PCB138, BUT NOT PCB153 AND PCB180, ACTS AS A WEAK ANTIANDROGEN IN VITRO

Anne Marie Vinggaard[1] and Eva Cecilie Bonefeld Jørgensen[2]

[1]Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Denmark
[2] Department of Environmental and Occupational Medicine, University of Aarhus, Denmark

Introduction

The polychlorinated biphenyls (PCBs) constitute a group of persistent environmental chemicals including 209 possible congeners exhibiting a variety of chlorine substitution patterns. Due to their lipophilic nature and resistance toward biotransformation, PCBs accumulate in the food chain and all environmental matrixes including human adipose tissue, blood and milk. 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. Depending on the position and number of chlorine substitutions, different classes of PCB congeners elicit a complex spectrum of biological and toxic responses in *in vivo* and *in vitro* models. Some PCBs exert dioxin-like activities mediated through the aryl hydrocarbon receptor (Ah receptor) giving rise to health risk such as organ toxicity and carcinogenesis. Although reports on interaction with other nuclear receptors are sparse, some congeners are hypothesized to possess endocrine disruptive potential through the Ah receptor and through interference with the biological activities of estrogens. Since most studies have been carried out using Aroclor technical mixtures, analysis of specific congeners are important to assess the risk in human exposure.

Several pieces of evidence indicate that environmental chemicals, which are able to bind to the androgen receptor (AR) may have an important impact on abnormalities associated with the developing male reproductive system. An inhibition of the action of the AR during the embryonic stage may lead to alterations in the development of the male external genitalia such as cryptorchidism and hypospadia. Thus, environmental antiandrogens may have contributed to the increasing incidence of reproductive abnormalities observed in the human male population.

The objective of the present study was to test the three PCBs which are most abundant in human breast milk (#138, #153, #180) for their ability to either agonize or antagonize the human androgen receptor (hAR) in a sensitive reporter gene assay. Comparison of these data to the potential Ah receptor activity of the PCBs was made.

Materials and Methods

Androgen receptor 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 % fetal bovine serum (BioWhitaker, Walkersville, MD). The assay was performed essentially as described in [1]. The cells were seeded in microtiter plates (Costar, Acton, MA) at a density of 5000 cells per well in DMEM/F12 containing 10 %

charcoal-treated fetal bovine serum (Hyclone, Logan, Utah) and incubated at 37°C in a humidified atmosphere of 5% CO_2/air. After 24 hr, test compounds ± 0.1 nM R1881 were added. The test solutions were prepared from stock solutions in ethanol (final ethanol concentration was 0.1-0.36 %). Each well was transfected with a total of 50 ng DNA consisting of the expression vector pSVAR0 and the 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 hr, the cells were lysed by adding 15 µl per well of a 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. Five µl was transferred to white Dynatech microtiter plates for measurement of luciferase activity in a BioOrbit Galaxy luminometer. Ten µl of a substrate containing 1 mM luciferin (Amersham Int., Buckinghamshire, U.K.) 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 sec interval after an incubation time of 2 sec.

Cytotoxicity test using AlamarBlue. Testing for cytotoxicity specifically on cell number was determined by measuring the reduction of AlamarBlue (Serotec,Kidlington,U.K) as described [1].

Ah receptor assay (CALUX assay). The **C**hemical-**A**ctivated **Lu**ciferase **E**xpression assay (CALUX assay) is based on H4IIE cells, stably transfected with a luciferase reporter gene, that were kindly provided by Dr. Abraham Brouwer, Waageningen University, The Netherlands [2]. Cells were maintained in MEMα medium supplemented with 5% FBS and 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were seeded at a density of 2.21 * 10^4 cells/well (100 µl/well) into Costar microtiter plates. After an incubation period of 24 h (80-90 % confluency of cells), PCBs (0.001 - 10 µM) and TCDD (0.003-3 nM) were added. The DMSO concentration was maximally 0.4 %. The following day, cells were washed twice with PBS and added 20 µl of lysis buffer (as used in the AR assay). Luciferase activity was determined as described above for the AR assay.

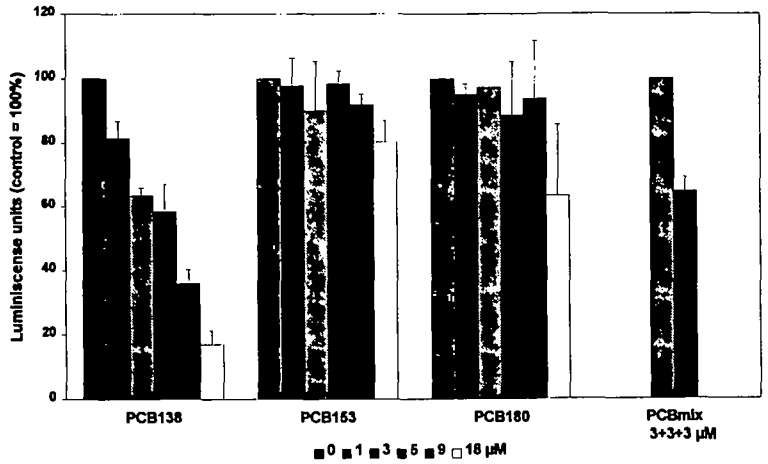

Fig 1. Antiandrogenic effects of PCBs

Data are presented as luminiscence units in %. The response to 0.1 nM R1881 was set to 100 %. Data represent the mean ± SD of three experiments performed in quadruplicate.

Results and Discussion

PCB138 showed statistically significant antagonistic effects on the hAR at all concentrations tested (Fig 1). No antagonistic effect of the other two PCB congeners was found. None of the PCBs excerted agonistic effects (*not shown*). Testing of a mixture of the three PCB congeners, each at a concentration of 3 μM, gave rise to a 40 % decrease compared to the control value. The magnitude of this response was comparable to the response caused by PCB138 3 μM alone, indicating that no complex interaction between the PCBs takes place at the receptor level.

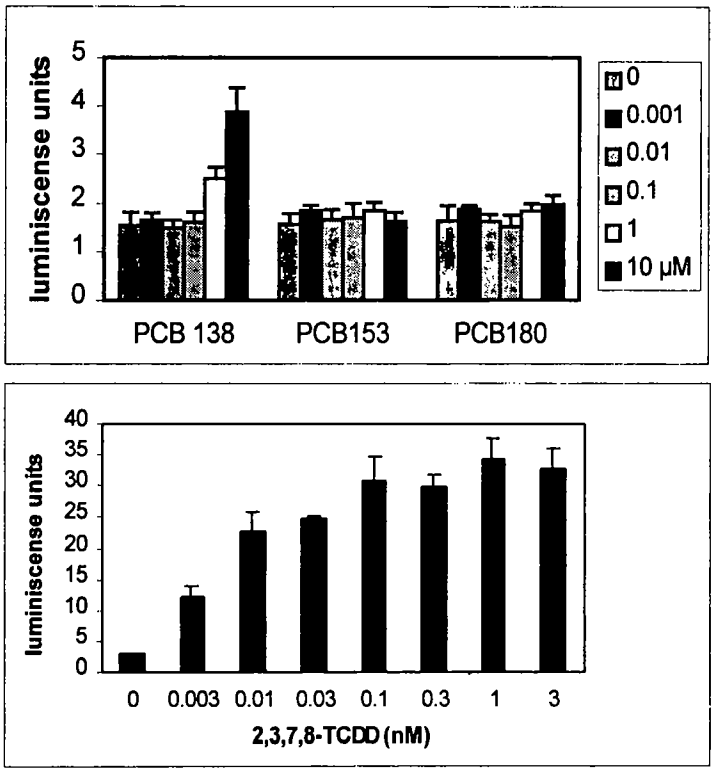

Fig 2. Activation of the Ah receptor by PCBs determined in the CALUX assay

Data are presented as luminiscence units relative to control values (=1). The actual luminiscence units for a control incubation was 495 ± 96 units (mean ± SD, n = 3). Results represent the mean ± SD of 4 independent experiments performed in quadruplicate.

Testing of the three diortho PCB congeners for Ah receptor activity in the CALUX assay, showed as expected that PCB153 and PCB180, which are known to cause phenobarbital-type, but not methylcholanthrene-type CYP450 enzyme induction, did not activate the Ah receptor at concentrations up to 10 μM. In contrast, a slight agonistic effect by PCB138 was observed at a concentration of 1 μM. This congener causes both phenobarbital-type and methylcholanthrene-type CYP450 enzyme induction. Thus, only PCB138 (or its metabolite) among these three congeners is capable of binding to the hAR and Ah receptor.
Kelce and coworkers have previously identified the pesticide (metabolites) vinclozolin, p,p'-DDE and procymidon as antiandrogenic compounds. In addition, we have shown that some PAHs are acting as weak antiandrogens [3]. This study demonstrates further evidence for the promiscuity of

the AhR to bind structurally different compounds, as the environmentally prevalent PCB138 or its metabolite(s) is capable of interacting *in vitro* with the hAR and inducing an AR-mediated effect.
Recently, data from a study involving developmental exposure of rats to a reconstituted PCB mixture (composed according to the congener-specific pattern in human breast milk) or Arochlor 1254 was reported. Adult male offspring with maternal exposure to either PCB mixture showed markedly reduced testis weights and serum testosterone levels, thus demonstrating persistent antiandrogenic effects [4]. Future experiments may show if PCB138 has any *in vivo* antiandrogenic effects and is affecting the endpoints measured in the above-mentioned study.

References

1. Vinggaard, A.M., Jørgensen, E.C.B. and Larsen, J.C.(1999) Rapid and sensitive reporter gene assays for detection of antiandrogenic and estrogenic environmental chemicals. Toxicol.Appl. Pharmacol. 155(2), 150-160.

2. Murk,A.J., Legler,J., Denison,M.S., Giesy,J.P., van den Guchte,C., and Brouwer,A. (1996) Chemical-Activated Luciferase Gene Expression (CALUX): A novel *in vitro* bioassay for Ah receptor active compounds in sediments and pore water. Fundam.Appl.Toxicol. 33,149.
3. Vinggaard, A.M., Hnida,C., & Larsen,J.C. (2000) Environmental polycyclic aromatic hydrocarbons affect androgen receptor activation in vitro. Toxicology, 145(2-3), 159-169.
4. Hany,J., Lilienthal,H., Sarasin,A.,Roth-Härer,A.,Fastabend,A.,Dunemann,L.,Lichtensteiger,W., and Winneke,G.(1999) Developmental exposure of rats to a reconstituted PCB mixture or Arochlor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol.Appl.Pharmacol. 158,231-243.

Acknowledgements

The support by the Danish Medical Research Council is greatly acknowledged. Thanks to Lonnie Sand for excellent technical assistance. Thanks to Dr. Albert Brinkmann, Dept. of Endocrinology and Reproduction, Erasmus University, Rotterdam for having provided the pSVAR0 and the MMTV-LUC constructs. The MMTV-LUC construct was originally developed by Organon BV. Thanks to Dr. Abraham Brouwer, Waageningen University, The Netherlands, for having provided the CALUX assay.