Special Review Article

Organotin-Induced Toxicity and Nuclear Receptor Signaling

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Abstract

Organotin compounds have been widely used as agricultural fungicides, rodent repellents, and molluscicides and in antifouling paints for ships and fishing nets. These widespread uses have resulted in the release of increasing amounts of organotins into the environment. In aquatic invertebrates, particularly marine gastropods, organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), induce irreversible sexual abnormality in females which is termed "imposex" at very low-concentrations. Although it has been theorized that these compounds act as potential competitive inhibitors for aromatase, which converts androgen to estrogen, and then increase levels of unconverted androgens in gastropods, their effective concentrations of aromatase inhibition are high. In addition to wildlife, organotins may have various undesirable effects on human health. In human ovarian granulosa cells, these compounds suppress aromatase activity at the nanomolar level. Contrary to this, in human choriocarcinoma cells, these compounds markedly enhance estrogen biosynthesis along with the increase of aromatase activity at the same low concentrations. Although there are many reports describing the potential toxicity of organotins, the critical target molecules for the toxicity of organotin compounds remain unclear. New data identify TBT and TPT as nanomolar agonist ligands for retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPAR) γ, which are members of the nuclear receptor superfamily. Here, we review the potential toxicity of organotin compounds via these nuclear receptors in mammals.

Keywords : organotin, aromatase, endocrine disruptor, retinoid X receptor (RXR), peroxisome proliferator-activated receptor (PPAR) γ

1. Introduction

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been widely utilized as biocides, agricultural fungicides, and wood preservatives, and as disinfecting agents in circulating industrial cooling waters and in antifouling paints for marine vessels [1, 2]. Human exposure to non-point sources of organotins occurs through contaminated dietary sources (seafood and shellfish), fungicides on food crops, and antifungal agents in wood treatments, industrial water systems, and textiles [3]. A variety of mono- and dialkyltins, which include significant contaminating trialkyl species, are also prevalently used as heat stabilizers in the manufacture of polyolefin plastics, bringing them into closer contact with drinking water and food supplies. Measured exposure levels of organotins, such as dibutyltin and tributyltin, in wildlife and human tissue samples are in the range of 3-100 nM [4-6]. The potential exposure of humans to organotins has therefore aroused great concern about their potential toxicity. Animal experiments suggested that the spectrum of potential adverse chronic systemic effects of organotins in humans is quite broad and includes primary immunosuppressive, endocrinopathic, neurotoxic metabolic, and enzymatic activity, as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary, gastrointestinal, blood dyscrasias, reproductive/teratogenic/developmental, liver, kidney, bioaccumulative, and possibly carcinogenic activity. Al-

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though many reports have described the potential toxicity of organotins, the critical target molecules for the toxicity of organotin compounds remain unclear.

Organotins as endocrine-disrupting chemicals

Organotin compounds are typical environmental contaminants suspected as endocrine-disrupting chemicals. Most of the toxic effects of organotin compounds on sexual development and reproductive function have been documented in mollusks. For example, very low concentrations of TBT and TPT induce female neogastropod snails to suffer irreversible sex organ alterations, a phenomenon known as "imposex" [7, 8]. These abnormalities are the result of a masculinization process by which male sex-organs develop, notably a penis and a vas deferens. In certain species, growth of a vas deferens disrupts the structure and function of the oviducts, preventing normal breeding activity and causing population decline. Imposex has been established as a form of endocrine disruption caused by elevated testosterone titers, leading to masculinization in organotin-exposed females [9-11].

The synthesis of sex steroids from cholesterol requires trafficking between mitochondria and smooth endoplasmic reticulum, and involves many enzymatic steps. Most of these steps use cytochrome P450 heme-containing enzymes, and the genes coding for these enzymes are abbreviated to CYP (Figure 1). The precise mechanism by which testosterone levels are increased in imposex-induced gastropods has not been fully elucidated, but the weight of evidence suggests that organotin compounds cause problems related to sterodogenesis [10] and/or testosterone metabolites [11], especially the inhibition of aromatase activity which converts androgen to estrogen.

In *in vitro* experiments, butyltins are demonstrated to exhibit structure-related inhibition of the catalytic activity of human aromatase protein from human placenta [12] or transfected cells [13]. In addition to aromatase, TBT inhibit the catalytic activity of human 5a-reductase I and II [14], rat 3β-hydroxysteroid dehydrogenase (3β-HSD) [15] and pig 17β-HSD I [16]. TPT also inhibit the catalytic activity of human aromatase, 5a-reductase II 17β-HSD I and III [17]. However, at concentrations effective (micromolar level) for the inhibition of these steroidogenic enzymes, TBT and TPT are generally toxic to mammalian cells because they cause apoptosis or necrosis [18-21]. In human choriocarcinoma cell lines, JAr, JEG-3, and BeWo, exposure to greater than 300nM TBT or TPT markedly decreases DNA and protein synthesis [18, 19]. Concentrations under 1 mM of either organotin compound did not significantly affect aromatase activity in microsomes isolated from human choriocarcinoma cells [18]. These results suggest that we have to consider the toxicity of organotin compounds in distinguishing between nonspecific toxicity to cells and the specific inhibition of steroidogenic enzymes.

In additon to these, sex steroid receptors and steroidogenic enzymes for sex steroid hormones have not yet been identified, and it remains unclear whether sex steroid hormones are critical factors for sexual development and reproduction in gastropods. Furthermore, homologues of both the estrogen receptor (ER) and androgen

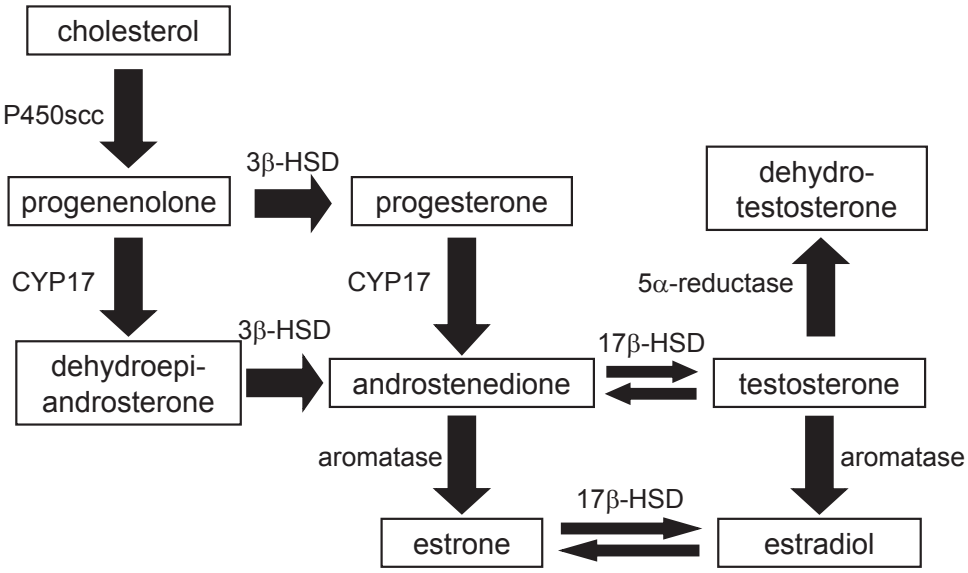

Fig. 1 The pathway of steroid hormone biosynthesis.

receptor (AR) have not been founds in invertebrates [22] and the composition of nuclear receptor family members is very different between vertebrates and invertebrates [22, 23]. Therefore, there is some doubt as to whether organotin compounds function as inhibitors of enzymes that metabolize androgens in gastropods, and this doubt led us to suspect that organotin compounds affect other target molecules in mammals.

Organotin compounds affect endocrine functions in human placenta and ovary

In a recent study, Nakanishi et al. investigated the effects of organotin compounds on aromatase activity [18, 24] and 17β-HSD I, which converts low-activity estrone to high-activity estradiol [19], in human choriocarcinoma cells. Both TBT and TPT increased the catalytic activity of aromatase and 17β-HSD I along with their mRNA expression in a dose-dependent fashion following exposure to non-toxic concentration ranges (3-100 nM). These results indicate that the observed organotin-induced alterations in human choriocarcinoma cells are due to the regulation of mRNA levels of both steroidogenic enzymes, not of these enzyme complex. In addition, these organotin compounds also markedly stimulated human chorionic gonadotropin (hCG) production in the same concentration ranges, along with its mRNA expression [18, 24]. These results suggest that organotin compounds are potent stimulators of human placental estrogen biosynthesis and hCG production *in vitro* and that the placenta represents a potential target organ in pregnant women for organotin compounds, the endocrine-disrupting effects of which might be the result of local changes in estrogen and hCG concentrations.

In opposition to the above results, however, Saitoh et al. reported that 20 ng/mL (about 60 nM) TBT and TPT suppressed both the activity and gene expression of aromatase in the human ovarian granulose-like cell line, KGN [20]. This discrepancy in the action of organotins on the gene expression of human aromatase is due to the tissue-specific expression of aromatase, which is strictly regulated (Figure 2). Human *CYP19* is a single-copy gene composed of 10 exons ; exons II to X encode aromatase protein, as well as the 3' untranslated region of mRNA common to all estrogen-producing tissues [25]. A number of variations of exon I exist. These encode the 5' untranslated regions of various *CYP19* mRNAs, which are selectively expressed in some tissues by alternative splicing [25-27]. The tissue-specific expression of *CYP19* in humans appears to be mediated by tissue-specific promoters lying upstream of the respective exon I sequences, and by transcription factors binding to specific regions of each promoter. In the placenta, *CYP19* is driven by the placental major promoter (I.1), and the transcript contains exon I.1, located approximately 89 kb

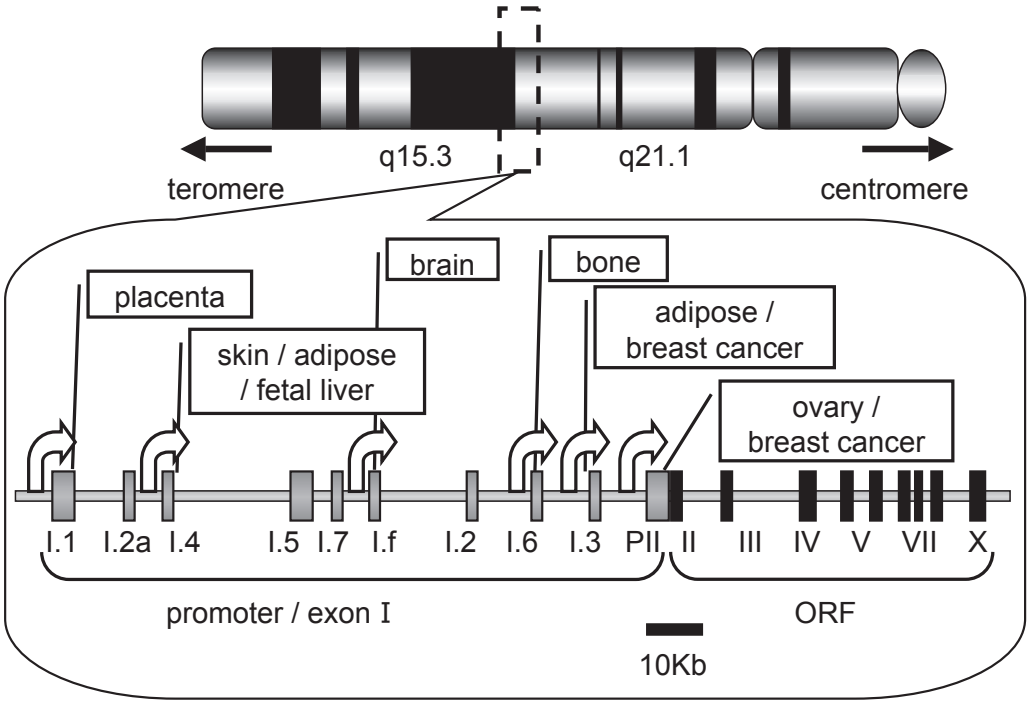

Fig. 2 Genomic organization of the human *CYP19* gene

upstream from exon II. On the other hand, ovarian transcripts contain a sequence at the 5'-end immediately upstream of the translation start site, because gene expression in the ovary uses a proximal promoter (II). In ovarian granulosa cells, the expression of *CYP19* is strongly regulated by the steroidogenic tissue-specific transcriptional factor, Ad4Bp/SF-1, via promoter II. In contrast, Ad4Bp/SF-1 is expressed at very low levels in the human placenta and may not play an important role in activation of the placental major promoter I.1 [28, 29]. Saitoh et al. suggest that the effects of organotin compounds in KGN cells are caused partly by association with Ad4Bp/SF-1 [20]. It is therefore likely that the action of organotin compounds in human placental cells is induced by a pathway clearly different from that in ovarian granulosa cells, giving rise to the promotion of aromatase activity and mRNA expression.

In human placental cells, all mRNA expressions of aromatase, 17β-HSD I and hCG are controlled by cAMP-dependent intracellular signal pathways, however, neither TBT nor TPT exerted any effect on intracellular cAMP production [18]. In addition, there is little possibility that these organotin compounds affect the cAMP-protein kinase A (PKA) pathway in the human ovary, because the cAMP-PKA pathway stimulates aromatase gene expression in the ovary through promoter II [30]. The possible target of these organotin compounds may be a signaling pathway common to the gene expression of aromatase, 17β-HSD I and hCG in the human placenta and ovary.

Organotin compounds are PPARγ and RXR agonists

Nuclear receptors play important roles in maintenance of the endocrine system, regulation of organ differentiation, and fetal development. Reproductive abnormalities in wildlife can be associated with exposure to environmental pollutants capable of mimicking the action of natural hormones. As the nuclear receptors of intrinsic hormone systems are likely to be targets of industrial chemicals, information on their ability to bind these chemicals is valuable for environmental risk assessment. Recently, Kanayama et al. reported assay systems for human nuclear receptors to determine whether suspected endocrine disruptors can bind to members of the nuclear receptor family on the basis of the previously described CoA-BAP system [31, 32]. Using these systems, they found that TBT and TPT were potential agonists of RXR and PPARγ [32]. In addition, these compounds also induced the transactivation function of RXR and PPARγ in mammalian culture cells. The effectiveness of each organotin compound was comparable to that of the natural ligand of RXR, 9-*cis* retinoic acid (9cRA) or the well-known PPARγ ligand rosiglitazone (Rosi) [32]. The dose ranges of TBT and TPT that induced transactivation were from 10 to 100 nM, which do not cause significant apoptosis or necrosis of mammalian culture cells in general. These results indicate that these organotin compounds function as RXR or PPARγ agonists in mammalian cells.

RXR stand out as unique members of the type II nuclear receptor subfamily and play dual roles in nuclear receptor signaling. On one hand, they can bind to their own response element (RXR response element) as a homodimer and activate transcription in response to their ligands, and on the other hand, they serve as partners for other nuclear receptors [33-35]. The existence of three types of heterodimers－fully permissive, conditionally permissive, and nonpermissive－has been described. In the first case, PPARs/RXR, farnesoid X-activated receptor (FXR) /RXR, and liver X receptor (LXR) /RXR heterodimers exhibit dual ligand permissivity, because they can be activated by the agonists of either RXR or its partner receptor, or both, in a more-than-additive fashion [36-42]. As an example of the second type, the RXR/ retinoic acid receptor (RAR) heterodimer exhibits conditional permissivity because a full response to RXR agonists occurs only in the presence of an RAR agonist [39, 43]. The third type is the nonpermissive heterodimer, such as the RXR/thyroid hormone receptor (TR) and RXR/vitamin D receptor, which cannot be activated by RXR agonists regardless of the presence (or absence) of the agonist of its partner receptor ; formation of the heterodimer is thought to actually preclude the binding of the ligand to RXR [44, 45]. TBT and TPT simulated the transactivation of an RXR homodimer and PPARγ/RXR heterodimer at non-toxic concentration ranges (10-100 nM), whereas they had no effect on the transactivation of RXR/TR and RXR/RAR heterodimers [24]. In particular, these organotin compounds activated PPARγ/RXR heterodimers more strongly than Rosi, because they may function not only as RXR agonists but also as PPARγ agonists (our unpublished data). Although the effects of organotin compounds on the transactivation of permissive RXR heterodimers other than PPARγ/RXR have not been determined, it is probably possible to stimulate the transactivation of other heterodimers because these compounds function as RXR agonists.

Regulation of aromatase gene expression by organotin compounds through RXR or PPARγ activation in human

Gene expression of human aromatase is regulated by the activation of PPARγ and/or RXR. In the human placenta, a selective RXR ligand stimulates aromatase gene expression, however, a selective PPARγ ligand has little or no effect on aromatase gene expression [24, 46]. In addition, the PPAR ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 [24], FXR ligand chenodeoxycholic acid [24] and LXR ligand T0901317 (our unpublished data), which are agonists of permissive heterodimer partners of RXR, all also failed to increase mRNA expression of aromatase in human choriocarcinoma cells. It is suggested that none of these pemissive heterodimers are involved in aromatase expression in the human placenta and that RXR homodimer may be required for the regulation of aromatase expression (Figure 3).

Unlike in the placenta, both RXR- and PPARγ-selective ligands suppress aromatase gene expression in the ovary [47-49]. However, it was suggested that PPARγ/RXR may inhibit promoter II lying upstream of the ovarian major exon I (PII) by an indirect mechanism because of the absence of a PPARγ/RXR response element in promoter II of aromatase [47]. A transcriptional factor, nuclear factor-κB (NF-κB), interacts with the ovarian promoter II sequence of aromatase and up-regulates its gene expression in the human ovary. In addition, activation of the PPARγ/RXR heterodimer interferes with the interaction between NF-κB and promoter II sequence of aromatase [49]. PPARγ/RXR, in the ovary, may regulate aromatase gene expression via the NF-κB signaling pathway (Figure 3).

In light of these findings, human aromatase expression regulated by organotin compounds may involve the activation of PPARγ and/or RXR [18, 20], because the aromatase expression pattern induced in the human placenta and ovary by activation of PPARγ and/or RXR is similar to that induced by organotin compounds (Figure 3). It has already been found, as supporting evidence, that organotin compounds stimulate the expression of a luciferase reporter construct containing the human placental promoter I.1 sequence of aromatase via a ligand-dependent signaling pathway of RXR [24].

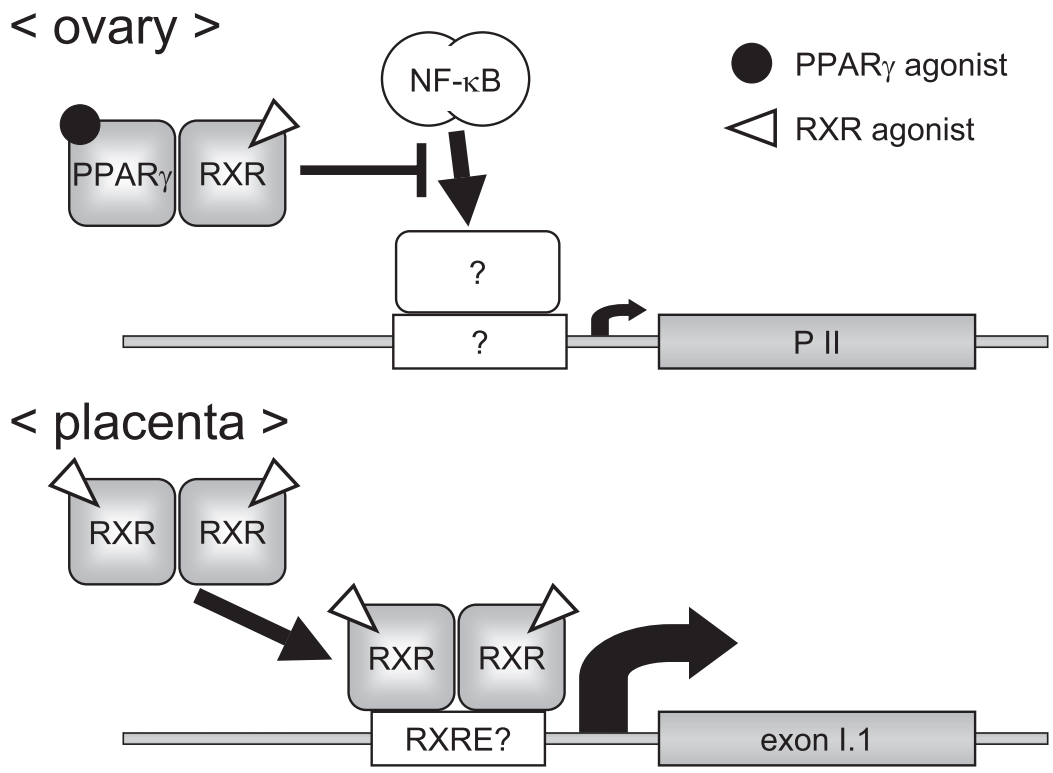

Fig. 3 Human aromatase promoter I.1 and II are regulated by the activation of PPARγ and/or RXR. In the ovary, organotins are ligands for PPARγ and RXR and may down-regulate aromatase gene expression through the suppression of NF-κB-dependent aromatase activation. On the other hand, in the placenta, organotins may induce aromatase gene expression by the transactivation of RXR homodimer, without involving PPARγ signaling pathway.

Potential toxicity by organotin compounds through RXR or PPARγ activation in mammals

PPARγ is activated by a variety of fatty acids and a class of synthetic antidiabetic agents, thiazolidinediones [50]. PPARγ agonists such as Rosi are used to treat type II diabetes and reverse insulin resistance in the whole body by sensitizing the muscle and liver tissue to insulin [51]. In addition, PPARγ also serves as an essential regulator of adipocyte differentiation and lipid storage in mature adipocytes [52]. Unfortunately, the adipogenic activity of PPARγ may result in undesirable effects such as obesity. RXR agonists also activate the PPARγ/RXR heterodimer and act as insulin-sensitizing agonists in rodents [42], underscoring the potential effects of both PPARγ and RXR agonists on diabetes and obesity. In light of these previous findings, Kanayama et al. evaluated the effects of TPT and TBT on adipogenesis and found that these organotins stimulate the differentiation of preadipocyte 3T3-L1 cells into adipocytes [32]. These results suggested that organotin compounds are a potential obesogen. A recent study from Grün et al. showed that, *in vivo*, acute exposure to TBT in adult mice resulted in coordinate regulation of lipogenic PPARγ/RXR target gene expression in adipose tissue and liver, and modulated adipocyte differentiation factors such as a members of the CCAAT/enhancer binding protein family and sterol regulatory element-binding protein 1c [53]. Furthermore, developmental exposure *in utero* led to a fatty liver (hepatic steatosis) phenotype and enhanced lipid staining of neonatal fat deposits, resulting in a significant increase in the epididymal fat pad size of mice later in life [53]. Whether this occurs through increased lipid storage, an increase in adipocyte number, or a combination of both is currently unresolved. However, activation of PPARγ/RXR induced by organotin compounds represents a compelling mechanistic example of a class of environmental pollutants that have the ability to impact key adipogenic factors, fat deposit size, and function.

In addition, exposure of rats *in utero* to TBT induces a dramatic increase in the incidence of low-birth-weight fetuses because of maternal hypothyroidism [54]. Furthermore, the RXR agonist bexarotene causes clinically significant hypothyroidism in patients with cutaneous T-cell lymphoma [55], and experimental exposure of rats to LG 100268 (a selective RXR agonist) induces the acute phase of hypothyroidism [56]. Similarities between the toxicity of TBT and selective RXR agonists suggest that at least some of the toxic effects of organotin compounds are mediated by RXR.

Yamabe et al. reported that TBT and TPT enhance the proliferation of androgen-dependent human prostate cancer cells and the transactivation of AR [57]. However, the AR antagonist flutamide cannot inhibit organotin-mediated AR transactivation [57], and these organotin compounds do not function as AR agonists in a yeast two -hybrid system (our unpublished data). Only recently, RXR was found to function as a novel co-regulator of AR, and 9cRA was found to inhibit AR activity through the activation of RXR [58]. It remains unclear whether the co-regulators recruited by organotin-activated RXR are different from those recruited by 9cRA, but RXR activation by organotins might be involved in the AR transactivation induced by them.

Taken together, these compounds may cause adverse effects on mammals through the activation of PPARγ and/or RXR because of the above-described toxic effects of organotin compounds in human cells and experimental animals.

Conclusions

Although organotin compounds inhibit the enzymatic activity of aromatase, their effective concentration is toxic for mammalian cells. In this review, we have proposed the activation of PPARγ and/or RXR as a novel mechanism for organotin-induced toxic effects in mammals. In addition, Nishikawa et al. recently reported that RXR plays an important role in the development of gastropod imposex, by showing the cloning of an RXR homolog from a marine gastropod, binding of organotins to that receptor, and imposex induction by injection of 9 cRA [59]. These findings indicated that RXR activation is also a critical event for endocrine disruption of organotins in gastropods. However, it is possible that organotin compounds affect target molecules other than PPARγ and RXR. For instance, organotin compounds have been shown to enhance histone acetyltransferase activity [60]. Further studies are needed to clarify the precise action mechanism of the toxicity of organotin compounds in mammals *in vitro* and *in vivo*, because they appear intricate.

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