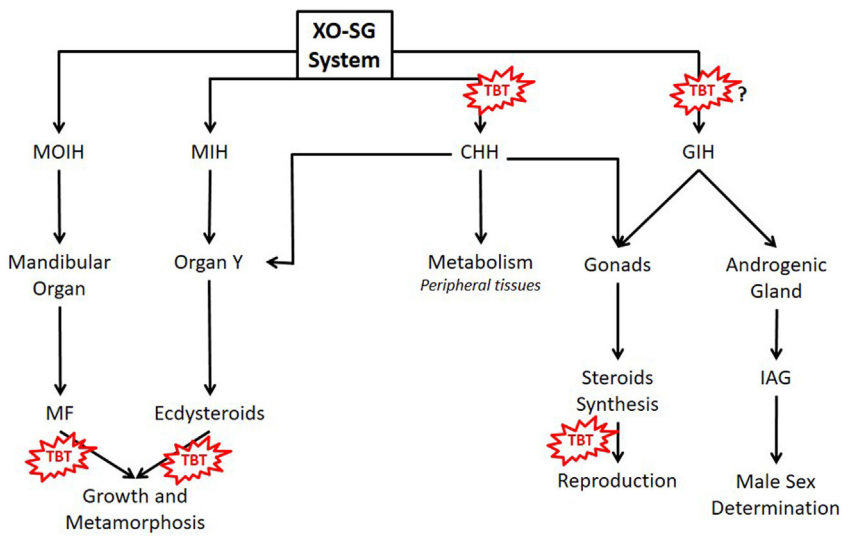

FIGURE 1 | Main hormones controlling metabolism, growth, and reproduction of crustaceans and possible TBT's action sites. MF, methyl farnesoate; MIH, molt-inhibiting hormone; MOIH, mandibular organ inhibitory hormone; CHH, crustacean hyperglycemic hormone; GIH, gonad-inhibiting hormone; IAG, insulin-like androgenic gland hormone; TBT, tributyltin; XO-SG, X organ–sinus gland system.

xenobiotics, useful to assess environmental risks, usually evaluate endpoints parameters such as mortality, egg hatching, development, growth, and reproduction (16, 25, 37, 38). These endpoints are usually expressed as median-lethal or median-effect concentrations (LC_{50} and EC_{50}) and no-observed-effect-level, which can be compared with predicted environmental concentrations in exposure media for purposes of risk assessment (17, 19, 39). Decapod crustaceans exhibit higher LC_{50} values to TBT than mysidacid shrimps, copepods, amphipods, and branchiopods (16, 26, 35, 40). This higher tolerance to TBT of decapods can be related to a faster rate of TBT elimination and/or activation (16). However, larval forms of decapods are highly sensitive to TBT (41). The LC_{50} for TBT of the shrimp *Penaeus japonicus* increased progressively during initial larval stages (nauplius to mysis) and sharply after metamorphosis (41). When the larvae were exposed to hyperosmotic or hypo-osmotic stress, the osmoregulatory capacity was compromised by TBT (41).

Organotins can enter crustacean's hemolymph from water, sediment, or food *via* gills and stomach (28, 42). Once inside the animal, their fate depends on the processes of accumulation, biotransformation (metabolism), and elimination (16, 28, 42, 43). In the hermit crab *Clibanarius vittatus*, assimilation of a single dose of TBT from food was higher than from water, and the levels of TBT in the tissues decreased progressively after 15 days, reaching null values after 75 days (44). In this study, dibutyltin (DBT) was also detected indicating an active metabolism of TBT (44). The hepatopancreas of crustaceans is an important metabolic organ that accumulates functions equivalent to vertebrate pancreas and liver: digestive enzyme synthesis, uptake and storage of nutrients, and xenobiotic's metabolism (42, 45–49). According to their physicochemical properties, xenobiotics can be metabolized in two distinct phases: phase I—oxidation, reduction, and hydrolysis of the substance by the cytochrome P-450 (CYP) system family of proteins; and phase II—conjugation of polar groups to become soluble (28, 42, 50). Crustaceans' hepatopancreas have an active CYP-dependent monoxygenase system that oxidizes TBT to a series of hydroxylated derivatives that are dealkylated to form DBT and/or monobutyltin (MBT) (42, 50–53). When blue crabs *Callinectes sapidus* were fed with TBT-contaminated food, TBT levels in the whole abdomen peaked to 0.12 μg g^{-1} after 4 days of feeding, while DBT and MBT peaked to 0.39 and 0.35 μg g^{-1} after 8 and 12 days of feeding, respectively (54). In another study in which *C. sapidus* were fed TBT-contaminated food, TBT levels were higher in hepatopancreas compared to gills and muscle (43). In a third study in which *C. sapidus* was fed TBT-contaminated food, the respiration rate, the expression of P-450 3A (CYP3A), and heat shock proteins (HSPs) in the hepatopancreas increased, indicating that the crabs were stressed by TBT (51). An active heat shock response, specially with increased HSP70 expression, occurs when crustaceans are exposed to many types of environmental stress such as heat (55–58), metals (59, 60), and salinity alterations (61, 62). Therefore, increased expression of HSPs could be a useful indicator of BTs/TBT contamination that should be studied in other crustacean species (**Figure 2**).

Reactive oxygen species (ROS), byproducts of cellular respiratory chain, are kept at physiological levels by a balance between oxidant and antioxidant agents (63, 64). Liver phase I metabolism also generates ROS as byproducts, leading to oxidative stress (OS) (37). Many drugs, pesticides, and metals induce OS in crustaceans, either by altering the expression and activity of antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) or by decreasing non-enzymatic antioxidants such as glutathione (37, 65, 66). In mammals, BTs increase ROS by decreasing the concentration and activity of SOD, GPx, and glutathione reductase (GR), while simultaneously increasing lipid peroxidation in liver, testis, and kidney (67). Since decapod crustaceans, such as the green crab *Carcinus maenas*, *C. sapidus*, and *Macrobrachium rosenbergii*, are considered good sentinel species, OS biomarkers should be monitored in bioassays with sublethal concentrations of BTs.

Stressed animals usually develop hyperglycemia. In vertebrates, it is considered a secondary response to the increase in catecholamine and corticosteroids' blood levels (68, 69). In crustaceans, the main hormone responsible for triggering hyperglycemia during stress is CHH (29, 34, 70, 71). Injection of 10 μmoles of tripalmitin, fentin, and fenbutatin increased glucose levels in the hemolymph of the crab *Oziotelphusa senex senex* (72). Since this effect did not occur in the eyestalk-ablated crabs, it is possible that OTs injection caused CHH secretion (72). In *M. rosenbergii*, the treatment with TBT (10, 100, and 1000 ng L^{-1}) dissolved in water for 90 days also increased glucose levels in the hemolymph (73). Therefore, synthesis, release, and secretion of CHH and its signaling are processes that could be disrupted as the result of OTs exposure and needs to be further investigated.

In mammals, TBT disrupts both glucose and lipid homeostasis: increases body weight, inflammation, adipogenesis, and blood glucose and insulin levels (2, 74, 75). These effects are mediated by alterations in insulin signaling cascade and of nuclear receptors such as estrogen receptor, peroxisome proliferator-activated receptor γ (PPARγ), and retinoid X receptor (RXR) (2, 74, 75). RXR can form both homodimers or heterodimers with many other nuclear receptors, including PPARs, and therefore bind to DNA response elements inducing the transcription of genes involved in xenoprotection, lipid homeostasis, and development (19, 76). Since TBT is recognized as a potent agonist of RXR, this binding can be considered a key step of TBT's mechanism of action (19, 77).

The main sites of glycogen and lipid storage in decapod crustaceans are the hepatopancreas, gonads, and muscle, and these energetic reserves fluctuate in distinct species according to seasonality, reproductive stage, molt cycle, type, and regularity of the diet (46, 49, 78). These metabolites are distinctively mobilized during diverse types of stresses, reflecting homeostasis alterations that can be used as biomarkers of health and stress condition (31, 37, 46, 47, 79). In the freshwater prawn *M. rosenbergii*, TBT (10, 100, and 1,000 ng L^{-1}) treatment reduced hepatosomatic index (HIS) and the content of proteins, glycogen, and lipids in the hepatopancreas in a dose-dependent manner (73). In the cladoceran *Daphnia magna*, lipids are stored in spherical lipid droplets scattered throughout the body, and treatment with 0.036 or 0.36 μg L^{-1} increased lipid fluorescent stain (80). In female *D. magna*, both doses of TBT decreased the levels of triglycerides, cholesteryl esters, and phosphocolines and increased diacylglycerol levels and altered the expression of many genes, including RXR (**Figure 2**) (80).

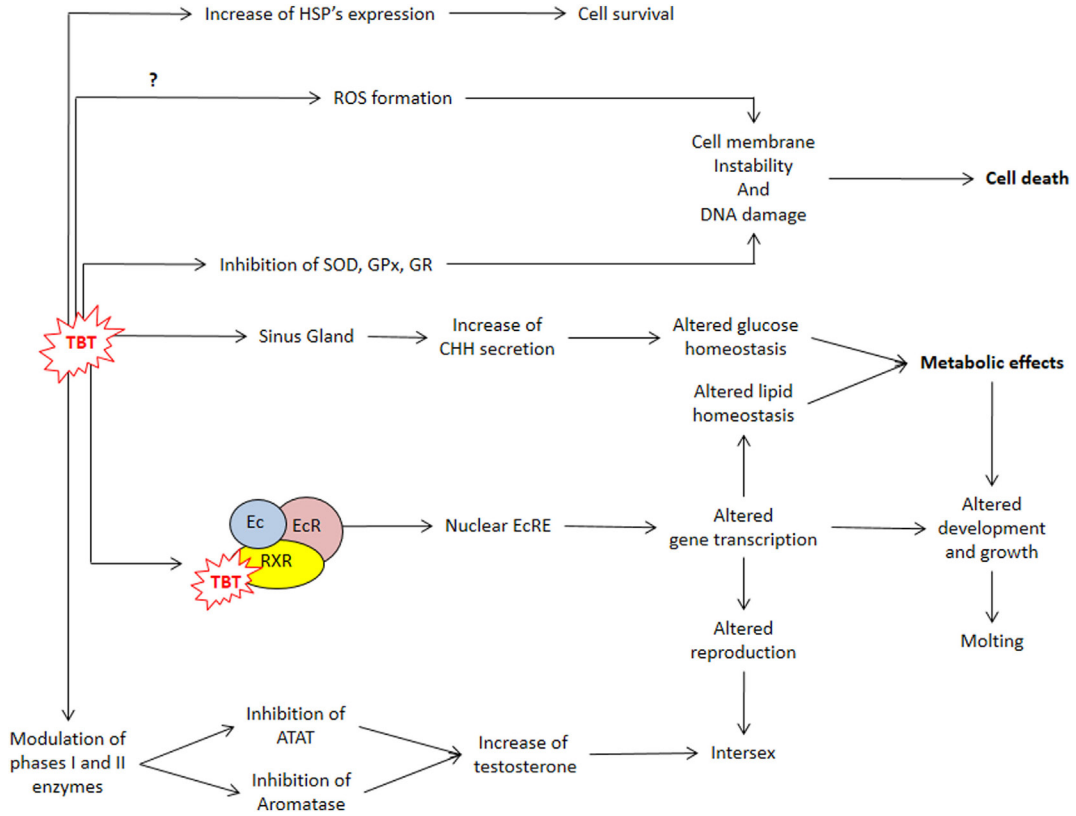

FIGURE 2 | TBT actions impairing metabolism, cell protection, and reproduction. ATAT, acyl-CoA:testosterone acyltransferase; CHH, crustacean hyperglycemic hormone; Ec, ecdysteroid; EcR, ecdysteroid receptor; EcRE, ecdysteroid responsive element; GPx, glutathione peroxidase; GR, glutathione reductase; *HSP*, heat shock protein gene; ROS, reactive oxygen species; RXR, retinoid X receptor; SOD, superoxide dismutase; TBT, tributyltin.

OTs EFFECTS ON GROWTH

Crustacean growth, as in other ecdysozoans, occurs by the recapitulated molting process (81). Molting is regulated by a negative feedback mechanism involving CHH, MIH, and ecdysteroids (**Figure 1**) (81, 82). Ecdysone and 25-deoxy-ecdysone, inactive ecdysteroids, are secreted by the Y-organ and converted to 20-hydroxyecdysone (20-HE) and ponasterone A, the active forms, in peripheral tissues (33, 81). Ecdysteroids bind to arthropod ecdysteroid receptor (EcR) that complex with RXR (22, 80). The heterodimer EcR:RXR binds to ecdysteroid response element regulating the transcription of genes involved in development, growth, reproduction, and the genes involved in the pathways of ecdysone synthesis (17, 22, 80). Incomplete ecdysis leading to death occurs when *D. magna* is exposed to exogenous 20-HE (22). TBT alone do not alter the incidence of incomplete ecdysis; however, when in combination with 20-HE, this incidence is increased. Therefore, TBT synergizes with 20-HE leading to mortality associated with molting (22). In TBT-treated daphnids, the expression of RXR and EcR increase, disrupting the ecdysteroids' pathways (22, 80). In the brown shrimp *Cangron cangron*, it was demonstrated that TBT fits in the ligand binding pocket of RXR, affecting the expression of RXR and EcR and probably of downstream genes (83). This genomic action of TBT was also demonstrated in the larvae of an insect *Chironomus riparius*, where TBT also increased the expression of RXR, EcR, as well as estrogen-related receptor gene and E74 (84).

Besides ecdysteroids, the sesquiterpenoids methyl farnesoate (MF) and juvenile hormone are also important during arthropod's growth and metamorphosis (85). MF, synthesized in the MOs, is the main sesquiterpenoid of crustaceans (**Figure 1**) (86). The major function of MF in crustaceans is regulation of reproductive maturation (86). MF binds to methoprene-tolerant (MET), which forms a heterodimer with steroid receptor coactivator (SRC), activating the transcription of downstream genes, such as sex-determining genes involved in oocyte maturation (87). In *D. magna*, TBT also affected the expression of genes related to MF signaling pathway such as MET and SRC (80). Considering that TBT may also affect MF signaling in other crustaceans, and therefore alter their growth and development, serious impact on both planktonic and benthic communities can be expected.

OTs EFFECTS ON REPRODUCTION

Imposex in female gastropods is one of the better-known effects caused by TBT on invertebrates. Imposex is characterized by the formation of male sexual organs such as penis and vas

deferens in these females (19, 86). Although some studies show an early sexual reversal (intersex) in crustaceans exposed to TBT, these changes are less marked than those occurring in mollusks (31, 88). Nevertheless, other detrimental effects on the reproductive system of different species of crustaceans were found in both females and males (27, 88–90). The mechanism by which TBT causes these damages is still unclear, and there are different possible sites of action (80, 86, 89).

Unlike mollusks, when female crustaceans are exposed to TBT, there is no formation of complete male sex organs (31). Nevertheless, in *M. rosenbergii*, the treatment with TBT (10, 100, and 1000 ng L^{-1}) for 45 days altered ovarian morphology and induced spermatogonia and ovotestis (with spermatocytes and structures similar to seminiferous tubules) (88). In the hermit crab *C. vittatus*, TBT induced several degrees of ovarian disorganization with follicular atresia and irregular oocytes although there was no formation of male sexual structures (27). Besides damage to reproductive organs, TBT may impair reproductive rates in further generations. Juvenile female *D. magna* exposed to TBT (100 and 1,000 ng L^{-1}) produced smaller newborn neonates than those of unexposed females and suffered a higher mortality during their adulthood, which resulted in lower reproductive output and fitness. The reproductive rates of exposed female's first clutch were also lower than control (80).

Although the main described effect of TBT is the masculinization of females, it also causes damage to male reproductive organs. In *M. rosenbergii*, exposure to TBT (10, 100, and 1,000 ng L^{-1}) for 45 or 90 days caused several damages to the gametes and to the gonadal tissue itself. The gonadosomatic index of the testes reduced, and the seminiferous tubules architecture was compromised by an increase in connective tissue and immature cells (spermatogonia and spermatocytes) (73, 90). Spermatozoa count and length reduced (73, 90). The activity of the antioxidant enzymes SOD, GPx, and GR reduced in the testes, while DNA damage increased (89). These results are in line with studies in mammals such as the hamster *Mesocricetus auratus*, where TBT also caused alterations in testicular histology and reduction in spermatogenesis and in enzymatic and non-enzymatic antioxidants (67).

Since sex steroids are the major regulators of vertebrate reproduction, many steroidogenic enzymes and steroid receptors seem to have co-evolved (91, 92). However, the role of vertebrate-type sex steroids on invertebrate reproduction is not well determined (19). In mollusks, TBT-induced imposex correlates with increased free testosterone (T) levels, probably induced by inhibition of acyl-CoA:testosterone acyltransferase, which conjugates T with fatty acids, and/or CYPs, reducing T clearance (19, 93). The stimulatory effects of steroids on crustacean reproduction are well recognized; however, it was only with the development of modern omics technology that genes of steroidogenic enzymes and putative steroid receptors were identified (31, 39, 94–98). In female *M. rosenbergii*, TBT reduced 17β-estradiol in the hemolymph and ovary and increased T levels in the ovary (88), while in males, TBT reduced T levels in testis (73, 90) (**Figure 2**) (53, 94). In crustaceans, an alternative action proposed was that TBT could block T excretion, but results are still inconclusive (18, 93, 99, 100).

The synthesis and release of steroids in crustaceans is controlled mainly by GIH and CHH, released from the ES-SG system (**Figure 1**) (32, 39). As already mentioned, OTs can stimulate CHH release and probably also interfere with other peptides of the CHH family such as GIH (72). Gonad-stimulating hormone, released from the brain and thoracic ganglion, monoamines, and MF also participate in the control of crustacean reproduction (32, 33, 39). GIH and MIH also regulate a peptide hormone called insulin-like androgenic gland hormone, synthesized by the androgenic gland, which is responsible for male sexual differentiation (39, 97). Therefore, there are many sites where TBT may affect the neuroendocrine regulation of crustacean's reproduction.

CONCLUSION

Crustaceans form a large group of aquatic animals that are important from both the economic and the ecological perspectives. They are important members of zooplankton and benthic communities and have vital roles in food chains, so the endocrine-disrupting effects of TBT on crustaceans can affect other organisms. They are also important fisheries worldwide. Therefore, human consumption of TBT-contaminated crustaceans can pose risks to human health. In summary, TBT can disrupt carbohydrate and lipid homeostasis of crustaceans by interacting with RXR and CHH signaling and can interact with other nuclear receptors, such as EcR, MET, and SRC, disrupting MF and ecdysteroid signaling, thereby altering growth and sexual maturity, respectively. This compound also interferes in cytochrome P450 system disrupting steroid synthesis and reproduction. Both macrocrustaceans and microcrustaceans are good models to study the effects of sublethal TBT contamination, usually found in natural environments. Multibiomarkers studies focusing on TBT's effects on molecular, biochemical, cellular, morphological, physiological, and behavioral endpoints can be developed with crustaceans. The recent advances in omics technology, with the development of transcriptomes, lipidomes, and proteomes, are providing a novel set of information. The knowledge of the genes involved in the growth, development, and reproduction of crustaceans will certainly provide novel insights about TBT effects.

AUTHOR CONTRIBUTIONS

EV wrote Sections "Introduction," "OTs Effects on the Metabolism," and "OTs Effects on Growth." JM wrote Sections "OTs Effects on Reproduction" and "Conclusion" and elaborated figures. AV reviewed the manuscript.

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MINI REVIEW
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Organotin Exposure and Vertebrate Reproduction: A Review

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Organotin (OTs) compounds are organometallic compounds that are widely used in industry, such as in the manufacture of plastics, pesticides, paints, and others. OTs are released into the environment by anthropogenic actions, leading to contact with aquatic and terrestrial organisms that occur in animal feeding. Although OTs are degraded environmentally, reports have shown the effects of this contamination over the years because it can affect organisms of different trophic levels. OTs act as endocrine-disrupting chemicals (EDCs), which can lead to several abnormalities in organisms. In male animals, OTs decrease the weights of the testis and epididymis and reduce the spermatid count, among other dysfunctions. In female animals, OTs alter the weights of the ovaries and uteri and induce damage to the ovaries. In addition, OTs prevent fetal implantation and reduce mammalian pregnancy rates. OTs cross the placental barrier and accumulate in the placental and fetal tissues. Exposure to OTs *in utero* leads to the accumulation of lipid droplets in the Sertoli cells and gonocytes of male offspring in addition to inducing early puberty in females. In both genders, this damage is associated with the imbalance of sex hormones and the modulation of the hypothalamic–pituitary–gonadal axis. Here, we report that OTs act as reproductive disruptors in vertebrate studies; among the compounds are tetrabutyltin, tributyltin chloride, tributyltin acetate, triphenyltin chloride, triphenyltin hydroxide, dibutyltin chloride, dibutyltin dichloride, diphenyltin dichloride, monobutyltin, and azocyclotin.

Keywords: organotin compounds, reproduction, vertebrates, endocrinology, environmental pollutants

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INTRODUCTION

Organotins (OTs) are organometallic compounds that are widely used in industry, such as in the manufacture of plastics, pesticides, paints, and others (1, 2). Despite being easily degraded in the environment, several studies have shown the toxicological effects in different trophic levels of the food chain (3, 4). In 2008, the World Health Organization decreed a ban on the use of OTs in paints on vessels. However, many countries did not adopt this ban. OTs are classified as endocrine-disrupting chemicals (EDCs), leading to inappropriate endocrine system functioning in various species (5, 6). Thus, their exposure can cause damage, sometimes irreversibly, such as the process of *imposex* in which female gastropods develop male sex organs (3). For humans and other vertebrates, the major route of OTs exposure is by the intake of contaminated seafood, and studies evaluating their toxicological risks are limited (7–11). OTs impair reproductive functioning, and the damage is associated with the imbalance of sex hormones and with improper modulation of the hypothalamic–pituitary–gonadal axis function of rodents (12–14). Here, we report that OTs act as reproductive disruptors in vertebrate

TABLE 1 | Summary of vertebrate reproductive changes induced by OTs.

	Animal models/dose/OTs						Reference
	Fish		Frog	Rodents		Monkey	
	(0.01–25 µg/kg)		(0.5 µg to 0.5 g/L)	(100 ng to 125 mg/kg)		(2.5–3.8 mg/kg)	
	TriOTs	Azocyclotin	Azocyclotin	TriOTs	DiOTs	DiOTs	
Adult male							
Organ weights (g/bw)							
Testes, epididymis	NR	NR	NR	↓	NR	NR	(20, 21)
Prostate, seminal vesicle	NR	NR	NR	↓	NR	NR	(21)
Sn accumulation	NR	NR	NR	↑	NR	NR	(22)
Histopathology							
Spermatocytes/spermatids	NR	↑	NR	↓	NR	NR	(20, 24)
Sperm viability/number	↓	↓	NR	↓	NR	NR	(20, 23, 24)
Seminiferous tubules	NR	NR	NR	↑/↓ Lumen	NR	NR	(21, 22)
Testes	Fibrosis	NR	NR	Edema	NR	NR	(15, 22)
Leidig cells (number)	NR	NR	NR	↓	NR	NR	(22)
Sex hormones	↓ FSH	NR	↑ T	↑/↓ LH, ↓ T	NR	NR	(13, 15, 24, 26, 27)
***In utero*effect**							
Cryptorchidism	NA	NA	NA	↔	NR	NR	(12)
Preputial separation	NA	NA	NA	↔	NR	NR	(54)
Testes, epididymis and prostate weight	NA	NA	NA	↓/↔	NR	NR	(12, 54)
Gonocytes, Sertoli cells (number)	NA	NA	NA	↓	NR	NR	(55)
Spermatid and sperm (number)	NA	NA	NA	↓	NR	NR	(12, 54)
Sperm motility	NA	NA	NA	↓/↔	NR	NR	(12, 54)
Sex hormones	NA	NA	NA	↑T, ↑LH, ↓E2	NR	NR	(12, 53, 54)
Adult female							
Estrous cyclicity	NR	NR	NR	Impaired	NR	NR	(14, 31)
Ovary weight (g/bw)	NR	↓	↓	↓/↑	NR	NR	(21, 24, 27, 29, 32)
Sn accumulation	NR	NR	NR	↑	NR	NR	(14)
Histopathology							
Ovarian follicles	NR	NR	NR	↑ Apoptosis, ↑ Atretic	NR	NR	(14, 31, 34)
Folliculogenesis	NR	Impaired	NR	↓ Mature follicles	NR	NR	(14, 24, 31, 37)
Sex hormones	NR	↑T, ↓E2	NR	↑/↓ E2, ↑T	NR	NR	(14, 24, 29, 31)
***In utero*effect**							
Vaginal opening	NA	NA	NA	↑/↓	NR	NR	(26, 56)
Estrous cyclicity	NA	NA	NA	Impaired	NR	NR	(26, 56)
Ovary morphology	NA	NA	NA	Impaired	NR	NR	(55)
Fertility							
Loss pre- and postimplantation	NR	NR	NR	↑	↑	↑	(40–43)
Number of live fetuses	↓	NR	NR	↑	NR	NR	(41, 45)
Sex ratio (female/male)	↔/↑/↓	NR	NR	↔	NR	↔	(19, 23, 41, 43–45)
Litter size	NR	NR	NR	↓/↔	NR	NR	(39, 41)
Hatchability/egg viability	↔/↓	NR	NR	NR	NR	NR	(19, 44)

OTs, organotins; TriOTs, triorganotins (tributyltin chloride, tributyltin acetate, triphenyltin chloride, triphenyltin hydroxide); DiOTs, diorganotins (dibutyltin chloride, dibutyltin dichloride, diphenyltin dichloride); Sn, tin; ↑, increased; ↓, decreased; ↔, unchanged or similar to control; NR, not reported; NA, not applicable; bw, body weight; LH, luteinizing hormone; T, testosterone; FSH, follicle-stimulating hormone; E2, estrogen; Fish, zebrafish (Danio rerio), rockfish (Sebastiscus marmoratus), and Oryzia latipes; frog, Xenopus laevis, Rodents, rats and mice; monkey, Macaca fascicularis.

studies (**Table 1**); among them are tetrabutyltin (TeBT), tributyltin chloride (TBTCl), tributyltin acetate (TBTAc), triphenyltin chloride (TPTCl), triphenyltin hydroxide (TPTOH), dibutyltin chloride (DBTCl), dibutyltin dichloride (DBTCl2), diphenyltin dichloride (DPTCl2), monobutyltin (MBT), and azocyclotin.

REPRODUCTIVE TOXICOLOGY

Male Reproductive Function

Several studies have evaluated OT exposure in male vertebrates, highlighting the dose-dependent impairment by OTs in different experimental models (15–19). Male mice at postnatal day (PND) 21 exposed to TBTCl at concentrations of 0.5, 5, and 50 µg/kg for 3 days presented a reduction in testis weight (20). There was also a decrease in testis weight in Swiss Webster mice on PND 15 exposed to a dose of 15 mg/kg/30 days of TPTCl as well as a reduction in epididymis, prostate, and seminal vesicle weight (21). Mitra et al. (22) reported that TBTCl exposure for 3 days at doses of 10–30 mg/kg caused an accumulation of tin in rat testes. Thus, the OTs were able to accumulate in the male reproductive tract, leading to morpho-functional abnormalities.

Studies have reported a consensus that OTs are very harmful to vertebrate reproduction and the quality of spermatozoa (20, 23, 24). A reduction in the numbers of spermatocytes and spermatids as well as sperm viability and an increase in abnormal gametes were observed in male rats after exposure to TBTCl in a dose-dependent manner (20). Similar data were observed in zebra fish (*Danio rerio*); when exposed to different doses of TBTCl from the first day of incubation of the eggs to PND 70, they exhibited effects such as reduced or completely lost sperm motility, absence of flagella, and the presence of only abnormal spermatozoa in semen (23). Similarly, zebra fish exposed to 0.09 and 0.45 μg/L of azocyclotin presented a reduction of 21.4 and 58.1% in the number of spermatozoa (24). However, there was an increase in spermatocytes with exposure to azocyclozine at levels of 0.09 (17.5%) and 0.45 μg/L (63.8%) in these fish at 5–6 months of age (24). Several histological studies have reported that OTs dramatically affect the reproductive apparatus cells in vertebrates (15, 21, 22, 25). Exposure to doses of TBTCl (10, 20, 30 mg/kg) for 1,100–1,300 h affected spermatogenesis, increased the lumen size of the seminiferous tubules, and caused testicular interstitial edema along with evident Leydig cell loss in male rats (22). The seminiferous tubule in male mice that received doses of TPTCl ≥3.75 mg/kg body weight (bw)/day presented a smaller tubule diameter and germinal epithelial reduction, suggesting that TPTCl exposure impaired spermatogenesis (21). Severe interstitial fibrosis was also observed in the interlobular septum of the testis with exposure to 10 ng/L TPTCl in rockfish (*Sebastiscus marmoratus*), and there was testicular vacuolization at 48 days of exposure (15).

The hormones associated with reproductive control are also affected by the presence of OTs (13, 15, 26, 27). *S. marmoratus* exposed to 10 ng/L TPTCl exhibited a decrease in follicle-stimulating hormone (FSH) mRNA expression (15). In rats, exposure to 6 mg of TPTCl/kg resulted in increased levels of luteinizing hormone (LH) (13). However, mice exposed for 3 days at 0.05 and 0.5 mg/kg TBTCl exhibited a reduction in serum LH levels of approximately 50% on PND 84 (26). In other studies, rats and hamsters exposed to TBTCl at 15 mg/kg/30 days and 100–150 ppm/kg/65 days, respectively, presented a reduction in testosterone levels (13, 24). By contrast, testosterone levels increased in the treatment of frogs (*Xenopus laevis*) with 0.5 μg/L of azocyclotin (27). In addition, studies have shown a reduction in serum estrogen levels and/or testes weight upon exposure to various OTs (TBTC1, TPTC1, or azocyclotin) in different rodent, toad and fish species (15, 20, 26, 27).

Female Reproductive Function

Organotins can also affect the female reproductive function in different animal models (12, 16, 19, 28–30). Rats exposed to 100 ng/kg of TBTCl have abnormalities in the estrous cycle and present increased ovary and serum tin levels (14, 31). In addition, OTs led to a decrease in the weight of the reproductive organs of rodents in a dose-dependent manner (21, 32–35). By contrast, Grote et al. (29) found an increase in rat ovarian weight when exposed to doses of 2–6 mg/kg/day of TPTCl for 30 days. Ma et al. (24), when exposing zebra fish to 0.09 and 0.45 μg/L of azocyclotin for 21 days, reported a reduction in the gonadosomatic index. Li et al. (27) demonstrated that adult frogs exposed to 0.05 and 0.5 g/L azocyclotin for 28 days also presented a reduction in the gonadosomatic index. The study also reported an increase in the number of hermaphroditic frogs after exposure to azocyclotin (27).

Furthermore, it has been reported that exposure to OTs causes impairment of the release and production of sex hormones (14, 24, 27, 29, 33). Grote et al. (29) demonstrated that rats treated with 6 mg/kg of TPTCl had increased serum estrogen levels. By contrast, other studies have shown a reduction in the serum estrogen levels and increased testosterone in TBTCl-treated rats (14, 27, 31, 36). Ma et al. (24) demonstrated that azocyclotin treatment caused an increase in testosterone levels and a decrease in estrogen levels in the ovaries of female zebra fish. It is also known that these xenobiotics cause abnormalities in uterine and ovarian morphology, impairing ovarian follicular development and increasing the number of atretic ovarian follicles in rodents (14, 31). Lee et al. (34) treated rats with 1–10 mg/kg bw of TBTAc for 7 days and observed an increase in ovarian follicular apoptosis. Shen et al. (37), by administering TPTCL *in vivo* (female mice: 5 or 10 mg/kg/day by oral gavage for 10 days) and *in vitro* (germinal vesicle oocytes: 100 mg/mL/1), found impairment in oocyte development *in vitro* and a reduction in the number of secondary and mature ovarian follicles *in vivo*. In zebra fish treated with azocyclotin, the development of the oocyte was also impaired (24). Thus, OT exposure impairs ovarian function in vertebrates, possibly leading to a loss of fertility.

FERTILITY

Exposure to OTs in vertebrates negatively affects fertility, impairing major reproductive indicators such as pre- and postimplantation, the number of live pups, litter size, and so on (14, 38, 39). Studies have shown that female rats exposed to 7.6 and 15.2 mg/kg TBTCl presented greater pre- and postimplantation loss and a reduction in bw and the number of live fetuses in the treated groups (40). In addition, female rats exposed to 20 mg/kg TBTCl at gestational days (GDs) 0–19 showed a significant increase in postimplantation loss (41). In the same model, female rats exposed to 15.2 and 30.4 mg/kg DBTCl for 3 days showed an increase in pre- and postimplantation embryo loss (42). This embryonic/fetal loss was also observed in cynomolgus monkeys (*Macaca fascicularis*) exposed *in utero* to 2.5 and 3.8 mg/kg of DBTCl by the organogenesis period but with no effects on morphological development (43).

Monkeys exposed to 2.5 and 3.8 mg/kg DBTCl for 30 days did not show any differences in the sex ratio (43). In female rats, exposure to 20 mg/kg TBTCl reduced the litter size and increased fetal numbers. However, the sex ratio did not show significant differences *in utero* exposure (41). Data show that the exposure of Swiss mice to 1.875, 3.75, or 7.5 mg/kg/day TPTCl did not result in changes to the litter size (39). Studies with the *Oryzia latipes* fish model showed that, when exposed to a diet of 5 and 25 μg/g TBTCl for 3 weeks, the fish produced eggs with a reduced hatch

capacity. However, no differences were observed in the sex ratio (44). In another study, using the same fish species but with exposure to TPTCl at different levels, reductions in the female birth rate and the number of eggs were observed for each female. In addition, the incubation capacity decreased, and many embryos died before hatching due to developmental defects (45). However, zebra fish exposed to TBT at 1 μg/g of diet showed no difference in hatchability and egg viability; however, a decrease in fecundity was observed, and the proportion of females was significantly higher (19). By contrast, zebra fish kept in tanks with a continuous flow of TBT of 0.1 ng/L from the post-hatch days 0–70 had a higher proportion of males (23).

PLACENTAL ASSESSMENT

Several studies have shown that placental functions are also affected by the toxicological actions of OTs (**Table 2**) (46–49). In a human placental tissue collected between 1997 and 2001 from Finland (Turku) and Denmark (Copenhagen), relatively infrequent detection of MBT (percentage of samples > limit of quantification (LOQ) ranging from 10 to 11%) and more frequent detection of TBTCl and TPTCl (percentage of samples > LOQ ranging from 31 to 99%) were reported. The levels of di- and triorganotins in the placental samples collected from Finland were higher than in the placental samples collected from Denmark, especially for TBTCl (99 versus 37%, respectively) (48, 49). Cooke et al. (50) found 650 ng/g TBTCl in the placenta of female rats exposed to 10 mg/kg bw/day and on GD 20, and the TBTCl levels in the placenta were approximately 5-fold higher than the levels in maternal blood and 10-fold higher than in the milk on PND 6.

Heidrich et al. (46) suggest that OTs alter the enzymes of the placental steroidogenic pathway. TBTCl was found to be a partial competitive inhibitor of human placenta cytochrome P450 aromatase activity with an IC_{50} value of 6.2 μM. The residual activity of TBTCl-saturated aromatase was 37%. DBTCl acted as a partial but less potent inhibitor of activity (65% residual activity), whereas TeBT and MBT had no effect. By contrast, human 3β-HSD (3β-hydroxysteroid dehydrogenase) type I activity was only moderately inhibited by TBTCl (80% residual activity) (46).

In the human choriocarcinoma cell line (JAR cells) used as a placental experimental model, the findings on aromatase were contrary to those by Heidrich et al. (46). TBTCl and TPTCl at a nontoxic level of 10^{-7} M for 48 h caused, through a cAMP-independent pathway, a dose-related increase in human chorionic gonadotrophin (hCG) secretion and an increase in aromatase activity; furthermore, this augmentation in enzymatic activity occurred concurrently with increases in mRNA expression and estrogen biosynthesis from androstenedione (28). Otherwise, neither of the mono-alkyltin compounds altered hCG production or aromatase activity (47). DBTCl2 stimulated aromatase activity at 30 nM but failed to induce hCG production. By contrast, DPTCl2 stimulated hCG production at 30 nM but not aromatase activity (47). Moreover, the changes in hCG and aromatase mRNA expression were nearly parallel to those in hCG secretion and aromatase activity (47).

These placental factors are both induced by specific ligands of retinoid X receptors (RXRs) (47). The treatment of an RXRα-transfected human choriocarcinoma cell line (JEG-3 cells) with 1–100 nM TBTCl for 24 or 48 h stimulated luciferase (LUC) expression from 1.5- to 9-fold, and exposing the cells to the same concentrations of TPTOH induced LUC expression from 1.8- to 19-fold, suggesting that low doses of these OTs activate RXR (47). The peroxisome proliferator-activated receptor gamma (PPAR-γ) ligand failed to increase the mRNA expression of aromatase in JAr cells, suggesting that PPAR–RXR is not involved in OTs-induced aromatase expression in the human placenta and that the RXR homodimer may be required for OTs-induced aromatase expression (47). By contrast, PPAR agonists, in addition to RXR agonists, stimulate mRNA expression of hCG, indicating that OTs-induced hCG expression might involve either PPAR–RXR heterodimers or RXR homodimers (47).

Exposure for 48 h to 100 nM of each OTs (TBTCl, TPTOH, and TPTCl) caused an increase in 17β-HSD I activity in JAr cells. TBTCl and TPTCl metabolites also altered 17β-HSD I activity, but the level of activation decreased in proportion to the dealkylation

TABLE 2 | Summary of placental changes induced by OTs.

	Animal models/dose/OTs									Reference
	Rodents		Human				JAr and JEG-3 cells			
	(100 ng to 125 mg/kg)		(0.25–20 mg/kg or 6.2 μM)				(1–100 nM)			
	TriOTs	DiOTs	TriOTs	DiOTs	TeBT	MBT	TriOTs	DiOTs	MBT	
Placenta										
Sn accumulation	↑	NR	↑	NR	NR	↑	NR	NR	NR	(48–50)
Aromatase activity	NR	NR	↓	↓	↔	↔	↑	↑/↔	NR	(28, 46, 47)
3β-HSD activity	NR	NR	↓	↔	↔	↔	NR	NR	NR	(46)
hCG secretion	NR	NR	NR	NR	NR	NR	↑	↑/↔	NR	(28, 47)
RXR activation	NR	NR	NR	NR	NR	NR	↑	NR	NR	(47)
17β-HSD I activity	NR	NR	NR	NR	NR	NR	↑	↑	NR	(51)
Progesterone	NR	NR	NR	NR	NR	NR	↑	↑	↑	(52)

OTs, organotins; TriOTs, triorganotins (tributyltin chloride, tributyltin acetate, triphenyltin chloride, triphenyltin hydroxide); DiOTs, diorganotins (dibutyltin chloride, dibutyltin dichloride, diphenyltin dichloride); TeBT, tetrabutyltin; MBT, monobutyltin; Sn, tin; ↑, increased; ↓, decreased; ↔, unchanged or similar to control; NR, not reported; Rodents, rats and mice; Human, placenta samples were obtained as reported in Ref. (46, 48, 49); JAr and JEG-3 cells, human choriocarcinoma cell lines.

or dearylation (mono- < di- < tri-) in JAr cells. The OTs that enhanced the catalytic activity of 17β-HSD I also increased its mRNA expression. However, the mRNA effects were much more pronounced than the changes in catalytic activity (51). Exposure at the same levels and time to TBTCl and TPTCl enhanced progesterone production in JAr cells (52). TBTCl and TPTCl metabolites also altered progesterone production. However, TeBT failed to stimulate this placental function at doses of <100 nM (52). Taken together, placental OT levels and hormonal changes should reflect the abnormal placental function, and these irregularities could be associated with the abnormal development and fetal exposure levels.

GENERATIONAL EFFECTS

Intrauterine exposure to TBTCl at different doses and routes did not alter the male:female ratio of pups in rats (50, 53). However, the exposure of zebra fish to 1 μg of TBTCl/g *via* the diet increased the proportion of females (19). Furthermore, in rats, gestational exposure to 125 ppm TBTCl did not alter the process of the descent of the male testis (12); nevertheless, in humans in Denmark, a positive correlation of the levels of DBTCl in the placenta with the occurrence of cryptorchidism in newborns was found (48). Furthermore, preputial separation in mice was not altered when exposed to 1, 10, and 100 μg/kg of TBTCl *in utero* (54).

Exposure to TBTCl by the diet with 5, 25, and 125 ppm of TBTCl/g of chow in the gestational period (GP) of rats induced a reduction in the epididymis, prostate, and testis weights in a dose-dependent manner (12). However, no significant changes in the weight of the male mouse sex organs were observed after exposure to 1, 10, and 100 μg/kg TBTCl (54), demonstrating different susceptibility/sensitivity to OTs according to the exposure model.

Studies have shown that testes can be a target organ for OTs action, as can be observed in the histological irregularities (12, 54, 55). Rat fetal testes exposed to 20 mg/kg TBTCl showed reduced numbers of gonocytes, Sertoli cells, and Leydig cells. In addition, there are differences in the expression of connexin 43 in Leydig cells, which may be reduced or completely absent (55). Moreover, seminiferous epithelial vacuolization, the retention of spermatids in the epithelium, and the retardation of spermatid maturation were observed in adult rats (12), while in mice, the sloughing of germ cells was observed in the seminiferous tubules (54); both were exposed to TBTCl in the GP.

In the parameters for sperm, OT–GP exposure has dose-dependent toxicological effects that vary according to the model of exposure used. In rats exposed to TBTCl *via* the diet, the spermatid and sperm counts were reduced, but no morphological changes or reduction in sperm motility were observed (12). In mice exposed to TBTCl in the GP until weaning, a dose-dependent reduction in sperm count was observed on both PND 49 and PND 152. A dose-dependent reduction in sperm motility at doses of 10 and 100 μg of TBTCl/kg bw (54) was also observed.

Rats exposed to TBTCl showed a dose-dependent increase in serum testosterone and LH levels as well as a reduction in the serum estrogen levels only in animals exposed to 125 ppm of TBTCl (12). In addition, mice exposed to a TBTCl dose of 10 mg Sn/kg in GD15 showed increased expression of the LH β-subunit mRNA (53). Meanwhile, mice exposed to TBTCl from the GP until weaning showed a reduction in intratesticular estrogen levels only on PND 49 (54). The most disturbing effects were observed in humans, where the LH levels in 4-month-old boys had a negative correlation while the inhibin B levels correlated positively with the levels of TBTCl in the placenta of women from Finland (48).

The effects of GP on OTs in the reproductive system of mammalian females have been underestimated until the present. Ogata et al. (56) reports that F1 and F2 generations of rats with a whole-life dietary concentration of 125 ppm of TBTCl showed a delay of approximately 6 days for vaginal opening and an impaired estrous cycle. In mice exposed to 10 or 100 μg of TBTCl/kg bw/day from GD 6 of pregnancy through the period of lactation, female offspring showed early vaginal opening and first day in estrus, thus presenting early puberty (26). In the same study, the animals showed no alteration in the weight of the female sex organs or hormonal levels. However, the animals showed a prolongation of the estrus and diestrus phases and irregularities in the estrous cycle (26). Intrauterine exposure to TBTCl at doses of 10 and 20 mg/kg bw altered the fetal ovarian morphology of rats with reduced germ cell numbers and increased apoptotic cells (55).

CONCLUSION

Organotins induce endocrine-disrupting effects in vertebrates, including humans, mainly by the exposure to OT-contaminated seafood intake. The effects of OTs have been associated with gender-specific changes in the morphological functioning of reproductive organs, including gonadal cell dysfunction and weight variation in the sex organs. Moreover, OTs are capable of crossing the placental barrier and thus accumulate in the placenta and in fetal tissues, generating congenital abnormalities. The toxicity level of OTs in various species may be related to their concentration and the timing or period of life of exposure. Thus, toxicological and bioavailability studies are needed for regulatory agencies to make informed decisions about the safety of OTs in food and for the environment in general.

AUTHOR CONTRIBUTIONS

The topics of the article were divided among the authors JA, PP, EM, IS, CC, ON, RF, LL, JG, who contributed with research and writing. In addition, JA and PP oversaw, assemble, and review the article. JA and PP contributed equally to the study.

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Overview of the Pathophysiological Implications of Organotins on the Endocrine System

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Organotins (OTs) are pollutants that are used widely by industry as disinfectants, pesticides, and most frequently as biocides in antifouling paints. This mini-review presents the main evidences from the literature about morphophysiological changes induced by OTs in the mammal endocrine system, focusing on the metabolism and reproductive control. Similar to other toxic compounds, the main effects with potential health risks to humans and experimental animals are not only related to dose and time of exposure but also to age, gender, and tissue/cell exposed. Regarding the underlying mechanisms, current literature indicates that OTs can directly damage endocrine glands, as well as interfere with neurohormonal control of endocrine function (i.e., in the hypothalamic–pituitary axis), altering hormone synthesis and/or bioavailability or activity of hormone receptors in the target cells. Importantly, OTs induces biochemical and morphological changes in gonads, abnormal steroidogenesis, both associated with reproductive dysfunctions such as irregular estrous cyclicity in female or spermatogenic disorders in male animals. Additionally, due to their role on endocrine systems predisposing to obesity, OTs are also included in the metabolism disrupting chemical hypothesis, either by central (e.g., accurate nucleus and lateral hypothalamus) or peripheral (e.g., adipose tissue) mechanisms. Thus, OTs should be indeed considered a major endocrine disruptor, being indispensable to understand the main toxic effects on the different tissues and its causative role for endocrine, metabolic, and reproductive dysfunctions observed.

Keywords: tributyltin, triphenyltin, impossex, endocrine disruptor, obesogen, metabolic disrupting chemicals, hypothalamus–pituitary axis

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INTRODUCTION

Organotins (OTs) belong to a class of pollutants described as organometallic used for various industrial purposes as disinfectants of water for industrial refrigeration, pesticides, biocides in antifouling paints, and wood preservatives (1–11).

Actually, tin-based compounds are known since the bronze age in the production of different metal alloys (12). However, the industrial use was consolidated only around 1940 as an efficient chemical stabilizer for plastic manufacture (5). Afterward, the biocidal effect of OTs was discovered and thus became intensively employed in a number of other commercial purposes. In this context, it is worth noting the use as an active principle of antifouling paints for boats and ships, reaching the apex in the 1990s, when about 80% of the boats worldwide used OT-based products (1, 3, 4).

Tin usually binds to non-polar radicals resulting in hydrophobic compounds; and due to their physicochemical properties, OTs are easily absorbed along the food chain. The effects depend greatly on the number and nature of radicals bound to the tin atom, being the tri-substituted (triorganostannic) forms, such as tributyltin (TBT) and triphenyltin (TPT) the most toxic. Fortunately, TBT is degraded in the environment to dibutyltin and then to monobutyltin (5, 12–16).

The TBT toxicology has become a major concern for the scientific community since the 1970s when toxic effects were discovered in different animal models, including mammals. As a result, researches were driven to better understand the actual impact of OT pollution for health and environmental risk (17, 18). These compounds can be easily assimilated by living organisms; in marine environment, for example, OTs are incorporated into soil and organic surface sediments such as phytoplankton, being absorbed by animals and plants of aquatic ecosystems (5, 19).

Figure 1 represents a visual summary of the main route of exposure to OTs for humans and the potential consequences for the endocrine system. Studies have shown that OTs cause several damages, including genetic, hepatic, renal, adrenal, neural, and immune toxicity (20–24). More importantly, recent reports indicate that TBT is a highly persistent chemical in the environment and food chain, being considered one of the largest existing endocrine disruptor with consequences to different hormonal functions (3, 20, 21, 25–27).

As illustrated at **Figure 2** and described in this mini-review, OTs are capable of altering the endocrine physiology at numerous levels: changing the pattern of hormone regulation, production, mechanisms of action or hormone elimination, and mimicking or blocking hormonal action (27–31). In this way, it is not possible to point out an exact toxic effect, whether acting directly on the endocrine glands, compromising hormonal receptors at the target cells, or both. Among all, one of the most iconic effects was noted in contaminated shellfish. These organisms undergo a phenomenon denominated "imposex," that is the superposition of male genital organs in female individuals (32, 33). Notwithstanding, this endocrine disruptor has been proven as able to reduce circulating estrogen levels and cause morphophysiological damages also in reproductive organs of vertebrates, including mammals (5, 20, 21, 26, 34).

Neuroendocrine Changes in Mammals Induced by Organotin: A Focus on the Metabolism and the Reproductive Function

In mammals, OTs administered at different doses induce morphological and functional changes in several tissues involved in the control of endocrine function and metabolism, such as the, hypothalamus, pituitary, pancreas, gonads, adipose tissue, adrenal, and thyroid glands (35–38).

The role of environmental pollutants such as OTs on the endocrine system also supports the metabolism disrupting chemical hypothesis (formerly termed "obesogen hypothesis"), which postulates that several environmental toxic chemicals, by altering the endocrine function, can induce metabolic changes related to obesity, impaired glucose metabolism, and dyslipidemia (39, 40). These endocrine and metabolic disorders caused by OTs, particularly obesity, may occur by central and peripheral mechanisms (41, 42). In fact, there are evidences that morphofunctional changes in both fatty tissues and central nervous system may contribute to the deleterious effects of

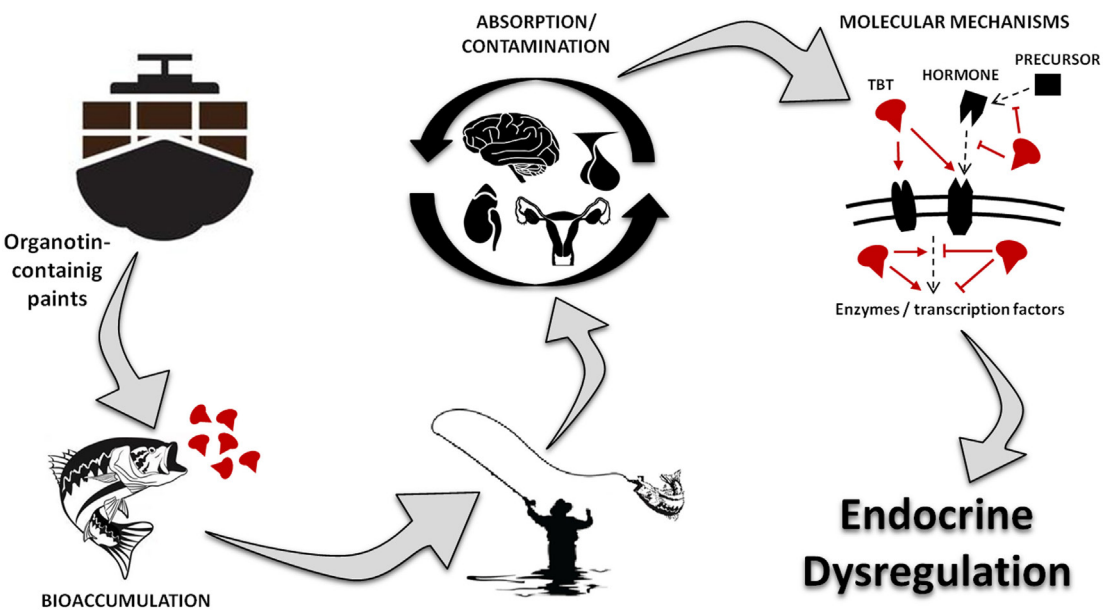

FIGURE 1 | Visual abstract showing the main route of exposure to organotins for humans, underlying toxic mechanism and the potential consequences for the endocrine system. TBT, tributyltin.

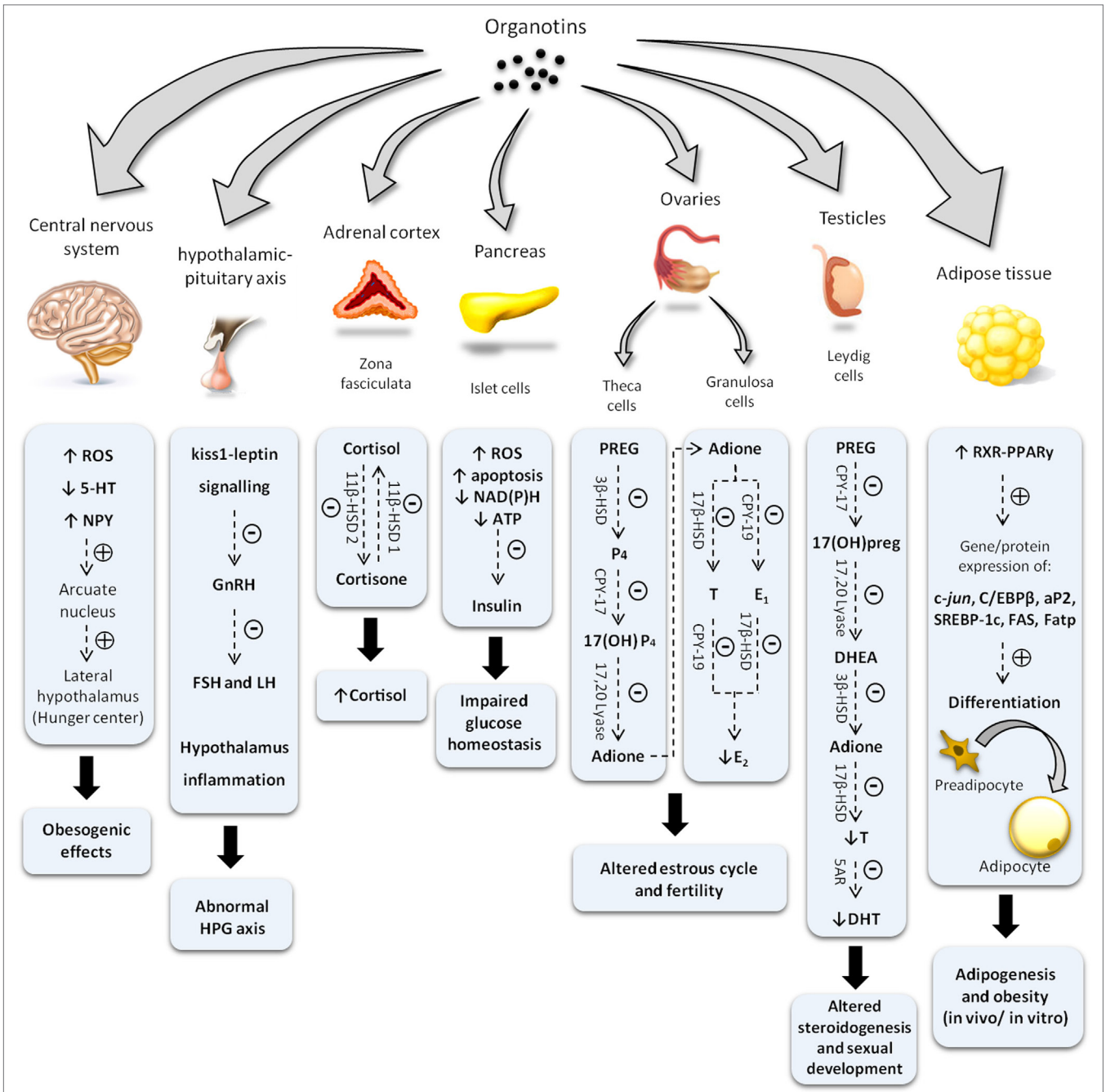

FIGURE 2 | Diagram illustrating changes in endocrine system induced by organotin and major mechanisms involved. Symbols (–) or (+) indicate inhibitory and stimulatory effects, respectively. ROS, reactive oxygen species; 5-HT, serotonine; NPY, neuropeptide Y; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; HPG, hypothalamus–pituitary–gonad; ATP, adenosine triphosphate; NAD(P)H, nicotinamide adenine dinucleotide phosphate; 11β-HSD, 11 β-hydroxysteroid dehydrogenase; PREG, pregnenolone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; P4, progesterone; E1, estrone; E2, 17-β estradiol; T, testosterone; CPY-17, 17α-hydroxylase; Adione, androstenedione; CPY-19, aromatase; 17(OH)preg, 17(OH) pregnenolone; DHEA, dehydroepiandrosterone; 5AR, 5α-reductase; DHT, dihydrotestosterone; C/EBPβ, CCAAT/enhancer binding proteins; SREBP-1c, sterol regulatory element-binding protein 1c; aP2, adipocyte-protein 2; FAS, fatty acid synthase; Fatp, fatty acid transport proteins.

these compounds related to obesity and metabolic syndrome complications (3, 20, 42–47).

In the central nervous system, OTs promote important neurotoxic effects with changes on behavior, metabolism, and neuroendocrine control (48–51). Experimental studies have found decreased levels of dopamine, noradrenaline, and serotonin in mice brains (48), reduced neuron counting and increased glutamate-induced calcium permeability in neuronal membrane

from rats (49), and an increase in reactive oxygen-derived species and oxidative damage associated with reduced antioxidant reserves in the nervous system exposed to TBT (50, 51). TBT also exerts its toxicity on other regions of the nervous system, as it is shown by disruption of the rat hypothalamic–pituitary–adrenal axis (22). In addition, the effects of OTs exposure on the brain are not restricted to general neurotoxic effects, but also to changes in neurohormonal control of metabolism and food intake. TBT administered acutely in mice activates the arcuate nucleus and the hunger center of the hypothalamus (46), and in rats increases neuropeptide Y (NPY) expression in the brain, in association with increased body weight, fat mass, and food intake (47). In addition, mice chronically exposed to low doses of TBT exhibited increased food efficiency and reduced leptin circulating levels associated with changes on the leptin–NPY–NPY–Y1 receptor axis in the hypothalamus (52). In this regard, it is well known the importance of leptin modulating the expression of NPY in the hypothalamus and, thus, the food intake. In fact, OT-induced changes on the leptin-NPY axis are associated with obesity due to increased food intake and decreased energy expenditure (53).

In relation to peripheral mechanisms involved in the obesogenic effect, it is well described the association between tin-based compounds and adipogenesis, through signaling between retinoid X receptor (RXR) and peroxisome proliferator-activator receptor gamma (PPARγ) (20, 25, 54–57). There are evidences that TBT increases adipocyte markers expression, lipid accumulation and glucose uptake in preadipocytes (36, 58, 59), and induces a differentiation to adipocytes by RXR/PPARγ activation (42–45). Notwithstanding, it is know that PPARγ plays also an orexigenic role, attributed to its central effects especially in both the NPY and agouti-related protein at the arcuate hypothalamic nucleus (60, 61). Since PPARγ is one of the peripheral targets of TBT, it is possible to speculate that this receptor may be also involved in the central effects related to OTs.

It is important to mention that there is a clear relationship between thyroid function and obesity, with changes in both thyroid-stimulating hormone and thyroxine (T_4) associated with changes in body weight and fat mass (62). In spite of few studies investigating the OT effects on the hypothalamic–pituitary–thyroid axis, there is a body of evidences indicating that TBT can also be considered a thyroid disruptor, thus contributing to the development of metabolic disorders and obesity (38, 63–65).

Furthermore, pancreas is a key target organ of metabolic disrupter chemicals not only for controlling glucose metabolism but also for modulating digestion (i.e., releasing digestive enzymes) and food intake (e.g., insulin can modulate hypothalamic center of hunger). In fact, in addition to the endocrine and metabolic changes described above, it is known that OTs affect both exocrine (66) and endocrine functions of the pancreas (20, 67–70). Regarding the later, it is proposed that the impairment in the glucose homeostasis occurs probably due to the ability of OTs to reduce insulin secretion and/or signaling, through inhibition of β cell proliferation, increased apoptosis, and decreased production of NAD(P)H and adenosine triphosphate (ATP) in pancreatic islet cells, associated with local oxidative stress (20, 67–70).

It is well known that the impacts on neuroendocrine control caused by OTs also interfere with reproductive endocrine function: TBT exposure was accountable for morphological changes, such as weight loss of the male and female reproductive organs (30, 31, 71) and abnormal steroidogenesis on gametes (72), as well as reproductive dysfunctions such as changes in ovary morphology and abnormal estrous cyclicity (26, 36, 73–75). Interestingly, Podratz et al. (26) demonstrated that the ingestion of seafood homogenate with imposex indeed provoke important alterations in the rat reproductive organs, strengthening the hypothesis that the ingestion of contaminated shellfish is an important source of exposure to OTs (26).

In female rats, TBT oral administration not only induced estrous cycle and ovary morphological abnormalities (i.e., increased apoptotic cells in the corpus luteum and granulosa cells and increased cystic follicles) but also reduced 17β-estradiol and elevate progesterone serum levels (74, 75). Moreover, studies demonstrate that, depending on the dose, TBT can activate estrogen receptors (ER) *in vivo* and *in vitro* having estrogenic and adipogenic activities (3); reduce ER function on metabolic and reproductive controls (20, 26); or even change ER expression in different sites of the hypothalamus–pituitary–gonadal (HPG) axis (75). Actually, the effects of TBT on the gonad function may be, at least in part, due to changes on the HPG axis. Recent studies reported significant alterations in pituitary and hypothalamic morphophysiology and reduced GnRH expression that was related to an impaired Kisspeptin/leptin signaling (22, 75).

Similarly, male adult rats exposed to TBT exhibit varied endocrine damages, including effects on the reproductive endocrine system (30, 71, 76–80). Using different doses, studies with rodents evidenced changes in gonad weight (71, 80), reduced level of luteinizing hormone and testosterone, and spermatogenic disorders associated with reduced Leydig (30, 79) and Sertoli cells (81).

In view of the changes described in the HPG axis and gonads from both genders, an impaired reproductive function should be expected. In fact, several studies have shown that exposure to OTs, in a dose-dependent manner, reduces fertility and embryonic implantation and causes teratogenesis (75, 82–87). Moreover, when administered to pregnant females, TBT-induced weight loss in mothers and their offspring, as well as growth retardation (76, 88). The *in utero* exposure to TBT also leads to an impaired sexual development by affecting germ cells, which may lead to permanent damage in the adult gonads (77). However, there are evidences that perinatal exposure to OTs in rodents affects differentially male and female pups: while male postnatal development was severely affected with decreased weight of reproductive organs, testosterone level and sperm motility, suggesting that impacts may persist throughout adulthood; female pups exhibited more discreet changes such as initiation of estrous cycling and opening of the vagina occurring at an earlier stage. If considering that the enzyme cytochrome P450 aromatase (P450arom) activity is differentially influenced by OTs in male and female organisms, these studies strengthen the hypothesis of the greater susceptibility of males in the pre- and postnatal periods (72, 89–92).

Taking together, the current literature presents strong evidences of OT-induced endocrine dysfunctions, including significant differences between genders following chronic exposure.

This is probably due to the ability of OTs causing not only general toxic effects but also specific molecular and cellular changes, thus altering cell signaling in different ways according to the physiology of each organism exposed.

Major Mechanisms on Metabolic and Endocrine Disrupting Induced by OTs

It is well known that OTs compounds induce their metabolic and endocrine-disrupting effects through interactions with transcriptional regulators such as nuclear and steroid receptors (42). Thus, OTs may affect different nuclear receptor signaling pathways inducing a variety of morphophysiological effects as reviewed herein. For example, as discussed above, OTs exerts obesogenic effect not only by stimulating adipogenesis as agonists of the PPARγ but also by central effects potentially *via* RXR/PPARγ signaling. Moreover, an equally well-described mechanism is to modulate the expression and/or activity of key enzymes for a number of biochemical processes involved in metabolism and endocrine function.

The synthesis of steroid hormones, for example, involves a number of steps catalyzed by enzymatic reactions that are potential targets for OTs including: (1) cholesterol metabolism, (2) chemical enzymatic conversions, and (3) trafficking of molecules between mitochondria and endoplasmic reticulum (93). Thus, OTs may induce biochemical and endocrine disorders due to this capability of up- or downregulate key enzymes of steroidogenesis (74, 75, 94–96). Studies have described a relationship between endocrine dysfunction induced by OTs and their effects on the enzyme P450arom, which converts androgens into estrogen (94, 96, 97). TBT is reported as a competitive inhibitor of P450arom by reducing its affinity for androstenedione, although this inhibitory effect depends on the exposed tissue, concentration, and time of exposure (94, 96, 97). Conversely, Nakanishi et al. (98) demonstrated that TBT and TPT can increase P450arom activity in a dose- and time-dependent manner in human placental choriocarcinoma cells (98). In addition, in male rats OTs increase P450arom activity and reduce testosterone levels, opposite effects to that found in females (30, 78, 89, 90). Thus, the effects of OTs on the enzymes activity vary not only with tissue or exposed cells, dose, and time of exposure but also according to gender, especially in the case of enzymes related to sex hormones. In animal studies are described an OTs-mediated inhibition of 17-hydroxylase, 3-β-HSD, and 17β-HSD, thus suppressing testosterone biosynthesis (99, 100). Furthermore, studies in human blood and tissue samples also evidenced an inhibitory effect on 5α-reductase 1 and 2, P450arom, 3β-HSD 2, 17β-HSD 1, 11β-HSD1, and 3 (101–103). In a molecular level, Lo et al. (101) suggest there is an interaction of OTs with critical cysteine residues of enzymes leading to disturbances in the steroid hormone levels (101).

It is worth noting that in addition to interaction with nuclear and steroid receptors or specific changes on enzymes involved with steroidogenesis as cited above, the endocrine dysfunction due to OTs exposure can be mediated also by general toxic effects, such as increased oxidative stress and damages to mitochondrial function and subsequent responses to cellular stress (104–107). In this regard, the inhibition of ATP synthesis evidenced by studies with OTs exposure could thereby trigger similar biochemical and/or endocrine dysfunctions (108–111).

Finally, based on studies with cells, tissues and living organisms including mammals exposed to OTs, there are strong evidences of the potential toxicity predisposing to metabolic syndrome complications and endocrine-reproductive disorders, due to changes in all components along the hypothalamus–pituitary axis and peripheral tissues. Notwithstanding, there are changes in different sites including adipose tissue, endocrine glands, neurohormonal, and metabolic control centers, which together can justify the role of OTs as an endocrine and metabolism disruptor in mammals.

AUTHOR CONTRIBUTIONS

LS and VM idealized the general structure of the text, RF and VM did the literature review, LS, RF, and VM wrote the text, idealized and designed the figures. LS did the final revision of the text.

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Tributyltin and Zebrafish: Swimming in Dangerous Water

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Zebrafish has been established as a reliable biological model with important insertion in academy (morphologic, biochemical, and pathophysiological studies) and pharmaceutical industry (toxicology and drug development) due to its molecular complexity and similar systems biology that recapitulate those from other organisms. Considering the toxicological aspects, many efforts using zebrafish models are being done in order to elucidate the effects of endocrine disruptors, and some of them are focused on tributyltin (TBT) and its mechanism of action. TBT is an antifouling agent applied in ship's hull that is constantly released into the water and absorbed by marine organisms, leading to bioaccumulation and biomagnification effects. Thus, several findings of malformations and changes in the normal biochemical and physiologic aspects of these marine animals have been related to TBT contamination. In the present review, we have compiled the most significant studies related to TBT effects in zebrafish, also taking into consideration the effects found in other study models.

Keywords: zebrafish, tributyltin, endocrine disruptors, imposex, obesogenic

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INTRODUCTION

Zebrafish, Danio rerio, is a native teleost to the southeastern Himalayan region that has emerged as a reliable model for studying not only embryogenesis and regeneration, but also disease. The main advantages of zebrafish when compared to other biological models refer to their small size, the easy maintenance characteristics, and relatively low cost (1). Zebrafish has a high fertility rate that is characterized by dozens of embryos per matching couple, which allow a significant number of genetic approaches, such as morpholino antisense oligonucleotide technology to knock down several genes, study their function, and generate new disease models (2). Zebrafish has also been used in the field of drug discovery with great success, since it can be used for target identification, pharmacokinetic/pharmacodynamic, and toxicology studies (3). Due to its large and traditional use in the drug discovery field, the expertise of zebrafish model has been transferred to the analysis of endocrine disruptor effects.

The anatomical structures are similar between zebrafish and human organs, which confirms that this model is versatile and useful. Compared to *Caenorhabditis elegans* and *Drosophila melanogaster* models, zebrafish has a greater number of genes with a higher homology to human genome (4). When it comes to *Mus musculus* comparison, zebrafish has about the same number of genes, although with less homology (70 versus 90%) but with a lower annual cost (4). Menke and coworkers showed the anatomic and histologic features of adult zebrafish, evidencing similarity in the hematopoietic system, spleen, thymus, heart, thyroid, kidney, gastrointestinal system, liver, pancreas, brain (with telencephalon, diencephalons, mesencephalon, metencephalon, and myelencephalon), hypothalamus, pineal gland, pituitary gland, eye, and musculoskeletal system tissues, besides reproductive organs (5).

Therefore, the use of zebrafish for toxicology investigation comprises reproductive, developmental, neuro, cardiac, ocular, endocrine, vascular, and carcinogenic toxicity with several end points to be analyzed that should be chosen carefully for each purpose (6). Thus, the use of zebrafish for studying the effects of endocrine disruptors and/or their mechanism of action is convenient.

Endocrine-disrupting chemicals (EDCs) are natural occurring or synthetic compounds that interfere with natural hormone synthesis, secretion, transport, binding, or elimination, leading to homeostatic imbalance (7). Gore et al. (2014) postulated that EDC can enter the human body by different routes of exposition, such as oral consumption of contaminated food or water, contact with skin and/or inhalation, intravenous administration, and biological transfer through the placenta or milk during lactation (8).

As one of the most widespread EDC, tributyltin (TBT) has gained special attention. TBT is an organotin (one or more covalent bonds between carbon and tin atoms) that is used as an antifouling agent in boat paints and is continuously released into the water. As a result, harbor areas are deeply affected by this compound, which causes changes in the endocrine system of marine organisms, such as the development of male sexual anatomical characteristics in female gastropods, leading to sterility and death (9). TBT is rapidly absorbed by marine organisms, incorporated and accumulated in different tissues; after absorption, TBT can be metabolized and can generate other tin molecules, with different toxic properties and mechanisms of action (10).

The studies regarding TBT effects in zebrafish are rare compared to other species and EDC. Li and coworkers showed that the exposure of common carp to TBT for 7 days leads to oxidative stress, the inhibition of antioxidant enzymes, and the inhibition of the Na^+/K^+ ATPase activity, acetylcholinesterase, and monoamine oxidase (11). Also, a diminished activity of Na^+/K^+ ATPase was found in *Sebastiscus marmoratus*, which corroborates with the idea of a toxic effect of TBT (12).

TBT, Gonads, and Sexual Bias

Regarding sexual development, intraperitoneal injections of 1 or 5 mg/kg TBT in adult zebrafish lead to the reduction in mRNA levels of *sox9* and *Dax1* in brain, which is a conflicting result (13). TBT as a male-biased population agent usually causes a severe shift in organism end point toward masculinizing phenotype (14). *sox9* gene encodes a transcription factor related to the male phenotype, while *Dax1* encodes a nuclear receptor that acts in the female development (15), so the presence of lower levels of *sox9* in the brain, together with a male phenotype animal, shows how complex EDC treatment effects could be (**Figure 1**).

Tributyltin promotes a dose-dependent increase in the masculinization rate of embryos treated for 70 days from hatching, reaching almost 100% of sex rate toward male with the concentration of 100 ng/L. These animals show abnormalities and a decreased motility of spermatozoid, because this population produces a higher quantity of spermatozoids that lack flagella (16). This is in agreement with other reports in the literature which suggest that TBT is an imposex-inducing agent in other species (17–23) and with the finding of aromatase inhibitory ability of TBT. Aromatase is the enzyme responsible for the conversion of androgens into estrogens in cells (**Figure 2**). Considering this, the human granulosa-like tumor cell line KGN displayed a significant suppressed aromatase activity when treated with TBT (24). Also, TBT might function as an agonist of the estrogen receptor alpha (ERα), since it has a proliferative effect on ER (+) breast adenocarcinoma cell line (MCF-7) (25). The treatment of HeLa cells transiently co-transfected with zebrafish estrogen receptors (zfERα, zfERβ1, and zfERβ2) with ethinyl estradiol results in a fourfold to sixfold increase in luciferase activity, an effect that was inhibited by TBT. By contrast, when cells were co-transfected with zebrafish androgen receptor and treated with testosterone, the treatment with TBT was not able to change luciferase activity, showing that imposex-inducing ability of TBT is widely complex and a multistep action (13).

The Obesogenic Role of TBT

Besides imposex, TBT is highly associated to increased adipogenesis and is considered as obesogenic (26). Little is known about TBT effects in brain, most of the studies being focused on

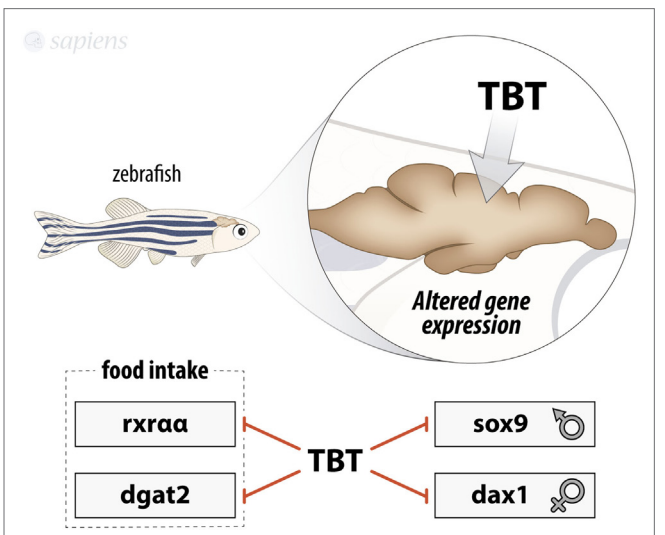

FIGURE 1 | Summary of tributyltin (TBT) effects on zebrafish brain. TBT is able to modulate gene expression in zebrafish brain by decreasing rxraa, dgat2, sox9, and dax1 mRNA expression.

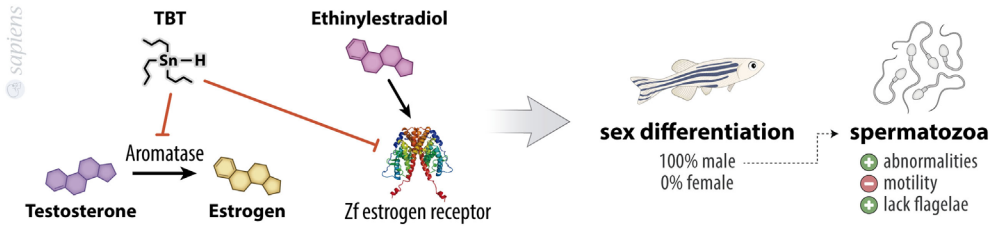

FIGURE 2 | Tribultytin (TBT) acting in sexual bias. TBT is an inhibitor of aromatase, the enzyme responsible for testosterone to estrogen conversion, besides inhibiting zebrafish estrogen receptor, decreasing the effects of ethinylestradiol. These molecular events point to male sexual differentiation of almost 100% of treated animals. TBT-treated animals also present increased spermatozoa abnormalities, increased lacking flagellae spermatozoa, and a decreased spermatozoa motility.

gene expression alterations concerning enzymes involved in lipid metabolism and sexual hormones (13, 27). Studies using 10 or 50 ng/L of TBT for 9 months in male and female animals showed the modulation of *R*etinoid *X R*eceptor alpha (RXRα/α)-nuclear receptor and *D*iacyl*G*lycerol O-*A*cyl*T*ransferase 2 (DGAT2)-lipogenic enzyme in both genders, with no modulation of PPARγ levels in brain, besides gender-specific alterations of gene expression (**Figure 1**). TBT might exert its lipogenic and adipocyte differentiation effects through the well-known RXR–PPARγ complex ligand ability (28, 29). These results confirm zebrafish as a good model for studying lipid homeostasis, since the complex mechanisms underlying food intake control and obesity development are similar to mammals.

The role of TBT as an obesogenic factor is well documented in the literature. Li and coworkers showed an activation of RXR-PPARγ heterodimer, triglyceride storage, and expression of adipogenic marker genes even in the presence of PPARγ agonist GW9662 in cultured preadipocytes (30). Indeed, TBT was shown to bind not only to RXR but also to PPARγ receptor (31), leading to weight gain, altered lipid homeostasis, lipid accumulation, raised expression of the adipocyte marker C/EBPα, reduced adiponectin expression, altered glucose metabolism, increased PPARγ expression, and hepatic inflammation (32–34).

Zebrafish treated with TBT shows an increase in adipogenesis at 15 days post fertilization and displays significantly increased adipocyte differentiation markers, with altered gene expression profile of adipogenic factors, like POMC (hypothalamic factor involved in feed behavior) and leptin (35). These data are consistent with the findings showing that female rats treated with TBT for 15 days present hyperleptinemia (36).

Exposure to TBT in the nanomolar range for 3 days increases the percentage of adiposity in larvae (by Nile red staining of adipocyte lipid droplets) with the induction of adipocyte hypertrophy despite fasting (37). Interestingly, human PPARγ antagonists did not block the *in vivo* obesogenic effect of TBT, but the human RXR antagonist UVI3003 fully abolished the effect, confirming that zebrafish adipose tissue is readily responsive to adipogenic molecules, even in a fasting state *via* RXR pathway (38). Zebrafish exposed to TBT for 9 months also presented altered body weight with increased triglycerides in male and the modulation of a range of lipogenic genes in liver, such as PPARγ, RXRα, C/EBPβ, and IGFIIα, all of them being adipogenic stimulators (27). Some recent work fully ratifies not only the zebrafish as an animal model for adipose tissue studies but also points to new techniques for assaying adipocytes dynamics in zebrafish (39–41) (**Figure 3**).

It was also reported that TBT could affect nutritional status by modifying yolk absorption. Yolk provides energy and nutrients for developmental phases in teleosts, since it is mainly composed of phospholipids and triacylglycerols packed into lipoprotein particles (vitellogenin) and surrounded by the yolk syncytial layer that functions to hydrolyze yolk molecules and transport them to embryos. TBT, as an obesogenic agent, causes a faster uptake of yolk (42).

Other TBT Effects in Zebrafish

Regarding behavioral aspects, there are only few studies and most of them point to altered end points. Male Wistar rats treated with various doses of TBT showed a dose-dependent decrease in spontaneous motor activity during dark phase and an inhibition in the acquisition of shock avoidance responses also in a dose-dependent manner, indicating that TBT exposure can cause a significant disturbance in rat behavior (43). Non-reproductive behavior alteration in teleost rare minnow was also documented, revealing that fish exposed to TBT had less group cohesion during the course of a 10min period of observation, altered shoaling in novel tank test, shorter latency before leaving shoal mates, and they spent more time away from shoal than control fish, with increased anxiety (44).

Considering the antioxidant ability and immunity, an 8-week treatment with TBT reduced superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities in a dose-dependent manner, with an increase in the relative expression of HSP70 and HSP90, IL-1β, IL-6, TNF-α, and NF-κB. Thus, TBT is an inducer of oxidative stress and plays an important role in the positive modulation of pro-inflammatory cytokines (45). This is consistent with data showing a decreased activity of SOD, CAT, and GPX in other species (46, 47), a higher expression of HSP70 in common carp (48), an increased IL-1β secretion by human immune cells (49), an increased IL-6 production in human peripheral blood mononuclear cells (50), and higher TNF-α levels in mouse serum (51).

It was also reported that TBT could affect nutritional status by modifying yolk absorption. Yolk in teleosts provides energy and nutrients for the developmental phase, being composed in majority of phospholipids and triacylglycerols packed into lipoprotein particles (vitellogenin) and surrounded by the yolk syncytial layer

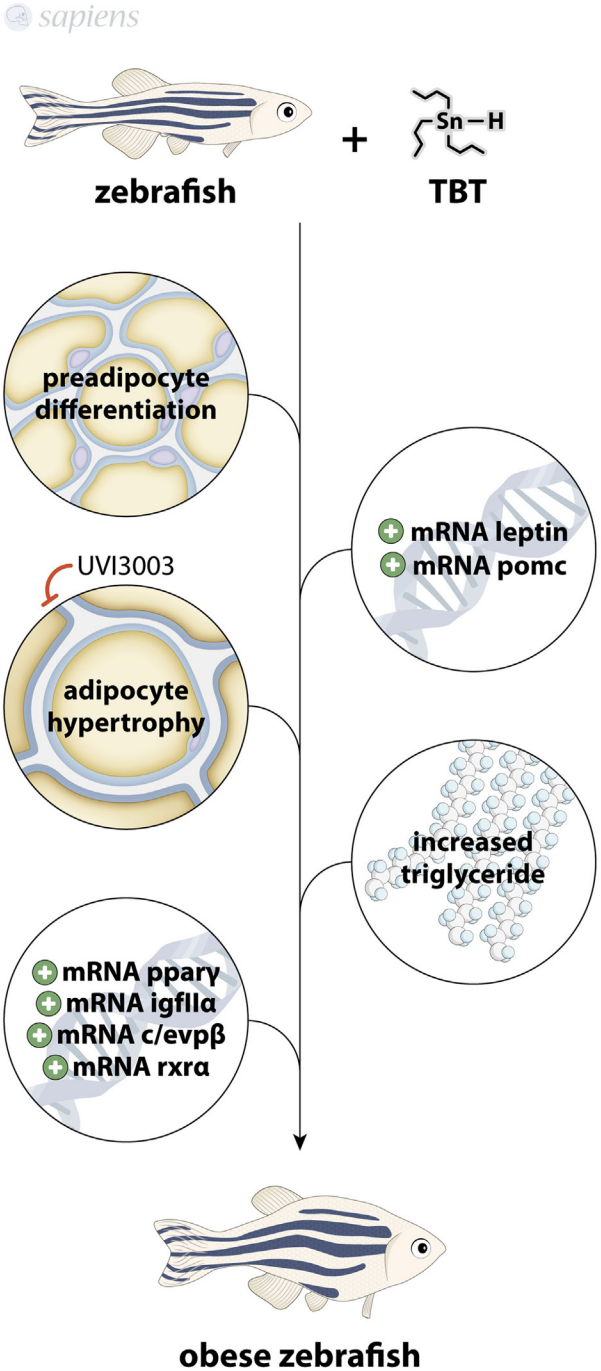

FIGURE 3 | Tributyltin (TBT) as obesogenic molecule. TBT-treated zebrafish presents increased preadipocytes differentiation, modulation of pomc, leptin, pparγ, c/evpβ, IGFIIα, and rxrα mRNA, increased adipocyte hypertrophy (that can be blocked by UVI3003) and increased triglyceride levels, culminating in an obese animal.

that functions hydrolyzing yolk molecules and transporting them to embryo. TBT, as obesogenic agent, caused a faster uptake of yolk in an automatic method to segment and quantify yolk areas in zebrafish larvae (42).

Zebrafish larvae treated with TBT (0.03 nM) show increased death with diminished hatch rates, an abnormal body curvature, a higher pericardial edema, and a dorsal curve rate. These data are controversial since Liang and coworkers (52) showed a higher hatch rate in embryos treated with higher concentrations of TBT (1 nM). Nevertheless, this could be due to EDC dose–response behavior that often show non-monotonic dose–response curve in a U-shaped or inverted U-shaped curves (0.03 or 1 nM), probably belonging to any point of the curve with a hatch rate as end point (53). Also, a decrease in heart rate was reported, with the differential expression of important genes related to cardiac function and development, such as *cav3* that encodes caveolin 3 protein and *cmlc1*, which encodes cardiac myosin light chain-1 (essential for zebrafish cardiogenesis) (54, 55). Other studies concerning cardiac function in TBT-treated animals were published revealing that this organotin induces cardiomyopathy in clam Ruditapes (56) and increased collagen deposition in heart interstice, impaired coronary vascular reactivity to estradiol, and enhanced the number of mast cells proximate to cardiac vessels in rats (57).

Unprecedented studies in zebrafish assessing TBT effects in systems not widely rummaged are also available. TUNEL staining of zebrafish embryos displayed TBT-induced apoptosis restricted to retinal neuronal cells and unidentified cells around trigeminal neurons with macrophage accumulation, probably by higher accumulation of TBT in the optic tract (58), showing selective apoptosis in this tissue (59). Also, genotoxicity using zebrafish erythrocytes was reported in an erythrocytic nuclear abnormality (ENA) frequency assay in animals exposed for 4 months, exhibiting a higher ENA frequency in TBT-treated conditions (60).

CONCLUSION

Studies concerning TBT as an EDC are rapidly growing every year based on its wide range of effects in humans and laboratory animals. These broad options of models comprising normal systems and diseases are of great importance for recognizing TBT actions due to its widespread usage in the world. Zebrafish is a reliable model for studying several diseases like cancer, obesity, and inflammation and has become a robust tool for assessing EDC effects. Studies using zebrafish as a biological model to access TBT effects are few but they corroborate the effects found in other classical animal models, such as murine ones. Brain effects of TBT related to behavior changes are well documented in the literature (44, 61–64) and absent in zebrafish, even though these animals possess similar structures and molecular complexity comparable to other models in order to test memory, anxiety, fear, and social behavior (65–67). Also, considering the hypothalamus–pituitary–thyroid axis, no study has been done yet to evaluate the effects of this compound in zebrafish, although an extensive and elucidating review described the action of TBT in other species (68).

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CB-J, DC, PS, and LM-A conceived and wrote the article.

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Organotin Compounds Toxicity: Focus on Kidney

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Organotin compounds (OTs) are synthetic persistent organometallic xenobiotics widely used in several commercial applications. They exert well-described harmful effects in brain, liver, adipose tissue, and reproductive organs, as they are endocrine-disrupting chemicals (EDCs), but the effects in the kidneys are less known. The kidneys are especially vulnerable to environmental contaminants because they are a metabolizing site of xenobiotics, therefore, pollutants can accumulate in renal tissue, leading to impaired renal function and to several renal abnormalities. Individuals chronically exposed to OTs present a threefold increase in the prevalence of kidney stones. These compounds can directly inhibit H^+/K^+-ATPase in renal intercalated cells, resulting in hypokalemia, renal tubular acidity, and increased urinary pH, which is a known risk factor for kidney stones formation. OTs effects are not only limited to induce nephrolithiasis, its nephrotoxicity is also due to increased reactive oxygen species (ROS). This increase leads to lipid peroxidation, abnormal cellular function, and cell death. Combined, the enzymatic and non-enzymatic antioxidant defense systems become deficient and there is a consequent uncontrolled generation of ROS that culminates in renal tissue damage. Still, few epidemiological and experimental studies have reported renal impact correlated to OTs exposure. This lack of investigation of the complete effect of OTs in renal function and structure led us to perform this review reporting the main researches about this subject.

Keywords: organotins, kidney, renal function, nephrotoxicity, pollutants, trimethyltin, tributyltin, triphenyltin

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INTRODUCTION

Organotin compounds (OTs) are synthetic organometallic chemicals with several commercial applications. The major one is in the plastics industry which utilizes these compounds particularly to produce polyvinyl chloride (PVC) (1). As PVC polymer is unstable under heat and light, OTs derivatives can be added as stabilizers (2). Methyltin stabilizers are made from monomethyltin (MMT) and dimethyltin (DMT) that are synthesized by a direct chemical reaction. Trimethyltin (TMT) is produced as a byproduct during this synthesis and it is more toxic than MMT and DMT. Methyltin-stabilized PVC is used in packaging, piping, coating, and window frames (1). OTs have been found to leach from PVC pipes and it can contaminate foodstuffs, beverages, drinking water, and sewage (3, 4).

Trisubstituted organotin species have biocidal properties and can be used as agricultural pesticides, wood preservatives, and antifouling paints on ships (5). The broad utilization of OTs allows sizable amounts of them to enter various ecosystems. Specially, tributyltin (TBT) and triphenyltin (TPT) have high complex toxic effect to aquatic life even at low levels (6, 7). They can act as endocrine-disrupting chemicals (EDC) in target and non-target organisms (8). In mollusks, TBT is able to lead to imposex development, which is an abnormal endocrine syndrome with imposition of male sex characteristics in female organisms (9). In mammals, abnormalities in metabolism and in neural, immune, hepatic, and reproductive systems are reported after TBT exposure (10–12). Widespread environmental contamination of marine ecosystems with TBT began in the 1960s and its use in antifouling ship paints was prohibited by the International Marine Organization in 2008 (13, 14). However, beyond its regular use in agriculture and other industrial processes, it is possible that TBT is still used in some parts of the world in countries that are not included in the International Convention on the Control of Harmful Anti-Fouling Systems on Ships and/or with poor environmental monitoring and fiscalization (15, 16).

For the human population, the major route of exposure to most OTs is ingestion, through the consumption of food and/or drinks either contaminated with OTs (17). Marine fishery products may contain high TBT levels (18), and different diets are expected to result in different OTs loads in human tissues (19–21). However, despite the evidence that such sources expose humans to OTs, limited data on deposition in humans are available. Thus, human risk assessment has mainly been based on experimental immunological studies and estimated human intake of seafood sources (18). OTs are detected in human blood at levels that range from 64 to 155 ng/mL, which leads to TBT tissue accumulation and immunological dysfunctions (22).

The impact of methyltin compounds on human health is primarily focused on its neurotoxic effects (23–27). Although the neurotoxic outcomes of OTs have been well documented, their nephrotoxic effects have received little attention. In 1987, Robertson and colleagues described TMT nephrotoxic effects and highlighted how undetected they were until then (28).

The kidneys play important roles in the maintenance of body homeostasis, such as regulation of extracellular fluid osmolality, volume, electrolytes, and acid–base balance (29). Furthermore, kidneys possess most of the common xenobiotic metabolizing enzymes contributing to the metabolism of drugs and foreign compounds, including environmental contaminants (30). In consequence, the kidneys tend to be more susceptible to those substances (31). Indeed, renal xenobiotic exposure leads to improper renal function (32). In this review, the renal outcomes related to OTs will be explored.

ORGANOTINS INDUCE STRUCTURAL AND FUNCTIONAL CHANGES IN KIDNEYS

Organotin compounds are acknowledged for its neurotoxic effects, producing a range of neurological symptoms and they are also known for its toxicity in liver and reproductive system (33–35). However, few studies have reported the renal toxicity of these compounds. In 1985, Dwivedi and collaborators were trying to discover the biological effects of OTs and demonstrated that several of these compounds can affect renal enzymatic activities in rats (36). The same study also demonstrated enzymatic alterations in liver and brain (36). Before that, hydronephrosis and vacuolar degeneration of renal tubules were described in rats exposed to TMT (37). Blood urea nitrogen (BUN) levels, tubular dilatation, and epithelial vacuolization were also shown to be increased by TMT exposure (38). However, these reports were conflicting with studies that described no significant effects in the kidneys that could be correlated to OTs exposure (39, 40).

Trimethyltin was first described as a potent nephrotoxicant in two studies where this compound was orally administered in rats (28, 41). TMT induced rats to a renal failure and there was a time-course relationship between the effects on the kidney and various neurological manifestations (28). TMT initially induced oliguria and renal lesions that progressed to acute renal failure. Proteinuria, increase in urinary pH and in BUN levels were also present (28). Histological abnormalities were observed as tubular focal effects especially in the outer medullary area with interstitium expansion and consequent obstruction of the vascular supply and swelling in the renal papilla. Another study demonstrated marked proximal tubular damage with dilation and loss of the brush borders. There was no clear evidence of glomerular damage, thus tubular lesions seem to be more important (28, 41).

Until 1993, neither nephrotoxicity nor pathological changes of the kidney induced by OTs had been reported in human studies. A case study of three patients admitted with acute TPT intoxication showed an increase in serum creatinine and BUN levels, which were compatible with the dysfunctional results of animal studies (42). A significant increase in proteinuria in all three patients could indicate severe tubular and mild glomerular injury (42).

Another important OT that has been investigated is TBT. Low subchronic oral doses of TBT exposure (2.0 or 6.0 μg/kg) were administered to rats once a week for over 30 or 60 days and showed no effect on kidney morphology (43). On the other hand, a higher dose of TBT (50 mg/kg diet) on a 30-month chronic toxicity study in rats resulted in decreased renal function weight (44). Despite the studies about TBT harmfulness, only a few studies evaluated its effects in renal morphology (45, 46). Mitra et al. showed morphological alterations in rats with a low and unique dose of TBT (5 mg/kg): the glomeruli appeared swollen with increased capsular space. Although kidney function was unaltered in this particular study, the oxidative stress, as well as reactive oxygen species (ROS), was increased in renal tissue (34). Thus, TBT presents a complex and contradictory toxicological renal effect. Furthermore, TBT was shown to lead to a reduced glomerular filtration rate (GFR) and increased proteinuria levels in female rats exposed to TBT (100 ng/kg/day) for 15 days (47). Renal structural abnormalities such as increased glomerular tuft area and tubulointerstitial collagen deposition were also observed. Additionally, TBT led to tin renal tissue accumulation associated with higher renal oxidative stress and apoptosis levels, leading to abnormal renal function (47).

Other striking features regarding OTs and their effects in the kidneys are the increase in oxidative stress, hypokalemia state, and kidney stones formation (47–49). They all will be discussed along these lines.

ORGANOTINS CHALLENGE KIDNEYS WITH OXIDATIVE STRESS

Organotin compounds have many biological impacts and are associated with endocrine and physiologic disruptor effects, acting as EDCs (50). It was recently demonstrated that they are able to bind nuclear receptor, such as glucocorticoid receptors and retinoid X receptor subtypes, forming a complex OTs-nuclear receptors with coactivators and inducing transcription of target genes (51, 52). This process promotes changes in the expression of proteins in addition to mitochondrial and cell dysfunctions (51, 52).

Oxidative stress is the main pathway involved in tissue damage induced by OTs in different organs, such as kidneys, testis, liver, lungs, adrenal gland, pituitary, and brain (34, 47, 53–55). It was described that TBT induces ROS production, lipid peroxidation, and cell death in rodent models (56). Moreover, it decreases the enzymatic and non-enzymatic antioxidant defense systems (catalase, superoxide dismutase, glutathione peroxidase, and vitamins C and E) (53). Indeed, oral administration of TBT for 65 days in Syrian hamsters led to high levels of serum creatinine, urea, bilirubin, and uric acid, with histopathological abnormalities in the testis, liver, and kidneys (53). TBT treatment induced a decrease in the activity of catalase, superoxide dismutase, glutathione peroxidase, and vitamins C and E, and an increase in lipid peroxidation in the same organs (53), demonstrating critical oxidative stress-induced damage by TBT action. ROS generation induced by TBT impairs cell function and culminates in tissue damage (53). Coutinho et al. (47) showed important renal function impairment induced by TBT in female rats, with renal inflammation and fibrosis, increased glomerular tuft area, reduced GFR, and increased proteinuria. TBT effects on renal dysfunction were shown to be due to the oxidative stress and apoptosis levels (47). TBT induced increases in ROS levels in the serum, liver, lung, and kidney of male Wistar rats after subchronic exposure to low doses of TBT for 1 month. In this case, kidney presented a 1.4-fold increase in the ROS levels after 1 mg/kg of TBT for 30 days, showing an important association between TBT exposure and renal ROS development (54).

Likewise the kidney, brain, and cardiovascular system damage induced by OTs are also due to ROS production (34, 55, 57, 58). Neurodegeneration in rats was shown to be *via* oxidative damage, mitochondrial membrane depolarization, DNA damage, and apoptosis in cortical cells, due to ROS overproduction (34). Hippocampus and hypothalamus in rats exposed to TBT develop inflammation, fibrotic process also due to increased oxidative stress (55, 59). Fibrosis also occurs in aortic rings as a consequence of oxidative stress increase induced by TBT exposure in female rats for 15 days (100 ng/kg/day), resulting in functional and morphological dysfunctions (58, 60, 61).

Studies of TBT effects in the kidney suggested that the oxidative stress is the main cause of renal damage induced by OTs (62, 63). Increased ROS induce mitochondrial dysfunction, caspase activation, DNA damage, and cell death, which in turn lead to an irreversible renal dysfunction. Similarly, a common feature of TBT toxicity on brain, testis, liver, lungs, adrenal gland, pituitary, and cardiovascular system was also shown to be due to ROS production (53–55).

Increased oxidative stress induced by OTs can bring damages to kidneys. Those compounds can affect kidneys in other ways like inducing hypokalemia that will be discussed next.

ORGANOTINS ARE POTENT HYPOKALEMIC INDUCTORS

Hypokalemia is a condition of low blood potassium (K^+) levels and it is one of the most common and dangerous electrolyte abnormalities observed in clinical medicine. It alters the functions of several organs, such as muscles, kidneys, and cardiovascular and neurologic systems (64). Clinical analysis of 76 cases from 13 poisoning accidents caused by TMT demonstrated that 81.6% (62 cases) presented hypokalemia, which persisted for more than 1 week in most cases (23). Urine K^+ levels were between 5 and 165 mmol/L in 47 patients. Accordingly, other clinical analysis of TMT intoxication also revealed low serum K^+ levels in 85.7% of the cases (48 from 56 patients) (65). These studies suggested that hypokalemia could be the main clinical indication of TMT intoxication (23, 65). Since no diarrhea or vomiting was observed in TMT intoxicated patients, which could justify blood K^+ levels reduction, it was postulated that TMT-induced hypokalemia could be due to its leakage in urine (48). Likewise, Guo et al. (63) analyzed 15 patients that were admitted to Sir Run Run Shaw Hospital from 2002 to 2007 with OTs poisoning and observed that most of patients presented elevated blood ammonia, metabolic acidosis, and decreased K^+ blood levels, as a result of renal OTs toxicity.

Unbound K^+ is freely filtered across the glomerulus and the majority of tubular K^+ is reabsorbed along the proximal tubule and the thick ascending limb of Henle's loop (66). Only 10% of filtered K^+ reaches the distal nephron and generally 10–20% of the filtered K^+ load is excreted (67), suggesting that this ion is secreted along the nephron. The control of K^+ secretion within the kidney occurs in the distal nephron (68). The collecting duct is composed of two cell types: principal and intercalated cells. Intercalated cells represent a small fraction of epithelial cells along the distal nephron that reabsorb K^+ *via* luminal-membrane H^+/K^+-ATPase. Inhibition of this ATPase prevents K^+ reabsorption and H^+ secretion and was suggested to be the mechanism underlying TMT-induced hypokalemia (48). Indeed, administration of 10 mg/kg of TMT in Sprague-Dawley rats induced hypokalemia and as K^+ serum levels were decreased, the K^+ leakage in the urine was increased (48). Although H^+/K^+-ATPase mRNA content and expression was not changed, TMT inhibited intercalated cells H^+/K^+-ATPase activity and K^+ reabsorption, decreasing H^+ secretion, inducing hypokalemia and acidosis, as reported in **Figure 1** (48).

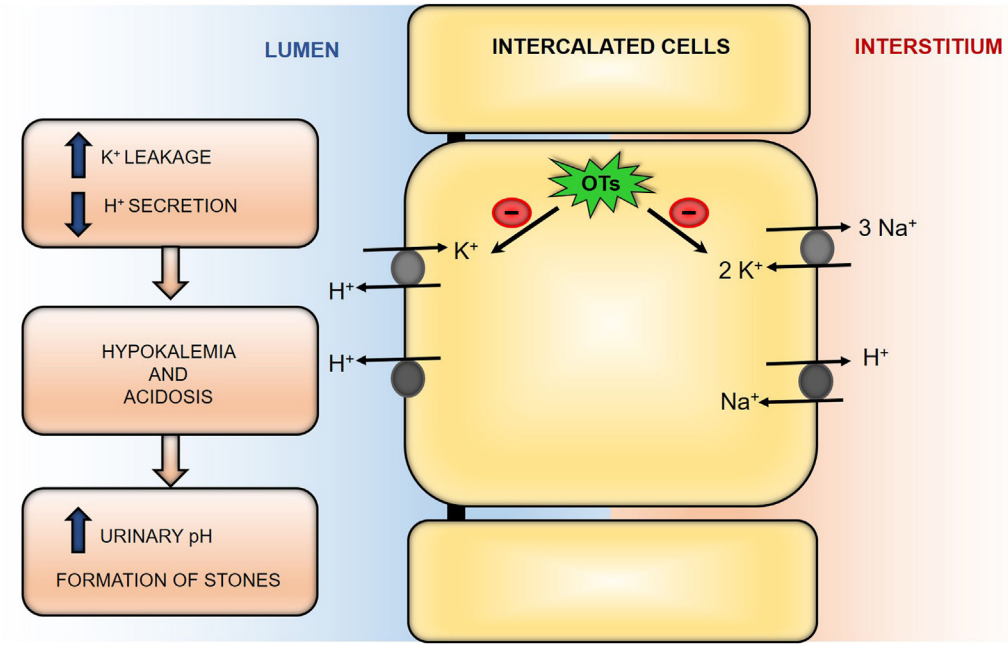

FIGURE 1 | Schematic representation of organotin compounds (OTs) effects in renal intercalated cells in mammalian models. OTs are able to inhibit both H^+/K^+-ATPase and Na^+/K^+-ATPase activities. This inhibition leads to hypokalemia and acidosis. Former is due to increased K^+ leakage to the lumen, and latter is due to decreased H^+ secretion. In consequence, urinary pH is increased, which is a known factor for formation of kidney stones.

It was demonstrated that other ATPase is involved with TMT-induced hypokalemia (48). Sprague-Dawley rats treated with 10 or 21.5 mg/kg of TMT for 11 days had a rapid and persistent decrease in plasma K^+ level, starting 30 min after the treatment and persisting until the end of the experiment at the 11th day. It was suggested that Na^+/K^+-ATPase modulation is the cause of TMT-induced hypokalemia, since its activity was decreased after TMT treatment, reducing renal K^+ reabsorption (48). Accordingly, Sprague-Dawley rats treated with 10 mg/kg of TMT presented a decrease in plasma K^+ level with the lowest dosage on day 6 (4.85 mmol/L) and recovering on day 28, and this TMT-induced hypokalemia was accompanied by Na^+/K^+-ATPase activity decrease (69). It was suggested that a rise of plasma aldosterone levels plays an important role on K^+ leakage resulting in TMT-induced hypokalemia, since it was tenfold increased after exposing rats to 46.4 mg/kg of TMT (69).

Not only TMT, but also other OTs are able to induce hypokalemia. Sprague-Dawley rats and Chinese Kun Ming mice treated *via* gavage and intraperitoneal injection with both DMT and TMT, presented a significant decrease in plasma K^+ level after 1 h of treatment, and the effect persisted for over 7 days (70). Both animal studies and clinical analyses of poisoned patients, demonstrated that OTs (TMT mostly) are powerful hypokalemia inductors, leading to decreased plasma K^+ levels, H^+ secretion, and consequent renal tubular cells acidity and urinary pH increase. This alkaline environment as result of OTs exposure favors the formation of various types of kidney stones and will be discussed in the next topic.

EXPOSURE TO ORGANOTINS IS ASSOCIATED WITH KIDNEY STONES

Kidney stones are a common health problem in industrialized countries affecting around 2–5% of the population during lifetime at least once (62, 71). The prevalence and incidence of nephrolithiasis is reportedly being increased globally. In the United States, overall stones prevalence has doubled over the past three decades. This increase has also been noted in most European countries and Southeast Asia (72, 73). The cause of these changes is unclear, but many factors predispose or contribute to the development of kidney stones, including genetic factors, diet, behavior, and environmental factors. It has been suspected that the latter is a potential major contributing cause. OTs such as TMT provoke hypokalemia likely due to H^+/K^+-ATPase inhibition which leads to urinary pH increase, as exposed above (48). The disruption of urinary pH and alteration of electrolytes levels may promote crystal deposition and stones formation in kidneys and urinary tract, as displayed in **Figure 1** (74).

As TMT potentially can induce nephrolithiasis, Tang et al. (70) examined 216 manufacturing workers exposed to TMT for at least 3 months and 119 control individuals that worked at the plant, but unexposed. Workers exposed to relative low levels of TMT in the air (<0.013 mg/m^3) were more likely to have kidney stones (threefold higher than the control group) and it was prevalent especially among those who were employed longer. This suggests a renal toxic effect that chronical TMT exposure may cause (70).

Another study investigated the long-term effect of TMT (14.7 mg/kg) submitting rats to this organotin in the drinking

water for 6 months (49). It was shown that different levels of TMT could induce a dose-dependent increase in kidney stones formation. As described in human poisoning cases, rats also presented inhibition of renal H^+/K^+-ATPase activity which leads to urinary pH increase. Alkaline urine favors the formation of calcium and phosphate stones; struvite stones can occur when urine pH is neutral or alkaline. TMT-treated rats presented the majority of stones composed of struvite in addition to calcium components, while the control group did not present any stones (62, 71).

Taking all these evidences in consideration, TMT exposure is positively associated with the development of kidney stones. The rising presence of this OTs in our daily environment may contribute to increase the risk of developing kidney stones and/or other renal abnormalities. Additional and comprehensive studies are necessary to corroborate with these findings and shed light on this subject.

CONCLUSION

Organotin compounds are a threat to human health and they are broadly used with various agricultural and industrial applications. Although their harmful effects have been better described in liver, reproductive, and nervous systems, their effects in the kidneys have not been widely investigated. The published data indicate that those contaminants have an impact in the kidney proper functioning, mostly, on its oxidative stress damage, on hypokalemia induction and on kidney stones formation. OTs toxicity depends on concentration, time of exposure, as well as the kind of species that it is being exposed. Therefore, OTs lead to an important renal toxicity that can be considered an important environmental risk for renal diseases development.

AUTHOR CONTRIBUTIONS

CB and FF contributed for data review and text writing. Editing and critical analysis were done by all three writers (CB, FF, and JG). CB prepared the figure.

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Tributyltin and Vascular Dysfunction: The Role of Oxidative Stress

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The organotin compounds (OT) are used as fungicides, stabilizers in plastics, miticides, manufacturing and agricultural biocides, wood preservatives and antifouling agents. Tributyltin (TBT) is an OT that was first used for antifouling because it was the most effective agent to prevent undesirable accumulation of marine organisms on solid surfaces, such as ships' hulls or mechanical components, immersed in saltwater. TBT can be easily absorbed by mammals through ingestion, and its cytotoxic effects have become a major concern since their discovery in the 1970s. Recently, it has been demonstrated that TBT exposure is detrimental to the cardiovascular system. TBT is a membrane active substance and its action seems to depend on the OT lipophilicity. As a result, TBT crosses the cell membrane and damages the endothelium and the smooth muscle cells. TBT exposure induces vascular dysfunction, most likely due to endothelial dysfunction and morphological changes in the vascular wall. In an experimental rodent model, small doses of TBT (100 and 500 ng/kg/bw/day for 15 days) modified the vascular reactivity in aorta, mesenteric and coronary arteries followed by smooth muscle cell atrophy, increased collagen deposition and fibrin accumulation. TBT exposure increases oxidative stress by inducing vascular superoxide anion production derived from NADPH oxidase and decreases nitric oxide (NO) production as well as eNOS protein expression. The goal of this review is to summarize the current state of the art regarding the mechanisms involved in the vascular and endothelial dysfunction induced by TBT.

Keywords: organotin compounds, tributyltin, vascular dysfunction, NADPH oxidase, endothelial dysfunction, nitric oxide

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INTRODUCTION

The organotin (OT) compounds have covalent bonds between tin (Sn) and carbon (C). Organotins are used as fungicides, as stabilizers in plastics, molluscicides, miticides, manufacturing catalysts, industrial and farming biocides, wood preservatives and antifouling agents (1–3). Tributyltin (TBT) was first used as an antifouling substance in the early 1960s and came to be recognized as the most effective agent used to prevent accumulation of aquatic organisms on solid shells, such as ships' hulls or motorized components, immersed in seawater. Over a decade ago, TBT copolymer (TBT-SPC) paints probably covered 70–80% of the world's fleet, leading to important economic benefits (4). Twenty years after TBT paints were introduced, it was demonstrated that they are deleterious to aquatic organisms. Even very low concentrations, such as 1 ng/L, were sufficient to cause imposex, as seen in noncommercial *Nucella lapillus* populations around Scottish oil ports, and along the south coast of England (5). Concentrations above 2 ng/L inhibited proper calcification of the commercial oyster, *Crassostrea gigas*, and concentrations above 20 ng/L inhibited larval growth (6).

Considering the harmful effects of TBT compounds on aquatic organisms, restrictions on use were imposed after the 1980s. The International Maritime Organization (IMO) approved a global prohibition on the use of TBT-based antifouling paints (4). However, countries lacking controlling national or regional legislation continue to use organotin compounds (OT) in coast-to-coast routes, mainly due to the lack of equivalent substitutes (4). In addition, TBT can be detected in marine biota and residue 20 years after initial contamination, due mainly to its high lipid-solubility. As a result, TBT residues can be found in organisms throughout the food chain, including mollusks, fish, seabirds and marine mammals (7, 8).

Humans are often exposed to TBT by the ingestion of contaminated seafood, water and beverages. TBT concentrations can vary in marine foods, so it is expected that different diets may cause different concentrations in human tissues and blood (9–11). Based on immune function studies, the World Health Organization adopted an Acceptable Daily Intake value for TBT of 250 ng/kg/day (4). However, due to uncertainty in human-rat toxicity extrapolation, a safety factor of one hundred was used for the final calculation of the daily intake value. The concentrations of TBT in human blood range from 20 to 50 ng/L in males and 170 to 670 ng/L in females and a study that analyzed blood samples from 38 volunteers from Michigan (USA) showed TBT concentrations ranging from below the detection limit up to 1,550 ng/L (12).

It is not clear in the literature what the TBT concentrations in the population are. There is a lack of clinical studies showing the TBT concentrations in human blood and tissues. Furthermore, as TBT can be easily absorbed by mammals, TBT cytotoxicity became a major concern since the discovery of the toxic effects in the 1970s. Consequently, investigators sought to better understand the impact of TBT pollution on the organism. In recent years, more focus has been put on the effects of TBT on the cardiovascular system. In addition, new evidence in the literature demonstrates that TBT exposures of 0.1–0.5 μg/kg/day, at or below the established Acceptable Daily Intake, are detrimental to the cardiovascular system (13–15).

The goal of this review is to summarize the current state of the art regarding TBT and vascular dysfunction, focusing on the mechanisms involved.

THE MECHANISMS WHICH BY ORGANOTIN INDUCES VASCULAR DYSFUNCTION: THE ROLE OF NOX AND OXIDATIVE STRESS

Vascular endothelial homeostasis is a tight balance between vasodilatation and vasoconstriction, pro-thrombotic, pro-inflammatory and anti-thrombotic, anti-inflammatory processes. Endothelial dysfunction can be defined as a shift of the endothelium toward reduced vasodilation, followed by increased vasoconstriction, increased platelet aggregation and adhesion leading to a pro-thrombotic state, enhanced smooth muscle proliferation, and increased vascular inflammation. Endothelial dysfunction is also characterized by reduced activity of key vasodilators such as NO; prostacyclin and EDHF are also recognized as vasodilators. On the other hand, reactive oxygen species (ROS) like the superoxide anion (O_2^-), peroxynitrite ($ONOO^-$), as well as endothelin-1, thromboxane A as well as angiotensin II are potent vasoconstrictors (16).

In recent years, vascular dysfunction attributed to TBT exposure has been thought to be manifest mainly in the above-described endothelial changes and also morphological changes in the vascular wall. **Figure 1** shows a summary of the main actions of TBT on the endothelium and on the smooth muscle cell. The endothelium is organized in a single layer of cells that are in direct contact with plasma, making these cells vulnerable to the molecules and ions there. As TBT is very lipophilic, it can easily cross the cell membrane and damage the endothelium as well as the smooth muscle cells.

In an experimental rodent model, a small dose of TBT (500 ng/kg) was shown to modify the vascular reactivity, increasing the vasoconstrictive response to phenylephrine in the aorta and in mesenteric arteries (13, 14), while an even smaller dose (100 ng/kg) of TBT decreased the vascular reactivity to phenylephrine in rat aorta (15). Specific TBT effects on vascular reactivity thus depend both upon its concentration and on the particular vascular bed examined.

Furthermore, manifestations of endothelial function have been demonstrated at both high and low TBT doses, in conductance and resistance arteries. Rodrigues et al. (15) showed that low dose TBT exposure (100 ng/Kg/day for 15 days) in female rats induced aortic atrophy, reduced wall thickness and reduced aortic wall surface area. The reduced vasoconstrictor response to phenylephrine, described just above, was characterized by an imbalance in NO bioavailability and an increase in ROS production. Although the authors did not validate this with a specific Nox inhibitor, their evidence suggests that NADPH oxidase is involved in the vascular dysfunction induced by TBT.

The same low dose (100 ng/kg) of TBT was able to induce vascular dysfunction in the coronary arteries in isolated rat heart (17). These hearts presented elevated interstitial collagen deposition, increased coronary pressure and decreased estradiol-induced vasodilation. The authors demonstrated that TBT induced endothelium denudation and platelet aggregation.

The toxicity of TBT was also demonstrated in cultured porcine aortic endothelial cells (18). TBT influenced the expression of markers involved in endothelial cell structure and function, indicating that TBT altered endothelial cells' shape, disrupted their assembly and interfered with their capability to interact with other cells (19). TBT also desensitized dose-dependent ANP-induced relaxation in isolated aortic rings of rats (20).

Ximenes et al. (13) exposed rats for 15 days to a TBT dose (500 ng/kg) that was larger but yet close to the Acceptable Daily Intake, and showed abnormalities in isolated aortic rings characterized by increased vasoconstriction to phenylephrine and KCl. TBT also decreased acetylcholine- and sodium nitroprusside-induced vasorelaxation, and increased oxidative stress. It seems that exposing rats to TBT increases superoxide anion production and hydrogen peroxide, for which the main sources are NADPH oxidase and xanthine oxidase,

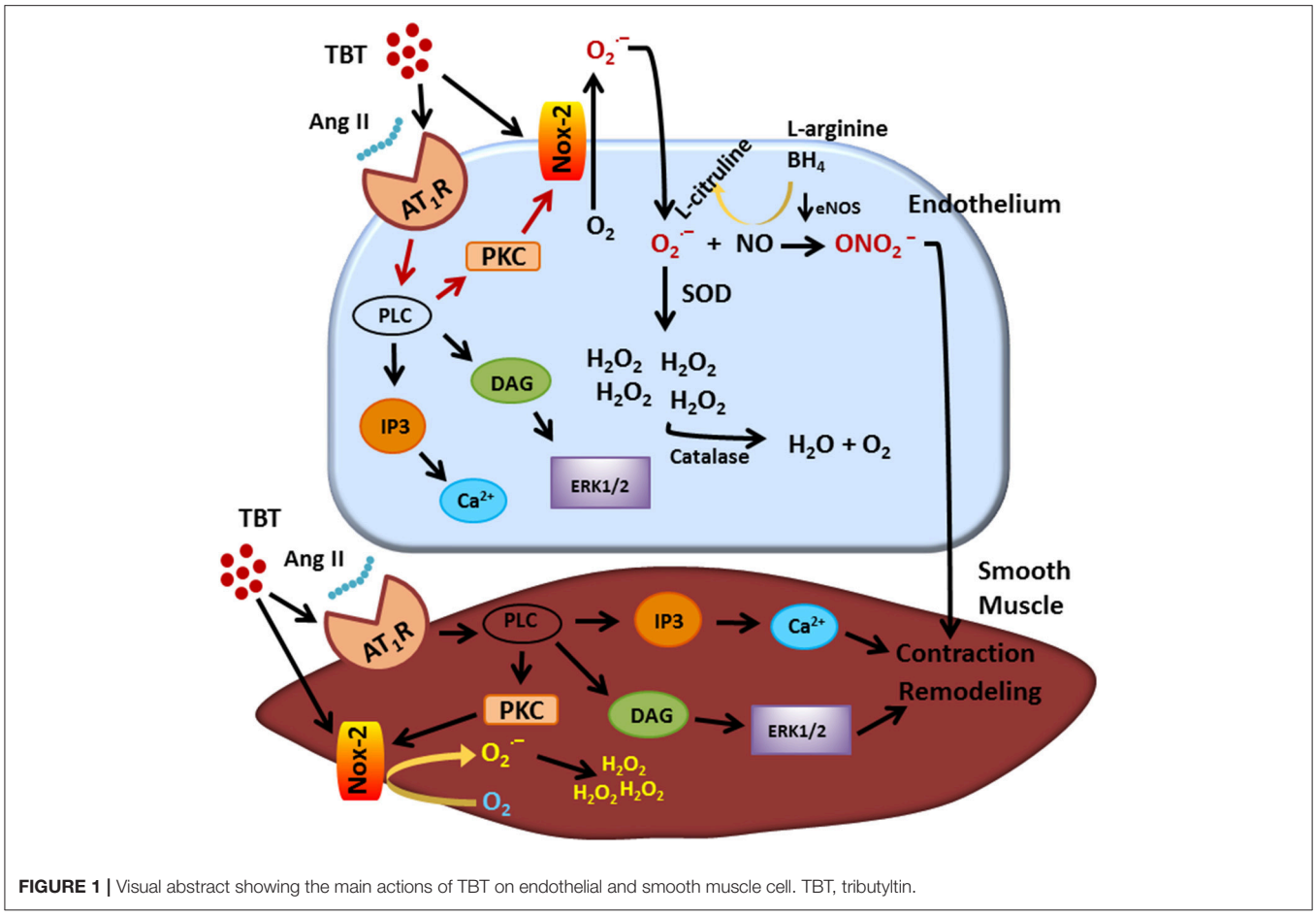

FIGURE 1 | Visual abstract showing the main actions of TBT on endothelial and smooth muscle cell. TBT, tributyltin.

respectively. Similar to the results in ref. Rodrigues et al. (15), these authors also demonstrated that animals exposed to TBT presented aortic atrophy, increased collagen deposition and fibrin accumulation.

This same dose of TBT has been shown to cause structural and mechanical abnormalities in mesenteric arteries. Resistance arteries have a central role in the maintenance of blood pressure and tissue perfusion, roles that are dependent on the capability of smooth muscle cells to contract and relax to vasoactive components (21, 22). Ribeiro Junior et al. (14), demonstrated that mesenteric arteries of rats treated with TBT (500 ng/kg) for 15 days showed increased phenylephrine-induced vasoconstriction and morpho-functional abnormalities. As shown in **Figure 2**, TBT exposure increased superoxide anion production derived from NADPH oxidase. It also decreased NO production as well as eNOS protein expression.

The vascular abnormalities induced by TBT in the mesenteric smooth muscle cells could involve angiotensin II receptor and gp91phox pathways. The increased artery collagen deposition could contribute to the enhanced vascular stiffness and increased pulse wave velocity in TBT-treated animals. Using Nox specific inhibitors, the authors characterized how NADPH oxidase induces vascular dysfunction in TBT-treated rats. Both VAS2870 and ML-171 decreased the vascular reactivity in mesenteric arteries from TBT-treated rats. The same response was not observed in control arteries. The authors also showed increased protein expression of Nox2, AT_1 receptor and ERK 1/2. It seems that TBT enhanced the angiotensin II downstream signaling pathway, leading to inward remodeling and vascular dysfunction. Oxidative stress is an important mediator of vascular remodeling in many vascular beds such as mesenteric and subcutaneous arterioles (23). Chan et al. used Nox-2 knockout animals to demonstrate that superoxide anion generated from the Nox2 isoform oxidase plays a main role in AngII-induced cerebral arteriolar inward remodeling (24).

The Nox family proteins are membrane-bound and they transfer electrons from NADPH to oxygen, generating superoxide anions (25). Among the seven members of the Nox family, Nox-1, Nox-2, Nox-4, and Nox-5 are expressed in vascular human tissue and are involved in regulation of vascular contractility (26, 27). The Nox enzymes are well known to be main players in mediating vascular dysfunction and disease (28–30). The Nox2 oxidase complex is widely distributed in lung, heart and vasculature (31). In addition, angiotensin II receptor activation further stimulates downstream PKC or Rho kinase pathways,

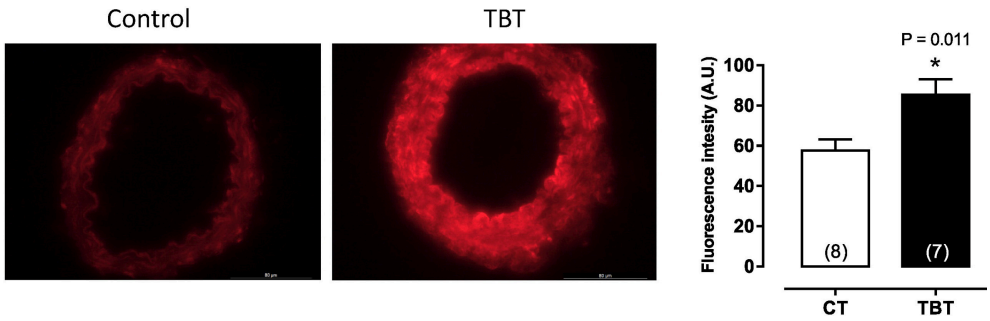

FIGURE 2 | Tributyltin increases vascular superoxide anion production in mesenteric arteries. Animals were treated with TBT (500 ng/Kg) for15 days. This figure is reprinted from Ribeiro Junior et al. (14).

leading to Nox-2 activation and superoxide anion production in smooth muscle cells (32–35), contributing to vascular dysfunction.

The mechanisms involving TBT adverse effects on the vascular system need to be better understood, toward eliciting vascular risks associated with even very low concentrations of TBT. According to the Acceptable Daily Intake value for TBT of 250 ng/kg/day adopted by the World Health Organization, doses below 250 ng/kg/day are tolerable. However, the literature we have reviewed here shows that even low doses of TBT induce vascular dysfunction in rodents. To date, effects of acute TBT exposure on vascular function and on cardiac muscle cells remain unexplored. Overall, data in literature are scarce and new studies are needed to understand how TBT affects the cardiovascular system. It is unquestionable that TBT is a risk factor for cardiovascular diseases.

AUTHOR CONTRIBUTIONS

KR, IS, and RR wrote the general structure of the text, KR, IS, and RR did the literature review, and designed the figures. RR read the final revision of the text.

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Populations Collapses in Marine Invertebrates Due to Endocrine Disruption: A Cause for Concern?

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In the beginning of the twenty first century, the International Program on Chemical Safety published a document entitled *Global Assessment of the State-Of-The-Science of Endocrine Disruptors.* The work indicated only weak evidence of endocrine-related effects in human populations, and in wild animal populations. This document was revised in 2012 (*State of the Science of Endocrine Disrupting Chemicals–2012*) (1). The new document and the extensive scientific evidence it provided showed clearly that ED effects could be a risk to human and wildlife health. These works, however, were focused in human health and related animal models, mainly vertebrates and particularly mammals. It can be argued that invertebrates and many other taxa are important parts of all ecosystems, and, in many instances, have been shown to be also vulnerable to endocrine disruption. Thus, this work is aimed to show some observations on important marine invertebrate taxa, from an ecological point of view. The most important example of endocrine disruption in marine wild populations is the imposex response of marine gastropods, known for more than 40 years, and worldwide used to evaluate marine antifouling pollution. Among the mollusks, other important natural resources are bivalve species, used as human food sources and cephalopods, free-living, highly specialized mollusks, and also human food sources. Effects derived from endocrine disruptors in these species indicate that consumption could bring these compounds to human populations in an almost direct way, sometimes without any form of cooking or preparation. While discussing these questions, this work is also aimed to stimulate research on endocrine disruption among the invertebrate taxa that inhabited our oceans, and on which these effects are poorly known today.

Keywords: endocrine disruption, marine invertebrates, ecological risk assessment, reproduction, environmental pollution

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INTRODUCTION

In the beginning of the twenty first century, the International Program on Chemical Safety (IPCS, a joint program with WHO—World Health Organization and UNEP—United Nations Environment Program and the International Labor Organization) published a document entitled *Global Assessment of the State-Of-The-Science of Endocrine Disruptors* (2). This work reunited the then available scientific information on endocrine disruption (ED). The results were indicative, not conclusive: it showed that some effects observed in wildlife could be attributed to chemical

compounds that can act as endocrine disruptor chemicals (EDCs), but the causal links are weak and effects related to highly polluted areas in most cases. Furthermore, the results indicated only weak evidence of endocrine-related effects in human populations. Among the studied compounds, most are POPs such as polychlorinated biphenyls (PCBs), dioxins and dichlorodiphenyltrichloroethane (DDT). The final remark was the need for broad, collaborative and international research efforts.

Against this background and putting forward a great sum of results from new research UNEP and WHO published a new document: *State of the Science of Endocrine Disrupting Chemicals—2012* (1). This document included three sections: the first explains the basic concepts and facts on endocrine disruption; the second discusses in detail the effects of endocrine disruptors in humans and wildlife in 12 chapters, *based in the fact that endocrine systems are very similar among vertebrate species and that endocrine effects manifest themselves independently of species*. This is an important remark for the further sections. The third and final section discusses exposure of humans and wildlife to EDCs and to potential EDCs. The key concerns derived from this impressive study are briefly showed below, as the original document is available at the WHO site (http://www.who.int/ceh/publications/endocrine/en/).

- Human and wildlife are dependent on the ability to reproduce and develop normally, what is not possible without a healthy endocrine system.
- Three evidence lines indicate concern on endocrine disruption: (i) high incidence and increasing trends of endocrine-related disorders in humans; (ii) the observation of endocrine disruption related effects in wildlife populations; (iii) identification of EDCs related to disease outcomes in laboratory studies.
- About 800 compounds are known or suspect to be able to affect: (i) hormone receptors, (ii) hormone synthesis, or, (iii) hormone conversion. Only a small fraction was thoroughly investigated. *The vast majority of chemicals in commercial use have not been tested at all*.
- Humans and wildlife are exposed to EDCs worldwide, and to more compounds than those that are POPs. However, there have been a failure in addressing the environmental causes to the increase of EDCs effects.
- The speed of disease incidence increases rules out genetic factors only as an explanation, indicating in the other hand environmental and non-genetic factors, as nutrition, exposition, and so on.
- There are critical exposure windows in the organism's development, such as fetal development or puberty, in which they are more susceptible to EDCs.
- Wildlife populations of different taxa have been affected by EDCs. In some instances, these EDCs were recognized as POPs, and *bans on these compounds have led to population's recovery* (a key remark for ecological risk evaluation).
- Internationally agreed and validated protocols to the identification of EDCs still may detect only a part of the known spectrum of ED effects. *This increases the likelihood of overlooking harmful effects in humans and wildlife*. Thus, *disease risk* (and ecological risk) *related to EDCs may be significantly underestimated*.

Even considering that some of these findings have been contested (3, 4), in this broad scenario the new document and the extensive scientific evidence it provided showed clearly that ED effects could be a risk to human and wildlife health, and that much effort is still required to a better understanding of these effects and to provide the measures required for avoiding this growing treat. While this study is a basic reference for those working in this field of research, this study was focused in human health and vertebrate models. Some instances of EDCs effects in invertebrate populations were indicated, and, in this case, with a focus in interference mechanisms and populations responses. The aim of this work is to advance a step further in the direction of the ecological risk evaluation state and requirements for the marine environments, from an ecotoxicological point of view. While being important for environmental health, these aspects are out of the scope of the original work.

ENDOCRINE DISRUPTION IN MARINE INVERTEBRATES: GENERAL ASPECTS OF THIS QUESTION

Invertebrates represent more than 95% of the known species in the animal kingdom, and large groups of these species are of ecological relevance in marine ecosystems (5–7). By 1999, compounds such as the common herbicides atrazine, simazine and Diuron, metals and organometallic compounds such as mercury, cadmium, or organotins, insecticides such as Toxaphene, DDT, or Endrin, alkylphenols such as nonylphenol or PCBs such as Aroclor 1242 or natural or synthetic vertebrate steroids such as diethylstilbestrol or testosterone were implicated in causing endocrine disruption in invertebrates (8). Evidence mounted ever since, and this kind of problem is being reported for several important groups of marine invertebrates, such as amphipods (9, 10), copepods, crabs, and hermit crabs (11, 12), barnacles (13), abalones (14), echinoderms (5), and polychaetes (8). In some species, intersexuality may include a simultaneous activity of both sexes gonads, in a true hermaphroditic condition (15) or could be induced by pollutants (14). Among decapoda and branchiopoda, intersexuality is fairly common, at typical background incidences of <1%, and many common pollutants have been shown to be capable to interfere with the hormonal responses (7, 11). While it is widely known that endocrine disruptors may play a key role in the conditions of marine

Abbreviations: CONAMA, National Environmental Council of Brazil; DDT, Dichlorodiphenyltrichloroethane; DOC, Dissolved Organic Carbon; ED, Endocrine Disruption; EDC, Endocrine Disrupting Compound(s); EQS, Environmental Quality Standard; IPCS, International Program on Chemical Safety; HPG, hypothalamic–pituitary–gonadal; Oct-GnRH, Octopus Gonadotrophin-Releasing Hormone; PAH, Polycyclic Aromatic Hydrocarbon(s); PCB, Polychlorinated biphenyls; POP, Persistent Organic Pollutant(s); POC, Particulate Organic Carbon; RPLI, Relative Penis Length Index; RPSI, Relative Penis Size Index; TBT, Tributyltin; TPT, Triphenyltin; UNEP, United Nations Environment Program; VDSI, Vas Deferens Sequence Index; WHO, World Health Organization.

invertebrate communities, it was often very difficult to make extrapolations from the results of studies did at cellular and sub-cellular levels to the individual and population levels for each tested species (10). A small compilation of some studies done in the last years with marine invertebrates can show the wide range of endocrine disrupting compounds and the variety of associated responses (**Table 1**, below). It should be noted that most of these studies do not focused in combined effects, a critical point in environmental monitoring.

Even as marked changes in marine invertebrate populations in some instances where demonstrated to occur, mainly molluscan populations, whole ecosystem, multitaxonomic environmental monitoring is seldom possible due to technical and funding questions. Only in some limited instances specific populations' distributions data are available, and mostly related to monitoring species, or those of great economic value (28). In many instances of toxicity assessment, single invertebrate species are being used to perform toxicity tests to evaluate potential responses of organisms of many different phyla, as pointed out by Depledge and Billinghurst (8). However, 20 years later this approach is still being used in many, if not most, instances.

In regard of population dynamics, some very important gaps in the available knowledge about environmental effects of pollutants are still present, turning the integration of ecologic and ecotoxicologic information even more difficult. Aspects such as habitat loss due to growing human pressure (29), the lack of specific knowledge of invertebrate endocrine systems, that are very different from vertebrate ones (6, 13), the assimilation pathways of pollutants such as water exposition, dietary exposition, feeding habits (8, 29) and also the very important questions of species responses at different development phases of the reproductive cycle and to mixtures of pollutants which may show similar/dissimilar effects (30). When the great variability of natural processes in marine communities is taken in account, it is not difficult to understand why seldom population's declines in marine invertebrates' communities have been shown to be derived from external forcing such as pollutant pressure. In the particular case of endocrine disruptors, the relative potency of each compound for the studied species is badly known, what makes the evaluation of combined toxicities a still more uncertain affair (6, 29).

As a concluding remark for this introducing section, I would argue that in the case of invertebrates the most impacting effects of pollutants, including endocrine disruptors, are those that could be strongly related to the occurrence of known pollutants affecting and, in some instances, eradicating or seriously compromising natural populations, and thus, affecting the marine ecosystems from an ecological point of view, or, also, compromising biological productivity. The most striking study cases are those related to molluscan species, and these will be the focus of the next sections.

Population decline, local extinctions or reduced reproductive capacity have been demonstrated to be directly related to endocrine disruption in three different conditions: the worldwide development of imposex in marine gastropod species (31), the occurrence of intersexuality in the abalone in Japan, which also led to some documented population reduction caused by reproductive failure (14) and the case of the Basin d'Arcachon, where bivalve commercial production collapsed during the peak of organotins application as biocides in marine antifouling paints and subsequently recovered as this application was restricted, France being the first country to exert this control (32, 33). As marine bivalves and cephalopods are also part of human diet in coastal areas (34), they are further discussed.

ENDOCRINE DISRUPTION: DETECTION AND EVALUATION OF EFFECTS IN MARINE GASTROPOD POPULATIONS IN A LOW ORGANOTINS EXPOSITION SCENARIO

In respect to ED in wildlife marine invertebrate populations, the most characteristic phenomena in organotins polluted areas is a syndrome that is called "imposex" in female gastropods. This syndrome consists in the imposition of male sexual characters, such as penis and/or vas deferens in female individuals. Smith (35), introduced this term after reports of a "penis like" growth of tissue behind the right tentacle of female gastropods, in the location of the male penis. Further research indicated the antifouling biocide tributyltin (TBT), then in intensive application in any kind of vessel as the main cause, and intensive boat and shipping activities areas as the most affected ones (36–39). By 1991, this problem had been reported in 132 gastropod species, this number rising to 192 by 2005 (40). The last extensive report raised again this figure to 268 species by 2009 (41). In the other hand, these last authors indicated some 42 gastropod species that does not develop masculinization when exposed to this compound. Species differential sensitivity, phylogeny—mesogastropods are remarkably less sensitive than neogastropods—and feeding habits are possible causes for these observations. There are many theories to explain the occurrence of this kind of DE syndrome, but the complete mechanism has not yet been totally explained (6, 41, 42).

However, as reports mounted in the literature, the problem of TBT antifoulings pollution was perceived to be global, as first indicated by Ellis and Pattisina (31). Many techniques were developed for imposex evaluation when field and laboratorial studies showed that the relative development of masculine characters on the females was dose-dependent for TBT and in some species also to TPT (triphenyltin, an alternative for TBT as biocide in the paints formulation). Development of these techniques resulted in the application of imposex development indexes, other than the simpler evaluation of the percentage of affected females in each given sampled population. These indexes were based on two approaches: those that compared the penis development in males and affected females, and those that followed the development of the vas deferens in affected females. In the first case, these indexes are the Relative Penis Length Index (RPLI) or the Relative Penis Size Index (RPSI) [(43), for a full description of measurements and application]. In the second case, the index is the Vas Deferens Sequence Index or VDSI [please refer to (40, 43–45) for particular applications of this approach].

TABLE 1 | Some examples of endocrine disrupting compounds and their range of effects in marine invertebrate species.

Compounds	Tested species	Effects	References
TBT, DBT	*Mya arenaria*	Lower progesterone levels, sexual maturation delay, "F"	(16)
TBT	*Mya arenaria*	Skewed sex ratio, vitellin reduction, oestradiol-17β production in gonad reduced "F"	(17)
TBT	*Ruditapes decussata*	Increase in testosterone, oestradiol decrease "F"	(18)
TBT	*Haliotis madaka*	Intersexuality, ovary spermatogenesis, "F" **populations reduction**	(14)
North Sea Oil (NSO)	*Mytilus edulis*	Ovarian follicle development, normal spermatogenesis	(19)
NSO + PAH + Alkylphenols	*Mytilus edulis*	Male gonadal melanomacrophage centers, degeneration ovary follicles	(19)
Bisphenol A	*Mytilus edulis*	Spawning induction for both sexes, ovocyte atresia	(20)
2,2′,4,4′-tetrabromodiphenyl ether	*Mytilus edulis*	Ovocyte atresia, male spawning induction	(20)
Diallyl phthalate	*Mytilus edulis*	Follicle and ovocyte reduction, male spawning induction	(20)
PAHs, TBT	*Mytilus galloprovincialis*	Intersexuality, oocite atresia "F"	(21)
Benzo(a)pyrene	*Portunus trituberculatus*	Reduced ovarian growth, testosterone, progesterone and 17βestradiol secretion reduction	(22)
Benzo(a)pyrene	*Chlamys farreri*	Reduced testosterone and 17βestradiol production, progesterone disruption in ovary, ovarian impairment, development delay	(23)
Bisphenol A, 17βestradiol	*Mytilus galloprovincialis*	Gene transcription	(24)
Testosterone	*Brachionus calyciflorus*	Increased swimming, fertilization rate and recognition ability in males	(25)
Progesterone, flutamide (non-steroidal anti-androgen)	*Brachionus calyciflorus*	Inhibited swimming speed, suppression of fertilization and reduced recognition ability in males	(25)
Dibutyl phthalate	*Galeolaria caespitosa*	Sperm dysfunction, impaired embryogenesis	(26)
Propylparaben	*Tigriopus japonicus*	Sex ratio alteration toward females	(27)

Most were laboratory studies, while those including field studies are indicated by the letter "F." Bold letters, population reduction observed.

These techniques provided the researchers with means to evaluate the relative intensity of the pollution and the extension of the affected areas with a very simple and cost effective monitoring tool.

Obviously, the ideal case is to have parallel chemical analysis for this monitoring, being these analyses of water (44), of sediments (46), or of the animals tissues (47). In the most ideal case, the intensity of imposex in gastropod populations or the organotin body burden of the animals could have provided a proxy of mean TBT water concentrations (44), but the environmental variability is such that these approaches were never thoroughly developed. Another combined monitoring approach, using imposex in gastropods populations to guide sediments sampling to the more critical areas is of easier application. Persistence of organotin pollution in conditions such as fine-grained, organic-rich, mostly anoxic coastal sediments (48, 49) has made TBT and other organotins legacy pollutants, being considered as POPs by WHO-UNEP (1). As a matter of fact, this is probably the most important reason that would explain why imposex is still being reported in European waters (50–53), even when clear instances of improvement are being reported (54). The same occurs in other areas were organotins uses were banned, such as Korea, for instance (55, 56). In the other hand, unregulated use of these compounds have been already demonstrated in some areas, for instance, Latin America (57–59) or North Africa (60–62).

From an ecotoxicological point of view, the work of Stroben et al. (44), being multispecific, clearly demonstrated that species sensitivity could be different even in the same genus, and thus, indicated that antifouling pollution could affect marine communities as a selective pressure. In **Figure 1**, below, some results of this study are presented and discussed.

As we can see from the original data, the species *Nucella lapillus* and *Ocenebra erinacea* are much more sensible, presenting a much more developed vas deferens than *Trivia* species or *Hinia* species at a given TBT concentration. Thus, exposed to similar conditions, the pollution effects on individuals and populations will differ greatly among the different species present at each site. Surely, not all species sampled occurred at all places at the same time. In any case, at the UK environmental target concentration of 2 ng(Sn). L^{-1} (vertical line 1), for instance, the four most sensitive species will present imposex, while both less sensitive *Hinia* species will not. All tested species that present imposex in this concentration range will have females presenting a small penis and/or a partially developed vas deferens, but no sterile individuals in the populations that reach these VDSI values (please refer to the original work for the details of each species VDSI development evaluation). However, at the 10 ng(Sn). L^{-1} concentration level, vertical line 2, that was then fairly common in coastal waters, the more sensitive species VDSI values would be above stage 4, indicating that populations began to show sterile females and thus were in danger by recruitment reduction. Local extinction of the most sensitive species was observed all around the world, sometimes eradicating part of the previous species population's distributions. For instance, some two thirds of the *Stramonita brasiliensis* populations area in Guanabara Bay, a highly polluted harbor area in Brazil, were lost between the sixties and the nineties (46). About half of this area was recovered by this species by 2012 [(45); see the details in **Figure 2** below for the area extension].

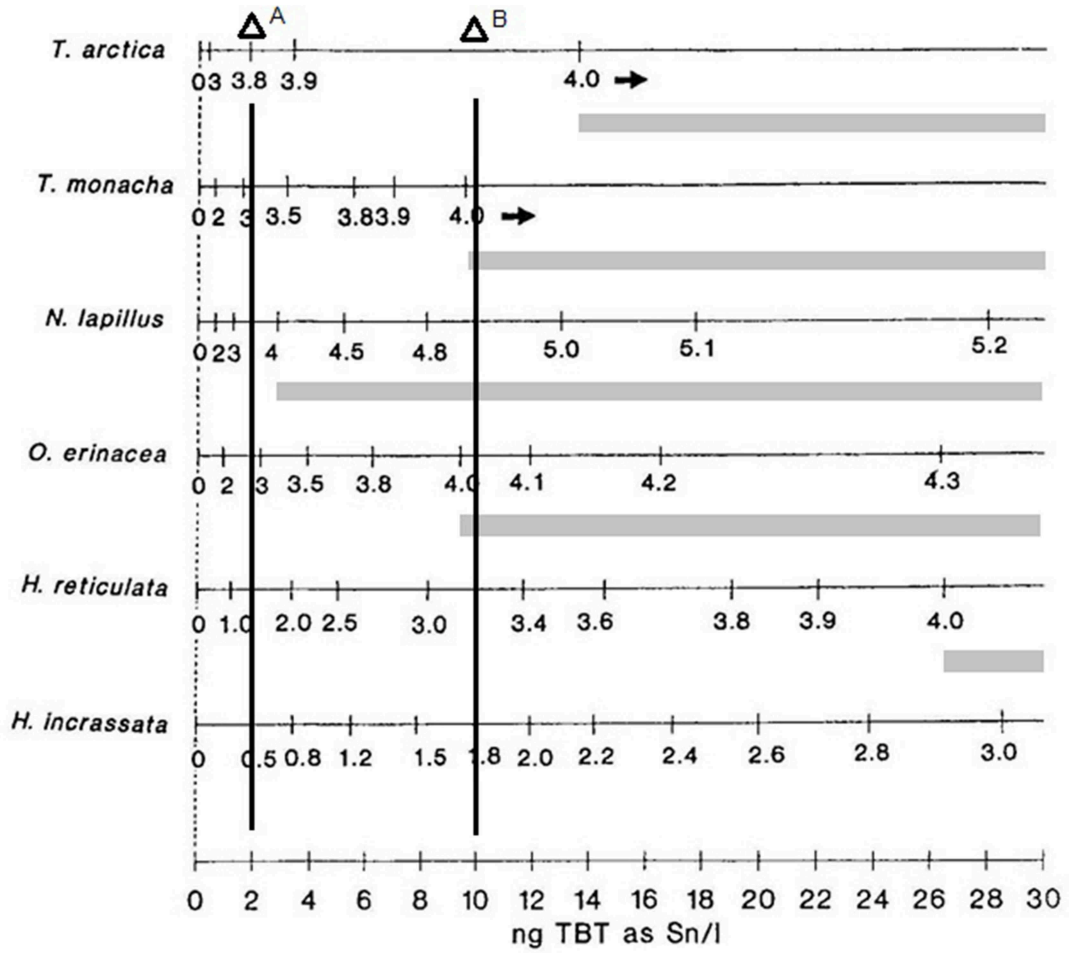

FIGURE 1 | Imposex development is different marine gastropod species at increasing water TBT concentrations. Imposex intensity: values of VDSI index measured for each species; TBT water concentrations in ng(Sn). L^{-1}. Ecological risk indicated by light gray bars indicating population damage by lack of recruitment due to female sterility. Vertical concentration lines: A: TBT EQS of the UK; B: Brazilian limit for sea waters, Class 1, CONAMA Resolution 357. Modified from Stroben et al. (44) by the author.

The recovery of affected populations after the controls on TBT application as biocide and further banning has being considered as a sure indication of pollution reduction (54). If only marine gastropods were affected by TBT, this would have been serious enough, but lack of knowledge of the response of other marine species to this compound makes the hypothesis of ecosystem recovery somewhat less consistent (63). Even in the case of gastropods, recent research indicated that resistance to TBT effects could control the distribution of two species with different organotin sensitivity of *Leucozonia* genus, at least in a heavily polluted, big harbor area (64).

Other important recent observations on imposex development are related to aphallic imposex expression, thus separating the two classic ways of imposex intensity evaluation (by females' penis lengths or by vas deferens development). Aphally in marine gastropods was first showed to occur in *Nucella lapillus* males, in a specific location in England, Dumpton Gap. This syndrome was reported as a genetic problem that caused male specimens to have undeveloped sexual characters, malformations or even to lack their penises. In the other hand, the syndrome caused a reduction in imposex development in the females, thus permitting an isolated population to survive in a heavily polluted coast. This particular condition was called "Dumpton syndrome" because it was discovered at Dumpton Gap (65, 66). By the late nineties, this syndrome has been described in Brittany (67, 68) and in the northwest coast of Spain (69). These last authors proposed a modified VDSI evaluation scheme, as sterile females *Nucella lapillus* were observed for the first time lacking penises. This observation made clear that penis development in imposex females may be independent of vas deferens development. Because of this observation, the authors pointed that in DS conditions, or, for instance, *at lower ambient organotins concentrations*, the VDSI so modified would be a better indicator of TBT pollution that indexes such as the RPLI or RPSI that would be meaningless for aphallic females. More recently, and under different experimental conditions,

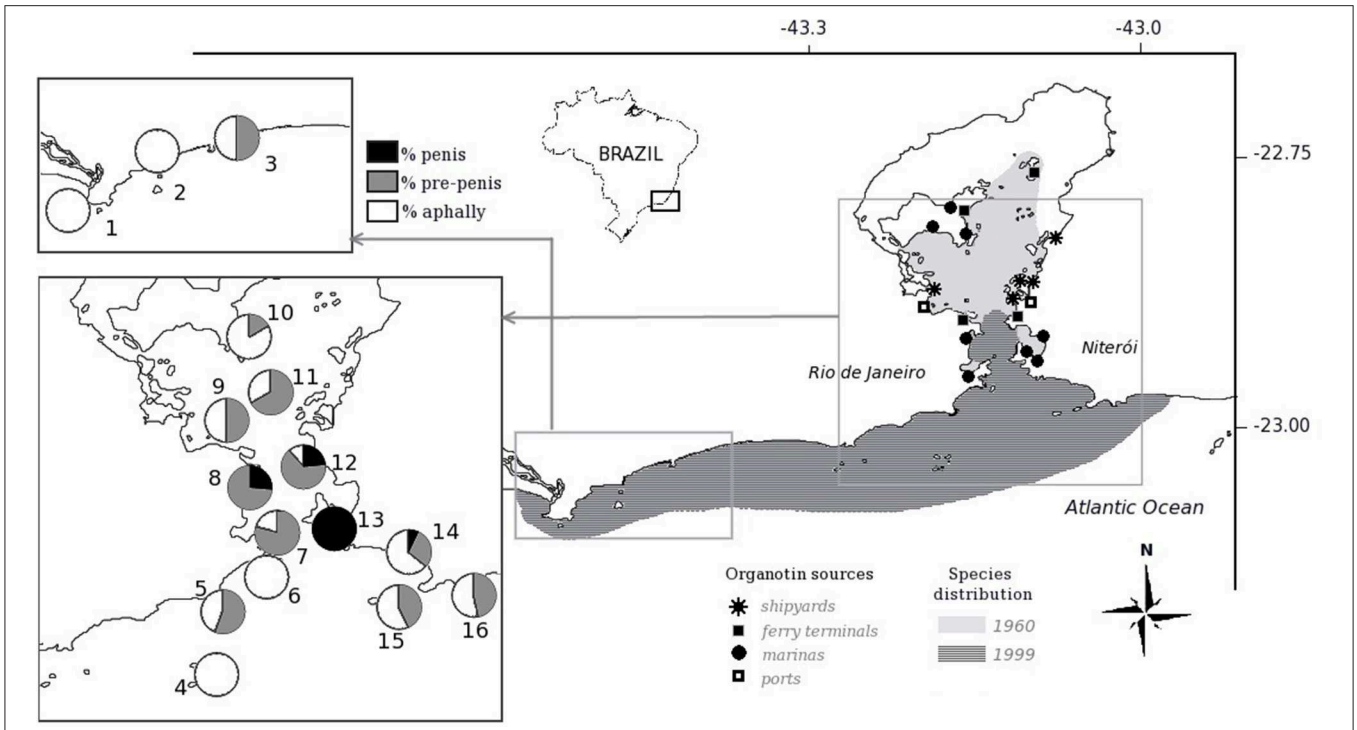

FIGURE 2 | Evolution of the marine gastropod *Stramonita brasiliensis* populations at Guanabara Bay, Brazil, between 1960 and 2013. Organotin sources and expression of imposex development are also shown. Modified from Toste et al. (45).

it was demonstrated that TPT (triphenyltin), a tri-substituted organotin used as biocide as substitute for TBT when this compound began to be controlled, mainly in Japan (70, 71) could induce aphallic imposex development in the same species (72). While these studies were related to *N. lapillus*, aphally is recorded in other gastropod species.

In the previously quoted work by Stroben et al. (44), the species *N. lapillus*, *Trivia arctica*, *T. monacha,* and *Hinia reticulata* were showed to present complete vas deferens development from near the base of the right tentacle, where the penis is located in the males, to the vulva opening, without penis development. In *Cantharus cecillei*, this same general development pattern was observed to occur, while presenting some specific differences [see (40), for the complete observations]. In these species, however, no observation was made about *male* aphally, what would indicate that these ways of imposex development were not related to DS. In another series of works with *Stramonita brasiliensis* in the Brazilian coast, female aphally was frequently observed (73, 74). The proportional incidence of female aphallic imposex development was showed to be related to the distance from the organotins sources at Guanabara Bay, along a sensible distance from the main sources area (to some 60 km distance of the organotins sources centroid, Spearman test $R = 0.6959$, $p < 0.05$), with no male aphally being observed (45). Thus, it became clear that at least for *S. brasiliensis* vas deferens development is independent of that of the penis and penis development occurred only closer to the organotin sources inside Guanabara Bay, and thus, at higher environmental concentrations.

What is more important, it was observed that imposex females could even be sterilized without the development of a penis, a very important observation for environmental monitoring using imposex response that was first demonstrated by Barreiro et al. (69) in *N. lapillus*. Based in these observations, a new imposex development scheme for VDSI in *Stramonita brasiliensis* was proposed, with low and high exposition routes, that is shown in **Figure 3**, modified from Toste et al. (45), below. These observations could be important for other imposex monitoring studies, as aphally in imposex females have been reported in some gastropod species, such as *Hexaplex trunculus*, Lahbib et al. (75); *Stramonita rustica*, Artifon et al. (76); *Thais brevidentata, Thais bisserialis, Thais kiosquiformis, Thais melones, Plicopurpura pansa, Plicopurpura columellaris*, Grimón et al. (77). These observations with other species confirmed that VDSI could be the only adequate approach for imposex intensity evaluation. With the global reduction of TBT pollution, worldwide demonstrated by dozens of published works of measured organotins concentrations worldwide in environmental matrices, the classic imposex evaluation approach seems to need a revision. The VDSI application as shown by Toste et al. (45), may even help discriminating higher from lower exposition conditions for the studied gastropod populations, what could be useful in evaluating the relative importance of the remaining organotins sources, or of their illegal use. This

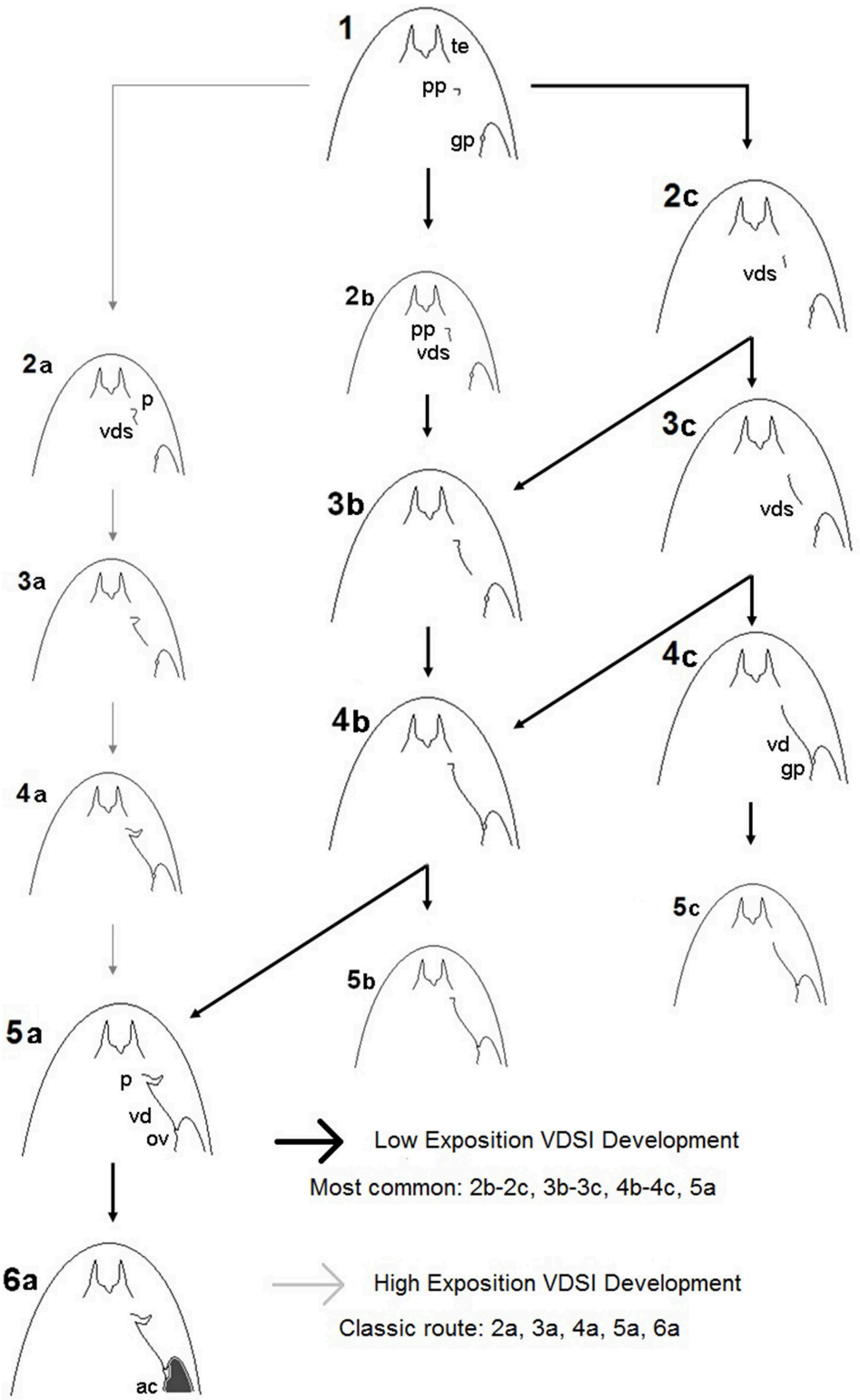

FIGURE 3 | Imposex development scheme for *Stramonita brasiliana (Stramonita haemastoma as originally published)* with high and low exposition routes, according to Toste et al. (45). Captions: te, tentacle; pp, penis primordium; gp, genital papilla; vds, vas deferens; ov, obstructed vulva; ac, aborted capsules. The inclination of the routes at the same stages is related to relative organotins exposition: the nearer to stage 1, lower expositions were indicated by distance for organotins sources in the field. Dark arrows indicate the more commonly observed routes in Southeastern Brazil.

approach, however, still requires the sacrifice of the studied animals, what would not be required by the use of a non-destructive approach such as the one developed by Fernandez et al. (73).

The widespread occurrence of imposex female aphally renders penis lengths indexes meaningless (for instance, total female aphally was recorded for 9 out of 19 stations for imposex monitoring in *Nucella lapillus* in 2014, causing values for RPLI in this species along the Portuguese coast to drop to almost zero, thus rendering its application unable to further indicate decreasing tendencies afterward but for one sampling station along the Portuguese coast [(53), **Table 1**]. The same observation was reported in *Hexaplex trunculus*, showing a temporal reduction pattern of phallic imposex development as observed in *Stramonita brasiliensis* in Brazil, by Lahbib et al. (78) in Tunisia. The use of vas deferens development only, without taking in account the penis development, for VDSI application was even indicated for *Nucella lapillus* by (79). In the other way, some recent studies done in more heavily polluted areas that still remain in Latin America, such as Peru (59, 80) and Chile (81), for instance, showed that penis-based indexes are still useful and could still provide relevant information for some time to come in the remaining hot spots areas. Anyway, the aphallic imposex development observed for *S. brasiliensis* could lead to female sterility, as previously pointed out, when the females presented only pre-penises or very small penises (45), thus making the VDSI the most relevant imposex development index to be applied in this new, mostly low exposure scenario.

There is still another complicating factor in the field imposex analysis: the quite relevant question of interference in the imposex response of the animals. This interference may arise by two different mechanisms: one is the complexation of organotins when high loads of organic matter are present in the waters at the same time. It was long known that organic matter has as very strong affinity to organotins (82) and that in anoxic sediments, degradation of organotins is very slow (83, 84). In any case, this same high affinity will be present in waters rich in POC (particulate organic carbon) and DOC (dissolved organic carbon). These organic compounds thus may act as a kind of "buffer," reducing the bioavailability of organotins to the biota, and, consequently, the imposex expression of gastropod populations. Frequently associated to organic rich waters in the coastal zone are direct sewage discharges, important artificial sources of POC and DOC. At the same time, these sewage discharges are also important sources of xenoestrogens to the same coastal areas. It has been demonstrated by bioassays with *Nucella lapillus* using sewage treatment plant effluents rich in xenoestrogens such as octylphenol, nonylphenol, and bisphenol A that exposition to these effluents could activate the estrogen receptor of this species (85) and at the same time were capable of reducing the imposex expression of TBT-treated females (86). The most affected response is, not unexpectedly, the RPLI, as we saw the growing relative importance of the aphallic vas deferens development routes before.

This interference mechanism was observed in the field for the first time at a small touristic city, called Paraty, which is located at the end of a small inlet in the southeastern coast of Rio de Janeiro state, Brazil. Coincidently, in this area, the organotin sources and organic matter sources are located very close to each other, in the inner inlet, and this particularity made possible to understand the different water residence times of each compound. Monitoring studies of imposex development in populations of *S. brasiliensis* were made in 2006 and 2011, and while a relative amelioration was observed in the inner inlet stations, the outer stations showed a relative aggravation of the imposex condition. The most likely reasons for this strange observation are that while organotins were present in the whole area, and recent input was showed to occur at the 2011 sampling by sediment analysis, the number of small boats, that are the only sources in the area, remained approximately constant while local human population has grown. Thus, organic matter and xenoestrogens inputs, aggravated by total lack of sewage treatment, also have certainly risen. Then, the "buffer" effect was enough to suppress part of the imposex development of the animals in the inner inlet. The populations recovered, while showing 100% imposex low expression incidence (only two penis bearing females recorded in the study). In the other hand, degradation of the organic matter and dispersion reduced the "buffer" effect in the outer inlet, and thus the remaining organotins are still able to produce a response in the animals. While somewhat speculative, the key to understand this process was the difference in water residence times of organotins, that are highly toxic to marine organisms, and of the sewage derived organic matter, that is highly nutritive, and thus, quickly degraded by aerobic bacteria. While not all these parameters were measured in occasion, the very color and particulate matter content of the waters from the inner inlet when compared to those of the outer inlet showed clearly where the problem was. A schematic conceptual description of this interference mechanism is shown in **Figures 4A,B**, below. For the original data and details, please refer to Borges et al. (87).

Putting all these information and ideas together, what seems clear is that in a new scenario of lower organotins inputs, in many instances derived from contaminated sediments, including in Europe (48, 88–90), the imposex response of marine gastropod populations should be evaluated with care. Under conditions of interference with this response, imposex development could be reduced in some areas, mostly in urbanized areas with parallel sewage discharge pollution. In these conditions, while the animals may show a low imposex response, the animals' body burden of organotins could still be high, even higher than in more pristine conditions. Some recent results seems to indicate this possibility with animals presenting high organotins body burden with showing low imposex responses (59, 91). Some aspects of the populations used for biomonitoring have influence on the results, such as genetics, temperature influence on metabolism or even the seasonality of the reproductive cycle, a basic aspect often not considered (92). In any case, the key to understand the animal's response would be the possibility of interference by other parameters in the water, which is dependent of each study area particularities. The conclusion that organotin pollution has been ameliorated may be doubtful in some situations. As biological monitoring is frequently used without bioassays or body burden analysis, the result of these studies must be evaluated with care. If interference is suspected,

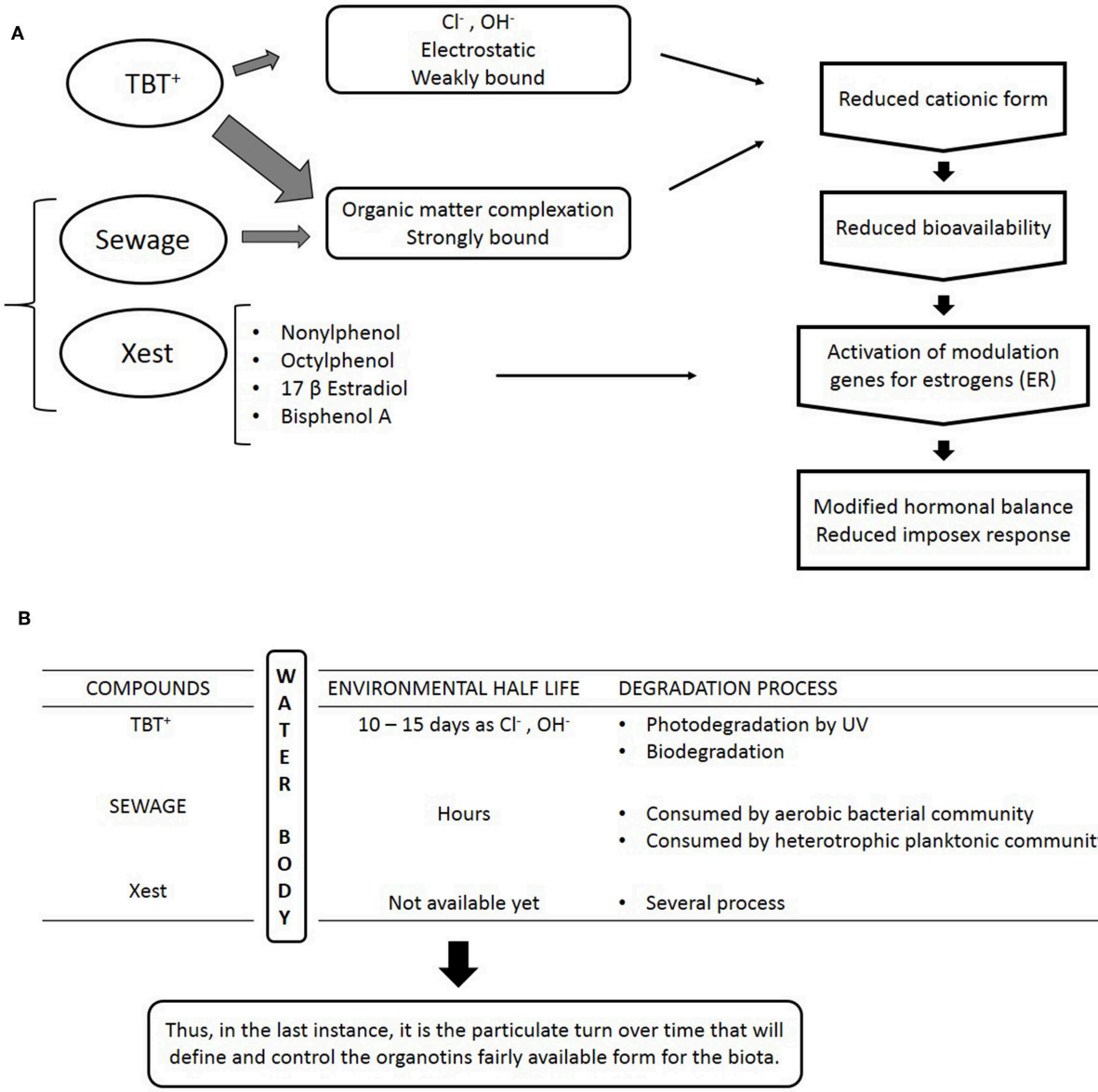

FIGURE 4 | (A) Schematic scheme for an interference mechanism in the imposex response of marine gastropod mollusk to organotin compounds. While cationic organotins are the most available form to biota, both particulate and dissolved organic matter from sewage can complex organotins, while sewage is also a source of xenoestrogens that may act as antagonists to imposex development. **(B)** Main environmental aspects of the interference mechanism proposed. Due to the multiple variables involved, only the general lines are indicated.

confirmatory chemical analysis is indicated, and occurrence of organotin pollution should be suspected. *In this case, the very occurrence of imposex in marine snails is a clue that biologically active organotin compounds are present in local waters.* It is important to remember that organotins could present a human health risk for some coastal populations, as previously reported (34, 76, 81, 93, 94). Imposex development is still the faster and cheaper biological monitoring method to evaluate the occurrence of remaining hotspots of organotin pollution, or to verify its illegal use.

ENDOCRINE DISRUPTION IN MARINE BIVALVE MOLLUSKS: GROWING IN IMPORTANCE AS BIOMONITORING ORGANISMS IN A NEAR FUTURE

Among all animal taxa, bivalve mollusks are probably the most important for monitoring the extension and intensity of marine pollution. Numerous programs of marine monitoring employed bivalve mollusks, among which the Mussel Watch was the most important. Being sessile, resistant, easily collected,

these are adequate organisms for monitoring studies. The fact that bivalves are also important components of many human populations diet in coastal areas, and the object of growing mariculture investments make these species important vectors of transference of pollutants introduced in these coastal areas to human populations. Marine bivalves have also been shown to present reproductive anomalies related to endocrine disruptors. When the bivalve *Ruditapes decussatus* was transplanted to TBT polluted areas, a rising on testosterone levels with estradiol reduction was observed (18). This same observation was made on *Mya arenaria* populations (16). This last species had shown male-biased populations in another study, by Gagné et al. (17), the first instance in which hormonal alterations were reflected at population level. The occurrence of intersexuality has been showed to occur in marine bivalves (21). On the subject of endocrine disruption, the most common studies were focused in the occurrence of vitellogenin proteins in males, for instance, of *Tapes philippinarum* (95–97) or *Mytilus edulis* (19, 20, 98). This occurrence is related to availability of xenobiotics such as nonylphenol, bisphenol A, ethinylestradiol, or PAHs, common in urban sewage (99–103). In spite of many instances of endocrine disruption being observed within populations of marine bivalves, the extinction of populations was not commonly observed. From this point of view, the most studied case of extensive populational damage was the Arcachon Bay, France, and the commercial farming of *Crassostrea gigas*, the Pacific oyster.

The Basin D'Arcachon is a closed roughly triangular tidal water body with some 156 km^2 area, in which some 10,000 to 15,000 tons of commercial oysters were produced each year. From 1975 to 1982, oyster production was severely reduced, due to absence of spatfall and anomalous growth, with shell calcification anomalies (32). While these problems were reported at the same time in other areas with this same species in England and also along the Spanish Mediterranean coast, the most important and studied area was Arcachon (104). In a very interesting review, Ruiz et al. (33), indicated that from initial water concentrations of TBT of <1 ng(Sn)L^{-1} by 1960, the increased use of this compound led these concentrations to rise above 100 ng(Sn)L^{-1} by 1981–1982, when controls on application were applied by the French government. After these measures, water concentrations of TBT decreased to about 10 ng(Sn)L^{-1} by 1987, reaching again about 1 ng(Sn)L^{-1} by 1993. These same authors have also shown that besides the oyster production collapse, other important ecological changes occurred in the same area. Simultaneously, it was observed a great reduction of the local populations of the gastropod *Ocenebra erinacea*, in which the first symptoms of imposex development were reported by 1973, earlier than the oyster production collapse. With the reduction of organotin pollution, gastropod populations recovered later. Also "green tides" of *Enteromorpha* spp. were reported to occur by 1982. While clearly indicating the gaps in the original data, the authors indicated that TBT effects on other invertebrate grazers may explain this anomalous observation, a first evidence of multispecific TBT ecological disturbance. Our research group has also noted excessive algal growth and apparent reduction of herbivorous species in some organotin polluted coastal areas in Brazil. This subject is now under a closer scrutiny, as our previous studies were designed for imposex evaluation only.

It should be pointed out, also, that dioecious bivalves did not have the internal fecundation of marine gastropods that made gastropods such useful species for environmental monitoring of endocrine disruption. Reproductive conditions evaluation in marine bivalves often requires histological analysis, what precludes a quick, fast, effective monitoring methodology such as imposex evaluation. With the phasing out of TBT, and the intensive use of new antifoulings that may act as xenoestrogens or estrogen agonists, such as Irgarol 1051, the "naval version" of atrazine [see (73) for a deeper discussion], imposex will be used only in the monitoring of the remaining hotspots of legacy organotin polluted areas. The relative importance of marine bivalves as indicators of marine endocrine disruption will probably rise in a near future, including in the evaluation of health risks for coastal human populations.

POSSIBLE NEW TARGETS FOR ED COMPOUNDS: MARINE CEPHALOPOD MOLLUSKS

Cephalopods are free-living predator mollusks with high mobility and very effective sensorial capabilities, almost completely opposed to the typical oyster species in respect of adequation for environmental monitoring studies. They are in some instances important and appreciated food items, and have been included in human risk analysis for coastal areas (34). From an endocrine disruption point of view, cephalopods have a more complex nervous system than the other mollusks. While in bivalves and gastropods neurohormones secreted by nervous ganglia and gonads are responsible for sexual maturation, showing first and second order control systems, cephalopods show a third order neuroendocrine control system that is comparable to the vertebrate HPG ax [see (105)]. In this sophisticated control system, the Octopus Gonadotrophin-Releasing Hormone (Oct-GnRH) has been shown to act as modulator in functions such as feeding, memory or sensorial, as well as in steroidogenesis in *Octopus vulgaris* (106). In the other hand, it has been shown that estradiol regulates Oct-GnRH and several functions of the nervous systems in the same species (107). As cephalopods are being considered excellent candidates for mariculture in Europe (105), a cycle may be closing on them: as xenoestrogens have been shown to be present in many instances in coastal areas and can affect these animals, their use in mariculture will turn them sessile for all environmental aspects such as bioaccumulation and pollutants transference for humans. Clearly more research is required on this subject.

ENDOCRINE DISRUPTION AND ECOLOGICAL RISK ASSESSMENT

The global aspects of endocrine disruption may be inferred by the ubiquity of detection of proven or suspect endocrine disrupting

compounds, even while the biological effects in invertebrate populations are not clearly shown yet. Simple and inexpensive monitoring tools as imposex induction was for organotins monitoring are still lacking, and tools as vitellogenin induction in males are not specific. Certainly a weight of evidence approach will be required at each location based on previous knowledge of possible ED compounds sources and loads. However, in the same way that imposex have been shown to occur worldwide, and to have caused many gastropod species local extinctions, there is no plausible reason today to suppose that the ubiquity of ED compounds is not producing population damage on many invertebrate species, damage that cannot be observed with current methods and approaches. It was recently demonstrated that male infertility could be induced by several environmental contaminants (108), and that even low incidence of female intersexuality in crustacean populations may have drastic effects at population level (10, 109).

Ecological risk assessment is one of the most difficult tasks today, because it requires a deep knowledge of at last three important fields that should be employed at the same time. These are: (i) the pollutant chemical properties and behavior in aquatic environments, that will define its speciation and reactivity; (ii) the physical components of dispersion and mixing that will also influence the compound's water residence time, which is the basic aspect to indicate the exposition of aquatic communities to pollutants: and (iii) the response of each individual species to the pollutant. This is the basic scenario for single pollutants exposition. Much of the current risk evaluation works relay on these three basic kinds of information. First, the available chemical data for each compound are not always complete or reliable. Second, the mean concentrations reported for the studied compounds in the literature were seldom based on modeled concentrations in any particular area, let alone thoroughly calibrated dispersion models (110–112). Third, the available database of biological effects of the ED tested compounds is very far from being complete.

While presenting today's best available technology, would these approaches be sufficient to understand the selective pressures posed by the actual sum of anthropogenic compounds present in coastal areas? I believe these are not. Experience has shown that as more studies developed, and as the legislations advance, still the biological communities change and productivity and biodiversity decreases, and the assessment of these changes depends on expensive ecological studies that are not usually made with the appropriate extension and frequency. To improve this situation, it would be important to advance along three separate lines of action at the same time:

Axis (i) the lack of knowledge on DE effects on invertebrates of many compounds in current use tool should be a priority in research. The importance of this point was shown in the particular study case of marine antifouling paints by interference these effects can induce in the evaluation of the most used biomonitoring approach.

Axis (ii) the problem of the "cocktail effect," not only by the way of sheer toxicity—what means acute or extensive effects -, but by the cumulative action of pollutants on the hormonal regulation mechanisms, should be another. This line is consistent with the results on human and vertebrate health discussed by WHO-UNEP (1).

Axis iii) the final considerations on the difficulty of tracing specific compounds effects on marine communities without knowing the "cocktail" composition, based on antifoulings examples previously discussed (112). This means a necessity for stronger simultaneous determination methods for multiple target compounds, and a relative potency scale for ED compounds in multiple marine taxa.

Certainly, there are other factors on these ecological risk evaluations, such as human population growth, that is greater in the coastal areas; climatic changes; the changing uses of littoral areas; overexploitation of marine resources (113). But we should always try to control the introduction in the environment of hazardous and potentially hazardous chemicals, including known and possible endocrine disruptors.

FINAL REMARKS

I guess in the near future it would be required to focus on the necessity of integrated studies, and on some measures required to make these studies easier to integrate. To reach this goal, we will need a relative potency scale for EDCs in marine species, an integrated database of EDCs with padronized doses and responses easily accessible to researchers and a combined chemical-ecotoxicological-ecological modeling and monitoring approach as the desired end-point. A growing number of works is appearing studying pollutants interactions to different taxa, and these efforts should be supported, because as pointed elsewhere, the interactions are not predicable. To finish this discussion, I would like to point out that specific bioindicators for ED in fieldwork in these new times would be much more probably the exception than the rule.

AUTHOR'S NOTE

My idea is that through the case of marine gastropod and bivalve mollusks to raise interest in research on the ecotoxicological and ecological effects of endocrine disruptors. Among marine invertebrates, endocrine disruption could be widespread, as I tried to show with the particular cases discussed. In the same way that the effects of endocrine disruptors are still poorly known in human and vertebrate populations, invertebrates could also be at risk, as several instances of populations extinctions and recuperation have been demonstrated. So, while by the point of view of human health research is much needed in this field, in the case of ecological damage and ecosystems functions much research is still required too. Perhaps even a specific topic may be raised on this subject.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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