EFFECT OF LOW DOSE MONO-ORTHO 2,3´,4,4´,5 PENTACHLOROBIPHENYL (PCB 118) ON THYROID HORMONE STATUS AND EROD ACTIVITY IN RAT OFFSPRING: CONSEQUENCES FOR RISK ASSESSMENT.

Sergio Kuriyama[1], Antonio Fidalgo-Neto[2], Wolfgang Mathar[3], Karen Friedrich[1] and Ibrahim Chahoud[1]

[1]Inst. of Clinical Pharmacology and Toxicology, Benjamin Franklin Medical Center, FU Berlin, Germany.
[2]Laboratory of Environmental Toxicology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.
[3]Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin Berlin, Germany.

Introduction

Polychlorinated biphenyls (PCBs), members of the halogenated aromatic group, are persistent environmental contaminants which are distributed throughout the ecosystem. The pattern of chlorine substitutions in the two phenyl rings gives each PCB congener its characteristic toxicological and biological properties[1]. PCB congeners with non-ortho chlorine substitution in the molecule adopt a coplanar configuration similar to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and can bind with high affinity to the aryl hydrocarbon receptor (AhR) [2]. As TCDD is the most competitive ligand for the AhR and almost all of the toxic effects are mediated by AhR, non-ortho substituted PCBs are also named dioxin-like PCBs[3]. However, congeners with ortho-substitutions (named non-dioxin like PCBs) do not interact with the AhR, but still elicit some toxic effects similar to TCDD such as neurotoxicity, carcinogenicity and endocrine disruption [3]. Although substitution at only one ortho position (mono-ortho PCBs) may achieve partial coplanarity and exhibit weak AhR agonist activity, a wide range of toxic effects are possible. The mono-ortho congener 2,3´,4,4´,5 pentachlorobiphenyl (PCB 118) is regularly detected in human tissue (including breast milk)[4] and it has been shown to alter thyroxine (T4) levels[5], induce 7-ethoxyresorufin-O-deethylase (EROD)[6] and to possess neurotoxic properties[7]. However, there is little information concerning the role of AhR as mediator of toxic effects of mono-ortho PCBs, especially at low dose levels.

Since PCB residues can still be detected in human tissues, the intention of our study was the exposure of rats to PCB 118 in a dose that corresponds with human exposure via breast milk. For that purpose, we calculate the dose in attempt to expose the rats to an amount of PCB 118 which is 100-fold higher than human concentration in breast milk. Thyroid hormone levels, EROD actvitiy and PCB concentration in hepatic and adipose tissue were evaluated using a single low dose of PCB 118 in dams and offspring rats. We used the induction of CYP1A-mediated 7-ethoxyresorufin-*O*-deethylase as a parameter of AhR activation.

Methods and Materials

Sprague-Dawley dams were treated on gestation day 6 by gavage with a single dose of 375 mg PCB118/kg body weight or peanut oil (control). On postnatal day (PND) 22 (weaning), all dams were sacrificed and male and female offspring were housed separately for the subsequent analysis. The levels of PCB 118 in fat tissue and liver were determined on PNDs 22 and 70. Fat extraction (8 g liver,

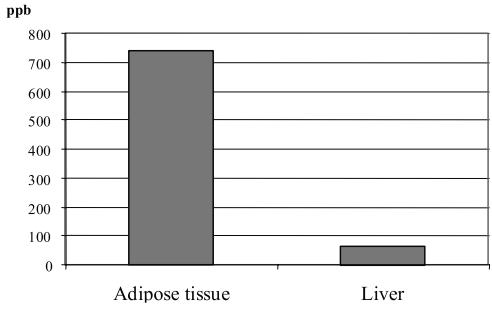

Figure 1. PCB 118 concentracion in dams tissue at weaning (PND 22).

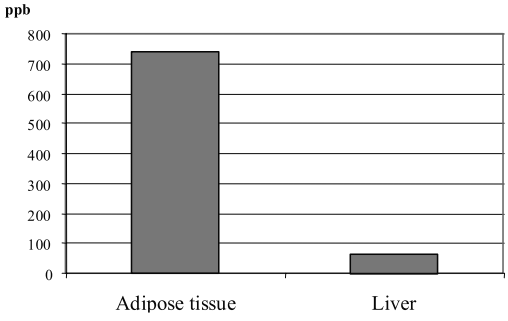

Figure 2. PCB 118 concentracion in offspring tissue at weaning (PND 22) and PND 70.

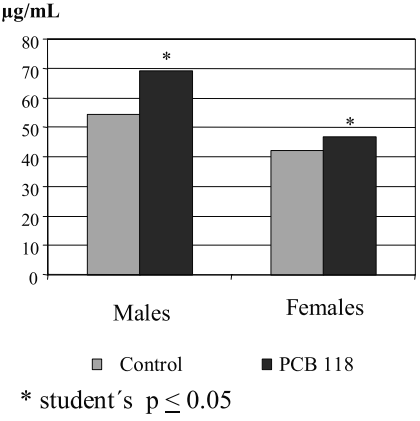

Figure 3. Total T4 Leveis in Offspring on PND 70.

Figure 4. Free T4 and TSH Levels in PND 70.

2 g fat tissue) was conducted by grinding the samples with equal amounts of sodium sulfate and sea sand followed by a 350 mL column extraction with hexane/acetone (2/1 = v/v). Ten to 20 % aliquots of extract were used for gravimetric fat determination. One to 10 % aliquots (addition of ^{13}C-PCB118 as internal standard) were transferred to a column (filled with 3 g silica/sulfuric acid and 1 g potassium silicate, separated by 0.5 g sodium sulfate) for elution with 25 mL hexane. After evaporation, the extracts were dissolved in 1mL i-octane and transferred to a column (filled with 1 g silica) for elution with hexane/toluene (65/35=v/v). After evaporation, the extracts (0.2 mL) were analyzed using HRGC-HRMS. Liver microsomes were obtained by ultracentrifugation (100,000 g) and ethoxyresorufin-O-deethylase (EROD) activities were determined on PND 22 (dams) and, PNDs 70 and 170 (offspring) as reported by Burke *et al.*, except for the use of an NADPH regenerating system which consisted of 0.25 mM ß-NADP, 2.5 mM $MgCl_2$, 5 mM glycose-6-phosphate and 0.5 units of glucose-6-phosphate-dehydrogenase per ml of incubation mixture. Reactions were initiated by the addition of the regenerating system and were carried out in a quartz cuvette at 37 °C. The reaction rate was measured

by increasing fluorescence caused by the accumulation of resorufin. The spectrofluorimeter settings were as follows: excitation wavelength 550 nm and emission wavelength 582 nm, with a 5-nm band slit width. Total serum thyroxine (T4), free serum thyroxine (FT4), total serum triiodothyronine (T3), free serum triiodothyronine (FT3) and TSH levels were determined from dams (weaning) and offspring (PND 70) using ELISA assay (DRG diagnostics GmbH - Germany).

Results and Discussion

The treatment with a single low dose of PCB 118 causes no change in microsomal protein concentration, liver weight and EROD activity in PCB-treated dams (weaning time) as well as in offspring on PNDs 70 and 170 compared to the control. Eventhough a high concentration of PCB 118 was found in livers of offspring and to a lesser extent in dams (fig 1 and 2), no induction of EROD was observed in these animals confirming that this mono-ortho congener is a very weak partial agonist of AhR.. However, the dose regimen used in our study changed the thyroid hormone status in dams and offspring significantly. At weaning, FT4 and TSH levels in dams were significantly depressed compared to controls. On PND 70, total-T4 concentration (male and female) and FT4 (male) levels were increased in offspring (fig 3 and 4). Hypothyroxinemia following PCB 118 administration has been previously reported and two different mechanisms were described. PCB 118 facilitates biliary excretion of T4 by UDP-glucuronyl transferase-induction. Alternatively, the congener binds with high affinity to transthyretin (the serum thyroxine transport protein) and inhibits the serum transport of T4[5]. The increase of thyroxine levels observed in offspring was first described by Davenport *et al.* (1976) who shows that perinatal hypothyroidism causes an age-dependent increase in T4 and TSH. This effect seems to be a “thyroid resistance” syndrome and additional investigation of the hypothalamic-pituitary-thyroid (HPT) and the hypothalamic-pituitary-adrenal (HPA) axes are underway to assess possible effects of PCB on both axes.

The use of the toxic equivalency factor (TEF) has been proposed to estimate the risk of mono-ortho PCBs, based on their ability to interact with AhR and elicit toxic effects similar to TCDD[1, 3]. However, there is little information about the mechanism of toxicity of mono-ortho congeners and the use of the TEF for risk assessment based only on the ability of PCB to interact and activate AhR is not reliable. The data presented in this study show that serum thyroid hormone level is a more sensitive endpoint than EROD assay for this mono-ortho PCB. We conclude that the calculation of TEF based only on EROD activity is not justified to assess toxicity of mono-ortho substituted PCBs.

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