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# Effects of single non-ortho PCBs on human sperm acrosome reaction *in vitro*

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## Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous pollutants, which are highly persistent in the environment. Due to their high lipophilicity they are universially found in human body fluids and tissues. Substantial amoumts of PCBs are also present in human body fluids associated with reproduction such as follicular fluid, seminal fluid and cervical mucus[1]. It was demonstrated that non- and mono-ortho substituted PCBs cause morphological lesions in cultivated rat tubuli seminiferi[2]. However, high concentrations of single non-ortho and di-ortho PCB congeners did not affect motility of ejaculated human spermatozoa in vitro[3]. Therefore, the purpose of the present study was to focus on human sperm function such as the acrosome reaction (AR). The AR is defined as receptor-mediated exocytosis involving fusion of sperm plasma and outer acrosomal membrane with formation of hybrid membrane vesicles. The AR is an essential step in the fertilization process because only acrosome-reacted spermatozoa can penetrate the zona pellucida and fuse with the plasma membrane of the oocyte[4]. Under physiological conditions the AR is induced by the zona pellucida after binding of spermatozoa to the oocyte. Therefore, it is of major interest whether non-ortho, "dioxin-like" PCB congeners may impair the AR of human spermatozoa.

## Materials and Methods

*PCB solutions:* Pure 3,3′,4,4′-tetracholorbiphyl (PCB 77) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), purchased from Promochem, were dissolved in DMSO/toluene [3/2] and added in final concentrations of 0.1 Vol.% DMSO/toluene and 0.05 Vol.% DMSO/toluene to human tubular fluid medium (HTFM), containing 5% of human serum albumin (HSA),. HTFM-HSA with 0.1 Vol.% DMSO/toluene was used as control. The final PCB concentrations in the media were determined by GC/MS analysis (table 1). First solid phase extraction of 1 ml medium was performed on 400 mg Adsorbex RP-19 columns (Merck, Germany). After washing and addition of the corresponding $^{13}C_{12}$-labeled PCB congener as internal standard, PCB were eluted with 1 ml n-heptane and analysed with HRGC/LRMS.

*Sperm preparation:* Fresh ejaculates from 2 to 4 healthy donors were used for each experiment. Motile spermatozoa were separated by glass wool filtration and washed twice with HTFM

containing 5% of human serum albumin. After the second washing procedure, motile spermatozoa were pooled and adjusted to a final concentration of $20x10^6$/ml.
*Experiment I:* Motile spermatozoa were treated with PCB 126 and PCB77 (0.1 Vol.% DMSO/ toluene) for 5 h at 37 $^0$C. Afterwards the spermatozoa were washed twice and incubated for another 16 h in HTFM-HSA at room temperature (spontaneous AR) or 4 $^o$C (induced AR)[5]).

*Experiment II:* Motile spermatozoa were incubated with two concentrations of PCB 126 (0.1 Vol.% DMSO/toluene and 0.05 Vol.% DMSO/toluene) or PCB 77 (0.1 Vol.% DMSO/toluene and 0.05 Vol.% DMSO/toluene) for 5 h at 37 $^0$C. Thereafter, the AR was determined.
The AR of living spermatozoa was determined by triple stain technique[6]). Sperm motility was evaluated using computer-assisted sperm motility analysis (Stroemberg-Mika).

## Results

**Table 1:** Initial PCB concentrations in the culture medium were as follows:

| | |
|---|---|
| PCB 77 (DMSO/toluene 0.1 Vol.%) | 3.27 µg/ml |
| PCB 126 (DMSO/toluene 0.1 Vol.%) | 0.87 µg/ml |

*Experiment I:*

**Table 2:** *AR after long term incubation at room temperature and low temperature*
Mean values ± standard deviation of the percentage of living acrosome reacted spermatozoa. Incubation in contaminated media for 5h and another 16h in HTFM-HSA at room temperature or at at 4 $^o$C (induced AR).
Fisher PLSD test: * signifikant increase ($p<0.05$)

| | AR in % after room temperature incubation n=7 | induced AR in % after low temperature n=7 | Vitality in % n=7 |
|---|---|---|---|
| PCB 126 (DMSO/toluene 0.1 Vol.%) | 27.8 ±9.0% * | 27.9 ±15.4% | 72 |
| PCB 77 (DMSO/toluene 0.1 Vol.%) | 21.7 ±11.1% | 25.0 ±11.9% | 73 |
| Control | 17.1 ±3.5% | 30.1 ±9.2% | 72 |

*Experiment II:*

**Table 3:** *Spontaneous AR after short term incubation*
Mean values ± standard deviation of the percentage of living acrosome reacted spermatozoa. Incubation in contaminated media for 5 h at 37°C.

Fisher PLSD test: ** highly signifikant increase ($p<0.001$)

| | AR in % n=7 | Vitality in % n=7 |
|---|---|---|
| PCB 126 (DMSO/toluene 0.1 Vol.%) | 10.4 ±1.9% ** | 84 |
| PCB 126 (DMSO/toluene 0.05 Vol.%) | 4.4 ±2.8% | n.d. |
| PCB 77 (DMSO/toluene 0.1 Vol.%) | 5.9 ±1.7% | 82 |
| PCB 77 (DMSO/toluene 0.05 Vol.%) | 5.5 ±0.9% | n.d. |
| Control | 3.2 ±1.2% | 80 |

## Discussion

The data clearly demonstrate that the non-ortho, "dioxin-like" PCB 126 is able to effect the acrosome reaction of human spermatozoa *in vitro*. The non-ortho PCB 77, which is considered to be less toxic[7] had no effect on the AR in this in vitro system. The vitality was unaffected within a incubation period of 24 h. in PCB exposed- and control groups. In contrast, the global motility decreased in treated groups as well as in controls after 24 h (data not shown). Therefore the percentage of living immotile spermatozoa increased. Only living acrosome reacted spermatozoa were evaluated. Since the effect of PCB 126 on acrosome reaction was observed before motility was affected, it can be hypothesized that (1) a degenerative acrosome reaction was induced or (2) PCB 126 activates one of the second messenger pathways dose-dependently.

In contrast to our results, Roediger et al. (1989) reported that PCB 54 causes a significant motility decrease and an induction of the AR in comparison to control at a much lower PCB concentration (0.1 ng/ml). The media used by Roediger et al. contained 1% DMSO. In our experiments a DMSO/toluene concentration of 0.1 Vol.% was not exceeded. Therefore the high DMSO concentration might be a possible explanation for the reduced motility observed by Roediger et al. (1989).

The background of PCB concentration in blood is between 5-7 ng/ml. The initial PCB concentration of the single "dioxin-like" non-ortho PCB 126 and 77 in our culture media were between 0.8-3.2 µg/ml. Based on wet weight concentrations sums of di- to heptachlorinated biphenyls in preovulatory cervical mucus as reported by Hanf et al. (1992) ranged from 3.18 to 10.9 µg/kg. The data presented here on non-ortho PCBs show effects at PCB concentrations far higher than those found in cervical mucus. Nevertheless negative effects on sperm function can not be totaly excluded since other organohalogen compounds can be identified in the genital tract and their synergistic effects are mostly unknown.

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