

Nuclear receptor profiling of bisphenol-a and its halogenated analogues.

Vanessa Delfosse, Marina Grimaldi, Albane Le Maire, William Bourguet, Patrick Balaguer

▶ **To cite this version:**

Vanessa Delfosse, Marina Grimaldi, Albane Le Maire, William Bourguet, Patrick Balaguer. Nuclear receptor profiling of bisphenol-a and its halogenated analogues.: Bisphenol-A is a ligand of several nuclear receptors. Vitamins and hormones, 2014, 94, pp.229-51. 10.1016/B978-0-12-800095-3.00009-2 . inserm-00942303

HAL Id: inserm-00942303

https://inserm.hal.science/inserm-00942303

Submitted on 5 Feb 2014

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Nuclear receptor profiling of bisphenol-A and its halogenated analogues

Vanessa Delfosse[1], Marina Grimaldi[2], Albane le Maire[1], William Bourguet[1*] and Patrick Balaguer[2*]

[1]Centre de Biochimie Structurale, Institut National de la Santé et de la Recherche Médicale U1054, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5048, Universités Montpellier 1 and 2, 34090 Montpellier, France. Tel: (33) 4 67 41 77 02, Fax: (33) 4 67 41 79 13, @: bourguet@cbs.cnrs.fr

[2]Institut de Recherche en Cancérologie de Montpellier, Institut National de la Santé et de la Recherche Médicale U896, Centre Régional de Lutte contre le Cancer Val d'Aurelle Paul Lamarque, Université Montpellier 1, 34298 Montpellier, France. Tel: (33) 4 67 61 24 09, Fax: (33) 4 67 61 37 87, @: patrick.balaguer@montpellier.unicancer.fr

- **Corresponding authors:**

  William Bourguet bourguet@cbs.cnrs.fr

  Patrick Balaguer patrick.balaguer@montpellier.unicancer.fr

Running title:

Bisphenol-A is a ligand of several nuclear receptors.

Abstract

Bisphenol-A (BPA) is one of the highest volume chemicals produced worldwide and the widespread exposure of individuals to BPA is suspected to affect a variety of physiological functions, including reproduction, development and metabolism. Its estrogenic activity has been well documented in the last fifteen years. In addition to estrogen receptors, BPA has been also shown to bind to and activate the estrogen related receptor γ and pregnan X receptor and inhibit the androgen receptor. Halogenated BPAs were also shown to activate the peroxysome proliferator activated receptor γ and inhibit thyroid hormone receptors. In this paper, we review recent studies shedding light on the structural and molecular mechanisms by which BPA and its halogenated derivatives interfere with nuclear hormone receptor signaling. These data provide guidelines for the development of safer substitutes devoid of hormonal activity and may help environmental risk assessment.

Key Words (5-10): bisphenol-A, halogenated bisphenol-A, nuclear hormone receptor, endocrine disruptor

I. Introduction

Human nuclear hormone receptors (NHRs) are a family of 48 transcription factors, many of which have been shown to be activated by ligands. NHRs regulate cognate gene networks involved in key

physiological functions such as cell growth and differentiation, development, homeostasis, or metabolism (Germain, 2006; Gronemeyer, 2004). As a consequence, inappropriate exposure to environmental pollutants often leads to proliferative, reproductive and metabolic diseases, including hormonal cancers, infertility, obesity or diabetes. NHRs are modular proteins composed of several domains, most notably an N-terminal domain which harbors a ligand-independent activation function (AF-1), a central DNA binding domain (DBD) and a C-terminal ligand binding domain (LBD) hosting a ligand-dependent transcriptional activation function (AF-2) (Gronemeyer, 2004). In the absence of the cognate ligand, some NHRs are located in the nucleus, bind to the DNA response elements of their target genes and recruit corepressors, while others are located in the cytoplasm in an inactive complex with chaperones. Ligand binding induces major structural alterations of the receptor LBDs leading to (1) destabilization of corepressor or chaperone interfaces, (2) exposure of nuclear localization signals to allow nuclear translocation and DNA binding of cytoplasmic receptors and (3) recruitment of coactivators triggering gene transcription through chromatin remodeling and activation of the general transcription machinery. The crystal structures of many NHR LBDs have been determined, revealing a conserved core of 12 α-helices (H1–H12) and a short two-stranded antiparallel β-sheet (S1 and S2) arranged into a three-layered sandwich fold. This arrangement generates a mostly hydrophobic cavity in the lower half of the domain which can accommodate the cognate ligand. In all hormone-bound LBD structures, the ligand binding pocket (LBP) is sealed by helix H12. This conformation is specifically induced by the binding of hormones or synthetic agonists and is referred to as the ‘‘active conformation’’ because it allows the dissociation of corepressors and favors the recruitment of transcriptional coactivators (Bourguet, 2000; Pike, 2006; Renaud, 2000). It is noteworthy that this conformational state can also be achieved by some constitutively active orphan receptors for which no natural ligand has been identified. In

this active-form, helices H3, H4 and H12 define a hydrophobic binding groove for short LxxLL helical motifs (L stands for leucine and x for any amino acid) found within coactivators. In contrast to agonist binding, interaction with antagonists prevents the correct positioning of helix H12, thus avoiding association with the LxxLL motifs of coactivators (Bourguet, 2000; Pike, 2006; Renaud, 2000). The LBD also contributes to the modulation of the N-terminal AF-1 through interdomain crosstalk so that both AF-1 and AF-2 domains can recruit a range of coregulatory proteins and act individually or in a synergistic manner (Benecke, 2000; Bommer, 2002; Wilson, 2011). The precise structural basis of this interdomain communication is unknown, as no 3D structure of an entire NR has been obtained.

Bisphenol-A (BPA) is one of the highest volume chemicals produced worldwide, with over 6 billion pounds produced each year. BPA enters into the composition of polycarbonate plastic, epoxy resins used in metallic food cans, dentistry sealants and thermal paper (Vandenberg, 2009).

Halogenated derivatives of BPA, which feature bromine or chlorine substituents on the phenolic rings, are used as flame retardants. Tetrabromobisphenol-A (TBBPA) is mainly used as a flame retardant to protect computer motherboards and other electronic equipment. Its production is currently estimated around 200,000 tons/year, and its presence has been reported in the environment (de Wit) and in wildlife (Darnerud, 2003). Tetrachlorobisphenol-A (TCBPA) has also been reported to be used as a flame retardant, but in much lower quantities than TBBPA (<10,000 tons/year) (Chu, 2005). The presence of TCBPA, as well as that of lower chlorinated analogues (monoCBPA, diCBPA and triCBPA) in environmental samples has been unequivocally demonstrated (Fukazawa, 2001; Gallart-Ayala, 2010; Liu, 2009). Contrary to TBBPA, and given the low levels of production of TCBPA, the origin of most chlorinated-BPA in the environment is more likely the chlorination of BPA than the dechlorination of TCBPA.

BPA or its halogenated derivatives have been shown to target several NHRs including the estrogen receptors (ERα, β), the androgen receptor (AR), the estrogen related receptor γ (ERRγ), the thyroid hormone receptors (TRα, β), the pregnane X receptor (PXR) and the peroxisome proliferator activated receptors (PPARα, γ, δ). The crystal structures of ERα and ERRγ in complex with BPA and of PPARγ bound to TBBPA or TCBPA have been reported recently. Here, we review recent studies showing the ways in which BPA and halogenated BPAs bind to and activate NHRs.

II. Estrogens receptors

Estrogen receptors (ERα and ERβ) are receptors for the sex hormone, 17β-estradiol (E_2), which play important roles in the growth and maintenance of a diverse range of tissues such as the mammary gland, uterus, bones or the cardiovascular system. Both ERs are widely distributed throughout the body, displaying distinct but overlapping expression patterns in a variety of tissues (Couse, 1999). ERα is expressed primarily in the uterus, liver, kidney, and heart, whereas ERβ is expressed primarily in the ovary, prostate, lung, gastrointestinal tract, bladder, and hematopoietic and central nervous systems (Kuiper, 1997). However, ERα and ERβ are coexpressed in a number of tissues including the mammary gland, thyroid, adrenal, bones and some regions of the brain. Although ERα and ERβ share similar mechanisms of action, several differences in the transcriptional abilities of each receptor as well as distinct phenotypes between gene-null animals have been identified, suggesting that these receptors may regulate distinct cellular pathways (Couse, 1999; Curtis, 1996).

ERs activity of bisphenols

BPA is considered as a weak environmental estrogen because of its relatively low affinity for the nuclear ERs compared with estradiol (10,000 to 100,000-fold lower affinity) (Delfosse, 2012; Kitamura, 2005; Paris, 2002). Several studies indicated that BPA is a partial agonist (Gould, 1998; Routledge, 2000). In a recent work, we have confirmed that BPA is a selective estrogen receptor modulator (SERM) that functions in a cell-selective manner (Delfosse, 2012). Our data reveal that BPA activity relies mostly on the AF-1 and depends on the cellular context. In this study, we compared BPA with different bisphenols for their affinity and activity using HeLa ERα and ERβ reporter cell lines. For ERα and ERβ affinity, the ranking order was BPC > BPAF > BPB > BPA > BPE > BPF > BPS. For activity, the ranking order is almost the same for ERα and ERβ. It is BPF =

BPE = BPS > BPA > BPC = BPAF = BPB, BPC being less active on ERβ than ERα. The potency likely originates from the differential synergy between AF-1 and AF-2 and this synergism is probably more important in the HeLa cellular context for ERβ than for ERα.

In two other studies (Riu, 2011a; Riu, 2011b), we have characterized the estrogenic activity of TBBPA and TCBPA together with lower halogenated BPA. For brominated analogues, the ranking order of estrogenic potency on ERα and ERβ was monoBBPA > diBBPA > triBBPA while TBBPA showed no estrogenic activity at all. Chlorinated BPA analogues are at least as potent agonists as BPA for ERα as already published by others groups (Mutou, 2006; Takemura, 2005). Contrary to brominated BPA, the halogenation degree of chlorinated bisphenols did not decrease their ER potency. Likely due to the lower ligand binding pocket size of ERβ compared to ERα, chlorinated analogues with the lowest molecular weight (mono- and diCBPA) exhibited the highest agonistic activity on ERβ.

Structures of BP-bound ERs

Structures of the complexes between ERα and BPA, BPAF, and BPC were recently reported (Delfosse, 2012) and their comparison with that of the E_2- and the OHT-bound ERα allowed the description of the binding mode of these BPs and the explanation of their different activities and binding affinities. The structures with BPA, BPAF and TCBPA display the canonical active conformation with H12 capping the LBP and the LxxLL-containing coactivator peptide bound to the AF-2 surface (Figure 2A). The structure with BPC displays an antagonist conformation similar to that observed in the OHT-bound structure (PDB code 3ERT) with H12 occupying the coactivator binding groove (Figure 2A). The structures also reveal two divergent binding mechanisms of BPs.

BPA adopts a binding mode reminiscent of that used by E_2 with the two phenol groups hydrogen bonded to three polar residues located at the two ends of the pocket, namely H524 (H11) on one side and E353 (H3) and R394 (H5) on the other side (Figure 2B and 2C). The remaining contacts involve van der Waals interactions. In contrast, BPC is positioned in the pocket so as to draw the phenol ring B into an alternate position as compared with that of the corresponding ring in BPA and TCBPA. A rotation by 180° around the main axis of phenol ring A orients ring B towards H12 (Figure 2D). Finally, in the BPAF complex each subunit of the ERα homodimer contains one BPAF molecule with the "BPA-like" or "BPC-like" positioning. The different activities of BPs could be attributed to the destabilization of the AF-2 surface as many of the previously recognized ligand – H11 stabilizing interactions observed in the E_2-bound structure are altered in the bisphenol-containing complexes (Bruning, 2010; Delfosse, 2012; Nettles, 2008). One of the key differences resides in the differential interaction of the various ligands with L525. In the E_2-bound structure, E_2 imposes a conformation of this leucine that strengthens a network of van der Waals interactions involving T347, L525 and L536 (Figure 2A). This cluster of interactions is of utmost importance because it holds together helices H3, H11 and the loop preceding H12, thereby stabilizing the AF-2 surface. In the structure with BPA (Figure 2B), the stabilizing interaction between the bisphenol and L525 is absent. This renders L525 more dynamic so that its side chain adopts different conformations in the two subunits of the homodimer. The situation is even worse in the structure with BPC where the side chain of T347 rotates by 180° to form a hydrogen bond with the hydroxyl moiety from the phenol ring B of BPC (Figure 2D). This loss of stabilizing contacts provoked by bisphenols renders the lower part of the ERα LBP more dynamic. Indeed, this portion of LBDs has been previously shown to have some of the characteristics of a molten globule and a large part of the agonistic properties of a ligand relies in its capacity to stabilize this region encompassing the H3/H11 docking surface for H12 in the

active conformation (Gee, 2001; Nagy, 2004). The complete disruption of the hydrophobic cluster by BPC probably accounts for the marked antagonistic character of this bisphenol. Modelling studies indicated similar binding modes in ERβ (Delfosse, 2012).

III. Estrogen related receptor γ

ERRγ is a member of the ERR subfamily of orphan receptors, which are closely related to ERs (Giguere, 2002) The ERR family includes three members, ERRα, ERRβ and ERRγ. The three receptors are very similar, with 90% sequence identity in the DBD and more than 60% in the LBD.

ERRγ is expressed in a tissue-restricted manner, for example very strongly in the mammalian brain during development, and then in the brain, lung and many others tissues during adulthood. In terms of structure, ERRγ is very close to ERs. Sequence alignment reveals a 60% identity in the DBD regions and a moderate similarity (<35%) of the LBDs, consistent with the incapacity of ERRγ to bind E_2 (Horard, 2003). Nevertheless, it has been demonstrated that ERRγ can interfere with estrogen signaling (Giguere, 2002). Indeed, ERs and ERRγ recognize the same DNA binding elements, share common target genes and are coexpressed in many tissues (Lu, 2001; Vanacker, 1999).

Exogenous overexpression of ERRγ in prostate cancer cell line results in inhibition of proliferation (Yu, 2007). Furthermore, treatment with an ERRβ/γ agonist has been shown to promote this antiproliferative effect, consistent with ERRγ being a favorable prognosis factor (Ariazi, 2006). Additionally, several lines of evidence suggest that ERRs play a central role in regulating energy metabolism. Genetic studies in mice reveal that their presence is essential for the generation of energy and related tissue-specific functions, and functional genomics/proteomics studies have

associated ERRγ with the control of vast metabolic gene networks, in particular those involved in mitochondrial biogenesis and function (Giguere, 2008). The rise in the incidence of metabolic syndromes correlates with the rise in the use and distribution of industrial chemicals that may play a role in generation of obesity (Baillie-Hamilton, 2002), suggesting that EDCs and ERRγ may be linked to this epidemic crisis. To date, the ERRγ have not been shown to interact with any physiologically relevant small molecules, suggesting that these receptors manifest constitutive activity (Greschik, 2002; Li, 2009), and, indeed, crystallographic analyses of ERRγ indicated that these receptors adopt the transcriptionally active conformation in the absence of any ligand (Greschik, 2002).

ERRγ activity of bisphenols

Compounds screening for activity on ERRγ have identified diethylstilbestrol and 4-hydroxytamoxifen as inverse agonists and BPA as agonist for ERRγ (Coward, 2001; Okada, 2008; Tremblay, 2001). Among bisphenols, BPA, BPE and BPB have been found to have the highest affinity for ERRγ (Li, 2009; Okada, 2008). Interestingly, these two studies have enabled to discriminate between ERα and ERRγ selective agonists. BPA could be considered as a pan agonist for ERα. BPAF and BPC could be considered as ERα selective agonists and BPE and BPF as ERRγ selective agonists. It is not surprising that these ligands can be used as tools to determine the respective roles of ERα and ERRγ in cells or organs co-expressing these two receptors.

Structural basis of BPs interaction with ERRγ

Although a natural ligand remains to be found for ERRγ, several synthetic ligands have been

identified for this receptor (Li, 2009; Okada, 2008). All display a conserved phenol ring such as BPs. The structure of ERRγ in complex with BPA and BPZ have been solved, revealing an overall protein conformation indistinguishable from that of the unliganded receptor (Abad, 2008; Matsushima, 2007; Matsushima, 2008) (Figure 3A). A close look at the LBP shows that, as previously anticipated, one of the two phenol-hydroxyl groups of BPA and BPZ forms hydrogen bonds with residues E275 (H3) and R316 (H5) while the second is hydrogen bonded to N346 in H7 (Figure 7). A hydrogen bond between Y326 and N346 holds N346 in position to interact with the second phenol group of BPs. Interestingly, this asparagine residue is not conserved in ERRα and β, thus accounting for the specific ERRγ-BPs interaction. Moreover, comparison of ERRγ and ERRα LBPs reveals a much smaller cavity for the latter (Kallen, 2004). The replacement of two alanine residues (A272 and A431) in ERRγ by a phenylalanine (F328) and a valine (V491) in ERRα accounts for this size reduction and further explains why BPA does not bind to the latter. BPs binding provokes only minimal LBP rearrangements (Figure 3B). E275, which appears disordered in the unliganded structure, adopts a unique conformation to make a hydrogen bond with one OH group of BPs, and the side chain of L345 moves away from the pocket upon BPs binding to open up the cavity and make room for the second phenol group of BPs. In summary, ERRγ possesses a LBP to which BPs can bind with high affinity and specificity while preserving the constitutively active conformation of the receptor. The limited impact of BPA binding on receptor structure is consistent with the fact that this ligand does not enhance or disrupt coactivator binding and thus appears as functionally silent ligand (Abad, 2008; Matsushima, 2007). Thus, the classical mechanism of NHR activation involving the re-localization and stabilization of helix H12 in the active conformation does not explain how ERRγ could mediate the estrogenic effects of BPs. Rather, thermal stability studies revealed that

BPA binding leads to global thermodynamic stabilization of ERRγ LBD, a phenomenon which could increase steady state levels of the receptor and impact both its cellular half-life and biological activity (Abad, 2008; Matsushima, 2007; Wang, 2006). A recent modeling study indicated that in ERRγ all bisphenols adopt the agonist BPA-like position observed in ERα due to the hydrogen bond with N346 from helix H7. This *in silico* result correlates both with functional data showing that bisphenols are ERRγ activators (Delfosse, 2012) and the crystal structure of ERRγ in complex with BPA (Abad, 2008; Matsushima, 2007).

IV. Androgen receptor

AR plays crucial role in the regulation of target gene expression in physiological processes like development and differentiation of the male embryo and spermatogenesis initiation and maintenance, as well as neuro-endocrine system functioning (Matsumoto, 2012). The central role of AR has been verified by AR-deficient mice (AR-KO) and by hereditary disorders of androgen insensitivity syndrome in human males. AR-KO mice display an array of phenotypes involving several organs and cell types. Reproductive tract and gonadal development as well as sexual behavior are severely impacted. In humans, AR mutations cause abnormalities from mild virilization defects to complete male to female sex reversal. These mutations lead to either complete (CAIS) or partial (PAIS) androgen insensitivity syndromes (McPhaul, 1999; Quigley, 1995). In the absence of ligands, AR is localized essentially in the cytoplasm. Binding to androgens enable HSPs dissociation and AR translocation to the nucleus. The AR LBD strongly contributes to the modulation of the N-terminal AF-1 through ligand-induced interdomain association. Furthermore, in AR, it appears that AF-1 predominates over AF-2 (He, 2000).

Androgens are thus male sex hormones that are critical for the development and maintenance of the

male reproductive system. The plasma androgen/estrogen ratio during fetal life creates a male-versus-female hormonal milieu (McLachlan, 1987). This delicate balance could be disrupted by environmental antiandrogens or estrogens and lead to hermaphroditism.

Finally, prostate development and prostate cancer are also critically dependant on androgens (McCarthy, 2011). Current therapies of prostate cancers are primarily based on androgen antagonists that interact with AR and completely or partially block the hormone-responsive pathways.

AR activity of bisphenols

BPA is considered a weak environmental anti androgen because of its relatively low affinity for AR (Li, 2010; Paris, 2002; Stroheker, 2004). In a recent work, we have confirmed these results (Delfosse, 2012). In this study, we compared BPA with different bisphenols for their anti-androgenic activity using an AR reporter cell line. The ranking order was BPC >> BPAF > BPA = BPB = BPE > BPF > BPS. It is noteworthy that BPC has the same potency than the vinclozolin metabolite M2 which is the most potent xenoantiandrogen (Kelce, 1997; Paris, 2002).

As it was observed for estrogenic activity, TCBPA was able to inhibit AR activity while TBBPA was not (Li, 2010) (P Balaguer , unpublished data). Interestingly, lower halogenated analogues were also AR inhibitors, mono- and di bromo and chloro-BPA being the most potent (P Balaguer, unpublished data). Interestingly, in a recent modeling study, all bisphenols appeared to bind to AR exclusively in the antagonist BPC-like orientation, in agreement with the observation that these compounds act as AR antagonists. This orientation appears to be stabilized by formation of a hydrogen bond between one hydroxyl group of the ligand and N705, a polar residue specific of AR (Delfosse, 2012).

Peroxysome proliferator activated receptor γ

PPARs are involved in the regulation of glucose, lipid, and cholesterol metabolism in response to fatty acids and their derivatives, eicosanoids, and drugs used in the treatment of hyperlipidemia and diabetes. The PPAR subfamily contains three members known as PPARα, PPARβ, and PPARγ. Each PPAR subtype shows a distinct tissue distribution and ligand preference.

PPARγ is highly expressed in adipose tissue, and is a central regulator of lipid storage and adipocyte gene expression and differentiation (Tontonoz, 1995) and is involved in various pathophysiologic disorders, including metabolic disease, insulin resistance, and diabetes (Rosen, 2001). PPARγ is the target for antidiabetic agents of the thiazolidinedione class, which includes troglitazone, pioglitazone, and rosiglitazone.

The LBD of PPARγ is rather large and the diversity of ligands that can be accommodated within its pocket, mainly presented by lipid derivatives, may contribute to the large array of roles that have been assigned to PPARγ.

PPARγ activity of bisphenols

There is consistency between the large PPARγ ligand binding pocket and the capacity to bind various compounds. Several reports have showed PPARγ activation by environmental pollutants such as organotins used as agricultural fungicides and anti-fouling agents in ship paints, phthalates as well as perfluorooctane-based chemicals found in industrial surfactants (Bility, 2004; Grun, 2006; Takacs, 2007). In two recent studies (Riu, 2011a; Riu, 2011b) we investigated the capability of the flame retardants TBBPA and TCBPA, of their biotransformation products and of their lower molecular weight analogues formed in the environment to activate PPARγ. The activity of the

different compounds was studied using reporter cell lines expressing PPARs. None of the compounds tested significantly impacted PPARα or PPARδ activity. In contrast, both TBBPA and TCBPA were capable of activating partially PPARγ, despite being approximately 100-fold less potent (EC50 values in the μM range) than the reference pharmaceutical compound rosiglitazone (EC50 values in the 10 nM range). We also demonstrated that TBBPA and TCBPA are human, zebrafish and xenopus PPARγ ligands while rosiglitazone does not bind zebrafish PPARγ. This work also provides evidence that activation of PPARγ depends on the halogenation degree of BPA analogues. The bulkier halogenated BPA analogues, the greater their capability to activate PPARγ. In addition, using PPARγ-based affinity column and crystallographic analysis, we clearly demonstrated that the sulfation pathway, usually considered as a detoxification process, leads for TBBPA and TCBPA, to the formation of sulfate conjugates which possess a residual PPARγ binding activity (Riu, 2011b).

Structural basis of halogenated bisphenols action on PPARγ

Despite the fact that the halogenated bisphenols are structurally unrelated to known PPARγ ligands, the crystal structures of TBBPA and TCBPA bound to the PPARγ LBD (Figure 4A) revealed the canonical tertiary fold of agonist-bound NHR LBDs (Bourguet, 2000; Riu, 2011a). The TBBPA and TCBPA complex structures are indistinguishable with a rmsd value of 0.29 Å for superimposed alpha carbons and nearly identical to that of PPARγ in complex with the agonist rosiglitazone (Nolte, 1998) with an rmsd value of 0.62 Å. PPARγ displays a large LBP that extends from the C-terminal helix H12 to the β-sheet S1/S2 so that halogenated BPA occupy a small portion of the LBP. Whereas rosiglitazone occupies a region of the LBP spanning from H11/H12 to the β-sheet S1/S2,

TBBPA and TCBPA occupy only the region between H3 and the β-sheet S1/S2, with one of its phenol cycle nestled between H3 and the β-sheet (Figure 4B). In contrast with rosiglitazone, a consequence of their smaller size is that halogenated BPA do not interact directly with H12. A close look at the LBP shows that the phenol groups of the BPA derivatives are involved in hydrogen bonds with S342 (β-sheet S1/S2) on one side and Y473 from H12 through a water-mediated hydrogen bond network on the other side. TBBPA and TCBPA contain four halogen atoms that contribute to ligand binding through van der Waals interactions. Additional interactions involving the ligand backbone are also observed. Interestingly, comparison of human, mouse and zebrafish PPARγ sequences reveals several residue differences which could explain the differential ligand specificity of the various species. In particular the replacement of human PPARγ G284 and C285 by larger serine and tyrosine residues in zebrafish PPARγ provides a rationale for the weak binding affinity of rosiglitazone for this receptor as compared to that observed for the human homolog. In contrast, the different binding mode of halogenated compounds allows both human PPARγ and zebrafish PPARγ to accommodate TBBPA and TCBPA. The structure of PPARγ LBD bound to the metabolite TBBPA-sulfate has been also solved (Riu, 2011b). Overall, the structure of PPARγ LBD is identical when bound to TBBPA or TBBPA-sulfate, with an rmsd value of 0.24 Å for superimposed alpha carbons (Figure 4C). The ligand is positioned almost identical to that of TBBPA, the sulfate group pointing towards H11/H12. Interestingly, accommodation of the sulfate group by PPARγ does not require any conformational change of the surrounding residue side chains. In this orientation, the sulfate moiety fills a portion of the ligand binding pocket occupied by water molecules in the TBBPA structure and is potentially involved in hydrogen bonds with Y327, K367, H449, Q286 and S289. The presence of a conserved water molecule mediates the indirect interaction

between TBBPA-sulfate and PPARγ helix H12. Overall, the crystallographic data validate the notion that this TBBPA metabolite is a PPARγ ligand with partial agonistic profile.

V. Thyroid receptors

Thyroid hormones (THs) are essential for the normal development, growth, and metabolism of all vertebrates (Zoeller, 2002), playing a major role in neurogenesis and brain function at all stages of development (Bernal, 2007). They are produced by the thyroid. THs, namely tetra-iodothyronine (thyroxine or T4) and tri-iodothyronine (T3) are the principal representative of circulating THs. In target cells, T4 is converted in T3 which is the most active TH. Moreover, THs are key developmental and differentiation hormones in all organs of the body, including the central nervous system and the skeleton. In amphibians, THs peak at birth and parallel metamorphosis that is totally dependent upon T3 (Leloup, 1977). The functions of THs are mediated by several isoforms of nuclear thyroid receptors, TRα and TRβ. The tissue distributions of TRs are relatively ubiquitous and the expression of these proteins begins early in development (Zoeller, 2002).

TRs activity of bisphenols

Several articles report *in vitro* studies revealing that BPA and its halogenated derivatives can alter thyroid hormone action by inhibiting T3 binding to and activation of TRs in the 1-10 μM concentration range (Freitas, 2011; Moriyama, 2002; Sun, 2009). Halogenated BPAs share more structural similarities with TH than BPA which could explain why in certain studies, TBBPA and TCBPA were found active at lower concentrations than BPA (Fini, 2012). Halogenated BPAs were also shown to bind to transthyretin (TTR), the carrier protein for T4 (Meerts, 2000), and their *in vivo* effects on thyroid signaling have been often observed in amphibians (Fini, 2007; Jagnytsch, 2006;

Shi, in press; Veldhoen, 2006).

VI. Pregnan X receptor

PXR is a broad-specificity sensor playing a critical role in the regulation of phase I (CYP), phase II (conjugating) and phase III (ABC family transporters) detoxifying enzymes, co-ordinately regulating steroid, drug, and xenobiotic clearance in the liver and intestine (Orans, 2005). Activated PXR binds to gene promoters as a heterodimer with RXR and induces the expression of target genes such as CYP3Aand CYP2B6. This receptor plays a prominent role as protector of the endocrine system from chemical perturbation by sensing increases in the concentration of a multitude of EDCs and inducing detoxification pathways to prevent other NHRs from interactions with these chemicals.

Activation of PXR can be positive, as it accelerates the detoxification process and consequently the elimination of xenobiotics. In contrast, the premature metabolism of active compounds such as hormones or drugs means that target responses will not be activated and can lead to harmful effects or adverse interactions. The metabolism of inactive compounds can also lead to the synthesis of active metabolites, such as the transformation of methoxychlor by CYP2C11, a PXR-induced enzyme, into HPTE, a compound sharing structural similarity with bisphenol-A (Mikamo, 2003).

Overall, it is difficult to conclude whether the xenobiotic activation of PXR is predominantly negative or positive, but clearly PXR plays an essential role in endocrine disruption (di Masi, 2009).

Pregnan X activity of bisphenols

Unlike most NHRs that tend to be specialized to bind few ligands with structural homologies, PXR is able to bind a large number of structurally diverse ligands with a wide range of affinities.. Human PXR binds a multitude of drugs such as the antibiotic rifampicin, the anti-cancer taxol, the anti-

cholesterol SR12813, the barbituric Phenobarbital, the St John's Wort anti-depressor hyperforine and many more, recently reviewed in (di Masi, 2009). Furthermore, numerous studies have focused on its ability to bind environmental compounds, such as pesticides (Lemaire, 2006), polychlorinated biphenyls (Jacobs, 2005; Tabb, 2004), brominated flame retardants (Pacyniak, 2007), antimicrobial triclosan (Jacobs, 2005) or natural and synthetic estrogens (Jacobs, 2005; Mnif, 2007; Xue, 2007) and among them, BPA in the 1-10 μM concentration range (Jacobs, 2005; Mnif, 2007; Sui, 2012; Takeshita, 2001; Xue, 2007). Interestingly, Sui et al (2012) found that BPA activated human PXR but not mouse PXR. Furthermore, they also showed that two others bisphenols, BPB and BPAF, are active on human PXR.

VII. Conclusions

Exposure to BPA is highly suspected to cause adverse effects in humans but controversy remains about its mechanism of action and low dose effects. Though ERα and ERβ are primary targets of BPA, alterations of other signalling pathways are probably implicated in the endocrine-disruptive effects of bisphenols. In particular, other members of the NHR family, including AR, ERRγ, PPARγ, TRs and PXR have been shown to correspond to secondary targets of bisphenols and crystallographic and modelling studies have provided structural insights as to how BPA and congeners interact with these NHRs. In addition, recent studies have revealed additional BPA targets through which bisphenols can stimulate rapid cellular responses at very low concentrations. These include the membrane-associated ERα and β (Alonso-Magdalena, 2012) and the G protein-coupled receptor 30 (GPR30) (Chevalier, 2012). These two types of receptors are capable of mediating the nongenomic effects of estrogens and xenoestrogens (reviewed in (Wetherill, 2007)) and it is now widely accepted that BPA not only has the efficacy of estradiol but is also equally potent regarding

several of its effects (Alonso-Magdalena, 2012; Chevalier, 2012; Wetherill, 2007). Thus is conceivable that BPA could act in a synergistic way through simultaneous action on various nuclear and membrane-associated receptors. Moreover, most of our current knowledge of BPA action is based on single molecule exposure in model systems *in vitro* or *in vivo*. These efforts have therefore taken little account of a more realistic situation in which humans are chronically exposed to low doses of multiple EDCs which very likely act also in a synergistic manner.

Both biological and structural data on EDC interactions with nuclear and membrane receptors, in addition to enable a better understanding of EDC activities will provide guidelines for the development of safer substitutes devoid of hormonal activity and may help environmental risk assessment. Alternatively, they will have potential applications in drug discovery by revealing unforeseen binding mechanisms.

VIII. Acknowlegments

This work was supported by Programme National de Recherche sur les Perturbateurs Endocriniens (P.B.), Agence Nationale de Recherches Contaminants, Ecosystèmes, Santé Project "BISCOT" 2010 Contaminants et Environnements: Métrologie, Santé, Adaptabilité, Comportements et Usages 004 02 (to P.B. and W.B.) and Programme Environnement Cancer Project "SynerPXR" 2012 (to P.B. and W.B.).

IX. References

Abad, M. C., Askari, H., O'Neill, J., Klinger, A. L., Milligan, C., Lewandowski, F., Springer, B., Spurlino, J., Rentzeperis, D. (2008). Structural determination of estrogen-related receptor gamma in the presence of phenol derivative compounds. *J. Steroid Biochem. Mol. Biol.* 108**,** 44-54.

Alonso-Magdalena, P., Ropero, A. B., Soriano, S., Garcia-Arevalo, M., Ripoll, C., Fuentes, E., Quesada, I., Nadal, A. (2012). Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. *Mol. Cell. Endocrinol.* 355**,** 201-207.

Ariazi, E. A., Jordan, V. C. (2006). Estrogen-related receptors as emerging targets in cancer and metabolic disorders. *Curr Top Med Chem* 6**,** 203-215.

Baillie-Hamilton, P. F. (2002). Chemical toxins: a hypothesis to explain the global obesity epidemic. *J Altern Complement Med* 8**,** 185-192.

Benecke, A., Chambon, P., Gronemeyer, H. (2000). Synergy between estrogen receptor alpha activation functions AF1 and AF2 mediated by transcription intermediary factor TIF2. *EMBO Rep.* 1**,** 151-157.

Bernal, J. (2007). Thyroid hormone receptors in brain development and function. *Nat Clin Pract End Met* 3**,** 249-259.

Bility, M. T., Thompson, J. T., McKee, R. H., David, R. M., Butala, J. H., Vanden Heuvel, J. P., Peters, J. M. (2004). Activation of Mouse and Human Peroxisome Proliferator-Activated Receptors (PPARs) by Phthalate Monoesters. *Toxicol Science* 82**,** 170-182.

Bommer, M., Benecke, A., Gronemeyer, H., Rochette-Egly, C. (2002). TIF2 Mediates the Synergy between RARalpha 1 Activation Functions AF-1 and AF-2. *J. Biol. Chem.* 277**,** 37961-37966.

Bourguet, W., Germain, P., Gronemeyer, H. (2000). Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. *Trends Pharmacol. Sci.* 21**,** 381-388.

Bruning, J. B., Parent, A. A., Gil, G., Zhao, M., Nowak, J., Pace, M. C., Smith, C. L., Afonine, P. V., Adams, P. D., Katzenellenbogen, J. A., Nettles, K. W. (2010). Coupling of receptor conformation and ligand orientation determine graded activity. *Nat. Chem. Biol.* 6**,** 837-843.

Chevalier, N., Bouskine, A., Fenichel, P. (2012). Bisphenol A promotes testicular seminoma cell proliferation through GPER/GPR30. *Int. J. Cancer* 130**,** 241-242.

Chu, S., Haffner, G. D., Letcher, R. J. (2005). Simultaneous determination of tetrabromobisphenol A, tetrachlorobisphenol A, bisphenol A and other halogenated analogues in sediment and sludge by high performance liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography A* 1097**,** 25-32.

Couse, J. F., Korach, K. S. (1999). Estrogen receptor null mice: what have we learned and where will they lead us? *Endocrine reviews* 20**,** 358-417.

Coward, P., Lee, D., Hull, M. V., Lehmann, J. M. (2001). 4-Hydroxytamoxifen binds to and deactivates the estrogen-related receptor gamma. *Proc. Natl. Acad. Sci. U. S. A.* 98**,** 8880-8884.

Curtis, S. W., Washburn, T., Sewall, C., DiAugustine, R., Lindzey, J., Couse, J. F., Korach, K. S. (1996). Physiological coupling of growth factor and steroid receptor signaling pathways: estrogen receptor knockout mice lack estrogen-like response to epidermal growth factor. *Proc. Natl. Acad. Sci. U. S. A.* 93**,** 12626-12630.

Darnerud, P. O. (2003). Toxic effects of brominated flame retardants in man and in wildlife. *Environment International* 29**,** 841-853.

de Wit, C. A., Herzke, D., Vorkamp, K. Brominated flame retardants in the Arctic environment--trends and new candidates. *Sci. Total Environ.* 408**,** 2885-2918.

Delfosse, V., Grimaldi, M., Pons, J.-L., Boulahtouf, A., le Maire, A., Cavailles, V., Labesse, G., Bourguet, W., Balaguer, P. (2012). Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes. *Proc. Natl. Acad. Sci. U. S. A.* 109**,** 14930-14935.

di Masi, A., Marinis, E. D., Ascenzi, P., Marino, M. (2009). Nuclear receptors CAR and PXR: Molecular, functional, and biomedical aspects. *Molecular aspects of medicine* 30**,** 297-343.

Fini, J., Le Mevel, S., Turque, N., Palmier, K., Zalko, D., Cravedi, J., Demeneix, B. A. (2007). An in vivo multiwell-based fluorescent screen for monitoring vertebrate thyroid hormone disruption. *Environ. Sci. Technol.* 41**,** 5908-5914.

Fini, J. B., Riu, A., Debrauwer, L., Hillenweck, A., Le Mevel, S., Chevolleau, S., Boulahtouf, A., Palmier, K., Balaguer, P., Cravedi, J. P., Demeneix, B. A., Zalko, D. (2012). Parallel biotransformation of tetrabromobisphenol A in Xenopus laevis and mammals: Xenopus as a model for endocrine perturbation studies. *Toxicol. Sci.* 125**,** 359-367.

Freitas, J., Cano, P., Craig-Veit, C., Goodson, M. L., Furlow, J. D., Murk, A. J. (2011). Detection of thyroid hormone receptor disruptors by a novel stable in vitro reporter gene assay. *Toxicol. in Vitro* 25**,** 257-266.

Fukazawa, H., Hoshino, K., Shiozawa, T., Matsushita, H., Terao, Y. (2001). Identification and quantification of chlorinated bisphenol A in wastewater from wastepaper recycling plants. *Chemosphere* 44**,** 973-979.

Gallart-Ayala, H., Nunez, O., Moyano, E., Galceran, M. T. (2010). Field-amplified sample injection-micellar electrokinetic capillary chromatography for the analysis of bisphenol A, bisphenol F, and their diglycidyl ethers and derivatives in canned soft drinks. *Electrophoresis* 31**,** 1550-1559.

Gee, A. C., Katzenellenbogen, J. A. (2001). Probing conformational changes in the estrogen receptor: evidence for a partially unfolded intermediate facilitating ligand binding and release. *Mol. Endocrinol.* 15**,** 421-428.

Germain, P., Staels, B., Dacquet, C., Spedding, M., Laudet, V. (2006). Overview of nomenclature of nuclear receptors. *Pharmacol Rev* 58**,** 685-704.

Giguere, V. (2002). To ERR in the estrogen pathway. *Trends Endocrinol. Metab.* 13**,** 220-225.

Giguere, V. (2008). Transcriptional control of energy homeostasis by the estrogen-related receptors. *Endocrine reviews* 29**,** 677-696.

Gould, J. C., Leonard, L. S., Maness, S. C., Wagner, B. L., Conner, K., Zacharewski, T., Safe, S., McDonnell, D. P., Gaido, K. W. (1998). Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Mol. Cell. Endocrinol.* 142**,** 203-214.

Greschik, H., Wurtz, J. M., Sanglier, S., Bourguet, W., van Dorsselaer, A., Moras, D., Renaud, J. P. (2002). Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3. *Mol. Cell* 9**,** 303-313.

Gronemeyer, H., Gustafsson, J. A., Laudet, V. (2004). Principles for modulation of the nuclear receptor superfamily. *Nat Rev Drug Discov* 3**,** 950-964.

Grun, F., Watanabe, H., Zamanian, Z., Maeda, L., Arima, K., Cubacha, R., Gardiner, D. M., Kanno, J., Iguchi, T., Blumberg, B. (2006). Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. *Mol. Endocrinol.* 20**,** 2141-2155.

He, B., Kemppainen, J. A., Wilson, E. M. (2000). FXXLF and WXXLF Sequences Mediate the NH2-terminal Interaction with the Ligand Binding Domain of the Androgen Receptor. *J. Biol. Chem.* 275**,** 22986-22994.

Horard, B., Vanacker, J. M. (2003). Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand. *J. Mol. Endocrinol.* 31**,** 349-357.

Jacobs, M. N., Nolan, G. T., Hood, S. R. (2005). Lignans, bacteriocides and organochlorine compounds activate the human pregnane X receptor (PXR). *Toxicol. Appl. Pharmacol.* 209**,** 123-133.

Jagnytsch, O., Opitz, R., Lutz, I., Kloas, W. (2006). Effects of tetrabromobisphenol A on larval development and thyroid hormone-regulated biomarkers of the amphibian Xenopus laevis. *Environ. Res.* 101**,** 340-348.

Kallen, J., Schlaeppi, J. M., Bitsch, F., Filipuzzi, I., Schilb, A., Riou, V., Graham, A., Strauss, A., Geiser, M., Fournier, B. (2004). Evidence for ligand-independent transcriptional activation of the human estrogen-related receptor alpha (ERRalpha): crystal structure of ERRalpha ligand binding domain in complex with peroxisome proliferator-activated receptor coactivator-1alpha. *J. Biol. Chem.* 279**,** 49330-49337.

Kelce, W. R., Lambright, C. R., Gray, L. E., Jr., Roberts, K. P. (1997). Vinclozolin and p,p'-DDE alter androgen-dependent gene expression: in vivo confirmation of an androgen receptor-mediated mechanism. *Toxicol. Appl. Pharmacol.* 142**,** 192-200.

Kitamura, S., Kato, T., Iida, M., Jinno, N., Suzuki, T., Ohta, S., Fujimoto, N., Hanada, H., Kashiwagi, K., Kashiwagi, A. (2005). Anti-thyroid hormonal activity of tetrabromobisphenol A, a flame retardant, and related compounds: Affinity to the mammalian thyroid hormone receptor, and effect on tadpole metamorphosis. *Life Sci.* 76**,** 1589-1601.

Kuiper, G. G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J. A. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 138**,** 863-870.

Leloup, Buscaglia (1977). La triiodothyronine, hormone de la métamorphose des amphibiens. *Comptes rendus hebdomadaires des scéances de l'académie des sciences.* Série D**,** 2261-2263.

Lemaire, G., Mnif, W., Pascussi, J. M., Pillon, A., Rabenoelina, F., Fenet, H., Gomez, E., Casellas, C., Nicolas, J. C., Cavailles, V., Duchesne, M. J., Balaguer, P. (2006). Identification of new

human pregnane X receptor ligands among pesticides using a stable reporter cell system. *Toxicol. Sci.* 91**,** 501-509.

Li, J., Ma, M., Wang, Z. (2009). In vitro profiling of endocrine disrupting effects of phenols. *Toxicol. in Vitro*.

Li, J., Ma, M., Wang, Z. (2010). In vitro profiling of endocrine disrupting effects of phenols. *Toxicol. in Vitro* 24**,** 201-207.

Liu, H., Zhao, H., Quan, X., Zhang, Y., Chen, S. (2009). Formation of chlorinated intermediate from bisphenol A in surface saline water under simulated solar light irradiation. *Environ. Sci. Technol.* 43**,** 7712-7717.

Lu, D., Kiriyama, Y., Lee, K. Y., Giguere, V. (2001). Transcriptional regulation of the estrogen-inducible pS2 breast cancer marker gene by the ERR family of orphan nuclear receptors. *Cancer Res.* 61**,** 6755-6761.

Matsumoto, T., Sakari, M., Okada, M., Yokoyama, A., Takahashi, S., Kouzmenko, A., Kato, S. (2012). The Androgen Receptor in Health and Disease. *Annu. Rev. Physiol.* Epub ahead of print.

Matsushima, A., Kakuta, Y., Teramoto, T., Koshiba, T., Liu, X., Okada, H., Tokunaga, T., Kawabata, S., Kimura, M., Shimohigashi, Y. (2007). Structural evidence for endocrine disruptor bisphenol A binding to human nuclear receptor ERR gamma. *J Biochem* 142**,** 517-524.

Matsushima, A., Teramoto, T., Okada, H., Liu, X., Tokunaga, T., Kakuta, Y., Shimohigashi, Y. (2008). ERRgamma tethers strongly bisphenol A and 4-alpha-cumylphenol in an induced-fit manner. *Biochem. Biophys. Res. Commun.* 373**,** 408-413.

McCarthy, N. (2011). Prostate cancer: Studying the classics. *Nat. Rev. Cancer* 11**,** 386.

McLachlan, J. A., Newbold, R. R. (1987). Estrogens and development. *Environ. Health Perspect.* 75**,** 25-27.

McPhaul, M. J. (1999). Molecular defects of the androgen receptor. *J. Steroid Biochem. Mol. Biol.* 69**,** 315-322.

Meerts, I. A., van Zanden, J. J., Luijks, E. A., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, A., Brouwer, A. (2000). Potent Competitive Interactions of Some Brominated Flame Retardants and Related Compounds with Human Transthyretin in Vitro. *Toxicol Science* 56**,** 95-104.

Mikamo, E., Harada, S., Nishikawa, J., Nishihara, T. (2003). Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. *Toxicol. Appl. Pharmacol.* 193**,** 66-72.

Mnif, W., Pascussi, J. M., Pillon, A., Escande, A., Bartegi, A., Nicolas, J. C., Cavailles, V., Duchesne, M. J., Balaguer, P. (2007). Estrogens and antiestrogens activate hPXR. *Toxicol. Lett.* 170**,** 19-29.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., Nakao, K. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J. Clin. Endocrinol. Metab.* 87**,** 5185-5190.

Mutou, Y., Ibuki, Y., Terao, Y., Kojima, S., Goto, R. (2006). Change of Estrogenic Activity and Release of Chloride Ion in Chlorinated Bisphenol A after Exposure to Ultraviolet B. *Biological and Pharmaceutical Bulletin* 29**,** 2116-2119.

Nagy, L., Schwabe, J. W. (2004). Mechanism of the nuclear receptor molecular switch. *Trends Biochem. Sci.* 29**,** 317-324.

Nettles, K. W., Bruning, J. B., Gil, G., Nowak, J., Sharma, S. K., Hahm, J. B., Kulp, K., Hochberg, R. B., Zhou, H., Katzenellenbogen, J. A., Katzenellenbogen, B. S., Kim, Y., Joachmiak, A.,

Greene, G. L. (2008). NFkappaB selectivity of estrogen receptor ligands revealed by comparative crystallographic analyses. *Nat. Chem. Biol.* 4**,** 241-247.

Nolte, R. T., Wisely, G. B., Westin, S., Cobb, J. E., Lambert, M. H., Kurokawa, R., Rosenfeld, M. G., Willson, T. M., Glass, C. K., Milburn, M. V. (1998). Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-[gamma]. *Nature* 395**,** 137-143.

Okada, H., Tokunaga, T., Liu, X., Takayanagi, S., Matsushima, A., Shimohigashi, Y. (2008). Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-gamma. *Environ. Health Perspect.* 116**,** 32-38.

Orans, J., Teotico, D. G., Redinbo, M. R. (2005). The nuclear xenobiotic receptor pregnane X receptor: recent insights and new challenges. *Mol. Endocrinol.* 19**,** 2891-2900.

Pacyniak, E. K., Cheng, X., Cunningham, M. L., Crofton, K., Klaassen, C. D., Guo, G. L. (2007). The flame retardants, polybrominated diphenyl ethers, are pregnane X receptor activators. *Toxicol. Sci.* 97**,** 94-102.

Paris, F., Balaguer, P., Terouanne, B., Servant, N., Lacoste, C., Cravedi, J. P., Nicolas, J. C., Sultan, C. (2002). Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit alpha and beta estrogen activities and antiandrogen activity in reporter cell lines. *Mol. Cell. Endocrinol.* 193**,** 43-49.

Pike, A. C. (2006). Lessons learnt from structural studies of the oestrogen receptor. *Best Pract Res Clin Endocrinol Metab* 20**,** 1-14.

Quigley, C. A., De Bellis, A., Marschke, K. B., el-Awady, M. K., Wilson, E. M., French, F. S. (1995). Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocrine reviews* 16**,** 271-321.

Renaud, J. P., Moras, D. (2000). Structural studies on nuclear receptors. *Cell. Mol. Life Sci.* 57**,** 1748-1769.

Riu, A., Grimaldi, M., le Maire, A., Bey, G., Phillips, K., Boulahtouf, A., Perdu, E., Zalko, D., Bourguet, W., Balaguer, P. (2011a). Peroxisome proliferator-activated receptor gamma is a target for halogenated analogs of bisphenol A. *Environ. Health Perspect.* 119**,** 1227-1232.

Riu, A., le Maire, A., Grimaldi, M., Audebert, M., Hillenweck, A., Bourguet, W., Balaguer, P., Zalko, D. (2011b). Characterization of novel ligands of ERalpha, Erbeta, and PPARgamma: the case of halogenated bisphenol A and their conjugated metabolites. *Toxicol. Sci.* 122**,** 372-382.

Rosen, E. D., Spiegelman, B. M. (2001). PPARgamma : a nuclear regulator of metabolism, differentiation, and cell growth. *J. Biol. Chem.* 276**,** 37731-37734.

Routledge, E. J., White, R., Parker, M. G., Sumpter, J. P. (2000). Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and ERbeta. *J. Biol. Chem.* 275**,** 35986-35993.

Shi, H., Qian, L., Guo, S., Zhang, X., Liu, J., Cao, Q. (in press). Teratogenic effects of tetrabromobisphenol A on Xenopus tropicalis embryos. *Comp Biochem Physiol C Toxicol Pharmacol* 152**,** 62-68.

Stroheker, T., Picard, K., Lhuguenot, J. C., Canivenc-Lavier, M. C., Chagnon, M. C. (2004). Steroid activities comparison of natural and food wrap compounds in human breast cancer cell lines. *Food Chem. Toxicol.* 42**,** 887-897.

Sui, Y., Ai, N., Park, S. H., Rios-Pilier, J., Perkins, J. T., Welsh, W. J., Zhou, C. (2012). Bisphenol A and its analogues activate human pregnane X receptor. *Environ. Health Perspect.* 120**,** 399-405.

Sun, H., Shen, O.-X., Wang, X.-R., Zhou, L., Zhen, S.-q., Chen, X.-d. (2009). Anti-thyroid hormone activity of bisphenol A, tetrabromobisphenol A and tetrachlorobisphenol A in an improved reporter gene assay. *Toxicol. in Vitro* 23**,** 950-954.

Tabb, M. M., Kholodovych, V., Grun, F., Zhou, C., Welsh, W. J., Blumberg, B. (2004). Highly chlorinated PCBs inhibit the human xenobiotic response mediated by the steroid and xenobiotic receptor (SXR). *Environ. Health Perspect.* 112**,** 163-169.

Takacs, M. L., Abbott, B. D. (2007). Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. *Toxicol. Sci.* 95**,** 108-117.

Takemura, H., Ma, J., Sayama, K., Terao, Y., Zhu, B. T., Shimoi, K. (2005). In vitro and in vivo estrogenic activity of chlorinated derivatives of bisphenol A. *Toxicology* 207**,** 215-221.

Takeshita, A., Koibuchi, N., Oka, J., Taguchi, M., Shishiba, Y., Ozawa, Y. (2001). Bisphenol-A, an environmental estrogen, activates the human orphan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. *Eur. J. Endocrinol.* 145**,** 513-517.

Tontonoz, P., Hu, E., Spiegelman, B. M. (1995). Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. *Curr. Opin. Genet. Dev.* 5**,** 571-576.

Tremblay, G. B., Kunath, T., Bergeron, D., Lapointe, L., Champigny, C., Bader, J. A., Rossant, J., Giguere, V. (2001). Diethylstilbestrol regulates trophoblast stem cell differentiation as a ligand of orphan nuclear receptor ERR beta. *Genes Dev.* 15**,** 833-838.

Vanacker, J. M., Pettersson, K., Gustafsson, J. A., Laudet, V. (1999). Transcriptional targets shared by estrogen receptor- related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. *EMBO J.* 18**,** 4270-4279.

Vandenberg, L. N., Maffini, M. V., Sonnenschein, C., Rubin, B. S., Soto, A. M. (2009). Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocrine reviews* 30**,** 75-95.

Veldhoen, N., Boggs, A., Walzak, K., Helbing, C. C. (2006). Exposure to tetrabromobisphenol-A alters TH-associated gene expression and tadpole metamorphosis in the Pacific tree frog Pseudacris regilla. *Aquat. Toxicol.* 78**,** 292-302.

Wang, L., Zuercher, W. J., Consler, T. G., Lambert, M. H., Miller, A. B., Orband-Miller, L. A., McKee, D. D., Willson, T. M., Nolte, R. T. (2006). X-ray crystal structures of the estrogen-related receptor-gamma ligand binding domain in three functional states reveal the molecular basis of small molecule regulation. *J. Biol. Chem.* 281**,** 37773-37781.

Wetherill, Y. B., Akingbemi, B. T., Kanno, J., McLachlan, J. A., Nadal, A., Sonnenschein, C., Watson, C. S., Zoeller, R. T., Belcher, S. M. (2007). In vitro molecular mechanisms of bisphenol A action. *Reprod. Toxicol.* 24**,** 178-198.

Wilson, E. M. (2011). Analysis of interdomain interactions of the androgen receptor. *Methods in Molecular Biology* 776**,** 113-129.

Xue, Y., Moore, L. B., Orans, J., Peng, L., Bencharit, S., Kliewer, S. A., Redinbo, M. R. (2007). Crystal structure of the pregnane X receptor-estradiol complex provides insights into endobiotic recognition. *Mol. Endocrinol.* 21**,** 1028-1038.

Yu, S., Wang, X., Ng, C. F., Chen, S., Chan, F. L. (2007). ERRgamma suppresses cell proliferation and tumor growth of androgen-sensitive and androgen-insensitive prostate cancer cells and its implication as a therapeutic target for prostate cancer. *Cancer Res.* 67**,** 4904-4914.

Zoeller, T. R., Dowling, A. L., Herzig, C. T., Iannacone, E. A., Gauger, K. J., Bansal, R. (2002). Thyroid hormone, brain development, and the environment. *Environ. Health Perspect.* 110 Suppl 3**,** 355-361.

Figure Legends:

Figure ER: Two different binding modes of BPs. (A) The whole structure of the ERα LBD in complex with SRC-1 coactivator peptide and BPA (grey) superimposed on that of ERα LBD bound to BPC (black). The dashed line denotes residues not visible in the electron density map. Interaction networks of E_2 (B), BPA (C), and BPC (D) with ligand-binding pocket residues in ERα. Hydrogen bonds are indicated by dashed lines. For clarity not all the protein – ligand interactions are depicted. Figures (B-D) also depict the interaction of the various ligands with L525. (B) E_2 strengthens a van der Walls interactions network involving T347 (H3), L525 (H11) and L536 (L11-12). (C) Due to a lack of contact with BPA, L525 is not stabilized and adopts two different conformations. (D) In the BPC-bound ERα structure, T347 rotates by 180° to form a hydrogen bond with the bisphenol, resulting in the disruption of the hydrophobic network.

Figure ERR: Structural determinants of BPs recognition by ERRs. (A) Overall structure of unliganded ERRγ LBD (PDB code 1KV6) in cartoon representation. The unoccupied LBP is highlighted in black and CoA stands for coactivator-derived peptide. (B) Superposition of ERRγ LBPs in absence of ligand (light grey, PDB code 1KV6) and in complex with BPA (dark grey, PDB code 2E2R). Residues involved in BPA binding are colored in dark grey and equivalent residues in the unliganded receptor are colored in light grey.

Figure PPAR: Crystal structures of PPARγ LBD in complex with TBBPA and TBBPA-sulfate. (A) Overall structure of the TBBPA-bound PPARγ LBD. The polypeptide backbone is illustrated as a grey ribbon and TBBPA is shown in stick representation. (B) Superimposition of the co-crystal structure of TBBPA-bound PPARγ LBD (in black) on the structure with rosiglitazone (grey, PDB code 2PRG). (C) Superimposition of the co-crystal structure of PPARγ LBD bound to TBBPA-sulfate (black) on the structure with TBBPA (grey). Roziglitazone, TBBPA and TBBPA-sulfate are shown in stick representation.

Figures

Tables