INDUCTION OF CYP1A ISOZYMES IN THE HUMAN HEPATOBLASTOMA CELL LINE HEPG2, THE RAT HEPATOMA CELL LINE H4IIE AND RAT PRIMARY HEPATOCYTES BY 'DIOXIN-LIKE' POLYCHLORINATED BIPHENYLS (PCBs). COMPARISON OF POTENCIES

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Introduction

Polychlorinated biphenyls (PCBs) are a family of 209 compounds (congeners) differing in extent and position of chlorination of their aromatic rings. They were extensively used in industrial applications because of their insulating and flame retardant properties, leading to widespread distribution in soil and water. Because of their lipophilic character, chemical stability and slow rate of degradation they tend to accumulate in adipose tissue of animals and humans, and although production and use have been terminated they are still present in the food chain and environmental matters[1].

Some of the PCB congeners, especially those with non- and mono-*ortho* chlorine substitution show patterns of toxicity resembling those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), including teratogenicity, endocrine disorders, tumor promotion and adverse effects on skin, immune system and reproduction[2]. There is sufficient evidence that these compounds share a common mechanism of action of toxicity, involving agonist binding to the cytosolic aryl hydrocarbon receptor (AhR)[3]. The ligand-activated receptor forms a heterodimer with the nuclear protein ARNT ('AhR nuclear translocator'), and after binding to specific DNA elements (XRE, xenobiotic responsive elements) increases transcription of several dioxin-depending genes (AhR gene battery)[4]. Among these genes, increased expression of cytochrome P450 1A1 (CYP1A1) is a well-understood example forming a parameter for measuring the potency of AhR agonists[5]. The 7-ethoxyresorufin-O-deethylase (EROD) activity of CYP1A isozymes is widely accepted to be used for measuring their induction by 'dioxin-like' compounds[6].

To describe the potency of dioxin-like compounds, the concept of Toxic Equivalency Factors (TEFs) has been developed with TCDD as one of the strongest AhR agonist referring to a TEF value of 1. The TEFs published by the World Health Organization (WHO) for risk assessment in humans and wildlife are consensus values derived from available experimental *in vivo* and *in vitro* data by scientific consideration and evaluation[7]. Following the recommendation of the WHO expert group, the values of our experiments determining the range of CYP1A-inducing potency of 'dioxin-like' PCBs are referred to as relative potencies (REPs).

Previously, we found considerable species differences in the potency of polychlorinated dibenzo-*p*-dioxins (PCDDs) to induce CYP1A isozymes[8-10].

In this work, investigations of the CYP1A-inducing potencies of most 'dioxin-like' PCBs were done in the human hepatoblastoma cell line HepG2, in the rat hepatoma cell line H4IIE, and in rat primary hepatocytes to complete the existing set of data. The data were used to compare the potencies in cells of human and rat origin.

Methods and Materials

Solutions for treatment of cell cultures were obtained by solving PCBs (Promochem, Wesel, Germany) in dimethyl sulfoxide (DMSO) and successive dilutions resulting in final DMSO concentrations of 0.5 % in the culture medium.

The human hepatoblastoma cell line HepG2, and the rat hepatoma cell line H4IIE were a kind gift from F. Wiebel (GSF, Munich, Germany).

Hepatocytes were prepared from male Wistar rats (Charles River, Kisslegg, Germany) as described earlier[11]. Preparations showing viability > 90 % were seeded on collagenated Petri dishes (6 cm diameter) at a density of 70 000 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum (FBS), 0.1 µM dexamethasone, 100 U/ml penicillin, and 100 µg/ml streptomycin. After 3 h, medium was replaced by fresh medium, PCBs were added, and the cells were incubated for additional 48 h. DMSO alone served as a negative control.

Cell lines were seeded on the same dishes at a density of 10 000 cells/cm^2 in the same medium. After reaching 50 % confluency, medium was replaced by fresh medium and cells were treated as described above.

After incubation, cells were washed with ice-cold saline and scraped of with a Tris-buffered glucose solution. After centrifugation, cells were homogenized by sonication at 50 W on ice. EROD activity in the homogenates were measured according to the method of Burke and Mayer[12].

Treatments were carried out in duplicate in three independent experiments. Dose-response curves, and EC_{50} values were calculated using a computerized log-probit procedure (Origin 5.0, Microcal, Northampton, USA).

Results and Discussion

The EC_{50} value for TCDD in HepG2 cells was found to be within the same order of mangnitude as the earlier published value, i.e. 0.68 nM vs. 0.31 nM[9].

The data show that the inducibility of CYP1A by TCDD in the human HepG2 line is one order of magnitude below that in the rat hepatoma cell line.

The PCBs also showed much higher EC_{50} values in human than in rat cells, i.e. they are less potent. The non-*ortho* congeners 126 and 169 elicit the most extensive species differences. The EC_{50} value of PCB 126 was found to be two orders of magnitude higher in human than in rat cells, whereas PCB 169 failed to induce EROD activity. The EC_{50} values for HepG2 were confirmed by Northern blotting, revealing that treatment with congeners not inducing EROD activity does not lead to an increase in CYP1A mRNA.

The reason for the highly reduced potencies of most 'dioxin-like' PCBs may either be related to differences in uptake, metabolism etc. or be based on a reduced ability to activate the AhR complex. The XREs of human CYP1A genes cannot hold fully responsible for reduced induction since the difference in the case of TCDD is only 10-fold.

Tab. 1. EC_{50} values ± S.D. (nM) for induction of EROD activity in HepG2, H4IIE, and rat primary hepatocytes, 48 h after treatment with TCDD and 'dioxin-like' PCBs

Inducer	HepG2	H4IIE	Rat PH
TCDD	0.68 ± 0.02	0.05 ± 0.013[a]	0.02 ± 0.005[a]
PCB 77[n]	2700 ± 230	530 ± 280[a]	140 ± 55[a]
PCB 126[n]	340 ± 10	0.28 ± 0.08[a]	0.22 ± 0.04[a]
PCB 169[n]	n.m.	17 ± 8.4[a]	7.1 ± 3.5[a]
PCB 105[m]	n.m.	4800 ± 2100[a]	270 ± 40[a]
PCB 114[m]	12000 ± 3700	253 ± 1	16 ± 0.5
PCB 118[m]	n.m.	13000 ± 4100[a]	660 ± 180[a]
PCB 123[m]	n.m.	17500 ± 347	1000 ± 73
PCB 156[m]	n.m.	690 ± 190[a]	74 ± 19[a]
PCB 157[m]	n.m.	361 ± 38	29 ± 1.4
PCB 167[m]	n.m.	n.m.	1670 ± 36
PCB 189[m]	n.m.	n.m.	12100 ± 6880

[n] non-*ortho* [m] mono-*ortho* [a] data published earlier[11]
n.m. not measurable PH primary hepatocytes

Tab. 2. EROD-specific REP values of 'dioxin-like' PCBs in HepG2, H4IIE, and rat primary hepatocytes, and corresponding WHO TEFs for human risk assessment and mammals[7]

Inducer	REP (HepG2)	REP (H4IIE)	REP (Rat PH)	WHO TEF
TCDD	1	1	1	1
PCB 77[n]	0.0003	0.00009	0.0001	0.0001
PCB 126[n]	0.002	0.2	0.09	0.1
PCB 169[n]	n.d.	0.003	0.003	0.01
PCB 105[m]	n.d.	0.00001	0.00007	0.0001
PCB 114[m]	0.00006	0.0002	0.001	0.0005
PCB 118[m]	n.d.	0.000004	0.00003	0.0001
PCB 123[m]	n.d.	0.000003	0.00002	0.0001
PCB 156[m]	n.d.	0.00007	0.0003	0.0005
PCB 157[m]	n.d.	0.0001	0.0007	0.0005
PCB 167[m]	n.d.	n.d.	0.00001	0.00001
PCB 189[m]	n.d.	n.d.	0.000002	0.0001

[n] non-*ortho* [m] mono-*ortho*
n.d. not determined for lack of EROD induction PH primary hepatocytes

The results demonstrate striking differences between rat and hepatoma cell lines with respect to REPs of EROD induction for most 'dioxin-like' PCBs. In particular, the non-*ortho*-substituted PCB 169 was inactive, and PCB 126 was less potent by two orders of magnitude in HepG2 cells.

In spite of the species differences between REPs in the cell systems investigated, it should be kept in mind that a tumor cell line like HepG2 may not be fully representative for the function of the normal organ it is derived from. Although own experiments showed a 10-fold higher EC_{50} value of EROD induction for TCDD in human compared to rat hepatocytes the corresponding REPs of PCBs may be different[10,11].
Our findings indicate the need for further investigations in experimental models from different species including humans in order to extend the data base of biochemical and toxic responses to PCBs.

Acknowledgements
The authors wish to thank Silke Ripp for expert technical assistance.

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