

# Disruption of Thyroid Hormone Function by Environmental Pollutants

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A great number of synthetic chemicals are released into the environment, many of which are known or thought to interfere with normal thyroid hormone (TH) function. THs play important roles in regulating growth and development and maintaining metabolic homeostasis. For example, amphibian tadpole metamorphosis is a TH-triggered and controlled developmental process, and has proven to be useful as a screening tool for environmental pollutants suspected of disrupting TH functions. TH disruption is thought to be caused through a variety of mechanisms, including increased thyroxine (T<sub>4</sub>) metabolism by uridine diphosphate glucuronyl transferases (UDPGTs), blocking TH signaling through TH receptors (TRs), and induction of mitochondrial membrane permeability transition (MPT). As our knowledge concerning the specific effects of these chemicals is very limited, further research is needed to obtain accurate information to be used in establishing guidelines for the protection of health in humans and wildlife.

**Key words** — amphibian metamorphosis, bisphenol A related compound, brominated flame retardant, environmental pollutant, polychlorinated biphenyl, thyroid hormone disruption

## INTRODUCTION

Global climate change is receiving particular attention because it is thought to cause habitat destruction, desertification and biodiversity loss, and also has a possible serious impact on human health and society. The problem of man-made chemicals released into the environment has also been of great concern in recent years. A large number of environmental chemicals including polychlorinated biphenyls (PCBs), bisphenol A (BPA) and brominated flame retardants (BFRs) have been shown to disrupt the normal action of endogenous hormones in wildlife and humans, leading to changes

in hormone-mediated responses.<sup>1–7)</sup>

The issue of endocrine disrupting chemicals (EDCs) traces its origin to reports that daughters born to mothers who had taken the synthetic estrogen diethylstilbestrol (DES) during pregnancy exhibited severe reproductive abnormalities, including a rare cancer of the vagina.<sup>8)</sup>

Accumulating evidence indicates that many xenobiotics have the capacity to affect thyroid system function.<sup>9)</sup> Thyroid hormone (TH) homeostasis plays an important role in vertebrate metabolism, growth and development.<sup>10–12)</sup> TH is also essential for normal brain development in the human fetus and newborn infant, since TH deficits in pregnant women result in neurological cretinism accompanied by severe cognitive and/or mental disorders in their offspring.<sup>13–22)</sup> The thyroid system operates in basically the same way in all vertebrates in-

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cluding humans.<sup>23,24</sup> The thyroid gland produces predominantly thyroxine ( $T_4$ ),<sup>25,26</sup> which is transported to target tissues by the serum transport proteins, transthyretin (TTR), thyroxine-binding globulin (TBG) and albumins.<sup>27</sup> TTR is important in mammals, while TBG is absent in all non-mammals.

One example of the importance of TH to vertebrate growth and development is amphibian metamorphosis.<sup>28–30</sup> Frog THs are structurally identical to mammalian THs,<sup>30</sup> while frog TH receptors (TRs) are highly homologous to other vertebrate counterparts. Type II deiodinase (DI-2) converts  $T_4$  into the much more biologically active 3,3',5-triiodothyronine ( $T_3$ ) through the removal of an outer ring iodine molecule, whereas type III deiodinase (DI-3) inactivates  $T_4$  and  $T_3$  by removal of an inner ring iodine molecule. The biological functions of  $T_3$  are mediated by the interaction with TR, which belongs to the nuclear hormone receptor families.<sup>31</sup> TR forms a heterodimer with a retinoid X receptor (RXR), thereby binding to the TH response elements (TREs) located in the regulatory region of target genes, followed by binding to  $T_3$  to enhance gene transcription.<sup>32</sup> Amphibian metamorphosis is triggered and controlled by THs, and thus it seems highly probable that TH synthesis, TH transport and TH metabolism are all potential target areas for the disrupting effects of environmental chemicals.

Tadpole tail resorption during anuran metamorphosis is a dramatic change resulting from apoptosis, or programmed cell death, and is controlled by TH.<sup>32</sup> Addition of THs to the water or medium used in rearing premetamorphic stage tadpoles results in an enhanced metamorphic process, and such TH-induced enhancement has proven to be a useful tool for investigating the effects of environmental chemicals.<sup>33</sup> The expression of key genes important for the TH signaling pathway can be easily monitored in real-time throughout development in transgenic tadpoles and frogs carrying enhanced green fluorescent protein (EGFP) transgenes relevant to thyroid axis and metamorphosis. TH function is regulated by a complex interplay between the hypothalamus, pituitary and thyroid gland.<sup>34</sup> For mammals the hypothalamic thyrotropin-releasing hormone (TRH) stimulates the biosynthesis and release of thyroid-stimulating hormone (TSH) in the pituitary gland, followed by release of  $T_4$  and  $T_3$  from the thyroid gland into the circulation. For developing tadpoles the hypothalamus controls TSH release

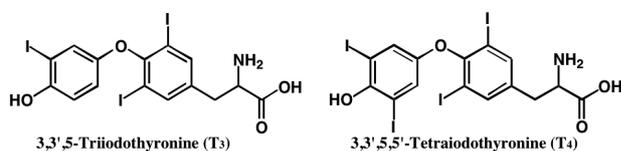
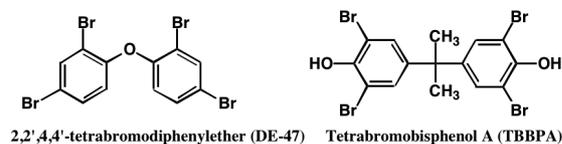
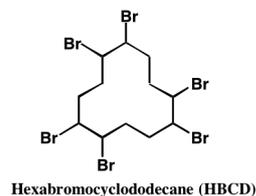
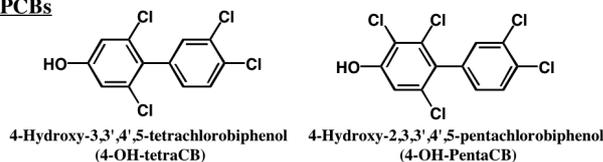
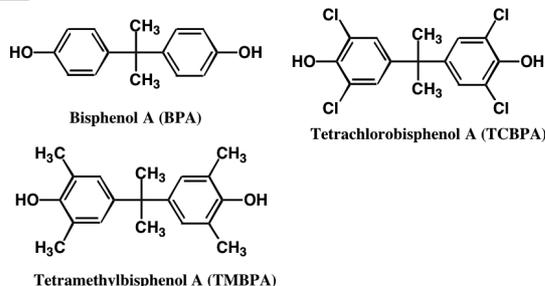
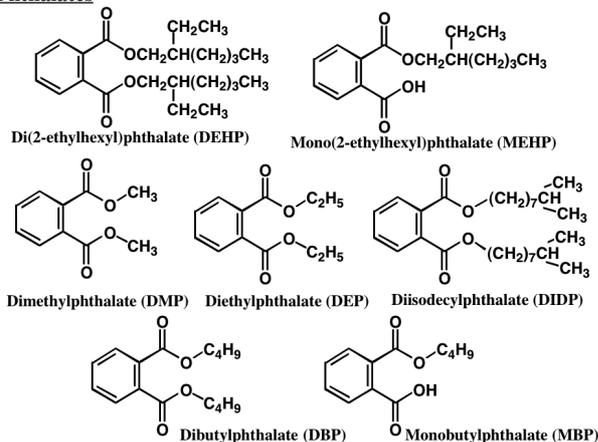
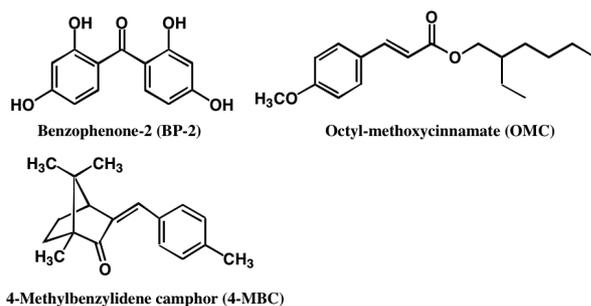
via corticotropin-releasing factor (CRF) rather than TRH.<sup>35,36</sup>

The present investigations focused on the effects of PCBs, BPA and related compounds, UV filters, BFRs, heavy metals and phthalates (Fig. 1) on TH homeostasis because these substances have been shown to be biologically active and often mimic TH and cause alterations in TH modulated actions.

## PCB

Although their production has been halted since 1977, PCBs are still present in the environment due to their high chemical stability.<sup>37,38</sup> PCBs contain 209 individual congeners, which differ according to the number and location of chlorine atoms on the two benzene rings.<sup>39</sup> PCBs are highly lipophilic, leading to their bioaccumulation in fatty tissues, and are biomagnified throughout the food chain.<sup>40,41</sup> Moreover PCBs are easily transferred to embryos and infants via the placenta and breast milk.<sup>22</sup> PCBs structurally resemble TH, and have been documented to disrupt normal thyroid function in laboratory animals.<sup>42</sup> Exposure to PCBs and related compounds causes a reduction in thyroid hormones in developing and adult animals.<sup>43–45</sup> PCB metabolite 4-OH-2,3,3',4',5-pentachlorobiphenyl (4-OH-CB107) was reported to be present in the blood plasma of humans and wildlife.<sup>46,47</sup> Exposure of pregnant female rats to 4-OH-CB107 induces a drastic decrease in TH level in their offspring, suggesting transgenerational effects of this substance.<sup>48</sup>

Aroclor 1221 (A1221; a low chlorinated PCB) and Aroclor (A1254; a high chlorinated PCB) are commercial PCB mixtures. Kiliç *et al.*<sup>49</sup> subcutaneously injected adult female rats with A1221 (10 mg/kg) and A1254 (10 mg/kg) for six weeks, and compared the effects of the two chemicals on serum TH concentrations and thyroid gland histology. The results showed that total  $T_4$  levels were significantly increased in A1254 and A1221-treated rats; A1254 exposure caused significant increases in free  $T_4$  levels, while A1221 administration significantly increased free  $T_3$  concentrations; distinct structure changes in the thyroid glands were observed in both PCB-treated groups in the thyroid glands, suggesting that PCB mixtures induce adverse effects on thyroid gland regardless of their degree of chlorination. Several studies have re-

**T<sub>3</sub> and T<sub>4</sub>****BFRs****PCBs****BPAs****Phthalates****UV filters**

**Fig. 1.** Structures of Thyroid Hormones, PCBs, BPAs, UV Filters, BFRs and Phthalates

ported however that PCBs decreased serum T<sub>4</sub> concentrations in adult and neonatal rodents.<sup>50–52</sup> In A1254-treated rats (200 µg/kg body weight; for 15 days), Anbalagan *et al.*<sup>53</sup> found that T<sub>3</sub> and T<sub>4</sub> levels were reduced. Hood and Klaassen<sup>54</sup> also found that treatment of adult male rats with A1254 for seven days significantly reduced serum T<sub>3</sub> and T<sub>4</sub> concentrations. Such differing effects of A1254 on TH induction may be explained by different treatment duration times. This is supported by the reports that changes in thyroid gland morphology were observed in A1254 long-term (six weeks) treated rats, but not in short-term (seven days) treated animals fed a diet containing A1254 at 25, 50, 100 or 200 ppm.<sup>49, 54</sup>

Previous studies have reported that PCBs do not seem to adversely affect TSH activity. For example, no effects of TSH were seen when developing rats were exposed to A1254 *in utero*, in spite of changes in plasma total and free T<sub>4</sub> levels, T<sub>3</sub> levels and deiodinase activity.<sup>44</sup> Exposure of mice to Kanechlor-500 (KC500) resulted in decreases in T<sub>4</sub> and T<sub>3</sub>, with no change in TSH.<sup>55</sup> Several investigations have shown that PCBs affect adversely amphibian metamorphosis. Jelaso *et al.*<sup>56</sup> reported that long-term dietary exposure to A1254 delayed progression through metamorphosis in developing *Xenopus laevis* (*X. laevis*). Lehigh Shirey *et al.*<sup>36</sup> found that exposure of *X. laevis* tadpoles to an environmentally relevant concentration (50 ppb) of

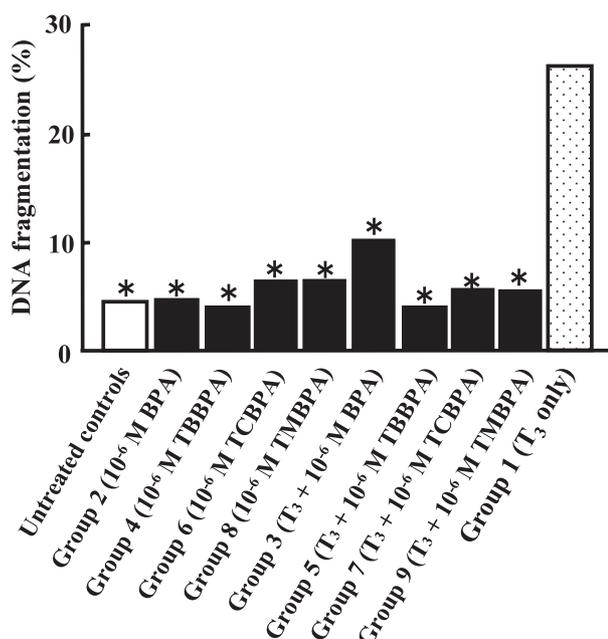
A1254 resulted in delayed metamorphosis and altered gene expression of TTR and DI-2 and DI-3. This change in DI-2 expression agrees with Morse *et al.*,<sup>44)</sup> who reported that conversion of T<sub>4</sub> into T<sub>3</sub> significantly decreases in the developing brains of rats after exposure to A1254 *in utero*. Long-term exposure (up to metamorphosis completion) of *X. laevis* tadpoles to the technical PCB mixture Clophen A50 reduced plasma T<sub>4</sub> levels and significantly delayed metamorphosis.<sup>57,58)</sup>

The following mechanisms have been proposed to explain how PCBs alter thyroid function. PCBs and related compounds are structurally similar to THs and therefore bind to TTR or TR to act as either a TH agonist or a TH antagonist.<sup>45,50,59)</sup> PCBs such as co-planar or dioxin-like PCB act through the aryl hydrocarbon receptor (AhR) mechanism. These PCBs can bind to the AhR and induce hepatic uridine diphosphate glucuronyl transferases (UDPGTs), leading to biliary excretion and elimination of T<sub>4</sub>.<sup>51,52,54,55)</sup> Miyazaki *et al.*<sup>22)</sup> suggest that PCBs induce a partial dissociation of TR/RXR heterodimer complex from the TRE, resulting in suppression of gene transcription and that PCB action site is TR present in many organs, including the central nervous system. Johansson *et al.*<sup>60)</sup> suggest that PCBs trigger apoptosis via activation of calcium-regulated calpains and lysosomal cathepsins, possibly by disrupting mitochondrial function and intracellular calcium signaling. Several workers have suggested that a pathway involving mitochondrial membrane permeability transition (MPT), cytochrome *c* release and caspase activation is involved in the mechanism of PCB-induced apoptosis.<sup>61)</sup> Moreover, our preliminary experiments have shown that 4-hydroxy-3,5,3',4'-tetrachlorobiphenyl (4-OH-TCB) enhances T<sub>3</sub>-induced *Rana rugosa* (*R. rugosa*) tadpole tail apoptosis in a concentration dependent manner. Recently Fujita *et al.*<sup>62)</sup> and Kashiwagi *et al.*<sup>63)</sup> reported that 4-OH-TCB induces MPT together with reactive oxygen species (ROS) generation, causing apoptosis.

## BPA AND RELATED COMPOUNDS

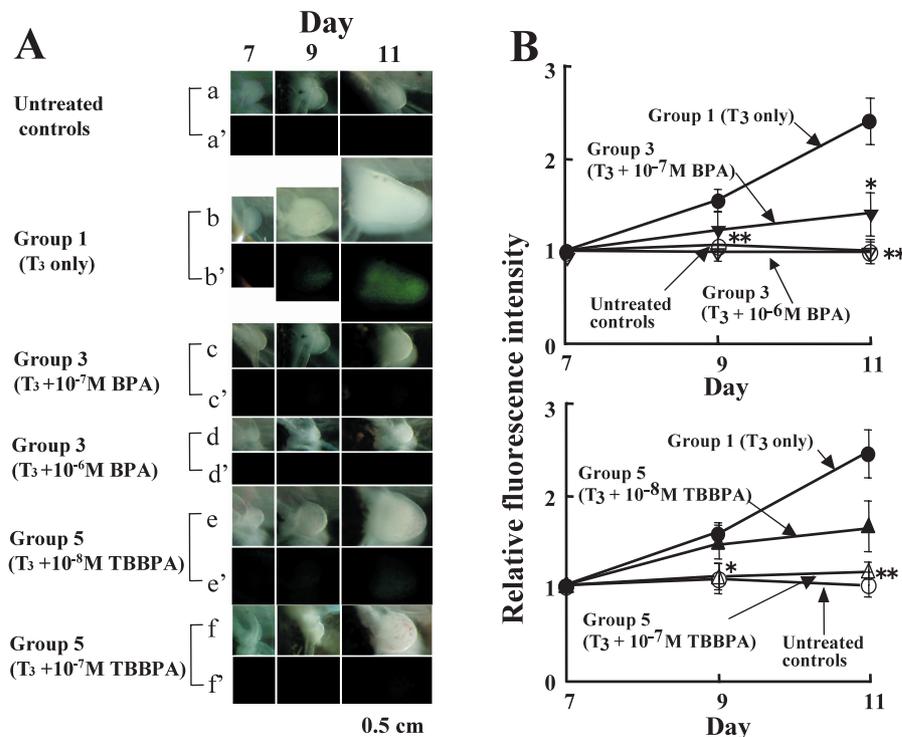
BPA has been widely used throughout the world as an industrial raw material for polycarbonate plastics and epoxy resins used in dental sealants, food containers, plastic baby bottles, food and beverage can linings, *etc.*<sup>64–66)</sup> Due to incomplete polymerization and to degradation of the polymers, BPA

has been reported to leach out of the plastic into food at higher temperatures.<sup>67–69)</sup> BPA is constantly released into the aquatic environment through various sources including the effluent from sewage treatment plants.<sup>70)</sup> The chlorinated structure analogue tetrachlorobisphenol A (TCBPA) is found in the effluent from waste-paper recycling plants,<sup>71)</sup> while the methylated structural analogue tetramethylbisphenol A (TMBPA) is used in polycarbonate resin. BPA and its analogs (BPAs) are common pollutants of rivers, lakes and seawater, resulting in chronic exposure by humans and wildlife. In fact, BPA has been detected in the plasma and placenta of pregnant women, in fetal plasma and amniotic fluid,<sup>72,73)</sup> as well as in the milk of nursing mothers.<sup>74)</sup> BPA administered to pregnant mice can be transferred to fetuses and alters postnatal development and sexual maturity at a low, environmentally relevant level (2.4 µg/kg).<sup>75)</sup> Maternal exposure to BPA in rats during pregnancy and lactation caused an increase in total serum T<sub>4</sub> in nursing pups.<sup>76,77)</sup> Goto *et al.*<sup>33)</sup> and Kashiwagi *et al.*<sup>63)</sup> reported that T<sub>3</sub>-treated *R. rugosa* tadpole tails displayed marked apoptotic features, including DNA fragmentation and ladder-like profiles, as opposed to essentially little or no fragmentation and ladder formation for T<sub>3</sub> + BPA, TCBPA and TMBPA-treated tails (Fig. 2). They also found that BPAs sup-



**Fig. 2.** Effect of BPA, TCBPA, TMBPA and TBBPA on DNA Fragmentation in Tails of T<sub>3</sub>-Treated and Untreated *R. rugosa* Tadpoles<sup>33)</sup>

\*Significantly less ( $p < 0.01$ ) than group-1 value.



**Fig. 3.** EGFP Activity in Hindlimbs of T<sub>3</sub>-Treated Transgenic *X. laevis* Tadpoles and Suppression by BPA and TBBPA<sup>33)</sup>

(A) Dorsal views of hindlimbs in reflected light (a–f) and EGFP fluorescence (a'–f'). (B) EGFP activity. \*Significantly less ( $p < 0.05$ ) than corresponding values for group-1 tadpoles. \*\*Significantly less ( $p < 0.01$ ) than corresponding values for group-1 tadpoles.

pressed spontaneous *Silurana tropicalis* metamorphosis controlled by endogenous circulating TH. These results indicate that BPAs act as TH antagonists. BPA was also found to block spontaneous and T<sub>3</sub>-induced metamorphosis in *X. laevis* tadpoles.<sup>78)</sup>

*In vitro* and *in vivo* studies have reported on the mechanism for the adverse effects of BPA and related compounds on TH action. TCBPA and TMBPA exhibited no antagonistic activity towards growth hormone production induced by T<sub>3</sub>.<sup>79)</sup> Dietary exposure to BPA by rats during pregnancy and lactation resulted in a elevation of serum T<sub>4</sub> in pups and up-regulated the expression of TH-responsive gene RC3/neurogranin in the developing rat brain.<sup>76)</sup> In contrast, BPA was found to limit the ability of TH to inhibit mouse oligodendrocyte differentiation.<sup>80)</sup> In human embryonic kidney cells and hepatoblastoma cells, Moriyama *et al.*<sup>81)</sup> found that BPA inhibited T<sub>3</sub> binding to TR, and by recruiting co-repressors nuclear receptor corepressor (N-CoRs) on the promoter, resulted in transcriptional suppression *in vitro*. Iwamuro *et al.*<sup>82)</sup> reported that BPA acts as a T<sub>3</sub> antagonist by suppressing TR  $\alpha$  and TR  $\beta$  gene expression in *X. laevis* tail tissue. Goto *et*

*al.*<sup>33)</sup> and Kashiwagi *et al.*<sup>63)</sup> reported that in transgenic *X. laevis* tadpoles carrying plasmid DNA containing TRE and 5'-upstream promoter regions of the TR  $\beta$  A1 gene linked to an EGFP gene, T<sub>3</sub> induced a strong EGFP expression in the hind limbs (Fig. 3). This expression was suppressed by BPA, TCBPA and TMBPA, suggesting that BPAs all act as antagonists to prevent the binding of T<sub>3</sub> to TR, resulting in suppression of TR-mediated gene expression. Protein disulfide isomerase (PDI) is observed in many organelles including endoplasmic reticulum, nuclear envelope, and plasma membranes, as well as in the cytosol of cells,<sup>83–86)</sup> and PDI action is regulated via T<sub>3</sub> binding to PDI. According to Hiroi *et al.*<sup>87)</sup> BPA inhibits T<sub>3</sub> binding to PDI, which adversely affects various cellular processes.

## UV FILTERS

UV filters are photostable substances used in cosmetic products to protect human skin from UV irradiation, and in plastics to prevent light-induced degradation.<sup>88–90)</sup> Being highly lipophilic and easily bioaccumulated are readily released into lakes

and rivers via swimming and wastewater,<sup>91,92)</sup> and then accumulate in fish.<sup>93,94)</sup> Humans are exposed to UV screens directly from cosmetics,<sup>95,96)</sup> or indirectly through the food chain, and residues of several UV filters have been detected in human milk.<sup>97)</sup> Schmutzler *et al.*<sup>98)</sup> reported that the UV filter benzophenone 2 (BP2) interfered with TH homeostasis in rats. They found that total serum T<sub>4</sub> was significantly decreased in BP2-treated rats, while serum TSH was significantly increased. Furthermore, thyroid peroxidase (TPO) activity, which plays an important role in TH biosynthesis in the thyroid gland, was also affected in BP2-treated animals. BP2 also reduced T<sub>4</sub>- and T<sub>3</sub>-concentrations in rats.<sup>99)</sup> Octyl-methoxycinnamate (OMC), one of the most frequently used chemical UV filters in the world, caused a dose-dependent decrease in serum TSH-, T<sub>4</sub>- and T<sub>3</sub>-concentrations in rats.<sup>100)</sup> In adult offspring born to 4-methylbenzylidene camphor (4-MBC)-treated rats, thyroid weight and T<sub>3</sub>-concentration were found to increase.<sup>101)</sup>

## BFR

Hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), polybrominated diphenyl ethers (PBDEs) and other BFRs are widely used as additive and reactive compounds in plastics, textiles, computers, electronic devices, televisions, building materials and many other consumer products. HBCD, PBDEs and polybrominated biphenyls (PBBs) are not chemically bound to the surface of the product, and are thought to separate and leach into the environment.<sup>102–104)</sup> TBBPA, on the other hand, is reactive and is chemically bound to the material.

PBBs are no longer produced. In 1973 feed containing PBBs was accidentally given to farm animals in Michigan,<sup>105)</sup> resulting in widespread contamination of milk, meat and eggs, as well as poisoning in animals.

Humans and wildlife are continually exposed to BFRs due to their persistent, lipophilic and bioaccumulating properties.<sup>5,6)</sup> Human serum concentrations of TBBPA and other BFRs increased from 0.44 ng/g lipids in 1977 to 3.3 ng/g lipids in 1999.<sup>106)</sup> For 0–4 year-old infants, however, the increase was another 1.5 to 3.5 times as great.

Evidence from investigations using laboratory animal models suggests that exposure to certain PB-

DEs results in disruption of TH action.<sup>6,107–114)</sup> Hallgren and Darnerud<sup>115)</sup> reported that exposure of female rats to the PBDE congener 2,2',4,4'-tetrabromodiphenylether (DE-47) for 14 days decreased the plasma level of T<sub>4</sub>, accompanied by a decrease in the *ex vivo* binding of <sup>125</sup>I-T<sub>4</sub> to TTR and the induction of hepatic ethoxy- and methoxyresorufin dealkylases (EROD and MROD) as well as UDPGT activity. Short-term exposure to the commercial PBDE mixtures DE-71 and Bromkal 70 caused hypothyroxinemia in both rats and mice.<sup>108,110–112)</sup> T<sub>4</sub> glucuronidation by UDPGT has been suggested as one of the mechanisms contributing to T<sub>4</sub> reduction by PBDEs and other polyhalogenated aromatic hydrocarbons (PHAHs).<sup>42,109,112)</sup> Zhou *et al.*<sup>113)</sup> orally administered DE-71 to female rats and examined the effects on THs and hepatic enzyme activity in them and their offspring. They found that serum T<sub>4</sub> was reduced in a dose-dependent manner in fetuses on gestation day 20 and in infants on postnatal day 4. Increased liver to body weight ratios in offspring were consistent with induction of EROD, pentoxyresorufin dealkylase (PROD) and UDPGT, suggesting that DE-71 acts as an endocrine disruptor in rats during development. Moreover commercial pentabromodiphenyl ethers (PentaBDEs) were found to affect TH homeostasis, and both technical products and pure tetra- and penta-congeners produced effects on serum T<sub>4</sub> in rats and mice.<sup>112,114,115)</sup> Chengelis<sup>116)</sup> reported that serum T<sub>4</sub>- and TSH-concentrations were affected in a 90-day HBCD toxicity study on rats. Exposure to PBBs resulted in decreases in serum T<sub>3</sub> and T<sub>4</sub> in rats and pigs.<sup>117,118)</sup>

Kitamura *et al.*<sup>119)</sup> reported that TBBPA suppresses T<sub>3</sub>-enhanced tadpole tail absorption. Goto *et al.*<sup>33)</sup> and Kashiwagi *et al.*<sup>63)</sup> reported that TBBPA suppressed spontaneous and T<sub>3</sub>-induced amphibian metamorphosis, including tadpole tail apoptosis (Fig. 2), and TBBPA inhibited a strong T<sub>3</sub>-induced EGFP expression in transgenic tadpole hind limbs (Fig. 3), suggesting that TBBPA acts as a T<sub>3</sub> antagonist. Exposure to TBBPA, however, results in accelerated metamorphosis in Pacific tree frog *Pseudacris regilla* tadpoles, suggesting that this chemical substance acts as a TH agonist.<sup>120)</sup> It is supposed that the effect of TBBPA on TH action will differ widely between species.

## HEAVY METALS

The heavy metals cadmium (Cd) and lead (Pb) have become widely distributed throughout the environment as a result of pollution from a variety of sources, and are highly toxic to humans and animals.<sup>121, 122)</sup> Cd has been classified as a human carcinogen by International Agency for Research on Cancer (IARC).<sup>123)</sup>

Swarup *et al.*<sup>121)</sup> compared differences in plasma hormone profile and liver function in adult cows living in lead-zinc polluted areas and non-polluted areas. They found that lead-exposed cows living in polluted areas showed significantly higher blood lead and T<sub>3</sub>- and T<sub>4</sub>-concentrations, as well as higher activities of alanine transaminase (ALT) and aspartate transaminase (AST), two serum biochemical parameters indicative of liver function. This suggests that lead burden in animals causes disturbances in TH action and liver function. In one investigation changes in serum T<sub>4</sub> and T<sub>3</sub> level and thyroid gland histomorphology were observed after subchronic exposure to a mixture of 16 organochlorines, Pb and Cd.<sup>124)</sup> Exposure to Cd has also been found to cause a decrease in serum concentrations of THs in male mice.<sup>125)</sup> Exposure to Cd in pregnant rats was found to reduce serum T<sub>3</sub> and T<sub>4</sub> levels, as well as cause ultrastructural abnormalities in the developing thyroid gland, including deterioration of the rough-surfaced endoplasmic reticulum in the follicular epithelium, large intracellular vacuoles and marked swelling of the mitochondria.<sup>126)</sup>

Viability and postnatal growth were decreased in the offspring of female rats exposed to Cd before and during gestation.<sup>127)</sup> In our investigation into the effects of cadmium chloride on amphibians, we<sup>128)</sup> found that a large number of *Rana japonica* tadpoles treated in Cd solutions at concentrations varying from 0.025 to 0.8 mg/l showed retarded growth and abnormal development of the forelimbs and hindlimbs. The influence on chromosomes was marked at all concentrations. Males and females developed from treated tadpoles were then mated with normal individuals. Five of the 17 males and 5 of the 10 females showed greatly reduced reproductive capacity and only 7–46% of all fertilized eggs grew into normal metamorphosed frogs.

## PHTHALATES

Phthalates are plasticizers which impart flexibility, pliability and elasticity to plastics, and are widely used in many consumer products, including building materials, clothing, children's toys, food packaging, automobiles, *etc.*, resulting in extensive exposure of humans and wildlife through multiple sources and routes.<sup>129, 130)</sup> Frequently used phthalate compounds include di(2-ethylhexyl) phthalate (DEHP), di-ethyl phthalate (DEP), diisodecyl phthalate (DIDP), di-methyl phthalate (DMP) and di-*n*-butylphthalate (DBP).<sup>131)</sup> DEHP has been detected in cow's milk and infant formula,<sup>132)</sup> and DEHP, DBP and DEP have been found in breast milk of suckling mothers.<sup>133)</sup>

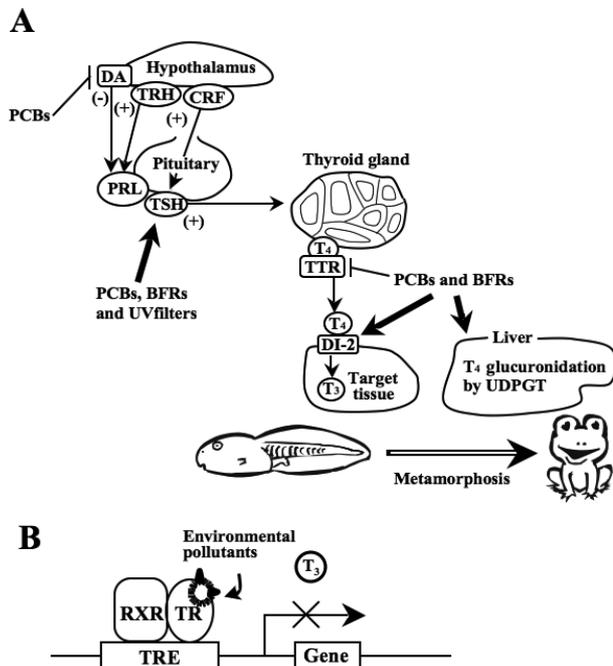
*In vitro* and *in vivo* investigations have reported the detrimental effects of phthalates on TH action. Wenzel *et al.*<sup>134)</sup> found that DEHP and other phthalates enhance iodine uptake in rat thyroid follicular cells. In rats fed DEHP-contaminated diets, histological changes in the thyroid and a decrease in plasma T<sub>4</sub> were seen, but T<sub>3</sub> level remained unchanged,<sup>135–138)</sup> while rats intravenously receiving DEHP showed increased concentrations of serum T<sub>4</sub> and T<sub>3</sub>.<sup>139)</sup> Exposure of male rats to DBP by oral gavage also caused a reduction in T<sub>4</sub> and T<sub>3</sub> levels in a dose-dependent manner.<sup>140)</sup> In a recent human study, Meeker *et al.*<sup>141)</sup> reported an inverse relation between mono(2-ethylhexyl) phthalate (MEHP, the hydrolytic metabolite of DEHP) concentrations and T<sub>3</sub> and free T<sub>4</sub> (FT<sub>4</sub>) serum concentrations in adult men. Huang *et al.*<sup>142)</sup> found that urinary concentrations of mono butyl phthalate (MBP) were associated with decreased T<sub>4</sub>- and FT<sub>4</sub>-concentrations in pregnant women at second trimester.

Zoeller<sup>2)</sup> suggests that phthalates and several other environmental chemicals bind to TRs, resulting in activation or inhibition of endogenous TH action.

## CONCLUSIONS

Humans and wildlife are continually exposed to many chemical substances released into the environment. A large number of these pollutants have been shown to disrupt thyroid homeostasis through various mechanisms (Fig. 4A and 4B).

Amphibian metamorphosis depends on the hypothalamic-pituitary-thyroid (HPT) axis, and anuran tadpoles are known to be affected differently



**Fig. 4.** Possible Mechanisms for Thyroid Homeostasis Disruption by Environmental Pollutants

A. Interference with hypothalamic-pituitary-thyroid (HPT) axis and hepatic catabolism of T<sub>4</sub>. B. Environmental pollutants bind to TR, resulting in inhibition of TR-mediated gene expression. Thick arrow = Activation; —| = Suppression. (+) = Stimulation; (–) = Inhibition. DA = Dopamine; PRL = Prolactin. HPT axis flow chart is based on Lehigh Shirey *et al.*<sup>36)</sup>

depending on whether the disrupting substance in question is TH agonist or TH antagonist. For this reason a metamorphosis assay using anurans is considered to be one of the best methods for detecting and assessing pollutant-induced TH disruption. The Organisation for Economic Co-operation and Development (OECD)<sup>143)</sup> is developing an amphibian metamorphosis assay as a screening test for chemical substances that disrupt the HPT axis. The general experimental design involves maintaining stage 51 *X. laevis* tadpoles in a flow-through system exposed to test chemicals for 21 days. At the end of treatment, hind limb length, body length (total body length) and wet weight are measured, and developmental stage and thyroid histology are recorded. In addition, transgenic *Xenopus* lines overexpressing TH enhanced gene sets have proved to be a useful bioassay for detecting TH agonists or antagonists.

According to DeVito *et al.*<sup>35)</sup> the process involved in the synthesis, storage, release, transport and metabolism of THs is complex and begins with the uptake of iodide ion by the thyroid gland followed by oxidation of iodide and iodination of tyrosine residues within thyroglobulin. Next iodotyrosine residues couple to produce iodothyronines, fol-

lowed by proteolysis of thyroglobulin and release of T<sub>4</sub> and T<sub>3</sub> into the blood, where they bind to serum transport proteins. Synthesis of T<sub>3</sub> from T<sub>4</sub> takes place in the target tissue, together with catabolism of T<sub>4</sub> and T<sub>3</sub> in peripheral tissues. Catabolism and biliary elimination of THs is conducted in the liver. T<sub>3</sub> action is mediated by interaction with TR. The manner in which environmental pollutants affect and alter these various processes needs to be clarified through further research.

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