# NON-DIOXIN-LIKE PCBs SUPPRESS THE AHR-DEPENDENT GENE EXPRESSION AND PROLIFERATION IN RAT LIVER CELLS

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## Introduction

Polychlorinated biphenyls (PCBs) are a group of hydrophobic chlorinated compounds characterized by high persistence, toxicity, bioaccumulative properties and their widespread distribution in the environment. The biological effects of individual PCB congeners strongly depend on the number and position of chlorine atoms. The non-*ortho*-substituted coplanar PCBs are known to elicit a set of adverse "dioxin-like" effects associated with the activation of the aryl hydrocarbon receptor (AhR), including reproductive toxicity, dermal toxicity, immunotoxicity, developmental toxicity, neurotoxicity, carcinogenesis and hepatotoxicity (1-3). Induction of CYP1 enzymes or AhR controlled luciferase reporter gene is commonly used for determination of AhR transactivation (4). Apart form regulating CYP1 expression, the AhR triggers also proliferation of confluent liver epithelial cells (5). Di-*ortho*-substituted PCBs, known as non-dioxin-like (NDL)-PCBs, exhibit a different spectrum of toxic modes of action, neurotoxicity, immunotoxicity, endocrine disruption or tumor promotion (6,7,8). In the present study we investigated the antagonistic potencies of NDL PCBs on AhR-mediated effects of 3,3´,4,4´,5-pentachlorobiphenyl (PCB 126) and/or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

## Material and methods

### Chemicals

PCBs were provided by Promochem (Germany). The NDL congeners were purified from dioxin-like compounds, as previously described (9). TCDD was from Cambridge Isotope Laboratories (Andover, MA, USA). DMSO was purchased from Sigma-Aldrich (Prague, Czech Republic).

### DR-CALUX®

The rat hepatoma H4IIEGud.Luc1.1 cell line, stably transfected with a luciferase reporter gene under the control of dioxin responsive elements, was used to detect the AhR-mediated activity in the DR-CALUX® assay (4). The assays were performed in 96-well cell culture plates. The cells were grown 24 h to 90-100% confluency and then exposed to the test compounds and 15 pM TCDD, both dissolved in DMSO, for 24 h. After 24 h of incubation, the medium was removed; cells were lysed in 30 µl of Triton-lysis buffer and luciferase activity was measured with a luminometer. AhR-antagonistic potencies of the individual PCB congeners were determined relative to 2,2',4,4'-tetrabrominated diphenyl ether (BDE-47), which was used as a reference AhR antagonistic compound (10).

### Cultivation and exposure of WB-F344 cells

Rat liver epithelial cells WB-F344 (kindly provided by Dr. J.E. Trosko, MSU, East Lansing, MI) were grown to confluency and exposed for 24 h to 5 nM PCB 126 or 50 pM TCDD with or without co-treatment with tested PCB congeners. Cytotoxicity was measured by the neutral red (NR) uptake assay after 24h exposure (11). None of PCB congeners were cytotoxic at the concentrations used in this study.

### Inhibition of WB-F344 cell proliferation

The inhibitory effect of selected NDL-PCBs on PCB 126-induced cell proliferation was determined in confluent WB-F344 cells, as previously described (12). Confluent cells were seeded at an initial density of 30,000 cells/cm$^2$ in 4-well cell-culture plates (Nunc, Roskilde, Denmark) and grown until confluency (after 72 h). Medium was refreshed and the cells were exposed to test compounds dissolved in DMSO for another 24 h. The final concentration of DMSO did not exceed 0.1% (v/v). After treatment, the medium was removed, cells were harvested with trypsinization and counted by Coulter Counter (Model ZM, Coulter Electronics, Luton, UK).

**Determination of CYP1A1 and CYP1B1 mRNA levels**

The mRNA levels of CYP1A1 and CYP1B1 were determined in WB-F344 cells by real-time RT-PCR (Tab. 1). Total RNA was isolated from cells using the NucleoSpin RNA II kit (Macherey-Nagel, Duren, Germany), including treatment with DNase I (Qiagen GmbH, Hilden, Germany). The amplifications of the samples were carried out using QuantiTect RT Mix (Qiagen GmbH, Hilden, Germany) according to manufacturer´s specifications. The CYP1A1 and **CYP11** probes were labeled with the fluorescent reporter dye 6-carboxyfluorescein (6-FAM) on the 5´-end and with either LC red 640 or the Black Hole 1 (BH 1) fluorescent quencher dyes on the 3´-end, respectively. The amplifications were run on the LightCycler (Roche Diagnostics, Mannheim, Germany). Changes in gene expression were calculated using the comparative threshold cycle method, using with porphobilinogen deaminase (EC 4.3.1.8; PBGD) as a reference gene (13).

**Table 1.** Primer and probe sequences used for quantitative real-time RT-PCR

| Genes | | Primes and probe sequences[a] |
|---|---|---|
| *CYP1a1* | F | 5´- TGAGAAGGGCCACATCC-3´ |
| | R | 5´- GGGTTGGTTACCAGGTACA -3´ |
| | P | 5´- TCACAACTGCTATCTCTTGGAGCCTC -3´ |
| | P | 5´- TTTGGAGCTGGGTTTGACACA-3´ |
| *CYP1b1* | F | 5´- CTCATCCTCTTTACCAGATACCCG-3´ |
| | R | 5´- GACGTATGGTAAGTTGGGTTGGTC-3´ |
| | P | 5´- CTCATGCAGGGCAGGCGGTCCCTCCCC-3´ |
| *PBG-D* | F | 5´-CCCAACCTGGAATTCAAGAGTATTCG-3´ |
| | R | 5´-TTCCTCTGGG TGCAAAATCTGGCC-3´ |
| | P | 5´-CCTCAACACCCGCCTTCGGAAGCT-3´ |

[a] The primers and probes are identified by letters designating the forward (F) or the reverse (R) primer, or the probe (P).

**Results and discussion**

First, suppressive effects of highly purified NDL-PCB congeners on TCDD-induced AhR transactivation were screened in the DR-CALUX® bioassay and expressed as relative potency (REP) values compared to the AhR-antagonistic reference compound BDE-47. Potencies of selected congeners to suppress AhR activation were then confirmed, using AhR-dependent induction of proliferation of confluent WB-F344 cells as an endpoint. Here, PCB 126 was used as AhR agonist (Tab. 2).

Second, suppression of TCDD-induced AhR-dependent *CYP1a1* and *CYP1b1* gene expression was determined in WB-344 cells for the most abundant PCB congeners, which were also active AhR antagonists in the DR-CALUX® and WB-F344 proliferation bioassays (Fig. 1). The suppression of CYP1A1 mRNA induction in WB-F344 cells closely correlated with potencies of NDL-PCB congeners to inhibit the AhR transactivation in the DR-CALUX® bioassay. In contrast, we observed only a partial suppression of CYP1B1 mRNA and cell proliferation, which occurred at higher doses, thus suggesting that these endpoints may reflect also regulation by additional signaling pathways.

Taken together, NDL-PCB congeners suppressed TCDD- or PCB 126-induced AhR-dependent events in two different rat liver cellular models, hepatoma H4IIE and WBF-344 cells. PCB 128, 138 and 170 were the most potent congeners, followed by PCB 28, 47 and abundant PCB 180. Significant AhR-antagonistic effects were also found for PCB 153 and other most abundant PCBs (Tab. 2).

**Table 2.** AhR-antagonistic responses of the 24 PCBs in the DR-CALUX® bioassay and in the WB-F344 proliferation test. Abbreviations: n, no suppression; w, weak suppression; BDE-47 REP, 2,2',4,4'-tetrabrominated diphenyl ether relative potencies; SD, standard deviation

| | TCDD induced AhR-transactivation (DR-CALUX) | | PCB-126 induced AhR-dependent proliferation in confluent cells WB-F344 | |
|---|---|---|---|---|
| **PCB congener** | **$IC_{50}$ (µM) ± SD** | **BDE-47 REP ± SD** | **$IC_{25}$ (µM)** | **LOEC (<90 % proliferation)** |
| **28** | 9.0 ± 2.9 | 0.43 ± 0.14 | 30 | 13 |
| **47** | 10 ± 3 | 0.38 ± 0.10 | 35 | 7 |
| **52** | 9.3 ± 1.8 | 0.40 ± 0.08 | N | - |
| **80** | >15 | <0.25 | N | - |
| **95** | >15 | <0.25 | W | 17 |
| **101** | - | - | N | - |
| **122** | 14 ± 5 | 0.27 ± 0.10 | 42 | 12 |
| **128** | 1.5 ± 0.6 | 2.6 ± 1.1 | 25 | 6 |
| **138** | 1.4 ± 0.1 | 2.7 ± 0.3 | 26 | 10 |
| **153** | 15 ± 0 | 0.25 ± 0.00 | 36 | 15 |
| **170** | 5.6 ± 1.9 | 0.67 ± 0.02 | 27 | <1 |
| **180** | 10 ± 3 | 0.38 ± 0.10 | 43 | 9 |

(A) (B)

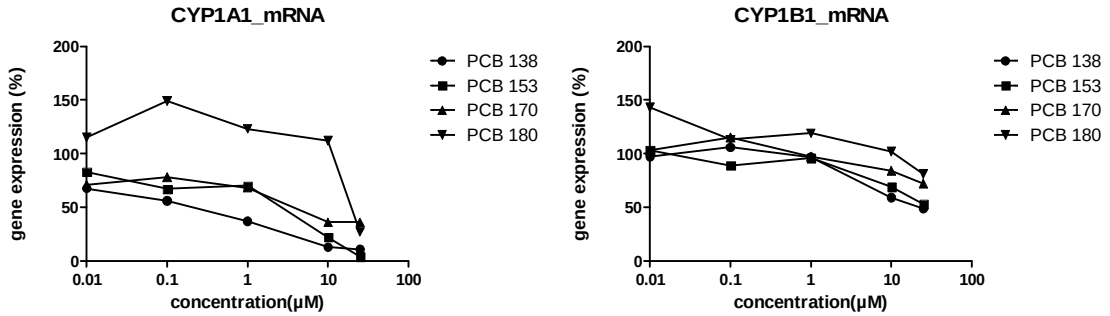


**Figure 1.** Inhibition of 50 pM TCDD-induced gene expression in confluent WB-F344 cell line after 24h coexposure with NDL-PCB congeners. (A) AhR-dependent gene expression of *CYP1a1*, and (B) AhR-dependent expression of *CYP1b1*

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