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Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women

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ABSTRACT: A sensitive HPLC method with fluorescence detection was developed for the determination of bisphenol A (BPA) and bisphenol B (BPB) in human blood serum. The detection limits of the method were 0.18 and 0.20 ng/mL for BPA and BPB, respectively. A single-step liquid–liquid extraction was used for the pre-treatment of serum samples. The recoveries of BPA and BPB spiked to sera were 85.6 and 87.7%, respectively. The analyses of sera from both healthy and endometriotic women emphasized the absence of bisphenols in all the control cases (11 women), whereas BPA was found in 30 sera (51.7%) and BPB was found in 16 sera (27.6%) in the group of 58 patients with endometriosis; in nine of such sera BPA and BPB were present simultaneously. Only relatively to the sera quantitated, BPA concentrations ranged from 0.79 to 7.12 ng/mL (mean concentration 2.91 ± 1.74 ng/mL), whereas BPB concentrations ranged from 0.88 to 11.94 ng/mL (mean concentration 5.15 ± 4.16 ng/mL). Therefore, the presence of at least one of the two bisphenols was verified in a percentage as high as 63.8% in the sera from endometriotic women, suggesting the existence of a relationship between endometriosis and BPA and/or BPB exposure. Indeed, it is well known that bisphenols can work as xenoestrogens, owing to their structural similarity to natural and synthetic estrogens (e.g. estradiol and dietilstilbestrol). However, further studies are necessary to confirm this hypothesis and to assess the actual dose at which exposures to bisphenols are able to increase the sensitivity of the endometriotic cells to estradiol.

Keywords: bisphenol A; bisphenol B; HPLC; serum levels; endometriosis

Introduction

Human endometrium changes during fertile life, pregnancy and menopause are regulated by cytokines and growth factors under the control of steroid hormones. Endometriosis is a common gynecological disorder, affecting at least 10% of women, and often causes pain or infertility. The disease is characterized by growth of endometrial tissue outside the uterus, with a spread that involves mainly ovaries, but also peritoneum, utero-sacral ligaments and recto-vaginal septum. Several theories have been proposed to explain the pathogenesis: retrograde menstrual flow through the fallopian tubes implants on the peritoneal surfaces, celomic metaplasia and hematologic and lymphatic spread (Sampson, 1940; Oral and Arici, 1997; Jenkins *et al*., 1986).

Emerging evidences have proposed a putative role for ubiquitous environmental contaminants in the occurrence of endometriosis. The mechanism of action of environmental substances may be carried out through the interaction with steroidal receptors, mimicking an estrogenic effect. More recently, the global concerns about the endocrine disruptors that mimic the effects of natural estrogens have also focused on bisphenols and their congeners (Gallart-Ayala *et al*., 2007).

Bisphenol A [2,2-bis(4-hydroxyphenyl)propane; BPA] is a small monomer (MW = 228.29) that is polymerized to produce polycarbonate, epoxy resins and other plastics. They are extensively employed for polycarbonate bottles and containers, food can linings and white dental fillings and sealants. Human exposure to BPA may arise through BPA leaching from these materials into foods (Sampson, 1940; Oral and Arici, 1997) and/or saliva (Jenkins *et al*., 1986; Krishnan E *et al*., 1993). BPA is also used as an additive in other types of plastics, such as polyvinyl chloride (PVC), used in medical tubing, toys and water pipes, and polyethylene terephthalates (PET). Bisphenol B [2,2-bis(4-hydroxyphenyl)butane; BPB] is a BPA derivative (MW = 242.32) with similar estrogen-like activity used in the manufacture of phenolic resins. Since a weak estrogen-like activity of BPA was reported by Krishnan *et al*. (1993), its effects on human health have become of growing concern. BPA shows estrogenic activity towards cell lines, such as estrogen-responsive breast cancer cell line MCF-7 cells and endocrine-disrupting effects *in vivo* (Rier and Foster, 2002; Zeyneloglu *et al*., 1997; Cobellis *et al*., 2003; Ashby *et al*., 1998; Ashby and Odum, 2004; Bergeron *et al*., 1999; Kim *et al*., 2002; Matthews *et al*., 2001).

The interaction of BPA and/or BPB with estrogenic receptor produces the activation of the same transcriptional-factor

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Abbreviations used: BPA, bisphenol A; BPB, bisphenol B.

(CREB) as 17-β-estradiol (Tinwell *et al*., 2000; Quesada *et al*., 2002), located in proximal promoter II of aromatase gene. Therefore, probably, this mechanism determines a greater activity of aromatase and, therefore, a greater production of estrogens, favoring the proliferative and inflammatory characteristics of endometriosis. Furthermore, previous studies have shown a relationship between BPA (and its metabolites) serum concentration increase, altered secretion of gonadotropic hormones and increase of androgenic hormones (Takeuchi *et al*., 2004, 2006).

It is useful to remember that androgenic hormones are the substrate of enzymes, codified from the genes CYP17 and CYP19 (Mlynarcikova *et al*., 2005), involved as agents in charge of the conversion of androstenedione and testosterone in estrone and estradiol, respectively; therefore, androgenic hormones are also likely to be involved in the onset of a mainly estrogenic climate. In conclusion, the way bisphenols work is still not clear, but it is assumable that they basically produce an alteration of the endocrine system that regulates the proliferation, the interaction and the cellular differentiation priming mechanisms favoring adverse health effects. Therefore, endometriosis having a multifactorial complex etiology, research in future should seek the meeting point between genetic predisposition and environmental factors able to modulate phenotypic expression.

Exposure estimates by the European Commission for BPA from various sources amount to less than 30 μg per day for an adult (or 0.05 mg/kg body weight) (Dekant and Volkel, 2008). However, it is now widely recognized that toxic effects of bisphenols can arise from chronic exposure to doses much lower than those reported for acute exposure by chemical corporations and regulatory agencies (vom Saal *et al*., 2006). Indeed, following a near-continuous daily exposure, and due to the high lipophilicity (clog *P* are 3.32 and 4.20 for BPA and BPB, respectively; clog *P* for Windows Version 2.0, Biobyte Corp., Claremont, CA, USA), they can accumulate in the adipose tissue, giving rise to persistent, although low, serum levels. Therefore, highly sensitive and selective analytical methods are needed for determination of low levels of BPA and BPB in serum samples.

The aim of the present study has been to set up a method for the simultaneous determination of BPA and BPB, for routine monitoring of serum levels in endometriotic women. Therefore, particular attention was paid to setting up a method characterized by a fast and easy extraction procedure of the active compounds from the biological matrix. In the present work we have used a simple one-step extraction procedure and a reversed-phase high-performance liquid chromatographic (RP-HPLC) technique with fluorescence detection. In order to avoid complex extraction procedures, with consequent possible poor recoveries, we selected as stationary phase a monolithic column because it is hardly affected by the presence of residual biological components of the samples.

Finally, we measured the concentration of both BPA and BPB in sera of women affected by endometriosis vs healthy controls, in order to establish a possible relationship between bisphenol presence in the serum and endometriosis.

Experimental

Reagents and Chemicals

Bisphenol A standard (minimum purity ≥99%) was purchased from Sigma-Aldrich (UK) and bisphenol B standard (minimum purity ≥99%) from TCI Europe (Zwijndrecht, Belgium). All chemicals and reagents were of either analytical or HPLC-grade and were purchased from Carlo Erba (Italy). Water was deionized, distilled and passed through a water purification system (Sartorius, Germany).

Subjects

The study was approved by the institutional review board of Second University of Naples; informed consent was obtained from the participants. A group of fertile women ($n = 69$), referred to the Department of Gynaecology, Obstetrics and Reproductive Medicine of the same university was enrolled. The patients were submitted to diagnostic or operative laparoscopy for the evidence of ovarian cysts or to investigate chronic pelvic pain and dysmenorrhea.

At the end of the operative procedure two different groups were established: endometriotic patients ($n = 58$; age 32.8 ± 6.7 years, mean ± SD; age range 21–42) and a control group ($n = 11$; age 34.5 ± 4.1 years, mean ± SD; age range 18–44). The latter group did not show any macroscopic evidence of disease and served in the study as age-matched controls. The endometriosis diagnosis was confirmed by histological examination of the endometriotic lesions, and patients were classified according to the revised American Fertility Society classification of endometriosis (Buttram 1985). Blood samples were collected in Vacu-test® tubes from the antecubital vein of each volunteer (5 mL each time). Glass syringes and glass tubes were employed throughout the sampling to avoid a contamination of BPA and BPB. Vacu-test® tubes were centrifuged at 3000 rpm, 20 min, promptly, then they were transferred to clean glass vials, labelled with a code identifying the patient and immediately frozen and stored at –20°C until assayed within one week after collection.

HPLC System

The HPLC system consisted of a pump (LC 10ADVP, Shimadzu, Japan), a 7725 injector with a 20 μL loop (Rheodyne, Cotati, USA) and a fluorescence detector (Waters 470; excitation wavelength 273 nm, emission wavelength 300 nm). The signal was recorded using PC software (Chromatoplus 2007, Shimadzu-Corporation, Kyoto, Japan). The mobile phase was a mixture of acetonitrile and phosphate buffer at pH 6.0 (35:65 v/v). The stainless-steel column was a reversed-phase Onyx Monolithic C_{18} column (100×4.6 mm i.d.; Phenomenex, USA). Chromatography was performed at a flow rate of 1.0 mL/min at room temperature (20 ± 2°C). Sample volumes of at least three fold the loop volume (20 μL) were injected into the chromatograph and concentrations of BPA and BPB were estimated on the basis of peak area from the calibration curve. The retention times (t_r) were 9.57 ± 0.30 min for BPA and 12.01 ± 0.40 min for BPB.

Mass Spectrometry Measurements

The mass spectrometric analysis was performed on samples obtained by collecting the chromatographic peaks corresponding to either BPA (t_r range 9.00–10.00 min) or BPB (t_r range 11.50–12.50 min) in 20 runs. Mass spectra were acquired using an API 2000 triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Applied Biosystem, MDS Sciex, Foster City, CA, USA).

All the mass spectra were recorded by infusion into the ESI source using acetonitrile as the solvent. For each analysis the

full-scan spectrum (mass range 100–600 *m/z*, scan time 1 s) was acquired for identification purposes. The ESI-MS was operated in the negative ion mode under the following conditions: ionspray voltage was kept at 5000 V; turbo gas temperature, 250°C; nebulizer gas (compressed air), 55 psi; curtain gas (N_2), 20 psi; declustering potential, 40 V; focusing potential, 200 V; entrance potential, 10 V.

Drug Analysis

The analyses of both standard solutions and serum samples were performed in triplicate according to the following procedure: 300 µL serum was added to 150 µL mobile phase; 150 µL perchloric acid 25% w/v was added to precipitate proteins and the mixture was vortex-mixed for 10 s and centrifuged at 3000 rpm for 5 min. The filtered supernatant was collected for the analysis.

The samples of control patients were considered as blanks; they were used (i) to confirm the assignment of peak identity to BPA and BPB, (ii) to verify the absence of interfering peaks, (iii) to assess the accuracy of the method and (iv) to determine the recovery.

Recoveries were calculated by comparing the peak areas of the spiked sera at 2.0, 4.0 and 10.0 ng/mL, subjected to the procedure for the sample pre-treatment, with those of standard solutions at the same concentrations of BPA and BPB. Both the analytes showed similar recoveries: 85.6 and 87.7% for BPA and BPB, respectively.

BPA and BPB methanol standard solutions were both at the following final concentrations: 0.5, 1.0, 5.0, 10.0 and 20.0 ng/mL. Calibration curves of peak area vs concentration (ng/mL) were obtained by injecting into the chromatograph the standard solutions (at least three fold the loop volume), corresponding to bisphenol amounts ranging from 10 to 400 pg. Straight lines ($r^2 = 0.989$ for BPA and $r^2 = 0.985$ for BPB) were observed within the range of concentrations considered. The limits of detection (LOD) of the analytical procedure are the lowest concentrations of analytes that can be measured with definable statistical certainty in a sample, and were calculated from the levels of the various analytes equivalent to three times the standard deviation of noise on analysis, whereas the limits of quantitation (LOQ) were calculated from the concentration of the analytes that provided signals equal to 10 times the noise signal of analysis. The LOQ of synthetic solutions were 0.50 and 0.60 ng/mL, for BPA and for BPB, respectively; the LOD were 0.15 and 0.18 ng/mL for BPA and BPB, respectively.

Standard solutions of BPA and BPB were injected in the HPLC immediately after preparation. No degradation product was observed in the HPLC analysis of standard solutions. Calibration procedures were repeated every two weeks. Within this period the coefficient of variation (CV%) was <0.5%.

Statistical Analysis

A commercially available statistical package for personal computer (Microsoft Excel 2000) was used. Data are expressed as mean ± standard deviation (mean ± SD).

Results and Discussion

The HPLC method we set up allowed us to perform rapid analyses of BPA and BPB in the sera with good detectability and quantification. Moreover, the extraction step of bisphenols from the biological matrix was very rapid and easy, due the use of a monolithic stationary phase. No interference from biological components was verified, since no signal interfering with the signals of BPA and BPB was observed, and other compounds and metabolites of serum samples were eluted within 4 min in chromatograms. The possible presence of either BPA or BPB in the sera was confirmed by comparison with the chromatograms of the same samples spiked with the respective pure compounds and through mass spectrometric analyses. For quantitative analysis, calibration procedures were performed on BPA and BPB standard solutions and repeated every 2 weeks; within this period the coefficient of variation (CV) was <0.5%. The solutions were injected in the HPLC immediately after preparation and no degradation product was observed in their analysis.

The method, after validation on spiked sera, was performed in the analyses of 69 sera from healthy and endometriotic women. Neither BPA nor BPB was found in any of the 11 control serum samples; for the 58 samples from endometriotic women, BPA was detected in the serum of 30 subjects (51.7%), but it was quantified in the serum of only 15 subjects, being under the LOQ in the other 15 subjects, i.e. in the subjects had serum concentrations under 0.50 ng/mL (this range accounts for both recovery percentage and serum dilution before HPLC analysis). In the 15 sera quantified, the mean concentration of BPA was 2.91 ± 1.74 ng/mL (mean ± SD), the minimal concentration being 0.79 ng/mL and the maximal concentration being 7.12 ng/mL. BPB was detected in the serum of 16 subjects (27.6%), but it was quantified in only 10 subjects, being under LOQ in six subjects, i.e. under 0.60 ng/mL. In the 10 sera quantified, the mean concentration of BPB was 5.15 ± 4.16 ng/mL (mean ± SD), with a minimal concentration of 0.88 ng/mL and a maximal concentration of 11.94 ng/mL. Since BPA and BPB were simultaneously present in nine serum samples, only 21 samples (36.2%) did not show the presence of BPA and/or BPB. The results are summarized in Table 1, whereas Fig. 1 shows the representative chromatograms

Table 1. Results of the analyses for BPA and BPB on serum samples of endometriotic women

	BPA	BPB
Number of serum samples containing bisphenol	30	16
Percentage of serum samples containing bisphenol	51.7%	27.6%
Percentage of serum samples quantitated	25.9%	17.2%
Range of concentration (ng/mL)	0.79–7.12 ng/mL	0.88–11.94 ng/mL
Mean concentration ± SD (ng/mL)	2.91 (±1.74) ng/mL	5.15 (±4.16) ng/mL

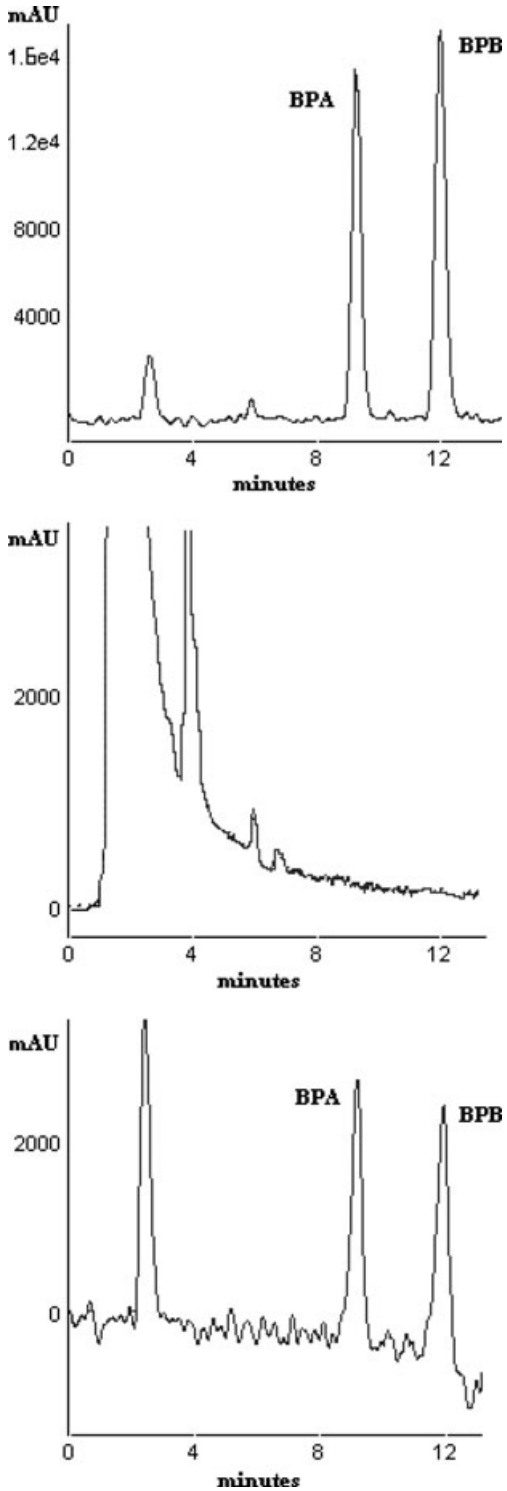

Figure 1. Chromatograms corresponding to (A) a standard solution of BPA and BPB 10.0 ng/mL; (B) blood serum of a healthy women; and (C) serum of an endometriotic woman containing both BPA and BPB. Figures on the axes are: time (min) on the abscissa, absorbance units on the ordinate.

obtained from (i) a standard solution, (ii) a reagent blank and (iii) a blood serum sample containing both BPA and BPB.

Precision is the measure of how close results are to one another, and it is evaluated by making repetitive measurements for the entire method and examined on serum samples. Very good inter-day precision data ($n = 6$) and intra-day precision data ($n = 3$) were obtained on samples of control patients spiked with 3.0 ppb of either BPA or BPB. The RSD (relative standard deviation) was 3.9 and 4.2%, respectively. The RSD% was calculated by dividing the standard deviation by the mean, and multiplying the value by 100. Figure 2(a, b) shows the mass spectra of two chromatographic peaks of a real sample, eluting at the retention times of BPA and BPB, respectively. The correspondence of the signals of molecular ions to those of BPA and BPB standards (*m/z* 227 $[M–H]^-$ for BPA and *m/z* 241 $[M–H]^-$ for BPB), together to the fragmentation pattern, confirmed their identity. Although the sensitivity of the proposed method is slightly lower than that of the other analytical methods reported in the literature for BPA in biological samples (Gallart-Ayala *et al*., 2007; Sun *et al*., 2001; Kuroda *et al*., 2003; Gallart-Ayala *et al*., 2008; Padmanabhan *et al*., 2008), the main advantage of the proposed method is that, differently from HPLC-MS methods; it does not require the use of non-routine procedures.

Conclusion

To date, the etiology of the endometriosis, an estrogen-dependent pathology, is still not clear, although in recent years many studies have addressed the possible implications of environmental factors in its pathogenesis. Increasing attention has been focused on some substances, defined as 'endocrine disruptors', that are able to interact with steroidal receptors and to reproduce such the endocrine responsiveness. BPA and BPB are two compounds that adequately reflect these properties, their ability to interact with estrogen receptors being fully justified by their structural similarity to the natural estrogens. Therefore, their implication in the estrogen-dependent pathologies is possible and the availability of rapid and simple analytical methods may be of great usefulness in the future investigations, which are expected to involve screenings on larger scale.

In the present study we have set up a HPLC method for the simultaneous determination of BPA and BPB in the serum. The method has been applied in an investigation about the possible relationship between serum levels of bisphenols and the occurrence of endometriosis.

The results can be summarized as follows: neither BPA nor BPB was found in any of the sera from healthy women (the control cases); in contrast, at least one of the bisphenols was found in 63.8% of sera of 58 patients with endometriosis. These findings strongly suggest the existence of a relationship between occurrence of endometriosis and the presence of BPA and/or BPB in the serum. Moreover, it is also important to underline that it is the first time that the sera of endometriotic women have been investigated for the presence of BPB. Its recognition (although less frequent than BPA, i.e. 27.6 vs 51.7%) supports its possible role in the occurrence of estrogen-dependent pathologies. The hypothesis that bisphenols are involved in the pathogenesis of endometriosis needs to be further confirmed by monitoring larger number of cases; however, our results are consistent with the hypothesis (Golden *et al*., 1998) that bisphenols enhance the sensitivity of the endometriotic cells to estradiol, working as xenoestrogens.

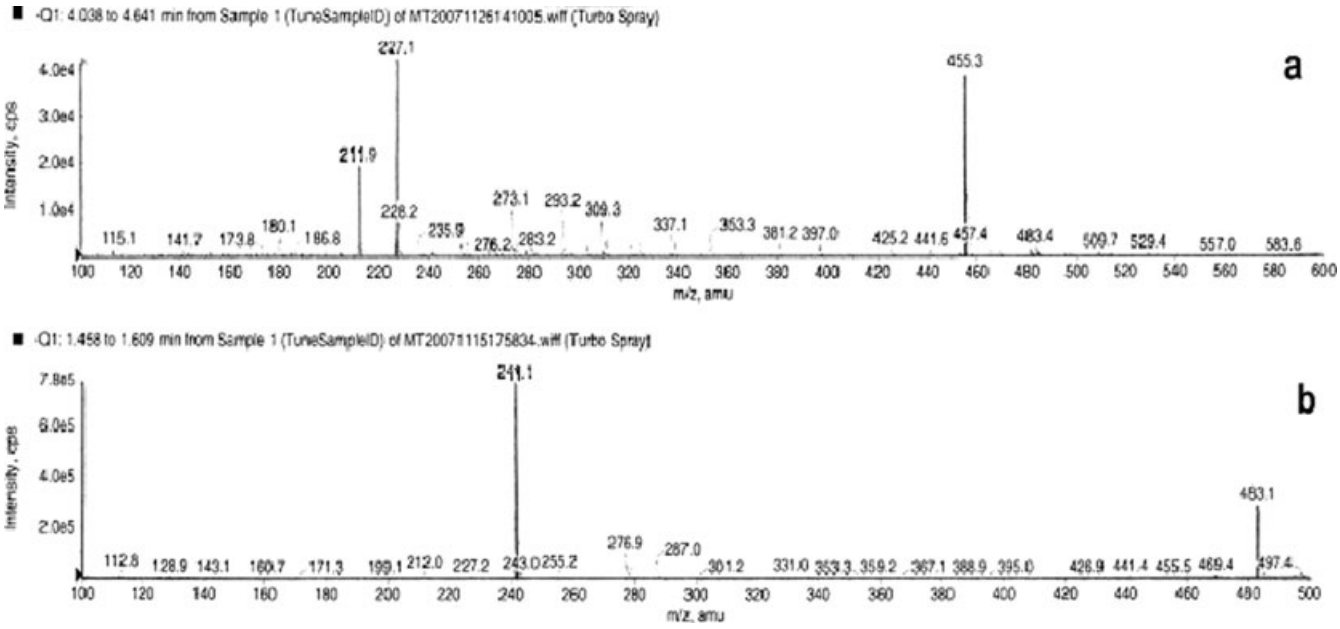

Figure 2. Mass spectra of BPA (a) and BPB (b) in a real sample of serum of endometriotic patients, acquired in full-scan ESI-MS in negative mode.

References

Ashby J and Odum J. Gene expression changes in the immature rat uterus: effects of uterotrophic and sub-uterotrophic doses of bisphenol A. *Toxicological Sciences* 2004; **82**: 458–467.

Ashby J and Tinwell H. Uterotrophic activity of bisphenol A in the immature rat. *Environmental Health Perspectives* 1998; **106**: 719–720.

Bergeron RM, Thompson TB, Leonard LS, Pluta L and Gaido KW. Estrogenicity of bisphenol A in a human endometrial carcinoma cell line. *Molecular and Cellular Endocrinology* 1999; **150**: 179–187.

Buttram VC Jr. Evolution of the revised American Fertility Society classification of endometriosis. *Fertility and Sterility* 1985; **43**: 347–350.

Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F, Mazzeo P and Petraglia F. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Human Reproduction* 2003; **18**: 1512–1515.

Dekant W and Volkel W. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. *Toxicology and Applied Pharmacology* 2008; **228**: 114–134.

Gallart-Ayala H, Moyano E and Galceran MT. Liquid chromatography/multi-stage mass spectrometry of bisphenol A and its halogenated derivatives. *Rapid Communications in Mass Spectrometry* 2007; **21**: 4039–4048.

Gallart-Ayala H, Moyano E and Galceran MT. Liquid chromatography/tandem spectrometry (highly selective selected reaction monitoring) for the analysis of isopropyithioxanthone in packaged food. *Journal of Chromatography A* 2008; **1208**: 182–188.

Golden RJ, Noller KL, Titus-Ernstoff L, Kaufman RH, Mittendorf R, Stillman R and Reese EA. Environmental endocrine modulators and human health: an assessment of the biological evidence. *Critical Reviews in Toxicology* 1998; **28**: 109–227.

Jenkins S, Olive DL and Haney AF. Endometriosis: pathogenetic implications of the anatomic distribution.*Obstetrics and Gynecology* 1986; **67**: 335–338.

Kim EJ, Kim JW, Lee SK. Inhibition of oocyte development in Japanese medaka (*Oryzias latipes*) exposed to di-2-ethylhexyl phthalate. *Environment International* 2002; **28**: 359–365.

Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* 1993; **132**: 2279–2286.

Kuroda N, Kinoshita Y, Sun Y, Wada M, Kishikawa N, Nakashima K, Makino T and Nakazawa H. Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. *Journal of Pharmaceutical and Biomedical Analysis* 2003; **30**: 1743–1749.

Matthews JB, Twomey K and Zacharewski TR. *In vitro* and *in vivo* interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chemical Research in Toxicology* 2001; **14**: 149–157.

Mlynarcikova A, Fickova M, Scsukova S. Ovarian intrafollicular processes as a target for cigarette smoke components and selected environmental reproductive disruptors. *Endocrine Regulations* 2005; **39**: 20–31.

Oral E and Arici A. Pathogenesis of endometriosis. *Obstetrics and Gynecology Clinics of North America* 1997; **24**: 219–233.

Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, Tao L and Kannan K. Maternal bisphenol-A at delivery: a looming problem? *Journal of Perinatology* 2008, **28**: 258–263.

Quesada I, Fuentes E, Viso-León MC, Soria B, Ripoll C and Nadal A. Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *FASEB Journal* 2002; **16**: 1671–1673.

Rier S and Foster WG. Environmental dioxins and endometriosis. *Toxicological Science* 2002; **70**: 161–170.

Sampson JA. The development of the implantation theory for the origin of peritoneal endometriosis. *American Journal of Obstetrics and Gynecology* 1940; **40**: 549–557.

Sun Y, Wada M, Kuroda N, Hirayama K, Nakazawa H and Nakashima K. Simultaneous determination of phenolic xenoestrogens by solid-phase extraction and high-performance liquid chromatography with fluorescence detection. *Analytical Sciences* 2001; **17**: 697–702.

Takeguchi T, Tsutsumi O, Ikezuki Y, Takai Y and Taketani Y. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. *Endocrine Journal* 2004; **51**: 165–169.

Takeuchi T, Tsutsumi O, Ikezuki Y, Kamei Y, Osuga Y, Fujiwara T, Takai Y, Momoeda M, Yano T and Taketani Y. Elevated serum bisphenol A levels under hyperandrogenic conditions may be caused by decreased UDP-glucuronosyltransferase activity. *Endocrine Journal* 2006; **53**: 485–491.

Tinwell H, Joiner R, Pate I, Soames A, Foster J and Ashby J. Uterotrophic activity of bisphenol A in the immature mouse. *Regulatory Toxicology and Pharmacology* 2000; **32**: 118–126.

vom Saal FS and Welshons WV. Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. *Environmental Research* 2006; **100**: 50–76.

Zeyneloglu H, Arici A and Olive DL. Environmental toxins and endometriosis. *Obstetrics and Gynecology Clinics of North America* 1997; **24**: 307–329.