Section 4
Genetic and Physiological Impacts of Organotin Compounds

Chapter 13
Genetic Impacts of Organotin Compounds

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Abbreviations AR: Androgen receptor; CYP: Cytochrome P450; 9cRA: 9 *cis*-retinoic acid; DBD: DNA binding domain; EDC: Endocrine disrupting chemical; ER: Estrogen receptor; hCG: Human chorionic gonadotropin; HSD: Hydroxysteroid dehydrogenase; LBD: Ligand binding domain; NR: Nuclear receptor; PKA: Protein kinase A; PPAR: Peroxisome proliferator-activated receptor; RXR: Retinoid X receptor; TBT: Tributyltin; TPT: Triphenyltin

13.1 Introduction

The concept of endocrine disruption was introduced at the Work Session on "Chemically Induced Alterations in Sexual Development: The Wildlife/Human Connection" in 1991. At this session it was pointed out that a number of environmental chemicals affect hormonal systems and have adverse health effects on wildlife and probably on humans. Such chemicals are referred to as endocrine disrupting chemicals (EDCs), and their effects have emerged as a major environmental issue. The nuclear receptors (NRs) of intrinsic hormone systems are likely to be targets of EDCs, because their intrinsic ligands are fat-soluble and low-molecular-weight agents, as are the environmental pollutants. Examples can be found among persistent organochlorine pollutants (DDT, PCBs), plasticizers (pthalates), detergents (alkylphenols) and birth control pills (ethynylestradiol) (McLachlan 2001). The effects of synthetic chemicals on sex hormone receptors such as the estrogen receptor (ER) and androgen receptor (AR) have attracted much attention, focusing on the reproductive failures observed in wildlife.

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Among them, the imposex phenomenon in marine gastropods provides one of the clearest examples of endocrine disruption in wildlife. While many field studies have demonstrated the adverse effects of organotins upon female gastropods, the mechanism underlying the imposex phenomenon has not been fully elucidated. Organotin compounds have been widely used as antifouling paints for ships and fishing nets since the 1960s and have thus been released into marine environments. Aquatic invertebrates, particularly marine gastropods, are extremely sensitive to organotin compounds and undergo changes in sexual identity in response to exposure. Most marine gastropods in organotin-polluted areas have shown reproductive failure due to oviduct blockage by vas deferens formation, resulting in population decline or mass extinction (Bryan et al. 1988; ten Hallers-Tjabbes et al. 1994). This phenomenon is called "imposex" as an abbreviation of "imposed sexual organs", because male genital organs, such as the penis and vas deferens, are imposed upon female organs (Smith 1971). Approximately 150 species of imposex-affected gastropods have been found in the world (Fent 1996; Matthiessen et al. 1999). Despite several hypotheses on the cause of imposex induction, such as aromatase inhibition, testosterone excretion-inhibition, functional disorder of the female cerebropleural ganglia, and involvement of neuropeptide APGWamide (Bettin et al. 1996; Ronis and Mason 1996; Oberdörster and McClellan-Green 2000, 2002), the mechanism through which they induce and promote the development of a penis-like structure and a vas deferens in female gastropods remains obscure.

It is well known that steroidal sex hormones such as 17β-estradiol and 5α-dihydrotestosterone exert important roles in physiological processes, including sexual development and reproduction in vertebrates. However, homologues of ER and AR, which mediate the action of sex steroids, have not been found in invertebrates (Escriva et al. 1997). Because gastropods are mollusks, they may not have a functional receptor for testosterone, suggesting that vertebrate-type sex hormones may not be involved in male sexual development in the gastropods. Recently, we and Grün et al. have shown, independently, that tributyltin (TBT) and triphenyltin (TPT) are high-affinity ligands for the human retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor (PPAR) γ, which are members of NR superfamily (Kanayama et al. 2005; Grün et al. 2006). In addition, functional homologues of RXR have been cloned from Japanese and European gastropods (*Thais clavigera* and *Nucella lapillus*). TBT binds to either of them at environmentally relevant levels and the natural ligand of RXR, 9-*cis* retinoic acid, induces imposex in both gastropods (Nishikawa et al. 2004; Castro et al. 2007). The mechanistic impacts of the overall findings are discussed.

13.2 Differences in Nuclear Receptors Between Invertebrates and Vertebrates

Members of the NR transcription factor function as concentration dependent sensors of cognate ligands to coordinate gene regulation of developmental and homeostatic hormone signalling pathways. NRs are structurally related proteins

that consist of a highly conserved DNA-binding domain (DBD) and a moderately conserved ligand-binding domain (LBD). The mammalian NRs mediate the actions of small molecular agents such as steroid hormones (e.g., estrogens, androgens, progesterone, glucocorticoids, mineralocorticoids), retinoic acids (all-*trans* and 9-*cis* isomers), thyroid hormone, 1.25 $(OH)_2$ vitamin D_3, and fatty acids. In addition to these receptors, the superfamily also contains a large number of so-called orphan NRs whose ligands do not exist or have not been identified (Giguére 1999). Phylogenetic study and extensive polymerase chain reaction (PCR) surveys have revealed that NR genes appeared very early on during metazoan evolution, but could not be found in fungi, plants, or unicellular eukaryotes (Escriva et al. 1997, 2000). By virtue of genome projects, we now know that *Homo sapiens*, *Ciona intestinalis*, *Drosophila melanogaster*, and *Caenorhabditis elegans,* respectively, have 48, 17, 21, and 284 kinds of NR genes (Maglich et al. 2001; Yagi et al. 2003). There is a striking difference between vertebrates and invertebrates with respect to their NR sets. For instance, receptors for sex and adrenal steroid hormones have not been found in ascidian, fruit fly and round-worm. Although distant ancestors of the ER have been found in octopus, snails, and other mollusks, these ER-like proteins do not bind to estrogens and were constitutive activated transcription factors like the orphan NRs (Thornton et al. 2003; Keay et al. 2006; Bannister et al. 2007). In addition to the absence of sex steroid receptors, cytochrome P450 (CYP) enzymes related to sex steroid biosynthesis have not been identified in invertebrates except for amphioxus *Branchiostoma belcheri*, which is considered to be evolutionarily closer to vertebrates than other invertebrates (Campbell et al. 2004; Rewitz et al. 2006; Mizuta and Kubokawa 2007). The data collected until now suggest that the existence or function of sex steroids is different between vertebrates and invertebrates.

13.3 Molecular Factors Related to Imposex

Imposex is induced by TBT at concentrations as low as 1 ng/l of tin (Sn) (Gibbs et al. 1987; Axiak et al. 1995) and is used extensively all over the world as a biomarker to monitor TBT pollution (ten Hallers-Tjabbes et al. 1994; Horiguchi et al. 1997a; Terlizzi et al. 1998, 2004). Not only TBT, but also TPT, has been shown to have a strong effect on the development of imposex in *Thais clavigera* (Horiguchi et al. 1997b). Historically, several hypotheses have been proposed to explain the chain of events and molecular factors leading to imposex development. The original work of Féral and Le Gall with transplantation experiments suggested that the hierarchic involvement of two illusive factors, termed the retrogressive factor (RF) and the penis morphogenic factor (PMF) (Féral and Le Gall 1983). They also suggested the fundamental role of two anatomical structures, the pedal and cerebropleural ganglia. Subsequently, the neuropeptide APGWamide was proposed as the putative PMF, because injection of APGWamide significantly induces imposex in the mud snail *Ilyanassa obsoleta* (Oberdörster and McClellan-Green 2000, 2002). They proposed that APGWamide is abnormally released by an external stimulus such

as TBT exposure and causes the development of male sex characteristics. Despite these observations, APGWamide failed to promote imposex in the prosobranch gastropod *Bolinus brandaries* (Santos et al. 2006).

Inhibition of aromatase, the key enzyme required for conversion of androgens to estrogens, has also been proposed as a potential driver for imposex development. An aromatase enzyme complex consists of the microsomal CYP19 and the flavoprotein nicotinamide adenine dinucleotide phosphate reduced-form reductase. Bettin et al. reported that TBT increases androgen levels through inhibition of aromatase activity in marine neogastropods at relatively high doses (Bettin et al. 1996). The TBT also inhibits the catalytic activity of human aromatase or a granulose cell-like tumor cell line (Cooke 2002; Heidrich et al. 2001; Saitoh et al. 2001). However, the CYP19 gene has not been found outside chordates (Mizuta and Kubokawa 2007). Therefore, it is doubtful whether the inhibitory effect of TBT on aromatase activity is a cause of the imposex in molluscs.

New insight is coming from a quite different direction. We reported that TBT and TPT are high-affinity ligands for RXR and PPARγ by comprehensive ligand screen with human NRs (Kanayama et al. 2005; Grün et al. 2006). We employed an *in vitro* molecular interaction screen between human NRs and coactivators, together with a yeast two-hybrid system, to test suspected EDCs for receptor-mediated activation (Kanayama et al. 2003). Surprisingly, organotins such as TBT and TPT act as potent nanomolar activators of both RXR and PPARγ. In addition, these compounds showed the transactivation function of RXR and PPARγ in mammalian culture cells (Kanayama et al. 2005; Grün et al. 2006). 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. 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 organotin compounds function as RXR or PPARγ agonists in mammalian cells.

13.4 Existence of the Retinoid X Receptor in Marine Gastropods

Although TBT and TPT apparently activate human RXR and PPARγ, the question is whether these receptors exist in gastropods or not. As described in section 13.2, the composition of members of the NR superfamily is quite different between vertebrates and invertebrates. The subgroup members of the thyroid hormone receptor, retinoic acid receptor, vitamin D receptor and PPAR appear to have been late acquisitions during the evolution of the NR superfamily (Escriva et al. 1997; Laudet 1997). Therefore, PPARγ might not be present in marine gastropods. In contrast, RXR is special among the NR superfamily. It is widely conserved in the evolutionary tree and its homologue, called ultraspiracle (USP), is found even in arthropods (Laudet 1997).

The RXR homologue has been cloned from *Thais clavigera* (Nishikawa et al. 2004) and more recently from *Nucella lapillus* (Castro et al. 2007). Either of these RXRs has a DBD composed of two C_2C_2-type zinc finger motifs and a putative LBD in the C-terminal region. The highest similarity with other species is in the DBD, where 85–90% of the amino acids residues are identical. The LBD of gastropod RXR also shows considerable similarity with that of vertebrate RXRs but has much less similarity with USP, the RXR homologue first found in *Drosophila melanogaster*. Although RXR binds 9cRA in organisms ranging from cnidarians (*Tripedalia cystophora*) to vertebrates, USP from arthropods is unable to do so (Heyman et al. 1992; Mangelsdorf et al. 1992; Henrich and Brown 1995; Kostrouch et al. 1998). As expected from the similarity of gastropod homologues to vertebrate RXR, the binding of gastropod RXR to 9cRA has been confirmed experimentally (Nishikawa et al. 2004; Castro et al. 2007). The dissociation constant in the binding of 9cRA to gastropod RXR is 15.2 nM, which is similar to the values reported for vertebrate RXRs (1–10 nM) (Heyman et al. 1992). Gastropod RXR also binds to organotin compounds, even though the 50% inhibitory concentration (IC_{50}) values are larger than for 9cRA (Nishikawa et al. 2004).

To examine the involvement of RXR in the development of imposex in *T. clavigera*, an *in vivo* injection experiment was carried out. Imposex was significantly induced in female *T. clavigera*, which received the injection of 9cRA, and substantial penis growth was observed in them after 1 month of 9cRA injections (Nishikawa et al. 2004). Through a combination of exposure experiments, Castro et al. (2007) also showed that 9cRA induces imposex in *N. lapillus* to the same degree as the positive control (TBT). Methoprene acid, a selective ligand for RXR, also induces imposex, albeit to lower degree than that observed for 9cRA and TBT (Castro et al. 2007). In this context, RXR plays an important role in the induction/differentiation and growth of male genital tracts in female gastropods. It is possible that sexual differentiation in primitive species is regulated by retinoid signaling instead of steroids. Meanwhile, we do not know whether gastropods inherently possess a pathway for the biosynthesis of 9cRA. Therefore, we do not know whether 9cRA is a real hormone or whether similar derivatives are. We need to identify the active compound from gastropods.

13.5 Possible Human Exposure to Organotin Compounds

The potent biocidal properties of organotins extended their uses to the production of high value food crops and industrial processes, in addition to antifouling biocides for marine vessels. Some organotins are used in food contact packing and drink containers. Human exposure to non-point sources of organotins may occur mainly through contaminated dietary sources, such as seafood, shellfish and food crops. Daily intakes of TBT oxide (TBTO) determined in Japan by the duplicated-position method were 4.7 ± 7.0 µg/day in 1991 (n = 39) and 2.2 ± 2.2 µg/day in 1992 (n = 40). Using the market based method, the daily intake was estimated at

6–9 μg/day in 1991 and 6–7 μg/day in 1992 (Tsuda et al. 1995). In Finland, TPT was detected as the predominant compound at levels up to 1.11 ng/g fresh weight in fish and seafoods (Rantakokko et al. 2006). In addition, a variety of mono- and dialkyltins, and significant contaminating trialkyl species, are also used extensively in the manufacture of polyolefein plastics (PVC) as a heat stabilizer during polymerization, bringing them into closer contact with drinking water and food supplies (Takahashi et al. 1999; Appel 2004).

The information on human exposure to organotin compounds is limited. In a study of eight volunteers from Germany, TPT was detectable in serum in the concentration range 0.17–0.67 mg/l (Lo et al. 2003). In a study of 38 volunteers from the USA, Kannan et al. reported that monobutyltin (MBT), dibutyltin (DBT) and TBT were detected in 53%, 81%, and 70% of the 32 blood samples tested. Blood concentrations of MBT, DBT and TBT were 8.17 ± 8.56, 4.94 ± 3.83, and 8.18 ± 15.4 ng/ml, respectively (Kannan et al. 1999). The toxicological significance of the concentrations of organotins measured in these studies is unknown. However, the potential exposure of humans to organotins has aroused great concern about their potential toxicity. Animal experiments suggested that the spectrum of potential adverse chronic systemic effects of organotins is quite broad and includes primary immunosuppressive, endocrinopathic, and neurotoxic effect, as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary, gastrointestinal, blood dyscrasias, reproductive/teratogenic/developmental, liver, kidney, bioaccumulative, and possibly carcinogenic activity. Although many reports have described the potential toxicity of organotins, the critical target molecules for the toxicity of organotin compounds remain unclear.

13.6 Enzyme Inhibition by Organotins

The synthesis of sex steroids from cholesterol requires trafficking process between mitochondria and smooth endoplasmic reticulum, and many enzymatic steps. In *in vitro* experiments, butyltins were shown to exhibit structure-related inhibition of the aromatase activity from human placenta (Heidrich et al. 2001) or transfected cells (Cooke 2002). However, effective inhibition of aromatase by organotins occurs only in the micromolar range. TBT and TPT are generally toxic to mammalian cells and they cause apoptosis or necrosis at micromolar levels (Saitoh et al. 2001; Nakanishi et al. 2002, 2006; Watanabe et al. 2003). In human choriocarcinoma cell lines, JAr, JEG-3, and BeWo, exposure to greater than 300 nM TBT or TPT markedly decreases DNA and protein synthesis (Nakanishi et al. 2002, 2006). In addition, a high concentration of TBT (above 1 μM) inhibits the catalytic activity of human 5α-reductase I and II (Doering et al. 2002), rat 3β-hydroxysteroid dehydrogenase (3β-HSD) (McVey and Cooke 2003) and pig 17β-HSD I (Ohno et al. 2005). At similar concentration ranges, TPT also inhibits the catalytic activity of aromatase, 5α-reductase II, 17β-HSD I and III (Lo et al. 2003). These observations suggest that enzyme inhibition by organotins is not specific to aromatase.

We should take into account the strong cytotoxicity and non-specific effects of organotin compounds, when measured *in vitro*.

13.7 Organotin Compounds Affect Endocrine Functions in Human Placenta and Ovary

In a recent study, we investigated the effects of organotin compounds on aromatase (Nakanishi et al. 2002, 2005) and 17β-HSD I, which converts low-activity estrone to high-activity estradiol (Nakanishi et al. 2006), 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 data indicate that organotins perturb the steroidogenic function through transcriptional regulation in human placental cells, not through direct enzyme inhibition. In addition, these organotin compounds also markedly stimulated hCG production in the same concentration ranges, along with its mRNA expression (Nakanishi et al. 2002, 2005). These results suggest that the placenta represents a potential target organ for organotin compounds in pregnant women and that endocrine-disrupting effects might be the result of local changes in estrogen and hCG concentrations.

In contrast to the above results, however, Saitoh et al. (2001) 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. This discrepancy in the action of organotins on gene expression is due to the tissue-specific expression of aromatase, which is strictly regulated. Human *CYP19* is a single-copy gene composed of 10 exons; exons II to X encode the aromatase protein, as well as the 3′ untranslated region of mRNA common to all estrogen-producing tissues (Simpson et al. 1994). 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 (Simpson et al. 1994; Sebastian and Bulun 2001; Bulun et al. 2003). The tissue-specific expression of *CYP19* 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 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 (Bamberger et al. 1996; Simpson et al. 1997). Saitoh et al. suggest that the effects of organotin compounds in KGN cells are caused partly by association with Ad4Bp/SF-1 (Saitoh et al. 2001). 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 (Nakanishi et al. 2002). In addition, there is little possibility that these organotin compounds affect the cAMP-protein kinase A (PKA) pathway in the human ovary, because it stimulates aromatase gene expression through promoter II (Michael et al. 1995). 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.

13.8 Regulation of Aromatase Gene Expression by Organotin Compounds Through RXR or PPARγ Activation in Humans

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 (Sun et al. 1998; Nakanishi et al. 2005). In addition, the PPAR ligand 15-deoxy-$D^{12,14}$-prostaglandin J_2, farnesoid X receptor ligand chenodeoxycholic acid and liver X receptor ligand T0901317, which are agonists of permissive heterodimer partners of RXR, all failed to increase mRNA expression of aromatase in human choriocarcinoma cells (Nakanishi et al. 2005). It is suggested that none of these permissive heterodimers are involved in aromatase expression in the human placenta and that RXR homodimer may be required for the regulation of aromatase expression.

Unlike in the placenta, both RXR- and PPARγ-selective ligands suppress aromatase gene expression in the ovary (Mu et al. 2000, 2001; Fan et al. 2005). 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 (Mu et al. 2001). A transcriptional factor, nuclear factor-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 (Fan et al. 2005). PPARγ/RXR, in the ovary, may regulate aromatase gene expression via the NF-κB signaling pathway.

In light of these findings, human aromatase expression regulated by organotin compounds may involve the activation of PPARγ and/or RXR (Saitoh et al. 2001; Nakanishi et al. 2002, 2005), 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. 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 (Nakanishi et al. 2005).

13.9 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 that are used to treat type II diabetes and reverse insulin resistance in the whole body by sensitizing the muscle and liver tissue to insulin (Lehmann et al. 1995). In addition, PPARγ also serves as an essential regulator of adipocyte differentiation and lipid storage in mature adipocytes (Tontonoz et al. 1994). 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 (Mukherjee et al. 1997), underscoring the potential effects of both PPARγ and RXR agonists on diabetes and obesity. In light of these previous findings, we evaluated the effects of TPT and TBT on adipogenesis and found that these organotins stimulate the differentiation of preadipocyte 3T3-L1 cells into adipocytes (Kanayama et al. 2005). 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 (Grün et al. 2006). Furthermore, developmental exposure *in utero* led to a fatty liver (hepatic steatosis) phenotype and enhanced lipid staining of neonatal fat deposits, and resulting in a significant increase in the epididymal fat pad size of mice later in life (Grün et al. 2006). 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.

Exposure of rats *in utero* to TBT induces a dramatic increase in the incidence of low-birth-weight fetuses because of maternal hypothyroidism (Adeeko et al. 2003). On the other hand, the RXR agonist bexarotene causes clinically significant hypothyroidism in patients with cutaneous T-cell lymphoma (Duvic et al. 2001), and experimental exposure of rats to LG100268 (a selective RXR agonist) induces the acute phase of hypothyroidism (Liu et al. 2002). Similarities between the toxicity of TBT and selective RXR agonists suggest that at least some of the toxic effects of organotin compounds may be mediated by RXR.

Yamabe et al. (2000) reported that TBT and TPT enhance the proliferation of androgen-dependent human prostate cancer cells and the transactivation of AR.

However, the AR antagonist flutamide cannot inhibit organotin-mediated AR transactivation (Yamabe et al. 2000), and these organotin compounds do not function as AR agonists in a yeast two-hybrid system (Nishikawa et al. 2004). Recently, RXR was found to function as a novel co-regulator of AR, and 9cRA inhibits AR activity through the activation of RXR (Chuang et al. 2005). Although it remains unclear whether the co-regulators recruited by organotin-activated RXR are different from those recruited by 9cRA, 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.

13.10 Conclusions

Although organotin compounds inhibit the enzymatic activity of aromatase, their effective concentration is cytotoxic. In this review, we have proposed the activation of RXR and/or PPARγ as a novel mechanism for organotin-induced negative impacts on invertebrates and vertebrates. We reported that RXR plays an important role in the development of gastropod imposex, by showing the cloning of RXR homologues from marine gastropods, binding of organotins to those receptors, and imposex induction by injection of 9cRA (Nishikawa et al. 2004; Castro et al. 2007). 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 (Osada et al. 2005). Further studies are needed to clarify the precise action mechanism of the toxicity of organotin compounds in mammals and gastropods.

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