Environmental Epidemiology and Toxicology (2000) 2, 24–31

www.nature.com/eet

Presence of environmental toxicants in semen and blood of young men from the Teplice region, Czech Republic

MIROSLAV MACHALA,[a] ROBERT ULRICH,[a] JAN VONDRÁČEK,[a,b] JIŘÍ RUBEŠ[a,c] AND RADIM J. ŠRÁM[c]

[a]*Veterinary Research Institute, 62132 Brno, Czech Republic*
[b]*Institute of Biophysics, Academy of Sciences of the Czech Republic, 612 65 Brno, Czech Republic*
[c]*Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic*

Concentrations of polychlorinated biphenyls (PCBs) and organochlorine contaminants in ejaculate and whole blood samples from young men of the Teplice district (the Czech Republic) were determined. In the ejaculate, concentrations of PCBs, calculated as the sum of indicator congeners, were in the range 1.19 to 8.15 ng/ml. In the blood, content of PCBs was between 2.3 and 11.9 ng/ml. Hexachlorobenzene was detected in all ejaculate and blood samples in a similar concentration range (under 1 ng/ml). In some ejaculate samples, high concentrations of *o,p′*-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethene) and especially *p,p′*-DDE and lindane (up to 25 ng/ml) were found. On the other hand, only low concentrations of DDT residues were determined in blood samples. No 4-nonylphenol and 17β-estradiol (potential xenoestrogen and major natural estrogen, respectively) were detected in selected semen and blood extracts. Using transgenic cell lines, the sample extracts were screened for estrogenic and dioxin-like activities *in vitro*. Both kinds of activities were detected in the samples. While the dioxin-like activity was generally quite low in both types of samples, we have detected relatively high estrogenic activity in several samples of semen and blood. Presence of estrogenic activity in samples of semen could suggest a possible mechanism of reproductive impairment in men. *Environmental Epidemiology and Toxicology* (2000) **2,** 24–31.

Keywords: *blood, dioxin-like activity, human, persistent organic pollutants, semen, xenoestrogenicity.*

Introduction

Polychlorinated biphenyls (PCBs) and chlorinated pesticides and their residues have been identified in human semen (Szymczynski and Waliszewski, 1981; Bush et al., 1986; Schlebusch et al., 1989) as well as in other human body fluids (Foster, 1995). Similarly, a presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other polychlorinated aromatic hydrocarbons have been documented in whole blood and semen samples (Schecter et al., 1996). Recently, an increased attention has been paid to the specific mechanisms of adverse effects of various contaminants, especially to the methods of their quantification and risk assessment. A novel *in vitro* reporter gene assay has been developed that can be used to directly measure the activity of contaminants on cellular responses mediated by the aryl hydrocarbon receptor (AhR). AhR can interact with a number of compounds such as polyhalogenated dibenzo-*p*-dioxins, dibenzofurans, biphenyls with coplanar molecules, etc. (Birnbaum and De Vito, 1995; van den Berg et al., 1998). The assay is based on AhR-mediated luciferase expression in genetically modified cell lines (Murk et al., 1997) and TCDD is used as a model compound to calculate the response as expressed by CALUX-TEQs (chemical-activated luciferase gene expression — TCDD equivalents).

Epidemiological studies in human population and wildlife suggest that reproductive impairment can be associated with environmental chemical contamination (Bush et al., 1986; Kavlock et al., 1996). Estrogen receptor-mediated perturbation by xenobiotics has been revealed as one of the major mechanisms of endocrine disruption (Kavlock et al., 1996; Gray et al., 1997; Ankley et al., 1998). Again, *in vitro* assays based on a reporter gene methodology can be used to assess estrogenic activities of complex mixtures of compounds, such as various environmental samples (Gray et al., 1997; Ankley et al., 1998). An assay employing MVLN transgenic cell line, derived from the human breast carcinoma cells MCF-7 (Pons et al., 1990), with luciferase activity as an endpoint was used in the present study.

Our aim was to determine the concentrations of persistent chlorinated pollutants in young men from a heavily polluted region in the Czech Republic (Šrám et al., 1996), and to reveal the presence of chemicals with potencies to cause reproductive impairment, i.e., estrogenic and TCDD-like activities. The study involved the following steps: 1) chromatographic determination of concentra-

1. Address all correspondence to: Dr. Miroslav Machala, Head, Department of Chemistry and Toxicology, Veterinary Research Institute, Hudcova 70, 62132 Brno, Czech Republic. Tel.: +420-5-4132-1241. Fax: +420-5-4121-1229. E-mail: machala@vri.cz

tions of PCBs and organochlorine compounds; chromatographic detection of 4-nonylphenol as potential xenoestrogen in extracts; immunoassay of presence of 17β-estradiol as natural estrogen in blood; 2) determination of *in vitro* estrogenic activity in extracts of ejaculate and whole blood samples; 3) determination of TCDD-like activity in extracts of semen and blood by measurement of Ah-receptor-mediated activity *in vitro*.

Methods

Sampling, Extraction and Purification Techniques

Twenty-five 18-year-old men from the district of Teplice (the Czech Republic) were recruited for the study. The samples of ejaculate and whole blood were stored frozen at -80°C. Samples of semen (200 μl) were diluted with distilled water (1:1, v/v), extracted and separated in one-step procedure on a solid phase extraction column (SPE C18, Applied Separations, USA) with chloroform. Extracts were then evaporated and dissolved in 100 μl of isooctane. Whole blood samples (2 ml) were mixed with 3 g Na_2SO_4, extracted 2 h on a Soxtec (Tecator, Sweden) by petrolether/acetone (1:1, v/v) and purified on a Florisil column. The extracts were then evaporated and dissolved in 200 μl of isooctane.

Chemical Analyses

Concentrations of PCBs and organochlorine contaminants were determined in aliquots of extracts by a GC/ECD method on a dual column system (DB-5 and DB-1705 columns, 60 m$\times$0.25 mm, 0.25 μm, Quadrex Corporation, USA). In selected samples (those with the highest estrogenic potentials), the presence of 4-nonylphenol and 17β-estradiol was determined by a GC/MSD (Varian 3600/DB-5 column, Finnigan MAT, USA) and ELISA method kit (Estradiol-6 II, Chiron Diagnostics, USA), respectively.

In Vitro Detection of the Dioxin-Like and Estrogenic Activities

Hepatoma H4IIE cell line, permanently transfected with a luciferase reporter gene under control of dioxin responsive enhancers, was used to screen for compounds able to elicit Ah receptor mediated gene expression (Aarts et al., 1995; Murk et al., 1997). The cells were kindly provided by Dr. A.J. Murk, WAU, Wageningen, The Netherlands. The cells were grown in minimal essential medium (α-MEM, Sigma, Czech Republic) supplemented with 10% heat-inactivated fetal bovine serum (FBS). The assays were performed in 96-well cell culture plates (Costar, USA). Briefly, 24 h after seeding (90% to 100% confluency), the cells were dosed with the extracts or TCDD calibration standards prepared in dimethyl sulphoxide (DMSO). Maximum concentrations of DMSO did not exceed 0.4%. After 24 h of exposure, the medium was removed, the cells were washed with 0.5$\times$ phosphate-buffered saline (PBS), luciferase was extracted with cell lysis buffer (Labsystems, Finland) and the plates were frozen at -80°C. The luciferase expression was then measured on a luminometer, using Luciferase Monitoring Kit (Labsystems, Finland).

MVLN cells, permanently transfected with the p-Vit-tk-Luc plasmid, were used for the detection of estrogenic activity. The cell line was obtained from Dr. Michel Pons, Montpellier, France. The cells were grown in a 1:1 mixture of Dulbecco's minimal essential medium:Ham's F-12 nutrient mixture (DMEM/F12, Sigma, Czech Republic) supplemented with 1 μg/ml insulin, 1 mM sodium pyruvate and 10% heat-inactivated FBS. The assays were performed in 96-well cell culture plates. Briefly, 24 h after seeding (90% to 100% confluency), the medium was changed to the

Table 1. Concentrations of PCBs in human ejaculate (ng/ml).

Sample no.	PCB congener no.							$\sum$[a]
	28	52	101	118	138	153	180	
1	0.71	0.30	0.73	0.56	0.77	0.90	0.38	4.35
3	0.76	0.50	0.65	0.28	0.66	0.74	0.64	4.23
6	0.10	0.10	0.39	0.10	0.73	0.63	0.12	2.17
7	0.05[b]	0.05	0.32	0.10	0.12	0.12	0.43	1.19
8	0.10	0.05	0.18	0.19	0.26	0.64	0.05	1.47
11	0.25	0.86	0.05	0.05	0.13	0.13	0.13	1.60
16	0.43	0.85	0.27	0.55	0.65	2.23	0.42	5.40
19	0.20	0.27	0.21	0.20	0.47	0.38	0.18	1.91
20	0.23	0.20	0.11	0.26	0.44	1.13	0.15	2.52
26	0.15	0.31	0.33	0.37	0.67	0.68	0.26	2.77
34	0.61	0.99	0.73	0.18	0.90	3.00	0.28	6.69
35	0.32	0.21	0.10	0.05	0.37	0.36	0.12	1.53
38	0.22	0.81	0.05	0.05	0.73	0.28	0.26	2.40
39	0.46	1.00	0.56	0.57	0.70	1.78	0.25	5.32
42	0.81	1.41	0.31	0.27	0.57	1.02	0.14	4.53
45	0.43	0.74	0.24	0.18	0.99	0.92	0.15	3.65
55	0.31	0.45	0.22	0.10	0.62	0.45	0.11	2.26
58	0.25	0.21	0.37	0.11	0.70	0.51	0.14	2.29
62	0.34	0.47	0.14	0.11	0.60	0.89	0.05	2.60
63	1.65	0.60	0.59	0.41	0.78	0.70	0.05	4.78
66	0.52	0.35	0.25	0.10	0.72	0.85	0.05	2.84
69	0.71	1.06	0.05	0.05	0.34	0.71	0.05	2.97
70	1.26	2.16	0.73	0.25	1.04	1.96	0.75	8.15
72	0.14	0.12	0.27	0.27	0.85	1.06	0.11	2.82
73	0.18	0.26	0.56	0.05	0.81	1.31	0.21	3.38
Mean	0.49	0.60	0.31	0.21	0.61	0.94	0.22	3.35
S.D.	0.40	0.50	0.21	0.15	0.24	0.67	0.18	1.69

[a]$\sum$, sum of concentrations of indicator PCB congeners (Nos. 28, 52, 101, 118, 138, 153, and 180).

[b]Values 0.05 correspond to one half of detection limit (0.10 ng/ml).

one supplemented with charcoal-stripped FBS and the cells were dosed either with the extracts or 17β-estradiol calibration standards prepared in DMSO. Maximum concentrations of DMSO did not exceed 0.1%. After 48 h of exposure, the medium was removed, the cells were washed with a serum-free medium and the luciferase expression was then measured on a luminometer, using LucLite™ kit (Packard Instruments, USA).

Results

Results of the Chemical Analysis of Ejaculate and Blood Samples

Concentrations of both PCBs and organochlorine pesticides in the ejaculate samples were determined. The results are summarized in Tables 1 and 2. Overall concentrations of PCB, calculated as the sum of indicator congeners, were between 1.19 and 8.15 ng/ml. Low concentrations of hexachlorobenzene (HCB) were detected in all ejaculate samples (between 0.13 and 1.54 ng/ml) with exception of samples 69 and 70, where its concentrations exceeded 2 ng/ml. Lindane was the prevalent isomer of hexachlorohexane (HCH), found in relatively high concentrations, especially in samples 1, 42, 69 and 70 (up to 21 ng/ml). High concentrations of both residual contaminants *o,p′*-DDE and *p,p′*-DDE, and surprisingly also of DDT, were found in samples 34, 39, and 63 (Table 2).

Concentrations of PCBs and organochlorine compounds detected in the whole blood samples are summarized in Tables 3 and 4. The content of PCBs was within the range 2.3 to 11.9 ng/ml. Thus, concentrations of PCBs in both types of samples were roughly similar, within the same order of magnitude. In all samples, higher-chlorinated congeners were the prevalent form of PCBs. However, contrary to the blood samples, several semen samples contained predominantly lower-chlorinated congeners (samples 42, 63, 69 and 70; see Tables 1 and 3). HCB was detected in all blood samples examined in the range similar to ejaculate samples. As for isomers of HCH, similarly to ejaculate samples, only increased levels of lindane were found (around 4 ng/ml in all samples). DDT

Table 2. Concentrations of organochlorine compounds in human ejaculate (ng/ml).

Sample no.	α-HCH	β-HCH	Lindane	δ-HCH	HCB	*o,p′*-DDE	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	*p,p′*-DDT
1	1.63	0.05	8.02	0.05	0.72	1.20	3.10	0.39	1.19	0.69	0.44
3	0.26	0.05	6.44	0.05	0.90	0.53	1.77	0.76	0.05	0.62	0.23
6	0.21	0.05	0.65	0.05	0.34	0.18	0.13	0.36	0.05	0.25	0.11
7	0.26	2.81	3.49	0.05	0.13	0.05	0.87	0.14	0.05	0.10	0.05
8	0.26	2.89	2.25	0.05	0.13	0.45	0.11	0.05	0.43	0.05	0.19
11	0.16	0.05	3.32	0.14	0.36	0.56	0.40	0.05	0.10	0.05	0.18
16	0.10	0.79	1.92	0.33	0.40	1.03	0.81	0.56	0.05	0.33	0.05
19	0.16	0.57	1.64	0.38	0.42	0.37	0.68	0.16	0.14	0.56	0.22
20	0.05	0.23	1.32	0.17	0.32	0.49	1.55	0.05	0.05	0.76	0.35
26	0.34	0.46	2.22	0.46	0.63	0.55	3.64	0.05	0.22	2.06	1.15
34	0.49	0.39	3.60	0.56	0.83	2.94	26.98	0.22	0.63	14.28	10.92
35	0.39	0.05	2.77	0.24	0.59	1.05	0.21	0.05	0.41	0.22	0.05
38	0.33	0.05	1.96	0.13	0.65	0.51	1.80	0.05	0.10	0.86	0.42
39	0.53	0.22	4.12	0.32	0.95	2.33	21.73	0.34	0.56	11.86	8.70
42	0.45	0.82	8.20	0.05	1.54	1.78	6.87	0.72	0.05	3.38	1.06
45	0.31	0.14	5.46	0.05	0.98	0.96	0.63	0.17	0.24	0.42	0.29
55	0.34	0.29	5.90	0.05	1.05	1.68	4.33	0.12	0.05	2.10	0.56
58	0.22	0.22	4.46	0.05	0.30	0.64	0.82	0.05	0.14	0.40	0.23
62	0.29	0.42	6.38	0.05	1.32	1.78	5.82	0.17	0.05	1.78	0.50
63	1.04	0.51	5.85	0.05	0.51	3.68	25.32	0.05	0.05	8.54	2.87
66	0.72	0.19	4.28	0.05	1.32	0.77	1.09	0.10	0.10	0.55	0.15
69	0.85	0.30	11.08	0.05	2.14	2.72	5.94	0.05	0.35	1.88	0.50
70	1.13	0.05	21.03	0.05	5.04	4.05	2.48	1.50	0.99	1.97	1.47
72	0.56	0.20	4.55	0.05	0.69	1.55	0.84	0.11	0.16	0.59	0.31
73	0.11	0.15	4.09	0.05	0.36	1.65	3.04	0.32	0.15	0.75	0.20
Mean	0.45	0.48	5.00	0.14	0.90	1.34	4.84	0.26	0.25	2.20	1.25
S.D.	0.36	0.73	4.06	0.15	0.96	1.06	7.60	0.32	0.30	3.64	2.61

Values 0.05 correspond to one half of detection limit (0.10 ng/ml).

Table 3. Concentrations of PCBs in human blood (ng/ml).

Sample no.	PCB congener no.							$\sum^a$
	28	52	101	118	138	153	180	
3	0.87	0.78	0.74	0.19	0.75	1.34	0.84	5.51
6	0.83	0.58	0.37	0.26	1.40	1.44	0.71	5.59
7	0.21	0.30	0.12	0.08	0.47	0.78	0.34	2.30
8	0.95	0.53	0.47	0.20	1.24	1.31	0.89	5.59
11	0.83	0.72	0.45	0.22	1.26	1.35	0.90	5.73
16	0.59	0.62	0.24	0.13	1.52	1.46	0.98	5.54
19	1.10	0.99	0.48	0.17	0.90	1.38	0.65	5.67
20	0.67	0.46	0.58	0.16	1.22	1.51	0.96	5.56
26	0.69	0.60	0.68	0.41	1.90	1.86	1.50	7.64
34	0.81	0.52	0.63	0.30	1.36	1.45	1.01	6.08
35	0.76	0.63	0.34	0.27	1.42	1.46	0.98	5.86
38	0.71	0.58	0.85	0.38	1.90	1.92	1.24	7.58
39	0.73	0.71	0.70	0.34	1.34	1.65	1.05	6.52
42	1.32	0.97	1.13	0.39	2.83	3.19	1.84	11.67
45	0.80	0.45	0.37	0.28	1.34	1.53	1.06	5.83
55	0.67	0.31	0.86	0.40	1.52	1.65	1.14	6.55
58	0.58	0.50	0.34	0.21	0.83	0.95	0.80	4.21
62	1.04	0.82	0.60	0.44	2.09	2.13	1.56	8.68
63	0.55	0.54	0.67	0.42	1.62	1.62	1.00	6.42
66	0.47	0.38	0.46	0.22	1.18	1.07	0.64	4.42
69	0.55	0.47	0.40	0.16	1.80	1.50	1.14	6.02
70	0.64	0.51	0.52	0.12	1.94	1.79	1.68	7.20
72	0.85	0.44	0.92	0.90	3.35	3.42	2.03	11.91
73	0.42	0.40	0.26	0.19	1.22	1.22	0.99	4.70
Mean	0.74	0.58	0.55	0.29	1.52	1.62	1.08	6.37
S.D.	0.23	0.18	0.24	0.16	0.61	0.58	0.39	2.05

[a]$\sum$, sum of concentrations of indicator PCB congeners (Nos. 28, 52, 101, 118, 138, 153, and 180).

and its metabolites were found in low concentrations in all blood samples, with *p,p′*-DDE present in the highest concentrations (Table 4).

Nonylphenol was not detected in the selected samples (detection limit 0.5 μg/ml, data not shown).

Dioxin-Like Activity in the Samples of Ejaculate and Whole Blood

The presence of compounds with potency to cause TCDD-like toxicity was examined in the extracts of semen (Figure 1). A weak induction of luciferase expression was detected in the majority of samples. However, the level of induction, with the exception of sample 19, was not high enough to calculate CALUX TEQs. The highest TCDD-like activity was found in the sample 19-TEQ, approximately 6.15 pg/μl of ejaculate.

An increased dioxin-like activity was found in several whole blood samples, especially in samples 42, 45, 62 and 72 (Figure 2). However, no correlation could be found between samples of semen plasma and blood coming from the same subject. The level of induction was too low to calculate CALUX TEQs.

Estrogenic Activity in Whole Blood and Ejaculate Samples

Relatively high estrogenic activity has been detected in a number of both semen and whole blood samples (Figures 3 and 4, respectively). The highest estrogenic activity was found in semen extracts 26 and 58 (between the activity caused by 6 and 10 pM of 17β-estradiol); some activity was also detected in samples 45, 62, 63, 72 and 73 (between the activity caused by 3 and 6 pM of 17β-estradiol). In the blood samples, the highest estrogenic activity was found in samples 58, 62, 63, 70, 72 and 73 (between the activity caused by 10 and 30 pM of 17β-estradiol); in samples 55, 66 and 69 estrogenic activity corresponded to the activity caused by 6 to 10 pM of 17β-estradiol. With the exception of sample 26, estrogenic activity detected in whole blood samples was very similar to that detected in semen plasma extracts.

In selected samples with a significant estrogenic activity, a content of 17β-estradiol was determined using immunoanalysis. However, this major natural estrogen was not detected in any sample examined (data not shown).

Discussion

It seems that to be able to interpret and evaluate risks of the presence of environmental contaminants in organism, it is necessary to combine both the data from chemical analysis and from biochemical monitoring of estrogenic and dioxin-like activity. There are at least two reasons in support of such an approach: (1) synergism and antagonism of xenobiotics inducing both endocrine and TCDD-like effects through interactions with specific receptors; (2) chemical analysis can always be used to determine only a limited selection of compounds. Xenobiotics belonging to different classes of contaminants can induce similar effects due to the similarities in size and structure. Thus, measurements of specific biochemical responses (dioxin-like or estrogenic activity) are complementary to the chromatographic analysis and may determine the total potential of a toxic effect of contaminants (Safe, 1994; Kavlock et al., 1996; van den Berg et al., 1998).

Parameters of human male fertility have been shown to be negatively associated with a burden of persistent environmental chemicals (Bush et al., 1986). However, only a limited number of studies from several regions exist that have reported trace levels of persistent chlorinated contaminants in human serum or seminal plasma. In human semen, DDE residues were found to be at the average level of 3 ng/g (Szymczynski and Waliszewski, 1981). Bush et al. (1986) have reported low concentrations of PCBs

Table 4. Concentrations of organochlorine compounds in human blood (ng/ml).

Sample no.	α-HCH	β-HCH	Lindane	δ-HCH	HCB	o,p'-DDE	p,p'-DDE	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT
3	0.13	0.29	4.40	0.06	0.34	0.08	1.14	0.08	0.21	0.20	0.54
6	0.10	0.48	4.22	0.06	0.37	0.26	0.78	0.10	0.08	0.22	0.38
7	0.05	0.13	2.70	0.02	0.17	0.01	0.38	0.09	0.06	0.10	0.19
8	0.24	0.04	4.34	0.15	0.35	0.09	1.15	0.17	0.06	0.22	0.38
11	0.16	0.63	4.03	0.04	0.30	0.12	0.85	0.13	0.09	0.16	0.28
16	0.22	0.21	3.71	0.04	0.37	0.13	2.76	0.12	0.08	0.18	0.51
19	0.76	0.38	3.71	0.19	0.82	0.40	1.56	0.18	0.08	0.22	0.25
20	0.11	0.04	3.64	0.07	0.22	0.12	1.82	0.12	0.03	0.16	0.32
26	0.21	0.28	3.52	0.03	0.88	0.19	2.90	0.12	0.16	0.32	0.38
34	0.13	0.28	3.98	0.06	0.28	0.11	1.63	0.09	0.06	0.28	0.36
35	0.12	0.34	3.97	0.06	0.45	0.10	1.74	0.11	0.05	0.18	0.41
38	0.14	0.39	4.21	0.08	0.38	0.12	2.36	0.12	0.10	0.42	0.56
39	0.09	0.43	3.90	0.06	0.50	0.10	1.79	0.05	0.04	0.23	0.32
42	0.35	0.40	4.53	0.07	0.85	0.30	2.14	0.22	0.12	0.82	1.30
45	0.19	0.22	4.22	0.05	0.45	0.24	1.22	0.03	<0.01	0.26	0.21
55	0.14	0.45	3.86	0.05	0.39	0.08	0.91	0.14	<0.01	0.30	0.41
58	0.11	0.31	3.42	0.07	0.31	0.10	0.63	0.15	0.03	0.18	0.28
62	0.13	0.36	4.14	0.04	0.56	0.08	1.33	0.40	0.17	0.30	0.83
63	0.05	0.15	3.24	0.06	0.12	0.12	0.80	0.27	0.11	0.30	0.74
66	0.22	0.05	3.87	0.10	0.30	0.13	1.18	0.11	0.20	0.24	0.30
69	0.13	0.07	4.58	0.11	0.47	0.10	2.56	0.11	0.27	0.37	0.53
70	0.09	0.02	4.36	0.06	0.35	0.07	1.14	0.12	0.10	0.48	0.51
72	0.13	0.15	5.07	0.18	0.50	0.18	2.56	0.11	0.18	0.59	1.57
73	0.07	0.02	3.08	0.07	0.29	0.07	1.17	0.09	0.12	0.37	0.43
Mean	0.17	0.26	3.95	0.07	0.42	0.14	1.52	0.13	0.11	0.30	0.50
S.D.	0.14	0.17	0.51	0.04	0.19	0.08	0.70	0.07	0.06	0.15	0.32

Values 0.01 correspond to one half of detection limit (0.02 ng/ml).

(mean total 32 PCB congeners of 5.8 ng/g wet weight) in seminal samples. Concentrations of several organochlorine compounds were determined in human semen samples in Germany and reported values include 2.4–146 ng PCBs/ml, 0.35–4.3 ng/ml of HCB, high concentrations of DDT (up to 16 ng/ml, while DDE residues were in the concentration range 0.4 to 6 ng/ml (Stachel et al., 1989). Organochlorine compounds in semen were found in the same concentration range as in blood. In our study, only a few samples showed the levels of PCBs or organochlorine

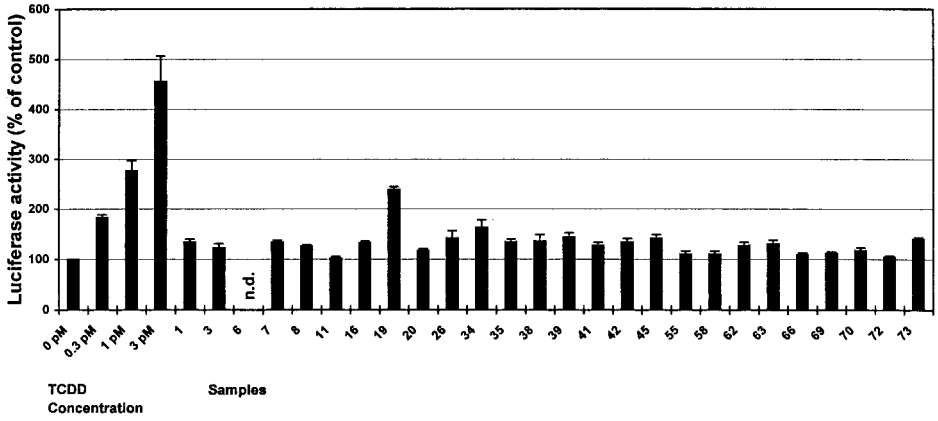

Figure 1. TCDD-like activity of extracts of human ejaculate (an equivalent of 0.008 μl of semen plasma per 1 ml of medium was applied to the cells). Results are expressed as mean ± standard deviation ($n = 3$) as a percentage of the solvent control. n.d. — not determined.

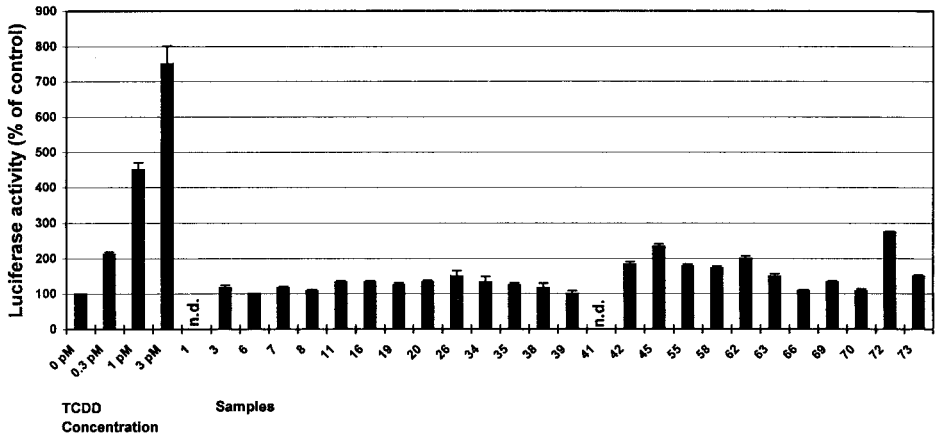

Figure 2. TCDD-like activity of extracts of whole blood samples (an equivalent of 0.04 μl of blood per 1 ml of medium was applied to the cells). Results are expressed as mean ± standard deviation ($n = 3$) as a percentage of the solvent control. n.d. — not determined.

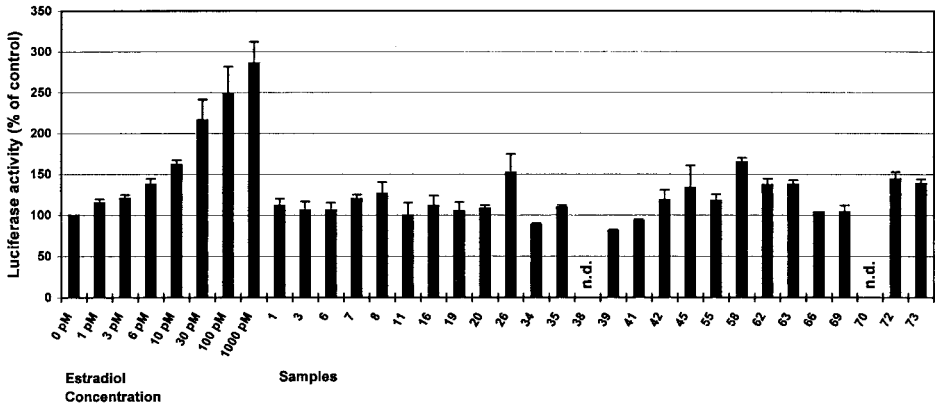

Figure 3. Estrogenic activity of extracts of human ejaculate (an equivalent of 0.002 μl of semen plasma per 1 ml of medium was applied to the cells). Results are expressed as mean ± standard deviation ($n = 3$) as a percentage of the solvent control. n.d. — not determined.

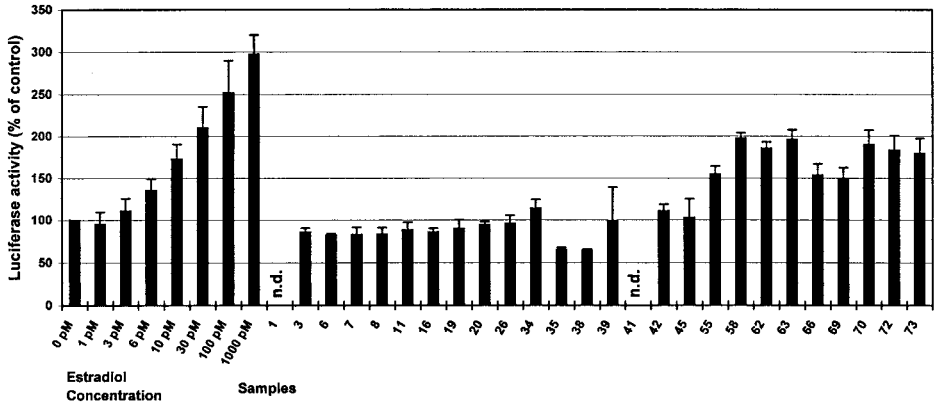

Figure 4. Estrogenic activity of extracts of whole blood samples (an equivalent of 0.01 μl of blood per 1 ml of medium was applied to the cells). Results are expressed as mean ± standard deviation ($n = 3$) as a percentage of the solvent control. n.d. — not determined.

pesticides exceeding average concentrations reported in blood (Bush et al., 1984; Phillips et al., 1989; Foster, 1995) or in semen (Szymczynski and Waliszewski, 1981; Bush et al., 1986; Schlebusch et al., 1989; Stachel et al., 1989; Foster, 1995). Nevertheless, high concentrations of PCB congeners, lindane, and *p,p′*-DDE (up to approximately 20 ng of individual compounds per ml) found in some ejaculates collected in the Teplice region, Czech Republic, could contribute to serious reproductive impairments and other adverse effects.

The presence of dioxin-like compounds represents a significant risk to human health and reproduction (Foster, 1995; van den Berg et al., 1998). The dioxin-like activity (results are summarized in Figures 1 and 2) can be induced with a number of compounds, such as TCDD or structurally related coplanar and mono-ortho-chlorinated PCBs, and other aromatic contaminants, including HCB (van Birgelen, 1998; van den Berg et al., 1998). Dioxins and dibenzofurans, including TCDD, were detected in the pooled semen samples in the U.S. population (Schecter et al., 1996). In our study, the dioxin-like activity was below the EC50 value for TCDD (approximately 10 pM), both in ejaculate and blood samples, although some activity was detected. Coplanar PCB congeners and mono-*o*-chlorinated PCB 105 and 156 were not detected in concentrations that could significantly affect the TCDD-like potential in extracts (data not shown). Also HCB could not induce TCDD-like activity, due to its low TCDD-like potency (TEF 0.0001) (van Birgelen, 1998). This was confirmed by the fact that in ejaculate samples 69 and 70 with the highest contents of HCB, dioxin-like activity was not detected. An increased dioxin-like activity detected in blood sample 72 could be associated with higher PCB concentrations. On the other hand, in several samples (e.g., in the semen sample 19) an increased expression of luciferase did not correspond with low concentrations of PCBs. TCDD-like potency could be attributed to some compounds not determined, such as chlorinated dibenzo-*p*-dioxins.

In a number of both ejaculate and whole blood samples, a significant estrogenic activity has been detected (Figures 3 and 4). In a majority of samples, estrogenic activity of ejaculate samples corresponded with respective blood samples. Since the natural estrogen, 17β-estradiol, was not found in these samples, their estrogenic activity can be attributed to xenobiotics. A number of compounds, including organochlorine pesticides and their metabolites — such as *o,p′*-DDT and *o,p′*-DDE may elicit estrogen-like effects (Gray et al., 1997). Others may act as antiandrogens — for example, *p,p′*-DDE (Kelce et al., 1995) that was present in some extracts in high concentrations. However, in this study, detected estrogenic activity was apparently not only related to this class of contaminants. Other compounds, such as metabolites of PCBs could also participate in xenoestrogenicity.

Evidence of xenoestrogens in human semen and blood is one of the major findings in this study. Detection of estrogen-like activity in samples of ejaculate could suggest a possible mechanism of reproductive impairment in men.

Acknowledgments

The authors thank Prof. William W. Au, The University of Texas Medical Branch, Galveston, USA, for critical reading of the manuscript and his valuable comments, and Dr. Milada Neumannová of the Clinic of Obstetrics and Gynaecology, Masaryk University, Brno, Czech Republic, for ELISA determination of 17β-estradiol. The project was supported by the Ministry of Environment of the Czech Republic (the Teplice II program, grant no. VaV340/1/97).

References

Aarts J.M.M.J.G., Denison M.S., Cox M.A., *et al.* Species-specific antagonism of Ah receptor action by 2,2′,5,5′-tetrachloro- and 2,2′,3,3′,4,4′-hexachlorobiphenyl. *Eur. J. Pharmacol.* 1995: 293: 463–474.

Ankley G., Mihaich E., Tillitt D., *et al.* Overview of a workshop on screening methods on detecting potential (anti-)estrogenic/androgenic chemicals in wildlife. *Environ. Toxicol. Chem.* 1998: 17: 68–87.

Birnbaum L.S., and De Vito M.J. Use of toxic equivalence factors for risk assessment for dioxins and related compounds. *Toxicology* 1995: 105: 391–401.

Bush B., Bennett A.H., and Snow J.T. Polychlorobiphenyl congeners, *p,p′*-DDE, and sperm function in humans. *Arch. Environ. Contam. Toxicol.* 1986: 15: 333–341.

Bush B., Snow J., and Kovlintz R. Polychlorobiphenyl (PCB) congeners, *p,p′*-DDE and hexachlorobenzene in maternal and fetal cord blood from mothers in upstate New York. *Arch. Environ. Contam. Toxicol.* 1984: 13: 517–527.

Foster W.G. The reproductive toxicology of Great Lakes contaminants. *Environ. Health Perspect.* 1995: 130 (Suppl. 9): 63–69.

Gray L.E., Kelce W.R., Wiese T., *et al.* Endocrine screening methods workshop report: Detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. *Reprod. Toxicol.* 1997: 11: 719–750.

Kavlock R.J., Daston G.P., DeRosa C., *et al.* Research needs for risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ. Health Perspect.* 1996: 104 (Suppl. 4): 715–740.

Kelce W.R., Stone C.R., Laws S.C., *et al.* Persistent DDT metabolite *p,p′*-DDE is a potent androgen receptor antagonist. *Nature* 1995: 375: 581–585.

Murk A.J., Leonards P.E.G., Bulder A.S., *et al.* The CALUX (chemical-activated luciferase expression) assay adapted and validated for measuring TCDD equivalents in blood plasma. *Environ. Toxicol. Chem.* 1997: 16: 1583–1589.

Phillips D.L., Pirkle J.L., Burse V.W., *et al.* Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch. Environ. Contam. Toxicol.* 1989: 18: 495–500.

Pons M., Gagne D., Nicolas A.-C., and Mehtali M. A new cellular model of response to estrogens: a bioluminescent test to characterize (anti)estrogen molecules. *BioTechniques* 1990: 9: 450–459.

Safe S.H. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses and implications for risk assessment. *Crit. Rev. Toxicol.* 1994: 24: 87–149.

Schecter A., McGee H., Stanley J.S., Boggess K., and Brandt-Rauf P. Dioxins and dioxin-like chemicals in blood and semen of American Vietnam veterans from the state of Michigan. *Am. J. Ind. Med.* 1996: 30: 647–654.

Schlebusch H., Wagner U., van der Ven H., Al-Hasani S., Diedrich K., and Krebs D. Polychlorinated biphenyls: the occurrence of the main congeners in follicular and sperm fluids. *J. Clin. Chem. Clin. Biochem.* 1989: 27: 663–667.

Šrám R.J., Beneš I., Binková B., Dejmek J., Horstman D., Kotešovec F., Otto D., Perreault S.D., Rubeš J., Selevan S.G., Skalík I., Stevens R.K., and Lewtas J. Teplice program — the impact of air pollution on human health. *Environ. Health Perspect.* 1996: 104 (Suppl. 4): 699–714.

Stachel B., Dougherty R.C., Lahl U., Schlösser M., and Zeschmar B. Toxic environmental chemicals in human semen: analytical method and case studies. *Andrologia* 1989: 21: 282–291.

Szymczynski G., and Waliszewski S.M. Content of chlorinated pesticides in human semen of a random populatin. *Int. J. Androl.* 1981: 4: 669–674.

van Birgelen A.P.J.M. Hexachlorobenzene as a possible major contributor to the dioxin activity of human milk. *Environ. Health Perspect.* 1998: 106: 683–688.

van den Berg M., Birnbaum L., Bosveld A.T.C., *et al.* Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* 1998: 106: 775–792.