Antagonistic effects of diortho PCBs (PCB 52 and 128) on Ah receptor-mediated induction of luciferase activity by 3,3',4,4'-tetrachlorobiphenyl (PCB 77) in Mouse Hepa-1c1c7 cells.

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Introduction

Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and related aromatic compounds are present in the environment as complex mixtures of congeners. Exposure of animals and man to these complex chemical substances occurs mainly via dietary intake. A risk assessment model to predict the potential toxic effects of this group of compounds has been developed, e.g., application of the toxic equivalency factor (TEF) concept to mixtures of PCDDs, PCDFs and PCBs[1]. In order to do this, one should have information on potential interactive effects between different congeners occurring in environmental samples.

With a view to monitoring dioxin-like toxicity of environmental samples, we have recently developed a mouse hepatoma cell line showing Ah receptor-mediated luciferase expression, as described elsewhere[2]. Here we report on a study on the interaction between the non-ortho-substituted, dioxin-like 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and two different diortho-substituted PCBs using our *in vitro* luciferase induction biological effect assay.

Materials and methods

Luciferase induction assay

pGudluc1.1-transfected Hepa-1c1c7 cells[2] were grown until confluent in 6-well plates

(Costar, 30 mm well diameter) in 3 ml of α-MEM supplemented with 10% (v/v) fetal calf serum ($\pm 1.4 \cdot 10^6$ cells/well). Pure, commercial PCB congeners (C.N.Schmidt B.V., Amsterdam, The Netherlands) were added to the culture medium in DMSO (Janssen, Cat. No. 16.785.04, Beersse, Belgium), the total concentration of which was kept constant at 0.2% in control and exposed wells. After 24 hours of exposure, luciferase activity was assayed using the Promega luciferase assay system, essentially according to the instructions of the manufacturer. Cells were harvested in 250 µl of Promega cell lysis reagent per well. Luminescence produced by 20 µl of sample (equivalent to about 10^5 cells) was quantified as the average counts per minute (cpm) detected during 10 subsequent 0.5 min measurements in a Canberra Packard 1600TR scintillation counter, operated in the single photon counting mode. When the linear response range of the counter was exceeded, the sample was diluted with cell lysis reagent (Promega) containing 1 mg/ml bovine serum albumin.

Results

Mouse Hepa-1c1c7 hepatoma cells stably transfected with a dioxin-inducible luciferase gene were exposed to PCB 77 alone and to PCB77 in combination with various concentrations of 2,2',3,3',4,4'-hexachlorobiphenyl (PCB128) (Fig. 1) or 2,2',5,5'-tetrachlorobiphenyl (PCB52) (Fig. 2).

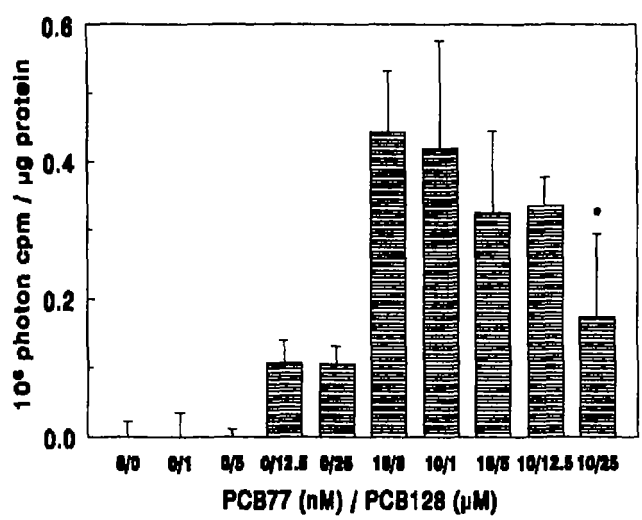

Fig. 1. Antagonistic effect of PCB128 on the Ah receptor-mediated response generated by PCB77 in the luciferase induction assay (* indicates a significant difference ($p<0.05$) with the level of luciferase expression induced by 10 nM of PCB77 alone).

In both cases, a clear dose-dependent antagonistic effect of the diortho substituted PCBs on the luciferase induction by the dioxin-like, non-ortho substituted PCB77 was observed. Although the higher concentrations of PCB 52 alone seemed even less active than low concentrations with respect to luciferase induction, this concentration effect relationship per se is obviously insufficient to explain the strong antagonistic interaction of PCB 52 with luciferase induction by PCB 77. Similar antagonisitic effects were observed when 2,3,7,8-TCDD was used in combination with PCB 52 and PCB 128 (data not shown).

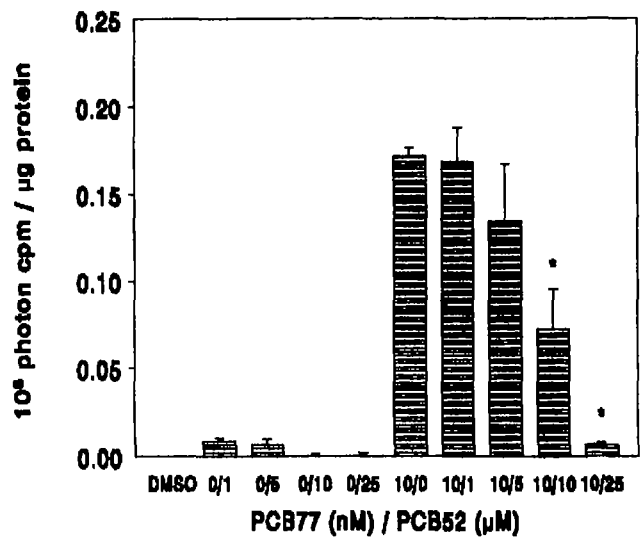

Fig. 2. Antagonistic effect of PCB52 on the Ah receptor-mediated response generated by PCB77 in the luciferase induction assay (* indicates a significant difference ($p<0.05$) with the level of luciferase expression induced by 10 nM of PCB77 alone).

Discussion

In addition to additive interactions reported for the toxic and biochemical effects induced by mixtures of 2,3,7,8-substituted PCDDs, PCDFs and the non-ortho substituted planare PCBs[1,3], both synergistic[4-6] and antagonistic[7,8] interactions have been reported for combinations with non-planar mono- and diortho PCBs and commercial PCB mixtures. The here reported antagonistic effects of diortho-PCB congeners (PCB 52 and 128) on Ah-receptor mediated luciferase induction by PCB 77 is probably not due to substrate inhibition as has been observed before in our laboratory for PCB 77 on EROD activity in hepatic microsomal suspensions (Morse, personal communication). Recent additional work on these antagonistic effects of diortho-PCBs on PCB-77 and TCDD-induced luciferase induction, indicates that the antagonistic effects are species-specific and also

occurs at the level of Ah receptor binding to dioxin responsive elements[9].

Acknowledgements

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