FERTILITY AND STERILITY®
VOL. 78, NO. 6, DECEMBER 2002

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Role of environmental estrogens in the deterioration of male factor fertility

Roya Rozati, M.D.,[a] *P. P. Reddy, Ph.D.,*[b] *P. Reddanna, Ph.D.,*[c] *and Rubina Mujtaba, Ph.D.*[a]

Mahavir Hospital and Research Center and University of Hyderabad, Hyderabad, India

Objective: To evaluate the role of the environmental estrogens polychlorinated biphenyls (PCBs) and phthalate esters (PEs) as potential environmental hazards in the deterioration of semen parameters in infertile men without an obvious etiology.

Design: Randomized controlled study.

Setting: Tertiary care referral infertility clinic and academic research center.

Patient(s): Twenty-one infertile men with sperm counts <20 million/mL and/or rapid progressive motility <25% and/or <30% normal forms without evidence of an obvious etiology and 32 control men with normal semen analyses and evidence of conception.

Intervention(s): Semen and blood samples were obtained as part of the treatment protocol.

Main Outcome Measure(s): Evaluation of semen parameters such as ejaculate volume, sperm count, motility, morphology, vitality, osmoregulatory capacity, sperm chromatin stability, and sperm nuclear DNA integrity.

Result(s): PCBs were detected in the seminal plasma of infertile men but not in controls, and the concentration of PEs was significantly higher in infertile men compared with controls. Ejaculate volume, sperm count, progressive motility, normal morphology, and fertilizing capacity were significantly lower in infertile men compared with controls. The highest average PCB and PE concentrations were found in urban fish eaters, followed by rural fish eaters, urban vegetarians, and rural vegetarians. The total motile sperm counts in infertile men were inversely proportional to their xenoestrogen concentrations and were significantly lower than those in the respective controls.

Conclusion(s): PCBs and PEs may be instrumental in the deterioration of semen quality in infertile men without an obvious etiology. (Fertil Steril® 2002;78:1187–94. ©2002 by American Society for Reproductive Medicine.)

Key Words: Male, fertility, idiopathic, infertility, environmental estrogens, xenoestrogens, polychlorinated biphenyls, phthalate esters

Received February 22, 2001; revised and accepted , .

Reprint requests: Roya Rozati, M.D., Assisted Conception Services Unit, Mahavir Hospital and Research Center, 10-1-1/A, Bhagwan Mahavir Marg, A.C. Guards, Hyderabad (A.P.) 500 028, India (E-mail: drrozati@rediffmail.com).

[a] Assisted Conception Services Unit, Mahavir Hospital and Research Center.

[b] Institute of Genetics and Hospital for Genetic Diseases, Begumpet, Hyderabad, India.

[c] Department of Animal Sciences, School of Life Sciences, University of Hyderabad.

0015-0282/02/$22.00
PII S0015-0282(02)04389-3

There is increasing circumstantial evidence that xenoestrogens may disrupt the development and function of the male reproductive tract (1–4). Manmade chemicals with known estrogenic effects include organochlorine pesticides, polychlorinated biphenyls (PCBs), phenolic compounds, and phthalate esters (PEs). These compounds come mainly from domestic and industrial effluents, solid waste disposal sites, and agricultural or urban runoff (5).

PCBs are halogenated, lipophilic, aromatic hydrocarbon mixtures of 200 or more congeners and are used extensively as insulators in electrical equipment, as plasticizers in polyvinyl carbonate (PVC) products, in carbonless copy paper, as de-inking solvents for recycling of newspaper, and as waterproofing agents. They have been detected as contaminants in almost every component of the global ecosystem including the air, water, sediments, fish, wildlife, and human adipose tissue, milk, and serum. PCB exposure has been associated with developmental, reproductive, dermal, and hepato-toxicity, adverse endocrine and carcinogenic effects, and the induction of diverse phase I and phase II drug-metabolizing enzymes. Significantly smaller penises have been reported in boys exposed to PCBs in utero (6).

PEs are among the most abundant industrial chemicals in the environment used in the production of various plastics. Prolonged exposure

to diethyl-hexyl and dibutyl phthalates has been associated with adverse effects on sperm motility (7) and mutagenic effects (8) in man.

Inadequate knowledge about the etiology of male factor infertility renders any effort to treat infertile men futile. The trauma and burden of an already distressed couple coping with infertility are often heightened by an unexplained diagnosis that eludes treatment. It is imperative that toxic factors in the environment that may result in this condition be identified and eliminated so that the appropriate therapy can be initiated.

Hence, the objective of this study was to evaluate the role of PCBs and PEs in the deterioration of semen parameters in infertile men without an obvious etiology and to identify the most susceptible group with regard to residential area and diet.

MATERIALS AND METHODS

Patients

Five hundred fifty-seven infertile couples were screened at the Assisted Conception Services Unit, Mahavir Hospital and Research Center, a referral center that receives cases from all over Andhra Pradesh, India. A complete clinical and case history evaluation was made, and laboratory investigations such as semen analysis were carried out as part of the fertility workup.

Institutional review board approval was obtained. Evaluation of semen parameters was executed as a part of the treatment protocol, thus obviating the need for an informed consent. Semen parameters such as ejaculate volume, sperm count, quantitative and qualitative motility, morphology, vitality, osmoregulatory capacity, nuclear chromatin stability, and sperm nuclear chromatin integrity were recorded for each sample after a 3-day period of abstinence as per the guidelines prescribed by the World Health Organization (9).

Sperm count was assessed by hemocytometry, morphology by Papanicolaou staining, sperm vitality by Eosin-Nigrosin staining, and sperm osmoregulatory capacity by the hypo-osmotic swelling test (10). Sperm nuclear chromatin stability was assessed by the sperm nuclear chromatin decondensation test. Sperm were treated with 1% SDS + 6 mM EDTA, Shorr stained, and classified according to the scheme of Rodriguez et al. (11). Sperm nuclear chromatin integrity was assessed by the chromatin condensation assay. Semen samples were treated with a citric acid buffer (pH 1.2) for 30 seconds and stained with acridine orange, and the DNA integrity was monitored under a fluorescent microscope. Green fluorescence was indicative of native (double-stranded) DNA, and red fluorescence was associated with denatured (single-stranded) DNA.

A male factor problem was identified in semen samples with a sperm count $<$20 mill/mL and/or rapid progressive motility (grade A) $<$25% or total progressive motility (grade A+B) $<$50% and/or $<$30% normal forms. A complete physical and urogenital examination was carried out in men with oligozoospermia (sperm count $<$20 million/mL) to rule out presence of a varicocele, congenital and acquired defects (congenital absence/ductal obstruction of the vas deferens and epididymides, cryptorchidism), inguinal surgery, testicular torsion, and sexually transmitted diseases.

Hormone analysis was carried out by radioimmunoassay to rule out hormonal disorders, semen culture and sensitivity to rule out infection, and karyotyping to rule out genetic abnormalities. The mixed agglutination reaction and immunobead test were carried out in specific cases to rule out an immunological factor. Infertile men with a history of systemic diseases, occupational exposure to reproductive toxicants, tobacco/alcohol consumption, iatrogenic causes involving a history of medical treatment (radiation, chemotherapy, drugs) or surgery (vas ligation) with possible adverse effects on fertility, family history of infertility/delayed conception, and febrile illness in the 6 months before analysis were excluded from the study.

Having ruled out the various causative factors, no obvious etiology could be observed in 52/300 (17.33%) infertile men who were either totally (exclusively male factor) or partially (combined factor) responsible for the infertility. PCBs and PEs were estimated in the seminal plasma of 21 such infertile men and 32 controls. Male partners of couples with [1] evidence of previous conception and normal semen analyses, [2] no history of tobacco or alcohol consumption, [3] no history of occupational exposure to reproductive toxicants, [4] no history of systemic diseases, [5] no history of medication or surgery, and [6] no history of febrile illness in the 6 months before analysis were selected as controls for the study.

On the basis of the documented source of xenoestrogens, men were further subcategorized into [1] urban fish eaters, [2] rural fish eaters, [3] urban vegetarians, and [4] rural vegetarians. Constant fish eaters consuming an average of at least seven fish meals/week were considered under the category of fish eaters. The total motile sperm count (TMC) was taken as a measure of semen quality. It was calculated as the product of grade A+B motility and the sperm concentration. For the sake of comparison of semen quality between these subcategories of men, two control groups were considered: control 1: fertile urban men with a mixed diet and no reported consumption of fish; and control 2: fertile rural men with a mixed diet and no reported consumption of fish. "Urban" was used to denote areas within the control of the Municipal Health Corporation; "rural" was used to denote areas not within municipal control.

Methods

The extraction procedure, high performance liquid chromatography (HPLC), was divided into five phases.

Phase 1: Isolation of Seminal Plasma

Semen was collected as a part of the treatment protocol, and after analysis of the semen parameters, seminal plasma was isolated by centrifugation at 2,000 × *g* for 20 minutes.

Phase 2: Removal of Sex Steroids by Charcoal-Dextran Treatment

Charcoal (Norit A, acid washed; Sigma, St. Louis, MO) was washed with cold sterile water immediately before use. A 5% charcoal and 0.5% dextran T-70 (D-4876; Sigma) suspension was prepared. Charcoal-Dextran (CD) suspension aliquots of a volume similar to that of the seminal plasma aliquots to be processed were centrifuged at 100 × *g* for 10 minutes. Supernatants were aspirated, and seminal plasma aliquots were mixed with charcoal pellets. The charcoal seminal plasma mixture was maintained by rolling at 4 cycles/minutes at 37°C for 1 hour. The suspension was centrifuged at 2,000 × *g* for 20 minutes. The supernatant was then filtered through a Nalgene filter with a pore size of 0.45 μm.

Phase 3: Extraction of Xenoestrogens

Extraction of xenoestrogens was performed by the method described by Burse et al. (12) with modifications. CD-treated seminal plasma was divided into three parts. One part was used as a blank, a known quantity of standard PCB (PCB mix 525; SUPELCO, USA) was added to the second part, and a known quantity of standard PE (Phthalate Esters Mix 48231; SUPELCO) was added to the third part to establish a comparison with the blank and to monitor the recovery of the added chemicals. The three aliquots were allowed to equilibrate at room temperature. Extracts from the blank preparation are devoid of absorbance at 280 nm and therefore do not interfere with the quantification of synthetic xenoestrogens. CD-treated seminal plasma lacks estrogenic activity.

The PCB mix was comprised of the following congeners: 2-chlorobiphenyl; 2,3-dichlorobiphenyl; 2,4,5-trichlorobiphenyl; 2,2′,4,4′-tetrachlorobiphenyl; 2,2′,3′,4,6-pentachlorobiphenyl; 2,2′,4,4′,5,6′-hexachlorobiphenyl; 2,2′,3,3′,4,4′,6′-heptachlorobiphenyl; 2,2′,3,3′,4,5′,6,6′-octachlorobiphenyl. The PE mix was comprised of the following congeners: dimethyl phthalate; diethyl phthalate; di-n-butyl phthalate; butyl benzyl phthalate; bis (2-ethylhexyl) phthalate; di-n-octyl phthalate.

Sample Extraction. One milliliter of methanol was added to each of the 2-mL aliquots of seminal plasma and mixed by vortexing, and then 3 mL of hexane:ethyl ether (1:1 by vol) (HPLC grade; Spectrochem, Ltd., Mumbai, India) was added to extract the mixture. The mixture was agitated on a rotary mixer for 15 minutes and then centrifuged at 2,000 × *g* for 5 minutes. The organic phase was collected, and the aqueous phase was extracted two more times. The organic phases were pooled and subsequently concentrated to 1 mL by evaporation under nitrogen steam.

Phase 4: Acid Cleanup of the Organic Phase Before HPLC

Concentrated sulphuric acid, 0.5 mL, was added to the concentrated 1-mL sample of organic phase. The organic phase was separated, and the aqueous phase was extracted two more times with 1 mL of hexane. The organic phases were pooled and dried completely under nitrogen, and the sample was resuspended in hexane before injection into the HPLC unit.

Phase 5: HPLC Analysis

Separation was performed by the method described by Medina and Sherman (13) and modified by Sonnenschein et al. (14) in a Shimadzu LC-6A liquid chromatograph solvent delivery system (Shimadzu SCL-6A system controller and Shimadzu SPD-6AV UV-Vis spectrophotometric detector equipped with a C-R6A chromatopac; Shimadzu, Japan).

Aliquots of 500 μL were injected into a 4 × 220 Partisil 5 silica column (Whattman, USA) equilibrated with 100% *n*-hexane (phase A) and *n*-hexane:methanol:isopropanol (40:45:15 by vol) (phase B) at a flow rate of 1.5 mL/minute and a pressure of 5×10^{-6} pascals. All of the organic solvents were of HPLC grade and were filtered with a 0.22-μm filter and degassed before use.

The gradient was developed as follows: 100% *n*-hexane was allowed to pass through the column for 2 minutes, after which the concentration of solvent B was increased to 10% in 10 minutes, 20% in the next 15 minutes, 50% in the next 5 minutes, and 100% in the next 5 minutes. Solvent B 100% was maintained in the column for 5 minutes before the concentration of solvent B was reduced to zero. The elution profile was monitored on a Shimadzu SPD-6AV UV-Vis spectrophotometric detector at 280 nm with an absorbance range of 0.001–2.56. The chromatogram was recorded on a C-R6A Chromatopac (Shimadzu).

Before standardization with the commercially available standards and the sample runs, a hexane blank was injected. Three samples were run for each patient: a seminal plasma blank, a seminal plasma sample to which a known amount of PCB was added, and a seminal plasma sample to which a known amount of PE was added. Four replicate runs were made for each sample. The peak HPLC fractions were collected in aliquots, and the spectrum of each fraction was confirmed spectrophotometrically with a UV-1601 visible spectrophotometer using a working wavelength of 210–320 nm.

Fractions with peak absorbances at the suggested wavelength were recorded to differentiate compound peaks from solvent peaks. The peak retention times of the blank sample were compared with those of the xenoestrogen- (PCB and PE) added sample and standard xenoestrogen retention

TABLE 1

Seminal xenoestrogens and semen parameters in infertile men and controls.

Characteristics	Controls (n = 32)	Infertile men (n = 21)	*t*
Age	32.5 ± 4.86	33.7 ± 3.45	0.98
PCB concentration (μg/mL)	0	7.63 ± 5.35	
PE concentration (μg/mL)	0.06 ± 0.02	2.03 ± 0.214	51.92[a]
Ejaculate volume (mL)	3.5 ± 1.38	2.5 ± 1.0	2.86[a]
Sperm count ($\times 10^6$/mL)	72.75 ± 17.61	17.04 ± 15.73	11.73[a]
Rapid linear progressive motility (grade A) (%)	53.0 ± 5.77	39.0 ± 34.08	2.28[a]
Total progressive motility (grade A+B) (%)	70.0 ± 18.98	52.0 ± 45.08	2.01[a]
Normal morphology (%)	63.46 ± 12.52	38.67 ± 23.86	4.94[a]
Head defects (%)	18.35 ± 1.23	35.67 ± 20.43	4.81[a]
Midpiece defects (%)	15.0 ± 10.61	23.33 ± 21.94	1.85
Tail defects (%)	3.0 ± 2.48	2.33 ± 1.15	1.16
Sperm vitality	79.48 ± 18.56	54.79 ± 26.97	3.95[a]
Sperm hypo-osmotic swelling test (%)	74.0 ± 13.39	53.98 ± 16.67	4.82[a]
Sperm nuclear chromatin decondensation (%)	19.58 ± 4.12	17.48 ± 1.95	2.17[a]
Single-stranded DNA (%)	4.3 ± 2.02	15.92 ± 6.02	10.10[a]

Note: Values represent mean ± SD.

[a] $P<.05$.

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times. After spectrophotometric estimation, fractions with peak absorbances at 280 nm were pooled, evaporated under liquid nitrogen, resuspended in hexane, and reinjected into the HPLC unit to purify the eluted compounds, and verify their peak retention times.

The concentration of the eluted compound was calculated from the area percentage under each peak. Congener-specific determination of PCBs and PEs was made based on the individual retention times of each fraction, and the respective concentrations were expressed in micrograms per milliliter. Individual PCB concentrations corresponding to the constituent congeners were added, and the total PCB concentration was expressed in micrograms per milliliter. Similarly, individual PE concentrations corresponding to the constituent congeners were added and the total PE concentration was expressed in micrograms per milliliter. The detection limits of PCBs ranged from 0.00323 to 14.97 μg/mL with a percentage recovery of 80.75%.

Xenoestrogen concentrations and semen parameters were compared between infertile men and controls, and their correlation was studied. Xenoestrogen concentrations and their TMCs were also compared among different categories of infertile men and controls.

Statistical Analysis

Differences in semen parameters between fertile and infertile men were analyzed by the Student's *t*-test. The correlation between seminal xenoestrogens and semen parameters was statistically analyzed by the coefficient of linear regression analysis. The Kolmogorov-Smirnov test was used for comparing semen quality among different categories of infertile men.

RESULTS

There was a significant deterioration in semen parameters (decreased ejaculate volume, sperm count, rapid and total progressive motility, normal morphology, vitality, sperm osmoregulatory capacity, nuclear chromatin decondensation, and sperm nuclear chromatin integrity) in infertile men without an obvious etiology when compared with controls. PCBs were detected in the seminal plasma of infertile men but not in that of controls. PE concentrations were significantly higher in the seminal plasma of infertile men than in that of controls (Table 1).

Significant negative correlations were observed between seminal PCB concentrations and the ejaculate volume ($r = -0.682$, $P<.001$), total progressive motility ($r = -0.477$, $P<.05$), sperm vitality ($r = -0.791$, $P<.001$), and sperm osmoregulatory capacity ($r = -0.754$, $P<.001$). There was a significant positive correlation between seminal PCBs and the percentage of single-stranded DNA in the sperm ($r = 0.564$, $P<.05$) but no significant correlation between PCBs and sperm count ($r = -0.022$), rapid linear progressive motility ($r = -0.403$), sperm normal morphology ($r = 0.124$), percentage of head defects ($r = 0.111$), or the percentage of sperm nuclear chromatin decondensation ($r = -0.076$) (Table 2).

Significant correlations were observed between seminal PEs and sperm normal morphology ($r = -0.769$, $P<.001$) and the percentage of single-stranded DNA in the sperm ($r = 0.855$, $P<.001$). However, there was no significant correlation between seminal PEs and the ejaculate volume ($r = -0.198$), sperm count ($r = -0.221$), rapid linear progressive

TABLE 2

Correlation of environmental estrogens and semen parameters in infertile men.

Semen parameters	Polychlorinated biphenyls (n = 21)		Phthalate esters (n = 21)	
	r	*t*	*r*	*t*
Ejaculate volume	−0.682	4.066[a]	−0.198	0.877
Sperm count	−0.022	0.099	−0.221	0.985
Rapid linear progressive motility (grade A)	−0.403	1.902	−0.046	0.199
Total progressive motility (grade A+B)	−0.477	2.357[a]	−0.142	0.624
Normal morphology	0.124	0.429	−0.769	5.36[a]
Head defects	−0.111	0.379	−0.436	2.11[a]
Vitality	−0.791	4.33[a]	−0.125	0.55
Sperm osmoregulatory capacity (%)	−0.754	5.02[a]	−0.165	0.73
Sperm nuclear chromatin decondensation (%)	−0.076	0.331	0.04	0.18
Single-stranded DNA (%)	0.564	2.787[a]	0.855	7.72[a]

[a] $P<.05$.

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motility ($r = -0.046$), total progressive motility ($r = -0.142$), sperm vitality ($r = -0.125$), sperm osmoregulatory capacity ($r = -0.165$), or percentage of sperm nuclear chromatin decondensation ($r = -0.04$) in infertile men (Table 2).

PCB and PE concentrations were the highest in infertile urban fish eaters, followed by infertile rural fish eaters, infertile urban vegetarians, and infertile rural vegetarians. They were higher in infertile fish eaters than in infertile non–fish eaters regardless of the place of dwelling and were higher in infertile urban dwellers than in infertile rural dwellers regardless of the diet. The TMCs in these infertile men were inversely related to their respective PCB and PE concentrations. TMCs were significantly lower in infertile urban and rural dwellers compared with urban and rural controls, respectively, and were lower in urban controls than in rural controls, but the difference was not statistically significant (Tables 3 and 4).

DISCUSSION

This study of infertile men is the first report from the Indian subcontinent that demonstrates the presence of xenoestrogens in semen samples. The significant presence of PCBs and PEs in the seminal plasma of occupationally unexposed infertile men and the significantly worse semen parameters in infertile men compared with controls suggest that these chemicals are ubiquitous in the environment and may be instrumental in the deterioration of sperm count,

TABLE 3

Xenoestrogen concentrations and semen quality in different categories of infertile men.

Patient characteristics	n	Mean polychlorinated biphenyl concentrations (μg/mL)	Mean phthalate ester concentrations (μg/mL)	Mean total motile sperm counts ($\times 10^6$/mL)
Control 1	13	0.0	0.064	25.11
Control 2	19	0.0	0.059	30.28
Urban dwellers	15	9.38	2.61	0.66
Rural dwellers	6	3.27	0.59	4.21
Fish eaters	15	9.44	2.65	0.59
Non–fish eaters	6	3.1	0.48	4.37
Urban fish eaters	12	10.49	3.13	0.51
Urban vegetarians	3	4.92	0.57	1.24
Rural fish eaters	3	5.26	0.77	0.92
Rural vegetarians	3	1.28	0.39	7.5

Note: Control 1: fertile men from urban areas with a mixed diet (excluding fish). Control 2: fertile men from rural areas with a mixed diet (excluding fish).

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TABLE 4

Kolmogorov-Smirnov test values for the difference in total motile sperm counts between infertile men and controls.

Groups	Values
Control 1 vs. control 2	0.115
Urban dwellers vs. control 1	0.68[a]
Rural dwellers vs. control 2	0.93[a]
Urban vs. rural dwellers	0.32[a]
Fish eaters vs. non–fish eaters	0.35[a]
Fish-eating urban dwellers vs. control 1	0.742[a]
Urban vegetarians vs. control 1	0.967[a]
Fish-eating rural dwellers vs. control 2	0.9[a]
Rural vegetarians vs. control 2	0.9[a]

Note: Control 1: fertile men from urban areas with a mixed diet (excluding fish). Control 2: fertile men from rural areas with a mixed diet (excluding fish).

[a] $P<.05$.

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motility, and fertilizing capacity. Evidence for the adverse effects of these chemicals is further substantiated by data from the correlation studies.

Also known as endocrine disruptors or hormone-mimicking chemicals, xenoestrogens have the potential to exert toxicities at many levels and by many molecular mechanisms (15). They may mimic, block, or both mimic and block a proper hormone response or bind to other receptors and create a novel reaction. Xenoestrogens may disrupt hormone-mediated events and inhibit normal signal transduction in the testis and epididymis by either binding to sex hormone–binding globulin and androgen-binding protein or by blocking the cell-surface receptors for these proteins (16), or they may disturb the hormonal milieu within the prostate and seminal vesicles by possibly interfering with androgen binding to the androgen receptors. They may also induce abnormalities in vascular function and structure and lead to reproductive disorders, impotence, and infertility by mimicking or antagonizing the vascular effects of E_2 (17). The testes and the sex accessory glands may be at a particular risk for the estrogenic effects due to the presence of receptors for male gonadal hormones (18).

The mechanism of action of PCBs is unclear but may involve antiandrogenic activity, modulatory effects on enzymes controlling sex hormone and receptor metabolism, an acute inhibition of enzymes involved in testicular steroidogenesis, a direct influence on hormone-producing organs such as the thyroid gland, pituitary gland, and adrenal gland (19), or the loosening of intercellular contacts between germ cells and Sertoli cells (20). The observation of a significant decrease in rapid and total progressive motility in men with unexplained infertility compared with controls is in agreement with reports citing an inverse correlation of PCBs with sperm motility in men with oligozoospermia (21).

Cytotoxic effects such as production of superoxide anion (O^{2-}) by human polymorphonuclear leukocytes (neutrophils) and activation of various intracellular signal transduction pathways (22) may explain the significant decrease in normal morphology, increased sperm damage, increased incidence of single-stranded DNA, and decreased fertilizing capacity in infertile men.

Negative correlations have been reported between phthalate concentrations in the cellular fraction of ejaculates and sperm production (23). Butyl benzyl phthalates (BBPs) may have an anti-androgenic effect (24) or may bring about the necrosis of the epididymal tubular epithelium (25). Highly significant reductions in testis size and corresponding decreases in daily sperm production have been reported after exposure of pregnant rats to relatively low levels of BBPs during the period of Sertoli cell multiplication (26).

Di-(2-ethylhexyl) phthalate is known to induce testicular atrophy accompanied by aspermatogenesis (27), increase peroxisome proliferation, and inhibit gap-junctional intercellular communication and replicative DNA synthesis (28). The increased intracellular rates of DNA-damaging reactive oxygen production after peroxisome proliferation (29) may be a mechanism contributing to the increased percentage of abnormal sperm in infertile men. Diethyl phthalate (DEP) may induce structural and functional alterations in Leydig cells, thereby affecting T output (30). A failure in androgen-dependent cytostructural modifications and biochemical changes involved in epididymal sperm maturation could result in a deterioration in sperm function.

Exposure to di-n-butyl phthalate has been associated with [1] reduced sperm counts and reproductive tract malformations (31); [2] adverse effects on the liver's mitochondrial energy-coupling processes, active $K+$ transport, and oxidative phosphorylation (32); [3] inhibition of testis-specific enzymes associated with postspermatogenic and premeiotic spermatogenic cells (33); [4] dissociation of germ cells from Sertoli cells, reduction in the levels of triglycerides, cholesterols, and phospholipids containing choline and ethanolamine residues, testicular fructose, glucose, iron, and zinc (34); and [5] depletion of glutathione and decrease in glutathione reductase activity (35).

Testicular zinc depletion causally related to the ensuing testicular and accessory sex organ atrophies (36) may significantly contribute to the deleterious effects of phthalates on spermatogenesis and sperm fertilizing capacity. The adverse effects of phthalates on the energy metabolism (32) could be an important mechanism involved in the reduction of sperm motility and could also interfere with the respiratory functions of Sertoli cells, thus inhibiting spermatogenesis.

Observations of higher xenoestrogen concentrations in fish eaters in this study are comparable to studies that have reported elevated concentrations of organochlorine com-

pounds in the tissues of people consuming large amounts of contaminated seafood when compared with the general population (37). PCBs that are associated with the fats of fish or animal flesh cannot be removed by washing and are only partially removed by cooking (38). Due to the low biodegradation and excretion in humans, these substances accumulate in the body fat and their concentrations reflect external exposure (39). The dietary intake of PCBs is expected to account for 89%–99% of human exposure to these compounds (40). The general population may be exposed to phthalates via food (baby milk formula, cheese, margarine, potato chips), drinks directly contaminated by plastic wraps containing phthalates, or polluted drinking water (41).

The past three to four decades has witnessed a tremendous increase in industrialization in Andhra Pradesh. With 25.72% urbanization and a literacy rate of 44.09%, the state has 208 industrially developed areas and estates. Environmental exposure to improperly disposed industrial effluents from major industries such as cement and cement products, synthetic drugs and pharmaceuticals, petrochemicals, plastic industries, heavy electricals, fertilizers, tobacco, and coal may account for observations of higher xenoestrogen concentrations and concomitantly lower TMCs in urban dwellers compared with rural dwellers.

The presence of xenoestrogens in rural dwellers with a vegetarian diet can be traced to the biomagnification of the chemicals after the consumption of edible plants grown on contaminated soil. Usually plants are too short lived to display responses to xenoestrogen toxicity because they are quickly consumed by either land animals or humans. Although guidelines for PCB tolerances exist only for food of animal origin, it has been estimated that leafy vegetables and unpeeled fruits from contaminated regions present a significant source of human exposure to PCBs (42). Furthermore, xenoestrogens usually enter water at the points of discharge of industrial and urban wastes into rivers, lakes, and coastal waters that may be used for irrigation. Xenoestrogens appear in measurable amounts in water used for irrigation (43).

From the correlation studies it is evident that the PCBs have significant dose-dependent relationships with most of the semen parameters studied (ejaculate volume, total progressive motility, sperm vitality, osmoregulatory capacity, and sperm DNA integrity) when compared with PEs, which suggests that the PCBs could perhaps be more instrumental than the PEs in the deterioration of semen parameters in infertile men without an obvious etiology.

Data indicating that a xenobiotic may affect multiple signaling pathways coupled with data showing synergistic effects of multiple xenobiotics on estrogen-responsive genes (16) reinforces the possibility that environmental xenobiotics, although present at low concentrations, may pose a threat to human health. Further studies are necessary to evaluate the epidemiological risk assessment in terms of minimum exposure limits.

Acknowledgments: The authors thank Prof. P. R. K. Reddy for scientific advice and researchers Dr. Srinivas Reddy, Dr. Narsa Reddy, Dr. V. Manjari, and Dr. B. Ramakrishna of the Department of Animal Sciences, University of Hyderabad, for their contribution to the study.

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