Concentrations and Congener Profiles of PCB in Human Preovulatory Cervical Mucus and Effects on Sperm Motility in vitro

Volker Hanf, Peter Behnisch*, Wolfgang Körner*, Claus-Peter Sonntag, Hans-Rudolf Tinneberg# and Hanspaul Hagenmaier*
University Women's Hospital Tübingen, Schleichstr. 4, D-72076 Tübingen;
Institute of Organic Chemistry*, Univ. of Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen and Krankenhaus Rosenhöhe#, D-33647 Bielefeld, Germany

1. Introduction

Polychlorinated Biphenyls (PCB) are ubiquitous pollutants derived from former technical use and formed de novo in combustion processes. Particularly the latter source leads to widespread human exposure. Although scientifically hard to prove it is a generally accepted belief in the public that human fertility has been declining over the last decades. Environmental pollutants, particularly persistent chlorinated hydrocarbons, such as polychlorinated dibenzo-p-dioxins (PCDD), -furans (PCDF) and biphenyls (PCB) have been discussed as possible reasons for the supposed decrease in infertility. However, little human data support the above mentioned hypotheses. In 1989 Wagner et al. (1) and in 1991 Schlebusch et al. (2) published first data on the concentrations of several chlorinated hydrocarbons in preovulatory cervical mucus. In 1991 Van der Ven et al. (3) examined the influence of the burden of these compounds in cervical mucus on the sperm-mucus interaction and sperm survival in human mucus. Unfortunately the data are available in abstracted form only, little details are given on the analytical methodology applied. Where exact results are reported, PCB are only quantitated as the sum of PCB 138/153/180. No congener specific analytical results are presented. Apart from these methodological limitations the concentrations appeared rather high. The authors found mean PCB concentrations between 32 and 96.5 µg/kg (wet weight). The results of experiments trying to show a detrimental influence of chlorinated hydrocarbons on sperm motility using Kremer's capillary test are inconclusive (3).

2. Objective

It was therefore the objective of this study to determine the congener-specific concentrations of PCB in human preovulatory cervical mucus and to extend earlier work on the influence of PCDD congeners on human sperm motility in vitro (4) to single PCB congeners.

3. Material and Methods

Influence of PCB 77 on human ejaculated sperm:

According to the principal method described earlier (4) several fresh human ejaculates were density fractionated using a two-step discontinuous Percoll gradient centrifugation.Sperm donors were healthy student volunteers aged 23 to 32 years. Highly motile spermatozoa from the different ejaculates were separately washed several times and then pooled. 100 µl of sperm cell suspension (9×10^7/ml) in Menezo B2 Medium and 100 µl of PCB 77 solution in Menezo B2 Medium containing

0.1 or 0.01 Vol% DMSO/toluene (1/1) were added to glass incubation vials, which were consequently transferred to a tissue culture incubator and kept at 37 °C, 5% CO_2. Control vials contained sperm suspension plus DMSO/toluene (1/1) at equivalent concentrations.
The addition of PCB 77 to the sperm cells was designated time zero hours (i.e. start of the incubation experiment). At regular intervals 5 µl samples were taken from the incubation vials and the sperm motility was analyzed using a automatized sperm motility analyzer (SM-CMA, Version 4.4, Stroemberg Mika, Bad Feilnbach, Germany) The motility was recorded as percent motile sperm in the samples and plotted against time after the start of the experiment. Each experiment was carried out in triplicate and repeated three times (n = 9). The PCB concentrations in the stock solutions of PCB 77 in Menezo B2 Medium containing 0.1 or 0.01 Vol% DMSO/toluene (1/1) were quantitatively analyzed before each experiment. The PCB 77 concentration in the sperm cells after the completion of the experiment was determined after washing of the cells, decanting the supernatant and performing a mini Soxhlet extraction followed by clean up and HRGC/LRMS.

Quantitative analysis of PCB in human cervical mucus:

Sampling procedure: After obtaining informed consent cervical mucus was gained from women under infertility treatment at the IVF clinic of the University Women's Hospital Tübingen. The mucus samples were aspirated from the cervical opening of the uterus (womb) around the time of ovulation after ovarian stimulation with HMG/HCG using glass pipettes. The samples were deposited in washed glass test tubes, capped and frozen until assayed.
Sample preparation: The samples weighed between 10 and 250 mg (wet weight before processing). To obtain weights of 680 to 880 mg per sample to be analyzed, mucus specimen from 6 to 9 women were pooled and dried under a nitrogen stream. The dry weight was recorded and the sample was homogenized after the addition of 3 ml heptane using an ultrasound rod.
Extraction and analytical method: After the addition of a $^{13}C_{12}$-labelled PCB standard mixture (12 ng each congener) the dried samples were treated in a capped tube for 12 hours at 80 °C. Clean up was carried out by chromatography on a column with 0.8g alumina Super I and 0.3 g silica gel/44% conc. H_2SO_4 . After preelution with 5 ml pentane PCB were collected eluting with 6 ml heptane/ dichloromethane (88/12), followed by collection of the PCDD/PCDF with heptane/dichloromethane 1/1.
Analysis and evaluation: Analysis was performed by HRGC/HRMS (resolution = 5000) using a 60 m DB-5 ms and/or a 30 m DB-Dioxin column. The mass spectrometer was run in the SIM (Selected ion monitoring) mode.

4. Results and Discussion

Results of sperm incubation with PCB 77:

Fig. 1 shows the decline of the percentage of motile cells in time after the addition of PCB 77 solution (or control medium). In comparison to pure control medium (Menezo B2) and medium with vehicle (DMSO/toluene (1/1): 0.1 or 0.01 Vol% resp.) no influence on sperm motility could be observed. The mean concentration of PCB 77 in medium with 0.1% DMSO/toluene was 6.57 µg/ml +/- 0.68 (SD); in

medium with 0.01% DMSO/toluene 0.45 µg/ml +/- 0.16 (SD). The PCB 77 concentration in the sperm cells after the completion of the experiment was 421.4 µg/g fat for cells incubated in 0.1%-solution and 7.9 µg/g fat for cells incubated in medium containing 0.01% DMSO/toluene (c.f. **Table 1**).
These analytical data prove that PCB 77 was bioavailable to the incubated sperm cells. While high concentrations were obtained in the cells no effect on sperm motility could be observed. This is in agreement with our earlier results on PCDD (4) and in contradiction to results by Roediger et al. (5), who observed a dose-dependent detrimental effect on sperm motility of PCB 54 even at concentrations as low as 0.1 ng/ml within 2 to 12 hours of incubation.

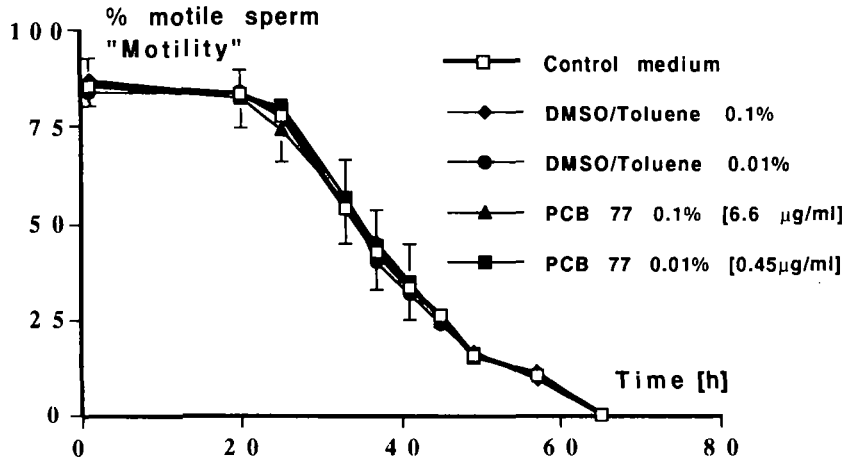

Fig. 1: Sperm motility on incubation with control medium, medium with vehicle (0.1 & 0.01%) and PCB 77 in DMSO/toluene. Bars indicate standard deviation for PCB 77 in 0.1% DMSO/toluene (n = 9)

Table 1: Concentrations of PCB 77 in media and sperm cells

examined sample	PCB 77 concentration
PCB 77 in medium with 0.1% DMSO/toluene	6.57 µg/ml +/- 0.68
PCB 77 in medium with 0.01% DMSO/toluene	0.45 µg/ml +/- 0.16
sperm incubated with PCB 77 in medium with 0.1% D/t	421,4 µg/g fat
sperm incubated with PCB 77 in medium with 0.01% D/t	7.9 µg/g fat

Results of PCB determination in preovulatory human cervical mucus:
Tables 2 lists the congener specific concentrations of PCB that should be analyzed according to recommendations by Ahlborg et al., 1994 (6). Based on the wet weight concentrations sums of

di- to heptachlorinated biphenyls ranged from 3.18 to 10.9 µg/kg (ppm), mean value was 6.42 ppm; on a dry matter basis concentrations ranged from 6.72 to 27.4 µg/kg (ppm) mean value was 14.62 ppm.

5. Conclusions:

For the first time we present a congener specific analysis of the PCB concentrations in human preovulatory cervical mucus. The concentrations are roughly one magnitude lower than those reported earlier by Wagner et al. and Schlebusch et al. (1/2). These concentrations are well comparable with those reported for human serum or follicular fluid and seminal plasma (7). Judging from our experimental results with human spermatozoa in vitro it appears unlikely that the motility of ejaculated human sperm in vivo could be compromised by PCB alone while the ejaculated sperm cells travel up the cervical canal thereby coming into contact with PCB at the concentrations measured. The data presented here and other unpublished data on PCB 101 show that PCB concentrations far higher than those found in cervical mucus do not reduce the motility of ejaculated human sperm cells in vitro. The experiments do however not exclude the possibility that PCB could interfere with the intratesticular sperm maturation thereby reducing male fertility. This possibility is presently under investigation in our laboratories.

6. References:

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Table 2 Concentrations and Congener Profiles of PCB in Human Preovulatory Cervical Mucus

	Levels in µg/kg [ppm] wet weight basis*			Levels in µg/kg [ppm] on dry matter basis*		
Total Polychlorinated Biphenyls	MIN	MAX	MEAN	MIN	MAX	MEAN
Total Dichlorobiphenyls	0,12	0,56	0,28	0,28	1,39	0,70
Total Trichlorobiphenyls	0,23	2,53	1,00	0,48	6,33	2,08
Total Tetrachlorobiphenyls	0,70	2,81	1,37	1,47	7,03	3,26
Total Pentachlorobiphenyls	0,50	1,42	0,92	1,16	3,55	2,13
Total Hexachlorobiphenyls	1,19	2,62	1,98	2,44	6,82	4,87
Total Heptachlorobiphenyls	0,56	1,45	1,15	1,17	3,63	2,28
Total Di- to Heptachlorobiphenyls [µg/kg]	3,18	10,83	6,42	6,72	27,36	14,62
Congener (IUPAC number)						
Di- *ortho*						
4,4'-DiCB (15)	0,07	0,07	0,07	0,06	0,16	0,13
2,4,4'-TriCB (28)	0,15	0,93	0,38	0,35	2,33	0,92
2,2',5,5'-TCB (52)	0,14	0,95	0,42	0,30	2,37	1,01
2,2',4,5,5'-PeCB (101)	0,09	0,15	0,14	0,24	0,39	0,31
2,2',3,4,4',5'-HxCB (138)	0,24	0,58	0,38	0,50	1,51	0,78
2,2',4,4',5,5'-HxCB (153)	0,15	0,55	0,33	0,30	1,44	0,78
2,2',3,3',4,4',5-HpCB (170)	0,06	0,07	0,07	0,13	0,18	0,16
2,2',3,4,4',5,5'-HpCB (180)	0,15	0,45	0,31	0,30	1,13	0,74
Mono-*ortho*						
2,3,3',4,4'-PeCB (105)	0,03	0,03	0,03	0,07	0,07	0,07
2,3,4,4',5-PeCB (114)	< 0,04	< 0,04	< 0,04	< 0,10	< 0,10	< 0,10
2,3',4,4',5-(118)\2',3,4,4',5- (123)-PeCB	0,06	0,06	0,06	0,12	0,12	0,12
2,3,3',4,4',5-HxCB (156)	0,04	0,15	0,05	0,08	0,31	0,18
2,3,3',4,4',5'-HxCB (157)	< 0,09	< 0,09	< 0,09	< 0,25	< 0,25	< 0,25
2,3',4,4',5,5'-HxCB (167)	0,04	0,04	0,04	0,07	0,16	0,16
2,3,3',4,4',5,5'-HpCB (189)	< 0,05	< 0,05	< 0,05	< 0,14	< 0,14	< 0,14
Non-*ortho*						
3,3',4,4'-TCB (77)	< 0,20	< 0,20	< 0,20	< 0,40	< 0,40	< 0,40
3,3',4,4',5-PeCB (126)	< 0,03	< 0,03	< 0,03	< 0,06	< 0,06	< 0,06
3,3',4,4',5,5'-HxCB (169)	< 0,04	< 0,04	< 0,04	< 0,06	< 0,06	< 0,06

* 5 samples were analysed, each consisting of a pooled sample from 6 to 9 women