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Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity

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Abstract

Polychlorinated biphenyls (PCBs) are ubiquitous environmental persistent contaminants giving rise to potential health hazard. Some PCBs exert dioxin-like activities mediated through the aryl hydrocarbon receptor. Although reports on interaction with other nuclear receptors are sparce, some congeners are hypothesized to possess endocrine disruptive potential. Here we present evidence that the three PCBs most abundant in biological extracts, 2,2′,3′4,4′,5-hexachlorobiphenyl (PCB # 138), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB # 153), and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB # 180) have pleiotropic effects on the estrogen- and androgen-receptor. In MCF-7 cells a slightly increased cell proliferation was observed at low concentrations (1–10 nM) in cells co-treated with 0.01 nM 17β-Estradiol, whereas the compounds inhibited cell growth significantly at 1 and 10 μM. In reporter gene (*ERE-tk-CAT*) analysis the three congeners exhibited a significantly estrogen receptor-ligand mediated decrease of the chloramphenicol transferase activity in both control and 10 nM 17β-estradiol induced MCF-7 cells. In addition, PCB # 138 elicited a dose-dependent antagonistic effect on androgen receptor activity in transiently co-transfected Chinese Hamster Ovary cells with an IC_{50}, of 6.2 μM. In summary, this study indicate that the di-*ortho*, multiple-chloro substituted biphenyls, PCB # 138, PCB # 153 and PCB # 180, can compete with the binding of the natural ligand to two nuclear receptors and thus possess the ability to interfere with sexual hormone regulated processes.

Keywords: PCB; TCDD; Estrogen receptor; Androgen receptor; AH-receptor; Breast cancer cells

Abbreviations: AHR, aryl hydrocarbon receptor; AR, androgen receptor; CAT, chloramphenicol transferase; E2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen-responsive-element; hexaCB and hepataCB, hexa- and heptachlorobiphenyl, respectively; LUC, luciferase; PB, phenobarbital; PCB, polychlorinated biphenyls; POC, persistent organochlorine; TCDD, tetradichlorodibenzo-*p*-dioxin; tk, thymidin kinase.

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1. Introduction

Persistent organochlorines (POCs) are industrial compounds, combustion products and pesticides found ubiquitous in the environment and residues are determined in fish, wildlife, and human. Many of these compounds can mimic sex steroid activities and are, therefore, potential endocrine disrupters giving increased risk of reproductive disorders and carcinogenesis. POCs which exhibit hormonal activity include polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxin/-furans (PCDD/PCDF) and insecticides (Safe, 1994; Safe and Zacharewski, 1997; Van den Berg et al., 1998).

PCBs are an important group of environmental pollutants which includes 209 possible congeners exhibiting a variety of chlorine substitution patterns (Fig. 1). These man-made compounds, first manufactured in 1929, are highly stable. Due to their physicochemical properties, PCBs have been used in numerous industrial products such as diluents, hydraulic fluids, heat transfer fluids, dielectric fluids for capacitors and transformers etc. Until the mid of 1970s, no disposal practice of PCB-contaminated waste have been employed, and this may be the major cause of the abundance of this group of environmental pollutants. Due to their lipophilic nature and resistance toward biotransformation, PCBs accumulate in the food chain and have been identified in practically all the environmental matrices including human adipose tissue, blood and milk (Borlakoglu and Walker, 1989; Asplund et al., 1994; Bates et al., 1994; Dewailly et al., 1994; Furst et al., 1994).

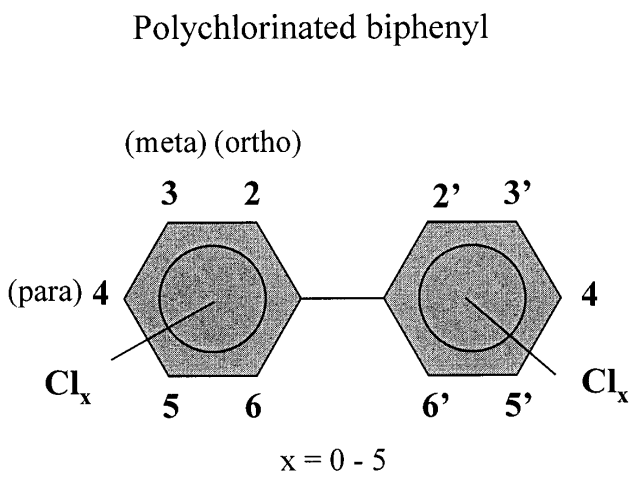

Fig. 1. Structure of PCB congeners.

The individual PCBs have different physical/chemical characteristics which influence their accumulation, uptake and metabolism in the environment and in organisms, giving rise to marked differences in PCB congener composition between the commercial PCBs (Aroclor) and biological extracts. However, in most biological extracts PCB # 138 (2,2′,3,4,4′,5-hexaCB), PCB # 153 (2,2′,4,4′,5,5-hexaCB), and PCB # 180 (2,2′,3,4,4′,5,5-heptaCB) are the dominating components (Safe, 1994; Cogliano, 1998). Depending on the position and number of chlorine substitutions, different classes of PCB congeners elicit a complex spectrum of biological and toxic responses in a number of in vivo and in vitro models (Safe, 1994). Many of the responses observed by the non- or mono-*ortho* coplanar PCBs correlate with their binding affinity to the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor believed to mediate several of the effects induced by these compounds including immuno-, reproductive-, neuro-, dermal- and hepatotoxicity, and carcinogenesis. These effects are similar to those reported for the prototype AHR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The di-*ortho* coplanar PCBs are weak AHR agonists (Safe, 1994). Some PCBs and dioxins have been shown to have potential estrogen and antiestrogen effects mediated via AHR which includes downregulation of the estrogen receptor (ER), interference of ligand activated ER binding to DNA response element and/or induction of cytochrome P450 monooxygenase 1A1, 1A2, or 1B1 activities, all involved in metabolism of estradiol (Spink et al., 1994; Krishnan et al., 1995; Kharat and Saatcioglu, 1996). The so-called 'mixed-type' monooxygenase induction are primarily exhibited by mono- and di-*ortho* PCB congeners that elicit both TCDD- and phenobarbital (PB)-like responses. The most active PB-type inducers contain at least two *ortho* and two *para* chlorine substituents with PCB # 153 as a prototypical PB-type inducer. Results have indicated that PB-type PCBs contribute to tumor promotion of the commercial mixtures (Safe, 1994). Less

is known about the biochemical and toxic effects of di- or multiple-*ortho* chlorine substituted PCBs despite their abundant occurrence in the biotic and abiotic environment (McFarland and Clarke, 1989).

In vivo and in vitro studies have shown that selected PCBs, PCB metabolites and their mixtures are capable of mimicking some of the biological activities of estrogens including precocious puberty, disrupted uterus and induction of estrogen responsive enzyme activities (Bitman and Cecil, 1970; Jansen et al., 1993; Safe, 1994; Li and Hansen, 1996). Mechanistic effects in vitro have been reported such as competition with 17β-estradiol (E2) for binding to ERα and ERβ, promotion of proliferation of the human breast cancer cell line MCF-7, and induction of gene expression (Nelson, 1974; Soto et al., 1995; Nesaretnam et al., 1996; Vonier et al., 1996; Arcaro et al., 1999). Based on their chemical structure and activities, it has been suggested that those PCB congeners that possess *ortho* substituents can elicit estrogenic responses following hydroxylation at a vacant *para* position (Korach et al., 1988; Gierthy et al., 1997). However, in addition to hydroxylated metabolites (Connor et al., 1997), non-*ortho* substituted PCB congeners (Nesaretnam et al., 1996) and selected parent PCB congeners may also exhibit estrogenic and antiestrogenic activities (Nesaretnam et al., 1996; Fielden et al., 1997; Nesaretnam and Darbre, 1997; Lind et al., 1999). Some environmental chemicals that antagonize the effects of androgens mediated via the androgen receptor (AR) may have serious impact on abnormalities associated with the developing male reproductive system (Kelce et al., 1997; Gray et al., 1999). Metabolites of the fungicide vinclozolin (Wong et al., 1995), the insecticide p,p′-DDT (p,p′-DDE) (Kelce et al., 1995) and the fungicide procymidone (Hosokawa et al., 1993; Ostby et al., 1999) are found as potent antagonist of the natural male sex hormone in vitro and in vivo.

Animal experiments have shown that bioaccumulated PCBs are more toxic than the technical mixture Aroclor (Cogliano, 1998). Since most studies have been carried out using Aroclor technical mixtures (Safe, 1994), analysis of specific congeners are important to assess the risk in human exposure. We have analyzed the effects on ER and AR activities of three very biologically relevant PCB congeners PCB # 138, PCB # 153, PCB # 180. In addition to cell proliferation in human breast cancer MCF-7 cells, the receptor actions were assessed by (i) transient reporter gene expression in ER positive (MCF-7) and ER negative (MDA-MB-231) human breast cancer cells, (ii) transient expression in Chinese Hamster Ovary cells (CHO K1) co-transfected with the AR and a reporter construct. The congeners PCB # 138, PCB # 153, and PCB # 180 are reported as weak AHR agonists in the developmental period (Crisp et al., 1998). In this study we show that these three PCB congeners possess the potential to interfere with sexual hormone receptor activities.

2. Materials and methods

2.1. Chemicals

PCB # 138, PCB # 153, and PCB # 180 were purchased from Dr Ehrensdorfer, Augsburg, Germany, ICI 182.780 from Zeneca Pharmaceutical, UK, and R1881 from NEN, Boston, MA, USA.

2.2. Transient gene expression assay in MCF-7 and MDA-MB-231 cells (ER assay)

MCF-7 cells (Breast Cancer Task Force Cell Culture Bank, Mason Research institute, Worcester) (passage 298–310) were maintained in phenol-free Dulbecco's modification of Eagle's Medium (DME) (GIBCOBRL, Denmark) supplemented with 1% fetal calf serum (FCS), 64 g/ml Garamycin, 2.5 mM glutamine, and 6 μg/l insulin and transfected as described (Jorgensen and Autrup, 1995, 1996) with the modification of using 1% charcoal treated FCS (CT-FCS) (HyClone, Belgium) in transfection media. The described (Bonefeld Jorgensen et al., 1997) p*ERE-tk-CAT* expression vector was used as reporter plasmid and pON249 (encoding the β-galactosidase enzyme) as an internal standard for transfection efficiency. Upon transfection the cells were treated for 48 h with solvent (ethanol) or 10 nM

17β-estradiol (E2) (Sigma, Denmark) with or without addition of the chemical to be tested. The chemicals were added from ethanol stocks (final ethanol concentration 0.1%) and used at nontoxic concentrations as deduced by a nonradioactive cell proliferation/cytotoxicity assay (Promega, Denmark). Chloramphenicol transferase (CAT) activities were normalized to transfection efficiency and protein content as described (Jorgensen and Autrup, 1995, 1996). The MDA-MB-231 cells (American Type Culture Collection, Rockville, MD, USA) were maintained as MCF-7 cells but with 5% FCS in cultivating media and 5% CT-FCS in transfection media. A chimeric p*ERE-LUC* reporter plasmid and the human hERα expression vector p*SG5-HEO* (Chambon, France) was used for transient co-expression assays in MDA-MB-231 cells. Transfection and luciferase activity measurements were carried out as described (Vinggaard et al., 1999).

2.3. E-screen based on proliferation of human breast cancer cells (MCF-7)

Stock cultures of MCF-7 BUS cells (passage 113–120), grown in DME (In Vitro, Denmark) supplemented with 10% CT-FCS in an atmosphere of 5% CO_2/95% air under saturating humidity at 37°C. MCF-7 cells, were seeded in 24-well plates (Linbro, McLean, Virginia) at a initial concentration of 10^4 cells per well. After 24 h the chemicals to be tested were added in experimental medium (phenol red-free DME, In Vitro, Denmark) supplemented with 10% CT-FCS (Biological Industries Co., Beit Haemek, Israel)). The chemicals were added from ethanol stocks (final ethanol concentration $\leq 0.1\%$) and were tested in the presence and absence of 10 pM E2. The bioassay was terminated on the 6th day (late exponential phase) and the cells were fixed and stained with sulforhodamine-B (SRB) as described in (Villalobos et al., 1995). Briefly, cells were treated with 10% trichloracetic acid and incubated at 4°C for 30 min, then washed five times with tap water and left to dry. The fixed cells were stained for 10 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Wells were washed five times with 1% acetic acid and air dried. Bound dye was solubilized with 10 mM Tris base (pH 10.5) in a shaker for 20 min. Finally, aliquots were transferred to a 96-well plate and read in a plate reader (Dynatech MRX, Chantilly, USA) at 492 nm.

2.4. Transient gene expression assay in Chinese hamster ovary cells (AR assay)

Chinese hamster ovary cells (CHO K1) were maintained in DMEM/F12 (Gibco, Paisley, UK) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma, St. Louis, MO) and 10% FCS (BioWhitaker, Walkersville, MD). The assay was performed essentially as described (Vinggaard et al., 1999). The cells were seeded in microtiter plates (Costar, Acton, MA) at a density of 5000 cells per well in DMEM/F12 containing 10% CT-FCS (HyClone, Logan, Utah) and incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air. After 24 h, the chemicals to be tested were added in culture media. The chemicals were added from ethanol stocks (final ethanol concentration in the media 0.1–0.36%). Shortly after addition of test compounds, each well was transfected with a total of 50 ng DNA consisting of the expression vector p*SVAR*0 (human AR) and the p*MMTV-LUC* reporter plasmid (both provided by Dr Albert Brinkmann, Erasmus University, Rotterdam) in a ratio of 1:100 using 0.15 μl of the non-liposomal transfection reagent FuGene (Boehringer Mannheim, Germany). After an incubation period of 24 h, the media was aspirated and the cells were lysed by adding 15 μl per well of a lysis buffer containing 25 mM trisphosphate, pH 7.8, 15% glycerol, 1% Triton X-100, 1 mM DTT and 8 mM $MgCl_2$, followed by shaking at room temperature for 10 min. Then 5 μl was transferred to white Dynatech microtiter plates for measurement of luciferase activity in a BioOrbit Galaxy luminometer. 10 μl of a substrate containing 1 mM luciferin (Amersham Int., Buckinghamshire, UK) and 1 mM ATP (Boehringer Mannheim, Germany) in lysis buffer was injected automatically and the chemiluminiscense generated from each well was measured over a 1 s interval after an incubation time of 2 s.

2.5. Cytotoxicity test using AlamarBlue

After incubation of the CHO cells for 24 h with compounds at the indicated concentrations, the cell number was determined by measuring the reduction of AlamarBlue (Serotec, Kidlington, UK). The assay is based on metabolic reduction of the AlamarBlue dye into a fluorescent species, which is detected after excitation of the reduced dye at 560 nm and subsequent emission at 590 run. Each well was added 5×10^3 CHO cells in black clear-bottomed microtiter plates (Costar). The day after, compounds dissolved in media was added, and the plates were incubated for 24 h before the addition of a 10% solution of AlamarBlue in PBS. Fluorescence was measured after 3 h at a slit width of 10 nm for both excitation and emission using a Perkin Elmer luminescence spectrophotometer LS50B, equipped with a microtiter plate reader.

2.6. Statistics

Statistical analysis of data on cell proliferation, estrogen and androgen receptor assays were analyzed by oneway analysis of variance. Multiple comparisons versus control were performed using a Bonferroni test for androgenic analyses and Sheffer's test for cell proliferation and estrogenic data. To study dose-response relations a linear regression was performed on the cell proliferation and estrogenic E2/PCB dose-response data. Significant differences were defined when $P \leq 0.05$.

3. Results

3.1. Estrogen receptor mediated transactivation of transiently transfected reporter gene in human breast cancer cells

Transient gene expression studies were carried out to determine the ability of PCB # 138, PCB # 153 and PCB # 180 to activate the estrogen responsive reporter plasmid *ERE-tk-CAT* (MCF-7) and *ERE-LUC* (MDA-MB-231). Toxicity tests confirmed the use of nontoxic PCB concentrations (data not shown). In ER-positive MCF-7 cells 10 nM 17β-estradiol (E2) increased the CAT activity 3-fold (100% response). Each of the three PCBs congeners exhibited a significantly dose-dependent decrease of E2 induced CAT activity (linear regression: # 138, # 153 and # 180; $R^2 = 0.72$, $R^2 = 0.70$, and $R^2 = 0.88$, respectively ($P \leq 0.000$ for each, Fig. 2). A significant decrease of basal CAT activity was observed upon exposure to the highest concentration tested, 9 μM (Fig. 2, solvent + PCBs). At 9 μM, PCB # 138 significantly inhibited the relative activity of the basal and E2 induced response by 61% ($P \leq 0.000$) and 36% ($P \leq 0.000$), respectively. At 9 μM, PCB # 153 and PCB # 180 repressed the basal activity by 50% ($P \leq 0.000$) and 79% ($P \leq 0.000$), and the E2 induced activities by 41% ($P \leq 0.000$) and 75% ($P \leq 0.000$), respectively. Treatment of the transfected cells with a mixture of the three PCBs (3 μM each) exhibited an antiestrogenic activity on ER function reducing the CAT activity by 42% (basal, $P \leq 0.000$) and 27% (E2 induced, $P \leq 0.01$). The repressing activity observed of these three congeners is comparable to the response of the pure ER antagonist ICI 182.780 (10^{-7} M) analyzed in parallel. In ER-negative MDA-MB-231 cells transfection of the *ERE-LUC* reporter vector alone elicited no inducible luciferase activity, upon exposure to 10 nM E2. However, co-transfection with the expression vector of the human ERα (p*SG5-HEO*) caused a 3-fold increase of the LUC activity upon exposure to 10 nM E2. Exposure of the co-transfected cells to 10 nM E2 and the PCBs or ICI 182.780 confirmed that the antiestrogenic effect of these compounds is mediated through ER (Fig. 3). Thus 9 μM of each of the three PCBs, # 138, # 153, and # 180, significantly decreased the ER dependent E2 induction of luciferase activity in MDA-MB-231 cells ($P \leq 0.003$, $P \leq 0.004$, and $P \leq 0.007$, respectively).

3.2. Effect on proliferation of human breast cancer cells

The effects of PCB # 138, PCB # 153, and PCB # 180 treatments on proliferation of MCF-7 BUS cells are shown in Fig. 4. E2 induced maximal cell yields at 100 pM (approximately 10-fold)

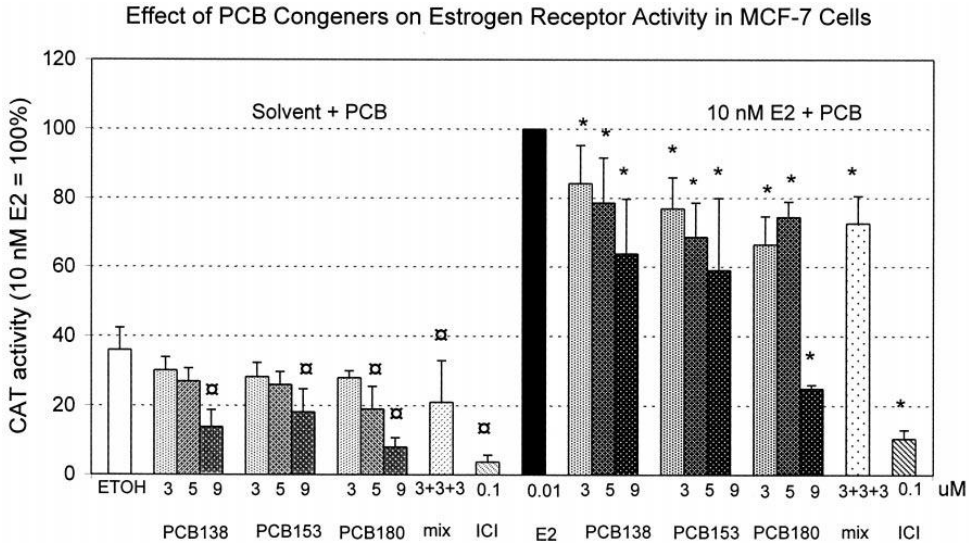

Fig. 2. Effects of PCB congeners on ER-mediated transactivation in MCF-7. Cells were transiently transfected with the p*ERE-tk-CAT* reporter plasmid. Upon transfection the cells were exposed to solvent (96% ethanol, $\leq 0.1\%$) or 10 nM E2 with or without addition of the given PCB concentrations (3, 5, or 9 μM; mix: 3 + 3 + 3 μM of each PCB; ICI 10^{-7} M). CAT activities were normalized to protein content and to transfection efficiency using pON249 encoding β-galactosidase enzyme, as an internal standard. The CAT activities of cells treated with 10 nM E2 were set to 100%. Data are expressed as the mean ± S.D., and are a pool of at least nine tests from three or four independent experiments, each performed in triplicate. ○ and * indicates a statistically significant ($P \leq 0.05$) difference from solvent (ETOH) treated or 10 nM E2 treated cells, respectively.

in the E-screen assay (Fig. 4a). None of the three PCBs had on its own nor as a mixture any effect on cell proliferation in the concentration range tested (0.001–10 μM (Fig. 4b–e), indicating that the working concentration range of PCBs are nontoxic to the cells. A significantly dose-dependent decrease of 10 pM E2 induced cell proliferation (half max response to E2, Fig. 4a) was observed for each of the three PCBs and their mixture (linear regression: # 138, # 153, # 180 and a mixture of the congeners; $R^2 = 0.53$, $R^2 = 0.83$, $R^2 = 0.57$, and $R^2 = 0.65$, respectively ($P \leq 0.000$ each, Fig. 4b–e). Co-treatment of cells with 10 pM E2 plus 0.001–0.1 μM of PCB # 138, PCB # 153 or PCB # 180 had no significant effect on cell proliferation compared to cells treated with E2 alone. However, exposure of the cells to 1 or 10 μM of the three PCB congeners exhibited an inhibition of the 10 pM E2 induced cell proliferation significantly at 10 μM for PCB # 138 and PCB # 153 ($P \leq 0.018$ and $P \leq 0.000$, respectively, Fig. 4b and c), and at 1 and 10 μM for PCB # 180 ($P \leq 0.000$ each, Fig. 4d). Cells exposed to 10 pM E2 and a mixture of the three congeners had no effect on cell growth at the lower concentrations (0.001–0.1 μM of each), whereas a decreased cell proliferation ($P \leq 0.024$) was observed following exposure to 10 μM of each congeners (Fig. 4e). The ER antagonist ICI 182.780 (0.0001–1 μM), analyzed in parallel, decreased cell proliferation to control level upon co-treatment with 10 pM E2 (data not shown).

3.3. Effect on androgen receptor activity

The androgenic and antiandrogenic effect of the three PBCs were analyzed in a transient co-expression assay using the *MMTV-LUC* reporter construct and the AR expression vector p*SVAR*0 in the Chinese Hamster Ovary cell line CHO K1. Except for a slightly toxic effect of 18 μM PCB # 180, no cytotoxicity was determined by the AlamarBlue method at the used concentration range (1–18 μM) (data not shown). None of the PCBs exhibited androgenic activity, neither did a mix of 3 μM of each PCB (data not shown). A dose-dependent increase of luciferase activity was observed upon treatment of the cells with the AR ligand R1881 at 0.01–100 nM (Fig. 5). PCB # 138 elicited a significantly dose-dependent antiandro-

genic effect on the 0.1 nM R1881 induced luciferase activity (set to 100%), reaching a 83% ($P \leq 0.001$) inhibition at 18 μM (Fig. 5). PCB # 153 and PCB # 180 showed no antiandrogenic activity at 1–18 μM. In the cells exposed to a mixture of the three PCBs (3 μM each) the R1881 induced luciferase activity was inhibited by 35%. This antiandrogenic effect is believed to reflect the response of PCB # 138 only, which at 3 μM caused an inhibition of 36%.

4. Discussion

Estrogens have known physiological functions such as influence on growth, differentiation, development, and function of several target tissues involved in reproduction, cardiovascular performance, bone maintenance, homeostasis, and behavior. Most of these responses are mediated as activities of the ER-ligand directed expression of estrogen responsive genes (Evans, 1988; Beato, 1989). Estrogens have also been implicated in the development of hormone-dependent cancers of breast, ovaries, endometrium, and prostate (Henderson et al., 1988; Yager and Liehr, 1996). The promotion of hormone-dependent tumors by estrogens is suggested to be due to induced genes such as growth factors, growth factor receptors, protooncogenes, and proteases, which contribute to cell proliferation, invasion, and metastasis (Lippman and Dickson, 1989; Dickson et al., 1990; Imagawa et al., 1990; Yager and Liehr, 1996). Xenobiotics capable of eliciting estrogenic activities are also hypothesized as a contributing factor in the development of hormone-related cancers and disruptive effects on reproduction in humans and wildlife (Colborn et al., 1993; Birnbaum, 1994; Wolff and Toniolo, 1995; Ahlborg et al., 1995a; Cogliano, 1998; Van den Berg et al., 1998). Estrogenic activity associated with PCB Aroclor mixtures in a uterotrophic model was reported 30 years ago (Bitman and Cecil, 1970). Subsequently, several studies have reported that commercial PCB mixtures or individual congeners and/or their hydroxylated metabolites exhibit anti- or estrogenic or activity (Jansen et al., 1993; Safe, 1994; Soto et al., 1995; Nesaretnam et al., 1996; Connor et al., 1997; Fielden et al., 1997; Gierthy et al., 1997; Moore et al., 1997; Nesaret-

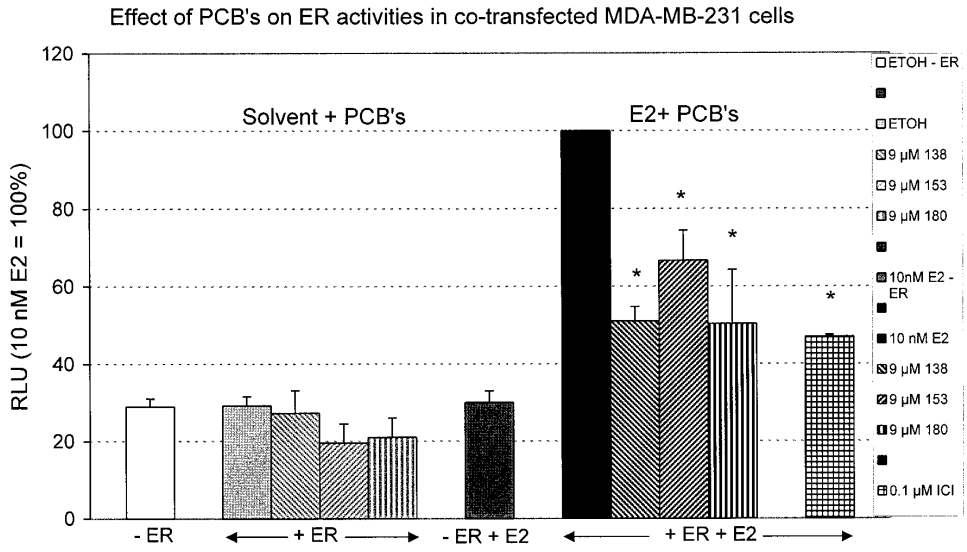

Fig. 3. Effects of PCBs on ER mediated transactivation in ER-negative MDA-MB-231 cells. Cells were transiently transfected with the p*ERE-LUC* reporter construct, or co-transfected with the p*ERE-LUC* and the p*SG5-HEO* expression vector encoding the human ERα. The treatment of cells are given in legend to Fig. 2 and Section 2. Data obtained from co-transfected cells upon co-treatment with E2 and PCBs or ICI are significantly ($P \leq 0.05$) different from the value of 10 nM E2 treated cells (100%). Data are expressed as the mean ± S.D., and are a pool of at least nine tests from three or four independent experiments, each performed in triplicate. RLU; relative luciferase units. *, indicates statistically significant ($P \leq 0.05$) difference from 10 nM E2 treated cells

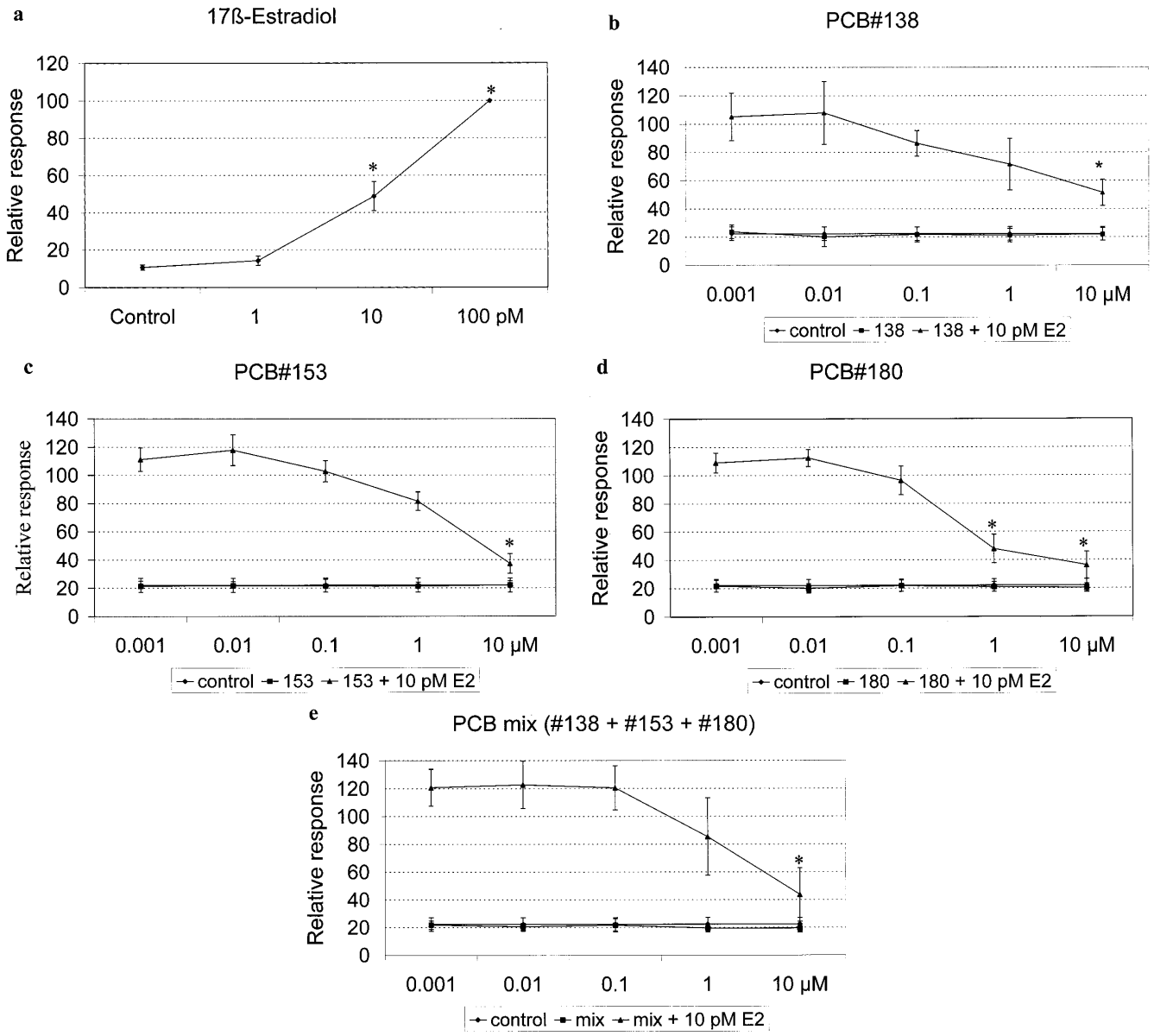

Fig. 4. Dose-response effects of the PCBs on proliferation of MCF-7 cells. Cell proliferation is expressed as relative cell number above hormone free control; (a) as dose-response of E2 concentrations (100 pM was set to 100%); (b–e) to treatment with 10 pM E2 (which was set to 100%, half max cell growth response to E2), and as a function of test compound concentrations, single exposure or co-treatments with 10 pM E2, (b) PCB # 138; (c) PCB # 153; (d) PCB # 180; (e) mixture of equal amounts of the three PCBs (the concentration given at the x-axis indicates the concentration of each of the three PCBs). * indicates statistically significant ($P \leq 0.05$) decrease relative to cells treated with 10 pM E2 alone. Data represent the mean ± S.D. of three independent experiments. Each experiment represents the average of the response data from eight wells per concentration.

nam and Darbre, 1997; Ramamoorthy et al., 1997). However, evidence for interaction of single PCB congeners with nuclear receptors has been sparse. Here we present evidence that three di-*ortho* substituted PCBs, # 138, # 153, # 180, which significantly bioaccumulate in mammal tissues (AMAP, 1998), exhibit effects on ER and AR functions. At the concentration range of 3–9 µM all the three congeners displayed a significant antiestrogenic effect on expression of the *ERE-tk-CAT* reporter plasmid, transiently transfected into MCF-7 cells, and the necessity of co-expression of hERα (HEO) in the ER negative MDA-MB-231 cells indicates that the observed responses were

mediated by the ER. The effect of the PCBs on basal reporter gene expression in MCF-7 reflects a low concentration of E2 in the cell culture medium despite the use of hormone-depleted serum. Reporter gene assays elicited a dose-dependent antagonistic effect of PCB # 138 on AR functions. In control cells an effect was seen on ER activity, whereas an effect on both ER and AR was observed in cells co-treated with their natural ligands, E2 or R1881, respectively. In cells co-exposed to 10 nM E2 or 0.1 nM R1881, the median effective competitive concentration (IC_{50}) values of PCB # 138 were 8.8 and 6.2 μM, respectively. Moreover, 1 or 10 μM of PCB # 180 and 10 μM of PCB # 138 or PCB # 153 significantly inhibited E2 induced MCF-7 cell proliferation. In the biological environment the PCBs will be in a mix of chemicals. Our analysis showed that each of these three di-*ortho* congeners acted on ER functions and cell proliferation, and PCB # 138 acted on AR activities as well. Neither PCB # 153 nor PCB # 180 had any effect on AR activities. Thus these single PCB congeners has the potential to compete with the natural ligand for binding to its receptor. In a recent study we showed that their relative effects on the estrogen receptor correlate well with their potency to affect mRNA expression of the estradiol responsive pS2 gene (Rattenborg, T and Bonefeld-Joergensen, EC, 1999, in preparation).

Estrogenic or antiestrogenic activity has been reported for both PCBs and hydroxylated PCBs (Bitman and Cecil, 1970; Korach et al., 1988; Soto et al., 1995; Li and Hansen, 1996; Nesaretnam et al., 1996; Fielden et al., 1997; Gierthy et al., 1997; Nesaretnam and Darbre, 1997; Ramamoorthy et al., 1997). Although these studies suggested that hydroxylation is important for interaction with ER, weak estrogenicity of PCBs has been observed in a number of studies (Soto et al., 1995; Fielden et al., 1997; Gierthy et al., 1997; Nesaretnam and Darbre, 1997; Arcaro et al., 1999). However, it is conceivable that in some cases the PCBs were metabolized in the test system to a hydroxylated PCB. In addition to hydroxylation, it has been hypothesized that the conformational restriction conferred by *ortho*-Cl-substitutions is correlated with estrogenicity (Korach et al., 1988). Arcaro et al. (1999) demonstrated that non-coplanar configuration

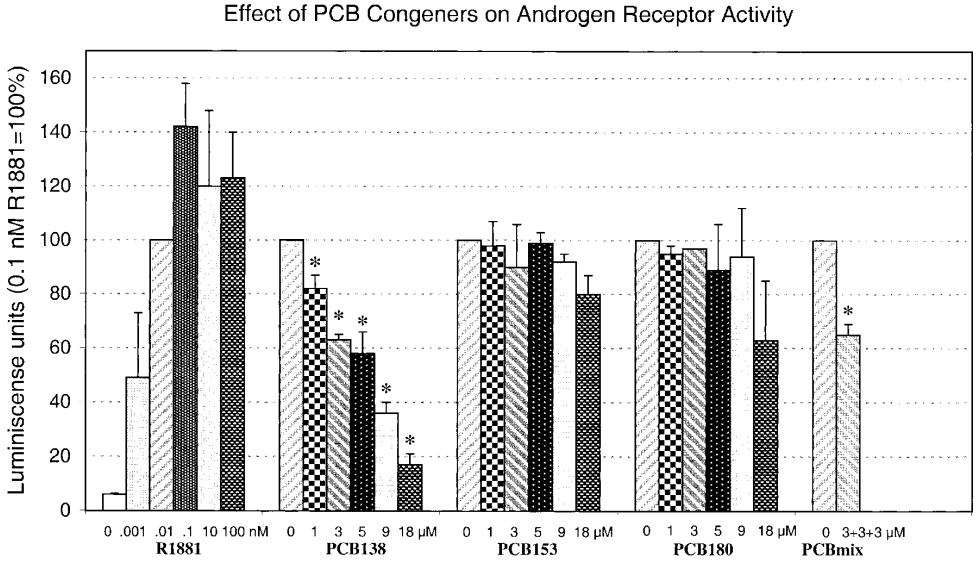

Fig. 5. Effects of PCBs on AR-mediated transactivation in CHO cells. Cells were transiently co-transfected with the p*MMTV-LUC* reporter plasmid and the p*SVAR*0 expression plasmid encoding the human AR. Upon transfection cells were exposed to either R1881 alone (0.001–10 nM) or to 0.1 nM R1881 plus the given PCB congeners at 1–18 μM. The dose-dependent antiandrogen effect of the three PCBs were determined by their ability to inhibit the response to 0.1 nM R1881 which was set to 100%. Data represent the mean ± S.D. of three independent experiments each performed in four wells. * indicates statistically significant ($P \leq 0.05$) decrease relative to cells treated with 0.1 nM R1881 alone.

obtained by saturation of the *ortho* position by Cl and *ortho* substitution on each of the biphenyl rings enhances estrogenic action (Arcaro et al., 1999). Here we show that di-*ortho*, multiple-Cl-substituted PCBs (two hexaCBs and one heptaCB) possesses antagonistic activities on ER functions. Recent studies of effects on reproductive hormones of PCB # 126 and PCB # 153 in male rats support the hypothesis that # 153 possesses estrogen-like properties (Desaulniers et al., 1999). To our knowledge the present study demonstrates for the first time the potential of PCB # 138, PCB # 153, and PCB # 180 to interfere with ligand mediated ER activities.

Some environmental chemicals have been identified to possess the ability to antagonize the effects of androgens such as the pesticides p,p′-DDE, vinclozolin, and procymidone (Hosokawa et al., 1993; Kelce et al., 1995; Wong et al., 1995; Gray et al., 1999; Ostby et al., 1999). Exposure to environmental antiandrogens of male fetuses in utero have been shown to result in abnormal development of genital tract such as cryptorchidism and hypospadia, and may potentially increase the risk of demasculinization in the human male population (Kelce et al., 1994; Foster, 1997). Moreover, estrogens at concentrations above physiological levels are antagonists of the AR (Wilson and French, 1976; Wade et al., 1997). To our knowledge no PCB congener are to date reported to possess antiandrogenic effects. We demonstrate that the PCB congener # 138 has significantly dose-dependent antiandrogenic activity at physiologic relevant levels. Thus this single agent have pleiotropic effects being able to bind to both the estrogen and androgen receptor. In a recent study (Hany et al., 1999) developmental exposure of rats to a reconstituted PCB mixture composed of 414 PCB congeners (including PCB # 138) according to the congener-pattern in human breast milk was investigated. Maternal exposure to the PCB mixture gave rise to reduced testis weight and serum testosterone levels in the male offspring thus demonstrating persistent antiandrogenic effects.

Humans are at the top of the food chain and will, therefore, contain relative by high amounts of xenobiotics which tend to bioaccumulate. POCs have been detected in human blood (Asplund et al., 1994), adipose tissue and breast milk worldwide (Bates et al., 1994; Dewailly et al., 1994; Furst et al., 1994; Sundhedsstyrelsen, 1999). In particular they accumulate in top rank predators of the aquatic food chain (Mulvad et al., 1996; AMAP, 1998) making the populations using these predators as food items especially exposed. Dewailly et al. (1994) reported that total TCDD equivalents (TEqs) for PCB were 3.5 times higher in milk samples from Inuit than from Caucasian milk samples, and coplanar PCBs make a larger contribution to TEq than dioxins and furans (Dewailly et al., 1994). For the three PCB congeners analyzed in this study, the following concentrations (mean of sum of PCB # 138, PCB # 153, PCB # 180) in the human breast milk collected in the late 80-ties have been reported (μg/kg lipid; n); Inuit (817; 109), Caucasian Quebec (90; 16) (Dewailly et al., 1994), New Zealand (91; 38) (Bates et al., 1994), North Rhine Westphalis, Germany (579; 286) (Furst et al., 1994), Denmark (1993–1994), (217; 37) (Sundhedsstyrelsen, 1999). Assuming milk with 3% fat and a daily intake of 800 ml for a baby of 5 kg, the babies will be exposed to a high level ranging from approximately 2–20 μg per day of these three PCB congeners. It has been suggested that the fetal as well as the neonatal and early-life is the most sensitive period of exposure (Safe, 1994; Cogliano, 1998). Since the transfer of PCBs over the placenta is almost 100% (AMAP, 1998) and the risk of developmental disturbances and cancer can be higher for persistent mixtures (Asplund et al., 1994; Safe, 1994), it is important to assess the exposure through human milk and other pathways. Moreover, it has been shown in animal tests that prenatal exposure to POCs, including PCBs is associated with toxicity and abnormal sexual development due to endocrine disruption (Colborn et al., 1993; Birnbaum, 1994; Wolff and Toniolo, 1995; Ahlborg et al., 1995b; Cogliano, 1998; Van den Berg et al., 1998). In summary, this study shows that three environmentally persistent and highly bioaccumulated PCB congeners are pleiotropic and possess receptor mediated antiestrogenic (PCB # 138, PCB # 153, PCB # 180) and antiandrogenic (PCB # 138) effects. Their environmen-

tal abundance and endocrine disruptive potential warrants further research to establish if these di-*ortho* PCBs should he incorporated in future human risk assessments of PCBs.

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References

Ahlborg, U.G., Lipworth, L., Titus Ernstoff, L., Hsieh, C.C., Hanberg, A., Baron, J., Trichopoulos, D., Adami, H.O., 1995a. Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: an assessment of the biological and epidemiological evidence. Crit. Rev. Toxicol. 25, 463–531.

Ahlborg, U.G., Lipworth, L., Titus-Ernstoff, L., Hsieh, C.C., Hanberg, A., Baron, J., Trichopoulos, D., Adami, H.O., 1995b. Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: an assessment of the biological and epidemiological evidence. Crit. Rev. Toxicol. 25, 463–531.

AMAP, 1998. Assessment report: Arctic pollution issues. Arctic monitoring and assessment programme (AMAP), Oslo, Norway.

Arcaro, K.F., Yi, L., Seegal, R.F., Vakharia, D.D., Yang, Y., Spink, D.C., Brosch, K., Gierthy, J.F., 1999. 2,2′,6,6′-Tetrachlorobiphenyl is estrogenic in vitro and in vivo. J. Cell. Biochem. 72, 94–102.

Asplund, L., Svensson, B.G., Nilsson, A., Eriksson, U., Jansson, B., Jensen, S., Wideqvist, U., Skerfving, S., 1994. Polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (p,p′-DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (p,p′-DDE) in human plasma related to fish consumption. Arch. Environ. Health 49, 477–486.

Bates, M.N., Hannah, D.J., Buckland, S.J., Taucher, J.A., van Maanen, T., 1994. Chlorinated organic contaminants in breast milk of New Zealand women. Environ. Health Perspect. 102 (Suppl. 1), 211–217.

Beato, M., 1989. Gene regulation by steroid hormones. Cell 56, 335–344.

Birnbaum, L.S., 1994. Endocrine effects of prenatal exposure to PCBs, dioxins, and other xenobiotics: implications for policy and future research. Environ. Health Perspect. 102, 676–679.

Bitman, J., Cecil, H.C., 1970. Estrogenic activity of DDT analogs and polychlorinated biphenyls. J. Agric. Food Chem. 18, 1108–1112.

Bonefeld Jorgensen, E.C., Autrup, H., Hansen, J.C., 1997. Effect of toxaphene on estrogen receptor functions in human breast cancer cells. Carcinogenesis 18, 1651–1654.

Borlakoglu, J.T., Walker, C.H., 1989. Comparative aspects of congener specific PCB metabolism. Eur. J. Drug Metab. Pharmacokinet. 14, 127–131.

Cogliano, V.J., 1998. Assessing the cancer risk from environmental PCBs. Environ. Health Perspect. 106, 317–323.

Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans [see comments]. Environ. Health Perspect. 101, 378–384.

Connor, K., Ramamoorthy, K., Moore, M., Mustain, M., Chen, I., Safe, S., Zacharewski, T., Gillesby, B., Joyeux, A., Balaguer, P., 1997. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: structure-activity relationships. Toxicol. Appl. Pharmacol. 145, 111–123.

Crisp, T.M., Clegg, E.D., Cooper, R.L., Wood, W.P., Anderson, D.G., Baetcke, K.P., Hoffmann, J.L., Morrow, M.S., Rodier, D.J., Schaeffer, J.E., Touart, L.W., Zeeman, M.G., Patel, Y.M., 1998. Environmental endocrine disruption: an effects assessment and analysis. Environ. Health Perspect. 106 (Suppl. 1), 11–56.

Desaulniers, D., Leingartner, K., Wade, M., Fintelman, E., Yagminas, A., Foster, W.G., 1999. Effects of acute exposure to PCBs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. Toxicol. Sci. 47, 158–169.

Dewailly, E., Ryan, J.J., Laliberte, C., Bruneau, S., Weber, J.P., Gingras, S., Carrier, G., 1994. Exposure of remote maritime populations to coplanar PCBs. Environ. Health Perspect. 102 (Suppl. 1), 205–209.

Dickson, R.B., Thompson, E.W., Lippman, M.E., 1990. Regulation of proliferation, invasion and growth factor synthesis in breast cancer by steroids. J. Steroid Biochem. Mol. Biol. 37, 305–316.

Evans, R.M., 1988. The steroid and thyroid hormone receptor superfamily. Science 240, 889–895.

Fielden, M.R., Chen, I., Chittim, B., Safe, S.H., Zacharewski, T.R., 1997. Examination of the estrogenicity of 2,4,6,2′,6′-pentachlorobiphenyl (PCB 104), its hydroxylated metabolite 2,4,6,2′,6′-pentachloro-4-biphenylol (HO-PCB 104), and a further chlorinated derivative, 2,4,6,2′,4′,6′-hexachlorobiphenyl (PCB 155). Environ. Health Perspect. 105, 1238–1248.

Foster, P.M.D., 1997. Assessing the effects of chemicals on male reproduction: lessons learned from di-*n*-butyl phalate. CIIT Activities 17, 1–8.

Furst, P., Furst, C., Wilmers, K., 1994. Human milk as a bioindicator for body burden of PCDDs, PCDFs, organochlorine pesticides, and PCBs. Environ. Health Perspect. 102 (Suppl. 1), 187–193.

Gierthy, J.F., Arcaro, K.F., Floyd, M., 1997. Assessment of PCB estrogenicity in a human breast cancer cell line. Chemosphere 34, 1495–1505.

Gray, L.E., Jr, Wolf, C., Lambright, C., Mann, P., Price, M., Cooper, R.L., Ostby, J., 1999. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p′-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol. Ind. Health 15, 94–118.

Hany, J., Lilienthal, H., Sarasin, A., Roth-Harer, A., Fastabend, A., Dunemann, L., Lichtensteiger, W., Winneke, G., 1999. Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol. Appl. Pharmacol. 158, 231–243.

Henderson, B.E., Ross, R., Bernstein, L., 1988. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation award lecture. Cancer Res. 48, 246–253.

Hosokawa, S., Murakami, M., Ineyama, M., Yamada, T., Yoshitake, A., Yamada, H., Miyamoto, J., 1993. The affinity of procymidone to androgen receptor in rats and mice. J. Toxicol. Sci. 18, 83–93.

Imagawa, W., Bandyopadhyay, G.K., Nandi, S., 1990. Regulation of mammary epithelial cell growth in mice and rats. Endocrinol. Rev. 11, 494–523.

Jansen, H.T., Cooke, P.S., Porcelli, J., Liu, T.C., Hansen, L.G., 1993. Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod. Toxicol. 7, 237–248.

Jorgensen, E.C., Autrup, H., 1995. Effect of a negative regulatory element (NRE) on the human CYP1A1 gene expression in breast carcinoma MCF-7 and hepatoma HepG2 cells. FEBS Lett. 365, 101–107.

Jorgensen, E.C., Autrup, H., 1996. Autoregulation of human CYP1A1 gene promotor activity in HepG2 and MCF-7 cells. Carcinogenesis 17, 435–441.

Kelce, W.R., Monosson, E., Gamcsik, M.P., Laws, S.C., Gray, L.E., Jr, 1994. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. Toxicol. Appl. Pharmacol. 126, 276–285.

Kelce, W.R., Stone, C.R., Laws, S.C., Gray, L.E., Kemppainen, J.A., Wilson, E.M., 1995. Persistent DDT metabolite p,p′-DDE is a potent androgen receptor antagonist [see comments]. Nature 375, 581–585.

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.

Kharat, I., Saatcioglu, F., 1996. Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor. Cross-talk between aryl hydrocarbon- and estrogen-mediated signaling. J. Biol. Chem. 271, 10533–10537.

Korach, K.S., Sarver, P., Chae, K., McLachlan, J.A., McKinney, J.D., 1988. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol. Pharmacol. 33, 120–126.

Krishnan, V., Porter, W., Santostefano, M., Wang, X., Safe, S., 1995. Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 cells. Mol. Cell. Biol. 15, 6710–6719.

Li, M.H., Hansen, L.G., 1996. Enzyme induction and acute endocrine effects in prepubertal female rats receiving environmental PCB/PCDF/PCDD mixtures. Environ. Health Perspect. 104, 712–722.

Lind, P.M., Eriksen, E.F., Sahlin, L., Edlund, M., Orberg, J., 1999. Effects of the antiestrogenic environmental pollutant 3,3′,4,4′, 5-pentachlorobiphenyl (PCB # 126) in rat bone and uterus: diverging effects in ovariectomized and intact animals. Toxicol. Appl. Pharmacol. 154, 236–244.

Lippman, M.E., Dickson, R.B., 1989. Mechanisms of normal and malignant breast epithelial growth regulation. J. Steroid Biochem. 34, 107–121.

McFarland, V.A., Clarke, J.U., 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Perspect. 81, 225–239.

Moore, M., Mustain, M., Daniel, K., Chen, I., Safe, S., Zacharewski, T., Gillesby, B., Joyeux, A., Balaguer, P., 1997. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicol. Appl. Pharmacol. 142, 160–168.

Mulvad, G., Pedersen, H.S., Hansen, J.C., Dewailly, E., Jul, E., Pederson, M.B., Bjerregaard, P., Malcom, G.T., Deguchi, Y., Middaugh, J.P., 1996. Exposure of Greenlandic Inuit to organochlorines and heavy metals through the marine food-chain: an international study. Sci. Total Environ. 186, 137–139.

Nelson, J.A., 1974. Effects of dichlorodiphenyltrichloroethane (DDT) analogs and polychlorinated biphenyl (PCB) mixtures on 17β-(3H)estradiol binding to rat uterine receptor. Biochem. Pharmacol. 23, 447–451.

Nesaretnam, K., Darbre, P., 1997. 3,5,3′,5′-tetrachlorobiphenyl is a weak oestrogen agonist in vitro and in vivo. J. Steroid Biochem. Mol. Biol. 62, 409–418.

Nesaretnam, K., Corcoran, D., Dils, R.R., Darbre, P., 1996. 3,4,3′,4′-Tetrachlorobiphenyl acts as an estrogen in vitro and in vivo. Mol. Endocrinol. 10, 923–936.

Ostby, J., Kelce, W.R., Lambright, C., Wolf, C.J., Mann, P., Gray, L.E., Jr, 1999. The fungicide procymidone alters sexual differentiation in the male rat by acting as an androgen-receptor antagonist in vivo and in vitro. Toxicol. Ind. Health 15, 80–93.

Ramamoorthy, K., Vyhlidal, C., Wang, F., Chen, I., Safe, S., McDonnell, D.P., Leonard, L.S., Gaido, K.W., 1997. Additive estrogenic activities of a binary mixture of 2′,4′,6′-trichloro- and 2,3′,4′,5′-tetrachloro-4-biphenylol. Toxicol. Appl. Pharmacol. 147, 93–100.

Safe, S.H., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. 24, 87–149.

Safe, S.H., Zacharewski, T., 1997. Organochlorine exposure and risk for breast cancer. Progr. Clin. Biol. Res. 396, 133–145.

Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., Serrano, F.O., 1995. The E-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ. Health Perspect. 103 (Suppl. 7), 113–122.

Spink, D.C., Hayes, C.L., Young, N.R., Christou, M., Sutter, T.R., Jefcoate, C.R., Gierthy, J.F., 1994. The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17β-estradiol 4-hydroxylase. J. Steroid Biochem. Mol. Biol. 51, 251–258.

Sundhedsstyrelsen, 1999, Indhold af dioxiner, PCB, visse chlorholdige pesticider, kviksølv og selen i modermælk hos danske kvinder 1993–1994. Danish Veterinary and Food Administration, The National Health Services.

Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

Villalobos, M., Olea, N., Brotons, J.A., Olea Serrano, M.F., Ruiz de Almodovar, J.M., Pedraza, V., 1995. The E-screen assay: a comparison of different MCF7 cell stocks. Environ. Health Perspect. 103, 844–850.

Vinggaard, A.M., Joergensen, E.C., Larsen, J.C., 1999. Rapid and sensitive reporter gene assays for detection of antiandrogenic and estrogenic effects of environmental chemicals. Toxicol. Appl. Pharmacol. 155, 150–160.

Vonier, P.M., Crain, D.A., McLachlan, J.A., Guillette, L.J., Jr, Arnold, S.F., 1996. Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environ. Health Perspect. 104, 1318–1322.

Wade, T.L., Chambers, L., Gardinall, P.R., Serlcano, J.L., Jackson, T.J., Tarpley, R.J., Suydam, R., 1997. Toxaphene, PCB, DDT, and chlordane analyses of Beluga whale blubber. Chemosphere 34, 1351–1357.

Wilson, E.M., French, F.S., 1976. Binding properties of androgen receptors. Evidence for identical receptors in rat testis, epididymis, and prostate. J. Biol. Chem. 251, 5620–5629.

Wolff, M.S., Toniolo, P.G., 1995. Environmental organochlorine exposure as a potential etiologic factor in breast cancer. Environ. Health Perspect. 103 (Suppl. 7), 141–145.

Wong, C., Kelce, W.R., Sar, M., Wilson, E.M., 1995. Androgen receptor antagonist versus agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. J. Biol. Chem. 270, 19998–20003.

Yager, J.D., Liehr, J.G., 1996. Molecular mechanisms of estrogen carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 36, 203–232.