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Reproductive Toxicology 19 (2004) 87–95
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Effects of gestational and lactational exposure to low doses of PCBs 126 and 153 on anterior pituitary and gonadal hormones and on puberty in female goats

Jan L. Lyche [a], Irma C. Oskam [a], Janneche U. Skaare [a,b], Olav Reksen [a], Torres Sweeney [c], Ellen Dahl [a], Wenche Farstad [a], Erik Ropstad [a,*]

[a] *Department of Production Animal Clinical Sciences, The Norwegian School of Veterinary Science, P.O. Box 8146 Dep., NO-0033 Oslo, Norway*
[b] *National Veterinary Institute, Oslo, Norway*
[c] *University College Dublin, Faculty of Veterinary Medicine and Conway Institute of Biomedical and Biochemical Research, University College, Belfield, Dublin, Ireland*

Received 6 November 2003; received in revised form 15 April 2004; accepted 11 May 2004

Available online 2 July 2004

Abstract

The aim of the present study was to investigate if environmental doses of PCB 153 and PCB 126 could produce effects in a controlled animal model. Possible adverse effects on the hypothalamic–pitutitary–gonadal axis were examined by measuring gonadotrophins and gonadal steroid hormone concentrations in goat kids exposed during gestation and lactation. The concentrations of PCB 153 and PCB 126 in adipose tissue in the goat kids 9 months post-partum were 5800 ng/g (fat-weight, range; 2900–12700 ng/g) and 0.49 ng/g (fat-weight, range; 0.28–0.80 ng/g), respectively. The pre- and post-pubertal plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (Prl) and progesterone (P4) were analysed. LH, FSH, Prl, and P4 were also measured during an induced oestrus cycle. The prepubertal LH concentration was significantly lower, the puberty was delayed and the P4 level during the luteal phase of an estrous cycle was higher in the group exposed to PCB 153. No significant effect of PCB 153 exposure was found on Prl and FSH. PCB 126 did not produce any effects at the exposure level tested in this study. In conclusion, perinatal exposure to PCB 153 affected the reproductive function and the puberty maturation in goats.

Keywords: PCB 153; PCB 126; LH; Progesterone; Delayed puberty; Estrus cycle; Estrogenic action; Gestational exposure

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent environmental chemicals that were manufactured for industrial use for about 50 years. Their resistance to degradation and strong affinity to adipose tissue allows them to accumulate in the lipid tissue of living organisms and biomagnify as they move through food chains. Although the production and use were banned 25 years ago, PCBs can still be identified in almost every component of the global ecosystem including air, water and soil, as well as in fish, animal and human tissues [1,2].

The PCB molecule consists of a biphenyl backbone on which chlorines are added in various numbers and at different positions that gives 209 possible congeners [3]. The different PCB congeners exhibit different physicochemical properties and biological activities, which influence their accumulation, uptake, and metabolism in the environment and in organisms, leading to marked differences in congener composition between the commercial mixtures and biological extracts [4].

The toxic responses of coplanar non-ortho and mono-ortho-substituted PCB congeners resemble those observed with 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD), and the mechanistic pathway for dioxins and dioxin like PCBs are proposed to be mediated via the aryl hydrocarbon receptor (AhR) present in target tissues [5]. Depending on the binding affinity to the AhR the different congeners have been given individual toxic equivalency factors (TEFs) [5]. By calculating the amount of the different congeners with their different TEFs the potential toxicity of a mixture can be

* Corresponding author. Tel.: +47 22964560; fax: +47 22597081.
E-mail address: erik.ropstad@veths.no (E. Ropstad).

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doi:10.1016/j.reprotox.2004.05.005

estimated. The TEF approach has been developed for risk assessment and regulatory control of exposure to complex PCDD, PCDF and PCB mixtures. On the other hand, the di-ortho-substituted PCB congeners have little or no affinity to the AhR, and the mechanisms behind toxicity are virtually unknown and they are not considered when toxicity of PCB mixtures is estimated [6].

PCBs have been shown to interfere with endocrine and reproductive functions in animals and humans [3,5,7,8]. Some of the effects may be explained by the fact that several PCBs exhibit estrogenic and antiestrogenic actions [9–13]. Observed changes in hormone levels and reproductive function following PCB exposure has been shown in cell cultures [14] laboratory animals [9,15,16], wild animals [17,18] and humans [8,19]. PCBs stored in the adipose tissue of the mothers pass to the developing fetus and the newborn progeny via placental transfer and mother's milk [20]. The question is whether background exposure can induce irreversible effects when animals and humans are exposed to PCB during critical windows of sexual differentiation or development [21,22]. During the differentiation of the endocrine/reproductive systems, hormones, growth factors and other endogenous substances regulate gene expression and direct differentiation [23]. One marked difference between exposure to endocrine disruptors during critical windows in development versus during adulthood is the irreversibility of an effect during development [24–27].

In order to assess possible endocrine disrupting effects following in utero PCB exposure, experiments with animals exposed to environmental relevant doses during gestation and lactation are needed. Most of the animal studies, which have investigated effects of PCBs, have been carried out with commercial mixtures [5,28]. The complexity of such mixtures, make it difficult to interpret the toxicity of single congeners. In order to detect such effects, studies with individual PCB congeners at relevant physiological levels are required.

The co-planar PCB 126 (3,3′,4,4′,5-pentachlorobiphenyl) has high acute toxicity, relatively strong affinity to the aryl hydrocarbon receptor (AhR) and has antiestrogenic properties [29]. The di-ortho-substituted PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) has low affinity to the AhR, low acute toxicity, shows estrogenic properties [7,9] and is the most highly concentrated PCB congener found in animal and human tissues (15–30% of sum PCB) [30,31].

The aim of the study was to investigate if doses of PCB 153 and PCB 126 comparable to exposures found in wild animals could produce effects in a controlled animal model. Possible adverse effects on the hypothalamic–pitutitary–gonadal axis were examined by measuring gonadotrophins and gonadal steroid hormone concentrations in a goat-kid model where the mothers were orally dosed PCB from day 60 of gestation until delivery. The goat kids were thus exposed in late gestation and in the suckling period, whereas female sexual function was studied during adolescence.

2. Materials and methods

2.1. Animals

Goat kids were studied from birth until sacrifice at 9 months of age. Their mothers were dosed orally with PCBs dissolved in corn oil from day 60 of gestation until parturition. Consequently, the offspring were exposed to PCB in utero and through mother's milk. Goats were chosen because they are easy to handle, the gestation is relative long compared to rodents giving the opportunity to assess fetal development by real time ultrasonography. Additionally, the newborn kid is more developed than the newborn rodent and may as such be more relevant as a model for large mammals including wildlife and humans. Another reason was that validated methodology on reproductive function in goats is available in our laboratory.

Forty-five does of the Norwegian breed were selected randomly, and estrus in these does was synchronized by treating for 18 days with EAZI-breed CIDR^{TM} G (Carter Holt Harvey, Agricultural Division, Hamilton, New Zealand) intravaginal device containing 30 mg progesterone. After withdrawal of the device, the does were mated twice a day with two intact males until the last doe came out of estrus. Pregnancy status and number of fetuses were determined by use of real-time ultrasonography from day 20 after mating until day 35, when all does were confirmed pregnant. The presence of a live fetus was diagnosed by the detection of heartbeat and provoked or spontaneous body movements.

Thirty pregnant goats were then allocated into three groups (10 goats/group) using block randomization. One group was exposed to PCB 153, a second group received PCB 126 and the third group served as a control. The animals in the experimental groups were orally administered PCB dissolved in corn oil three times a week (Monday, Wednesday, Friday) from day 60 of gestation until delivery (13 weeks), whereas the control group was given *only* corn oil. The doses of PCB 153 and PCB 126 were estimated to be 98 μg/kg body wt./day, and 49 ng/kg body wt./day, respectively. The animals were kept indoors, and the groups were held in separate pens in order to avoid contamination through the ingestion of PCB-contaminated feces. The does received standard hay and concentrate feeding.

The goats were clinically examined every day until kidding, and their weights were recorded once a week. Blood samples were collected three times weekly (Monday, Wednesday and Friday) from mating to parturition. The goats delivered indoors and every kidding was supervised. The gender, weight, condition and time of birth of the kids were recorded. The mothers and kids remained together until weaning (6 weeks post-partum). The body weights of the kids were recorded once a week. Blood samples from the kids were collected three times weekly (Monday, Wednesday, and Friday) from 4 months until sacrifice at 9 months of age. The oestrous cycles of the female kids were synchronized using a 14 days intravaginal progestagen

sponge treatment (40 mg Flugestone acetate; Chrono-gest®, Intervet, Boxmeer, Holland).

The procedures have been conducted in accordance with the laws and regulations controlling experiments/procedures in live animals in Norway, i.e. the Animal Welfare Act of 20 December 1974, no. 73, chapter VI, sections 20–22 and the Regulation on Animal Experimentation of 15 January 1996. In addition, Norway has signed and ratified The European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 18 March 1986. The Norwegian legislation conforms in all respects with the basic requirements of this Convention and guidelines prepared in pursuance of it.

2.2. Chemicals and drugs

3,3′,4,4′,5-Pentachlorobiphenyl (IUPAC no. 126) and 2,2′,4,4′,5,5′-Hexachlorobiphenyl (IUPAC no. 153) in pure (99%) powder form were acquired from Promochem (Ulricehamn, Sweden) The calculated amount of powder was dissolved in acetone, and corn oil was added as a vehicle. The acetone was evaporated, and the PCB and the corn oil were mixed using an ultrasonication bath for 3 min. The concentrations of PCB 126 and PCB 153 were then determined by gas chromatography. The solutions were stored at room temperature in order to remain homogeneous.

2.3. Blood sampling

Blood samples were collected from the jugular vein using a Vacutainer® (Leuven, Belgium). The samples were then stored for 1 h at room temperature, plasma was separated by centrifugation at 3000 × *g* for 15 min, and the sample was then stored at −20 °C until analysis.

2.4. Chemical analysis of PCB

Chemical analyses of PCBs 153 and 126 were performed at the Laboratory of Environmental Toxicology, Norwegian School of Veterinary Science, Oslo. The laboratory is accredited by Norwegian Accreditation as a testing laboratory for these analyses according to the requirements of NS-EN45001 and ISO/IEC Guide 25. Briefly, samples of adipose tissue (∼1–3 g) were weighed and internal standards PCB 29 and 112 were added. The samples were extracted twice with cyclohexane and acetone using an ultrasonic homogenizer followed by centrifugation. The samples were then cleaned up with ultra-pure sulfuric acid. Percent extractable fat was determined gravimetrically. For details on extraction and cleanup, see Andersen et al. [32].

Aliquots of the final extract were injected into a gas chromatograph/electron capture detection (GC-ECD) instrument equipped with a capillary column (SPB-5), 60 m, 0.25 mm i.d. and 0.25 μm film layer (Supelco Inc., Bellefonte, PA). The makeup gas was helium (flow 60 ml/min). The temperature program was as follows: start 90 °C (held for 3 min), 25 °C increase/min to 180 °C (held for 2 min), 1.5 °C increase/min to 220 °C (held for 2 min), and 3 °C increase/min to 275 °C (held for 15 min). Chromatographic data were calculated using the software HP GC Chemstation (Hewlett® Packard, Atlanta, USA).

Quantification was performed using PCB 29 and 112 as internal standards in each sample. For further details on equipment for the chromatographic separation, detection and quantification, see Anderson et al. [32].

2.5. Quality assurance

Standard procedures were used to ensure adequate quality assurance and control, and the results were within the laboratory's accredited requirements. Percent recoveries of OCs in spiked material (blood and tissue samples, respectively) varied from 95 to 128%.

Detection limits for individual PCB congeners were determined as three times the noise level and depended on the type of sample analyzed. Quantification level was set as three times the detection limit.

Detection limits in tissue (brain, liver, fat) ranged from 0.17 to 0.51 ng/g wet weight for PCB 153 and from 0.12 to 0.35 ng/g for PCB 126, depending on the tissue type. In plasma it was found to be 0.009 ng/g wet weight for both PCB 153 and PCB 126.

All calculations were done within the linear range of the detector's 5-level calibration curve.

The reproducibility was tested continuously by analyzing the PCB levels in the laboratory's own reference sample (seal blubber), which was within the mean coefficient of variance. The repeatability of the GC-performance was tested by repeated injection of standard components at regular intervals.

Blank samples were included in each series to test for interference.

2.6. Hormone assays

2.6.1. Progesterone

Progesterone concentration in plasma was measured using an enhanced luminescence assay (Amerlite®, Kodak) using horseradish-peroxidase as the enzyme label. The assay was validated for use with goat plasma by demonstrating parallelism between dilutions of plasma samples and the standard curve. Assay sensitivity was 0.2 nmol/l. The interassay coefficients of variation for samples containing 15.9 or 31.8 nmol/l were 5.6 and 6.3%, respectively. The intraassay coefficients of variation for the same samples were 3.7 and 4.0%, respectively.

2.6.2. Luteinizing hormone

Plasma LH concentrations were measured by a heterologous double antibody radioimmunoassay described and validated for goat plasma by Beckers et al. [33].

The interassay coefficients of variation for samples with 0.69, 1.95, and 6.68 ng/ml LH were 18.8, 9.7 and 9.0%, respectively. Standard curve ranged from 0.2 to 25 ng/ml. Assay sensitivity was 0.8 ng/ml.

2.6.3. Follicle-stimulating hormone

FSH concentrations were determined using a heterologous assay described by Crowe et al. [34] using the anti oFSH NIDDK-NIH antibody (AFP-C 5288113). The standard used as reference preparation was USDA-oFSH-SIAFP-RP-2 (AFP 4117A) and the sensitivity of the assay was 0.012 ng (oFSH-RP-2)/tube. The assay was validated for use with goat plasma by demonstrating parallelism between dilutions of goat plasma samples and the standard curve.

2.6.4. Prolactin (Prl)

Serum Prl concentrations were determined using the RIA method of McNeilly and Andrews [35]. Sensitivity of the assay, as defined as 95% binding, was 0.5 ng/ml. Mean inter- and intraassay coefficients of variation were 6.6 and 7.5%.

2.6.5. 17β-estradiol (E2)

Estradiol concentration were analysed using the kit. "Coat-a-count®, Estradiol" (Diagnostic Products Corporation, Los Angeles, USA). The detection limit was 8.0 pg/ml, corresponding to 95% binding of the labelled hormone, assuming the basic procedures were followed. The E2 concentration was low in the samples, so both the standard curve abd the samples were modified to bring the concentrations within the detection limit. The standards were diluted with 0.02 M borate-buffer, pH = 8.5 to concentrations ranging from 10 to 660 pg/ml. Goat plasma-samples were up-concentrated to bring E2 concentration within the linear range of the modified standard curve. 500 μl plasma from each sample was extracted with newly distilled diethyl ether, evaporated to dryness and added 250 μl borate-buffer (0.02 M, pH = 8.5), thus doubling the concentration of E2 in the plasma samples. Extraction recovery was 79.5 ± 4.1% (n = 6), determined from single recovery-test per run, spiked with known amounts of the hormone. All concentrations were adjusted according to the recovery sample. The inter assay CV from control samples with low (49.9 pg/ml), medium (83.1 pg/ml) and high (204.1 pg/ml) concentrations of E2 were 6.4%, 10.1% and 6.2%, respectively (n = 6). RIA-counts of E2 were performed by a 1470 Wizard™ Automatic Gamma-counter (Wallac Oy, Turku, Finland).

2.7. Statistical analysis

The effects of PCB on plasma LH-level was studied in a repeated measures mixed model analyses. PROC MIXED of SAS was used [36]. The dependent variable was the natural logarithm to the plasma LH-level + 0.05. The transformation of the dependent variable was conducted to approximate a normal distribution of the data and because of a number of zero level analyse results. The independent variables in the model were month, PCB exposure and an interaction term between month and PCB exposure, the latter to assess impact of exposure by time. The compound symmetry correlation matrix was used. Other correlation structures were also modeled, but compound symmetry resulted in the best model fit (Schwartze's Bayesian criterion was closest to zero).

Time to puberty and area under the progesterone curve was analyzed in variance analyses using proc GLM [37] in SAS. The area under the progesterone curve was calculated using the trapezium rule [38]. PCB exposure was used as the independent variable both when time to puberty and area under the progesterone curve was assessed. To normalize the data, both dependent variables were transformed by taking the natural logarithm. The least significant difference (LSD) approach was used in pairwise comparison to control for the type I comparison wise error rate. Statistical significance was assessed by the type III F-test, and $P < 0.05$ was considered statistically significant.

The effects of PCB on serum progesterone obtained from day 4 to 14 after peak LH-level was studied in a repeated measures mixed model analyses. PROC MIXED of SAS was used [36]. The dependent variable was the natural logarithm to serum progesterone concentration. The transformation of the dependent variable was conducted to approximate a normal distribution of the data. The independent variables in the model were day after peak LH-level, PCB exposure and an interaction term between day and PCB exposure, the latter to assess impact of exposure by time. The logarithmic correlation matrix was used, as it resulted in the best model fit (Schwartze's Bayesian criterion was closest to zero).

3. Results

In the does and their offspring there was no effect of exposure on pregnancy duration, body weight or observed health status during the experimental period. The male/female sex ratio was 0.54 for the PCB 126 exposed kids, 1.25 for the PCB 153 treated kids and 1 for the control animals. However, the sex ratio was not significant different between the treatment groups.

The PCB 153 concentrations in adipose tissue in the PCB 153 group at 40 weeks of age ranged from 750 to 9800 ng/g (mean: 5800 ng/g, lipid-weight). The adipose tissue levels of PCB 126 in the PCB 126 exposed group at 40 weeks of age ranged from 0.28 to 0.8 ng/g (mean: 0.49 ng/g, lipid-weight). The mean concentrations of PCB 153 in unexposed doe and kid adipose tissue were 40 ng/g (lipid-weight) and 18 ng/g (lipid-weight), respectively. The concentrations of PCB 126 in the adipose tissue in the unexposed animals were below the detection limit. For further details, see Lyche et al. [20].

Animals exposed in utero to PCB 126 reached puberty (day 203; range 194–214) at a similar time as the control animals (day 203; range 191–212), as determined by days from birth to the first progesterone rise. Animals exposed in utero to PCB 153 experienced delayed ($P < 0.05$; Fig. 1)

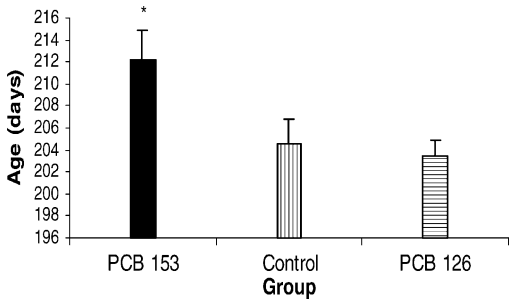

Fig. 1. Onset of puberty in PCB 153 ($n = 7$) and PCB 126 ($n = 10$) exposed kids compared to control animals ($n = 7$), as determined by days from birth to the first progesterone rise (±S.E.M.).

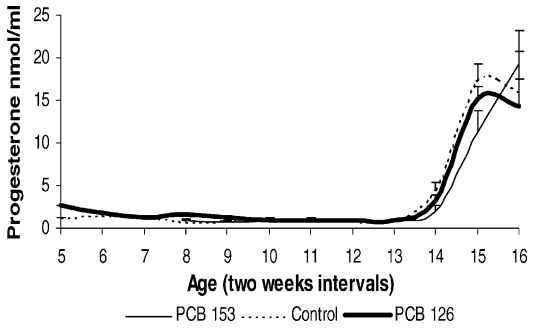

Fig. 2. Progesterone (±S.E.M.) level in blood expressed as mean of 2 weeks intervals from 10 to 32 weeks of age in goats exposed to PCB 153 ($n = 7$) and PCB 126 ($n = 10$) during foetal life and during the suckling period and in unexposed animals ($n = 7$).

onset of puberty (day 212; range 200–221) compared to control animals (Fig. 1). It must be noted that one doe exposed to PCB 153 reached puberty 8 weeks before the control animals and as a result this doe was omitted from the analysis. There was no difference in progesterone concentrations or in body weight at the time of the onset of the puberty between any of the experimental groups (Fig. 2). A significant lower pre-pubertal LH concentration ($P < 0.05$) was found in the PCB 153 exposed animals compared to the control animals (Fig. 3). No significant differences were shown between the PCB exposed groups and the controls in the pre- and post-pubertal concentrations of FSH and Prl.

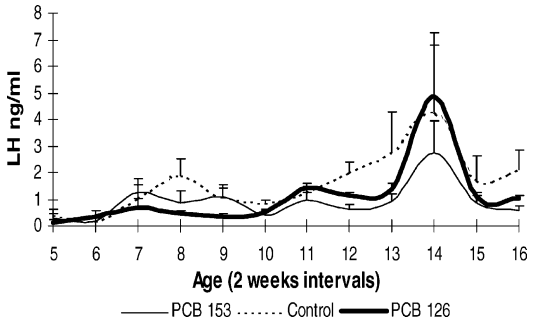

Fig. 3. Mean luteinizing hormone (±S.E.M.) level in blood expressed as mean of 2 weeks intervals from 10 to 32 weeks of age in goats exposed to PCB 153 ($n = 7$) and PCB 126 ($n = 10$) during foetal life and during the suckling period and in unexposed animals ($n = 7$).

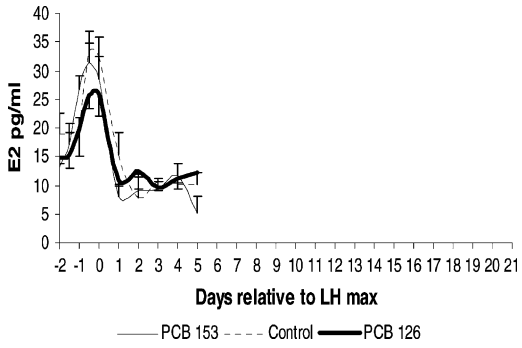

Fig. 4. Mean estradiol (±S.E.M.) concentration in blood during an induced oestrous cycle in 9 months old goats exposed to PCB 153 ($n = 5$) and PCB 126 ($n = 10$) during foetal life and during the suckling period and in unexposed animals ($n = 8$).

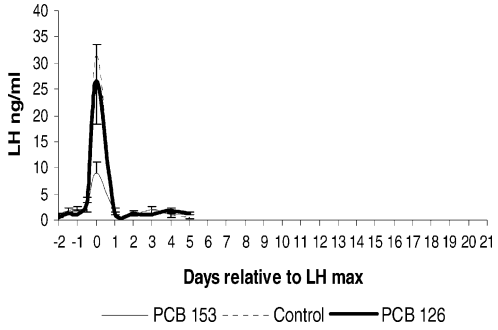

Fig. 5. Luteinizing hormone (±S.E.M.) concentration in blood during an induced oestrous cycle in 9 months old goats exposed to PCB 153 ($n = 5$) and PCB 126 ($n = 10$) during foetal life and during the suckling period and in unexposed animals ($n = 8$).

The oestrous cycles of the juvenile does were synchronized using a 14 days intravaginal progestagen sponge treatment. The concentrations of FSH, Prl, E2 (Fig. 4) and P4 (Fig. 6) are expressed on a time scale relative to the peak of the individual preovulatory LH surges (Fig. 5). Progesterone was significantly increased from days 4 throughout day 14 after the preovulatory LH surge in the PCB 153 treated animals compared to both control and PCB 126 exposed animals (Fig. 6). FSH, E2 and Prl were

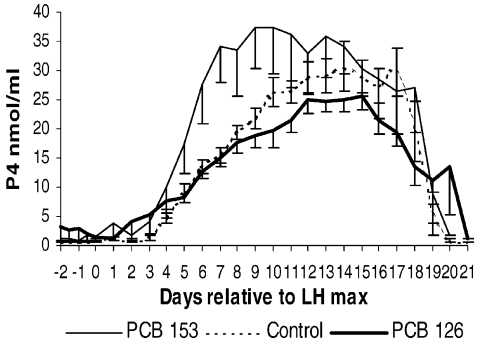

Fig. 6. Progesterone (±S.E.M.) concentration in blood during an induced oestrous cycle in 9 months old goats exposed to PCB 153 ($n = 5$) and PCB 126 ($n = 10$) during foetal life and during the suckling period and in unexposed animals ($n = 8$).

affected neither by day nor by treatment. Even though not significant, the peak LH surge in the PCB 153 group was numerically lower than in the control group (Fig. 5).

4. Discussion

The results of this experiment indicate that maternal exposure to low doses of the di-ortho-substituted PCB 153 during gestation and lactation suppressed prepubertal plasma LH concentrations and delayed the onset of puberty of the female offspring. The signifcance of these observations is highlighted by the fact that the levels of PCB 153 in the adipose tissue of the offspring (mean 5800 ng/g) were lower than those found in polar bears on Svalbard (mean 7080 ng/g, subadults) [30]. The PCB 153 concentration in goat kid plasma is 20-fold higher than the level found in a group of Swedish men [39]. In contrast, maternal exposure to PCB 126 at the low level tested in the present study did not affect any of the reproductive parameters measured in the female offspring. It should be noted that the PCB 126 concentration in goat kid plasma is at the same level as found in human blood [40] and based on the present study it is not possible to draw conclusions on possible effects at higher exposure levels. PCB 126 have been given a TEF-value of 0.1 due to its relative potential to activate the AhR compared with TCDD [5,41]. The concentration of PCB 126 times its TEF-value provides an estimate of the potential toxicity in terms of TCDD equivalents (TEQ). The TEQ-value for PCB 126 in the adipose tissue of the kids was estimated to be 49 pg/g TCDD equivalents. This value can be used to compare the negative results of the present study with data available in the literature for other AhR agonists.

The reduced prepubertal plasma concentration of LH and the delayed onset of puberty observed in this experiment, suggest that PCB 153 can influence sexual maturation in female goats. Impacts of PCBs on pubertal events after perinatal exposure have been reported in previous studies and include both delays and accelerations of puberty [2,42,43,44]. The timing of vaginal opening occurred earlier and the first oestrus was delayed in rats exposed perinatally to a commercial mixture of PCB congeners (Aroclor 1254) in comparison to control animals [42]. In contrast, Sager and Girard [2] identified a delayed vaginal opening and delayed puberty in rats exposed to Aroclor 1254. Guinea pigs exposed to PCBs (Clophen 50) in utero and via mothers milk showed a delayed onset of puberty [43]. In girls, an inverse relationship between breast development and dioxin like compounds has been reported [44]. In the same study, serum concentration of PCB 153 was inversely correlated with pubic hair growth and genital development in boys.

During the prepubertal period the hypothalamic-pituitary axis is highly sensitive to inhibitory effects of gonadal steroids, hence the circulating gonadotropin concentrations remain low. During the transition to puberty, the response to ovarian estradiol inhibitory feedback action is reduced and the GnRH pulse generator runs at high frequency. As a consequence the pituitary produces high-frequency LH pulses culminating in ovulation [45]. The timing of puberty is governed by both internal and external factors such as birth weight, nutritional state, photoperiod, circadian/circannual rythms and the hormone levels during fetal and prepubertal life [46].

Possible mechanisms for the action of PCB 153 in relation to puberty include alteration of endocrine feedback loops and changes in circulating hormone levels. The lower prepubertal LH concentration in the PCB 153 group may have influenced the observed delay in puberty.

Initiation of events leading to first ovulation is dependent of high frequency LH pulses and any condition that leads to deficiency in tonic LH secretion may delay the onset of puberty such as in the undernourished lamb [45]. A disturbance of the inhibitory feedback action of the small amounts of oestradiol secreted by the ovary in the immature female may disturb the normal pubertal development. Meikle et al. [47], exposed premature ewe lambs to exogenous oestradiol and showed that long time exposure had negative effect on LH secretion. Premature lambs showed lower LH secretion and delayed puberty following chronic treatment with exogenous oestradiol [48]. Reduced LH secretion is previously found in rats exposed to PCB 126 and PCB 153 [9]. However, the study exposed adult animals only.

The reduced LH concentration in the PCB 153 group may be due to effects upon the pituitary and/or hypothalamus or by a direct effect on the ovary. A direct effect of PCB153 on ovarian follicular oestradiol secretion was found in vitro by exposure of theca and granulosa cells using doses relevant to those used in the present experiment [14]. Direct effects of PCB on the pituitary and/or hypothalamus have also been reported [9,11]. The effects on gonadotrophin secretion caused by A1242 and PCB 52 (ortho-substituted; [11]) were similar to those observed following E2 treatment. In the Desauliners study [9], they found similar effects between PCB 153 and E2 on distribution of FSH isoforms, suggesting that PCB 153 may exert an oestrogen-like action.

One of the females in the PCB 153 group was regarded as outlier and excluded from the statistical calculations due to the fact that she reached puberty almost 8 weeks before the other does. It is difficult to explain the severe advancement of puberty in one goat in the PCB 153 group in contrast to the delayed puberty observed in the seven others. One possible explanation may be the concept of hormesis characterized by an U-shaped dose response relationship. Adverse effects do not increase monotonically with dose, but decrease initially as dose increases and then rise with higher doses [49–51].

In the present study, we investigated the effect of perinatal exposure to PCBs on oestrus cycle patterns by analysing the plasma levels of Prl, E2, LH, FSH and P4 during an oestrus cycle. A significantly higher plasma progesterone concentration during the luteal phase was found in the PCB 153 group compared to the control group. The measurements were conducted 8 months post-exposure indicating an irreversible

change in endocrine function. To our knowledge there are no studies that have investigated luteal progesterone concentrations following perinatal exposure to PCBs. A previous study performed on pregnant mink reported increased P4 concentrations following exposure to PCB 153 and decreased P4 concentrations following exposure to PCB 126 during pregnancy [52]. In contrast, P4 concentrations were increased by PCB 126 and unchanged by PCB 153 in adult female mink, which were not pregnant [53]. The cause of this apparent discrepancy in the results are unknown but may be explained by different time of exposure in relation to hormonal status. In an in vitro study, theca and granulosa cells in culture were exposed to increasing doses of PCB 153 and PCB 126, and a dose-dependent increase in progesterone levels were found in both cell types exposed to PCB 153. In the cell cultures exposed to PCB 126, increased progesterone production were only seen following exposure to the highest dose [14].

Increased LH-induced progesterone synthesis was found in immature rats following treatment with a commercial mixture of PCBs (Clorphen A-30; [54]). In female polar bears exposed to a mixture of environmental chemicals including PCBs, the progesterone concentrations were positively correlated with plasma sum PCB concentrations [17]. In both studies the animals were exposed to a mixture of possible harmful chemicals. The complexity of such mixtures, make it difficult to interpret the toxicity of single congeners. The clinical or biological importance of the increased progesterone levels during the luteal phase observed in the present study is unknown. However, several studies have demonstrated irregular oestrus cycles following PCB exposure [42,55–57]. Loss of regular oestrus cyclicity was found after prepubertal exposure to low doses of oestrogen [58].

The mechanisms behind PCBs' endocrine disrupting effects are not understood. However, PCBs may act as endocrine disruptors by affecting synthesis, secretion, transport or metabolism of endogenous hormones and/or bind to hormone receptors and agonise/antagonise actions caused by endogenous hormones [59]. PCB may also influence the endocrine homeostasis by disrupting the normal up and down-regulation of hormone receptors [52].

Estrogenic effects of PCBs have previously been related to the lower chlorinated diortho-substituted congeners and their parahydroxylated metabolites [10,60,61] whereas the dioxin-like coplanar congeners like PCB 126 have been shown to mediate antiestrogenic action [29,62]. PCB 153, which is a higher chlorinated di-ortho-substituted congener showed no ER binding affinity when tested in the "E-Screen test" [63]. On the other hand, Bonefeld-Jorgensen et al. [64] found that PCB 153 antagonized the effects of E2 in MCF-7 cells by competing with the binding of E2 to the ER. PCB 153 has also been shown to have estrogenic effects in rat [7,9]. Precisely how PCB 153 may interfere with oestrogen action is unclear. However, to determine if a chemical has estrogenic effect is difficult because of the complicated mechanisms involved. At present, there are two known intracellular ERs including ER α and ER β, differing in both tissue distribution and biological activity [65]. Recent cloning of ER β was followed by the discovery of a variety of its isoforms, and diversity in ER β isoforms may influence the E2 response [66,67]. In addition, increasing evidence indicates the presence of functional ERs on cell membranes involved in rapid effects of E2 [68,69]. It has also been shown that various estrogenic compounds cause differential activation of ERs [70,71].

The question is if such mechanisms of action can explain the lower LH-levels, the delayed puberty and the increased progesterone concentration during the luteal phase caused by perinatal exposure to PCB 153. Those results resemble effects observed after prepubertal exposure to exogenous E2 [47,48,58,72], suggesting that PCB 153 may interfere with estrogenic action. A strong evidence for the hypothesis is the studies by Bonefeld-Jorgensen et al. [64] and Rattenborg et al. [73] which showed that PCB 153 have the potential to interfere with ligand mediated ER activity.

In conclusion, low dose exposure to the highly bioaccumulated PCB 153 during gestation and lactation disrupt normal hormone production and puberty development in female offspring. The fact that the effects were caused by PCB 153 and not PCB 126 suggest that AhR independent mechanisms are involved. PCB 126 may have produced effects at exposure levels above the low level tested in this study. Conclusions regarding the mechanisms behind PCB 153's toxicity are not possible to draw based on the present study and warrants further research. The environmental abundance and the endocrine disruptive effects of PCB 153 showed in the present and previous studies raise the question if di-ortho-substituted PCBs should be incorporated in risk assessment of PCBs.

Acknowledgments

We wish to thank Jens Røhnebæk, Lise Evensen, Elin Røyset, Lill-Wenche Fredriksen, Anuschka Polder, Beate Carolin, Signe Haugen, Lise Lotte Torp and Niamh Hynes for excellent technical assistance. This work was partly supported by grant 127534/720 from Norwegian Research Council.

References

[1] DeRosa C, Richter P, Pohl H, Jones DE. Environmental exposures that affect the endocrine system: public health implications. J Toxicol Environ Health B Crit Rev 1998;1:3–26.
[2] Sager DB, Girard DM. Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. Environ Res 1994;66:52–76.
[3] Carpenter DO. Polychlorinated biphenyls and human health. Int J Occup Med Environ Health 1998;11:291–303.
[4] Cogliano VJ. Assessing the cancer risk from environmental PCBs. Environ Health Perspect 1998;106:317–23.
[5] Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 1994;24:87–149.

[6] Kodavanti PR, Tilson HA. Structure–activity relationships of potentially neurotoxic PCB congeners in the rat. Neurotoxicology 1997;18:425–41.
[7] Li MH, Zhao YD, Hansen LG. Multiple dose toxicokinetic influence on the estrogenicity of 2,2′,4,4′,5,5′-hexachlorobiphenyl. Bull Environ Contam Toxicol 1994;53:583–90.
[8] Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A, et al. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res 1995;38: 404–10.
[9] Desaulniers D, Leingartner K, Wade M, Fintelman E, Yagminas A, Foster WG. Effects of acute exposure to PCBs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. Toxicol Sci 1999;47:158–69.
[10] Gierthy JF, Arcaro KF, Floyd M. Assessment of PCB estrogenicity in a human breast cancer cell line. Chemosphere 1997;34:1495–505.
[11] Jansen HT, Cooke PS, Porcelli J, Liu TC, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod Toxicol 1993;7:237–48.
[12] Moore M, Mustain M, Daniel K, Chen I, Safe S, Zacharewski T, et al. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicol Appl Pharmacol 1997;142:160–8.
[13] Safe SH. Endocrine disruptors and human health—is there a problem? An update. Environ Health Perspect 2000;108:487–93.
[14] Wojtowicz AK, Gregoraszczuk EL, Lyche JL, Ropstad E. Time dependent and cell-specific action of polychlorinated biphenyls (PCB 153 and PCB 126) on steroid secretion by porcine theca and granulosa cells in mono- and co-culture. J Physiol Pharmacol 2000;51:555–68.
[15] Backlin BM, Madej A, Forsberg M. Histology of ovaries and uteri and levels of plasma progesterone, oestradiol-17beta and oestrone sulphate during the implantation period in mated and gonadotrophin-releasing hormone-treated mink (Mustela vison) exposed to polychlorinated biphenyls. J Appl Toxicol 1997;17:297–306.
[16] Li MH, Hansen LG. Enzyme induction and acute endocrine effects in prepubertal female rats receiving environmental PCB/PCDF/PCDD mixtures. Environ Health Perspect 1996;104:712–22.
[17] Haave M, Ropstad E, Derocher AE, Lie E, Dahl E, Wiig O, et al. Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. Environ Health Perspect 2003;111:431–6.
[18] Troisi GM, Mason CF. PCB-associated alteration of hepatic steroid metabolism in harbor seals (Phoca vitulina). J Toxicol Environ Health A 2000;61:649–55.
[19] Yu ML, Guo YL, Hsu CC, Rogan WJ. Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long-term follow-up interviews of the women from the Taiwan Yucheng cohort. Int J Epidemiol 2000;29:672–7.
[20] Lyche JL, Skaare JU, Larsen HJS, Ropstad E. Levels of PCB 126 and PCB 153 in plasma and tissues in goats exposed during gestation and lactation. Chemosphere 2004;55:621–9.
[21] Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, et al. Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs. Environ Health Perspect 1999;107(Suppl 4):639–49.
[22] Cooke PS, Zhao YD, Hansen LG. Neonatal polychlorinated biphenyl treatment increases adult testis size and sperm production in the rat. Toxicol Appl Pharmacol 1996;136:112–7.
[23] Cunha GR, Alarid ET, Turner T, Donjacour AA, Boutin EL, Foster BA. Normal and abnormal development of the male urogenital tract. Role of androgens, mesenchymal-epithelial interactions, and growth factors. J Androl 1992;13:465–75.
[24] Eriksson P, Viberg H, Jakobsson E, Orn U, Fredriksson A. A brominated flame retardant, 2,2′,4,4′,5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. Toxicol Sci 2002;67:98–103.
[25] Greco TL, Duello TM, Gorski J. Estrogen receptors, estradiol, and diethylstilbestrol in early development: the mouse as a model for the study of estrogen receptors and estrogen sensitivity in embryonic development of male and female reproductive tracts. Endocr Rev 1993;14:59–71.
[26] McLachlan JA, Newbold RR. Estrogens and development. Environ Health Perspect 1987;75:25–7.
[27] vom Saal FS. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses in utero. J Anim Sci 1989; 67:1824–40.
[28] Holene E, Nafstad I, Skaare JU, Krogh H, Sagvolden T. Behavioural effects in female rats of postnatal exposure to sub-toxic doses of polychlorinated biphenyl congener 153. Acta Paediatr Suppl 1999;88:55–63.
[29] Krishnan V, Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. Toxicol Appl Pharmacol 1993;120:55–61.
[30] Bernhoft A, Wiig O, Skaare JU. Organochlorines in polar bears (Ursus maritimus) at Svalbard. Environ Pollut 1997;95:159–75.
[31] Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. Environ Health Perspect 1998;106(Suppl 1):171–89.
[32] Andersen M, Lie E, Derocher AE, Belikov SE, Bernhoft A, Boltunov AN, et al. Geographic variation of PCB congeners in polar bears (*Ursus maritimus*) from Svalbard east to the Chukchi Sea. Polar Biol 2001;24:231–8.
[33] Beckers JF, Wouters-Ballman P, Ectors F, Derivaux J. Induction de l'oestrus chez les génisses en anoestrus fonctionnel. Ann Med Vet 1978;122:597–604.
[34] Crowe MA, Padmanabhan V, Hynes N, Sunderland SJ, Enright WJ, Beitins IZ, et al. Validation of a sensitive radioimmunoassay to measure serum follicle-stimulating hormone in cattle: correlation with biological activity. Anim Reprod Sci 1997;48:123–36.
[35] McNeilly AS, Andrews P. Purification and characterization of caprine prolactin. J Endocrinol 1974;60:359–67.
[36] Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS system for mixed models. Cary, NC: SAS Institute; 1996.
[37] SAS. SAS/STAT Users guide. Version 6 ed. Cary, NC: SAS Institute; 1992.
[38] Altman DG. Practical statistics for medical research. London: Chapman & Hall; 1995.
[39] Glynn AW, Wolk A, Aune M, Atuma S, Zettermark S, Maehle-Schmid M, et al. Serum concentrations of organochlorines in men: a search for markers of exposure, Sci Total Environ 2000;263 (1–3):197–208.
[40] Shadel BN, Evans RG, Roberts D, Clardy S, Jordan-Izaguirre D, Patterson Jr DG, et al. Background levels of non-ortho-substituted (coplanar) polychlorinated biphenyls in human serum of Missouri residents. Chemosphere 2001;43(4–7):967–76.
[41] van den Berg M, Peterson RE, Schrenk D. Human risk assessment and TEFs. Food Addit Contam 2000;17(4):347–58.
[42] Brezner E, Terkel J, Perry AS. The effect of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat—I. Comp Biochem Physiol C 1984;77:65–70.
[43] Lundkvist U. Clinical and reproductive effects of Clophen A50 (PCB) administered during gestation on pregnant guinea pigs and their offspring. Toxicology 1990;61:249–57.
[44] Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect 2002;110:771–6.
[45] Foster DL, Yellon SM, Olster DH. Internal and external determinants of the timing of puberty in the female. J Reprod Fertil 1985;75:327–44.
[46] Conte FA, Grumbach MM, Kaplan SL, Reiter EO. Correlation of luteinizing hormone-releasing factor-induced luteinizing hormone

and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in agonadal patients: relation to the restraint of puberty. J Clin Endocrinol Metab 1980;50:163–8.
[47] Meikle A, Tasende C, Garofalo EG, Forsberg M. Priming effect of exogenous oestradiol on luteinizing hormone secretion in prepubertal lambs. Anim Reprod Sci 1998;54:75–85.
[48] Foster DL, Ryan KD, Goodman RL, Legan SJ, Karsch FJ, Yellon SM. Delayed puberty in lambs chronically treated with oestradiol. J Reprod Fertil 1986;78:111–7.
[49] Calabrese EJ, Baldwin LA. Applications of hormesis in toxicology, risk assessment and chemotherapeutics. Trends Pharmacol Sci 2002;23:331–7.
[50] Calabrese EJ, Baldwin LA. Hormesis: the dose-response revolution. Annu Rev Pharmacol Toxicol 2003;43:175–97.
[51] Kohn MC, Melnick RL. Biochemical origins of the non-monotonic receptor-mediated dose-response. J Mol Endocrinol 2002;29:113–23.
[52] Patnode KA, Curtis LR. 2,2′,4,4′,5,5′- and 3,3′,4,4′,5,5′-hexachlorobiphenyl alteration of uterine progesterone and estrogen receptors coincides with embryotoxicity in mink (Mustela vision). Toxicol Appl Pharmacol 1994;127:9–18.
[53] Aulerich RJ, Bursian SJ, Breslin WJ, Olson BA, Ringer RK. Toxicological manifestations of 2,4,5,2′,4′,5′-, 2,3,6,2′,3′,6′-, and 3,4,5,3′,4′,5′-hexachlorobiphenyl and Aroclor 1254 in mink. J Toxicol Environ Health 1985;15:63–79.
[54] Fuller GB, Knauf V, Mueller W, Hobson WC. PCB augments LH-induced progesterone synthesis. Bull Environ Contam Toxicol 1980;25:65–8.
[55] Arnold DL, Mes J, Bryce F, Karpinski K, Bickis MG, Zawidzka ZZ, et al. A pilot study on the effects of Aroclor 1254 ingestion by rhesus and cynomolgus monkeys as a model for human ingestion of PCBs. Food Chem Toxicol 1990;28:847–57.
[56] Barsotti DA, Marlar RJ, Allen JR. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aoroclor 1248). Food Cosmet Toxicol 1976;14:99–103.
[57] Masuda Y. Fate of PCDF/PCB congeners and change of clinical symptoms in patients with Yusho PCB poisoning for 30 years. Chemosphere 2001;43:925–30.
[58] Nass TE, Matt DW, Judd HL, Lu JK. Prepubertal treatment with estrogen or testosterone precipitates the loss of regular estrous cyclicity and normal gonadotropin secretion in adult female rats. Biol Reprod 1984;31:723–31.
[59] Vos JG, Dybing E, Greim HA, Ladevoged O, Lambre C, Tarazona JV, et al. CSTEE opinion on human and wildlife healt effects of endocrine disrupting chemicals, with emphasis on wildlife and ecotoxicology test methods. Ref Type: Report, 1999.
[60] Kramer VJ, Helferich WG, Bergman A, Klasson-Wehler E, Giesy JP. Hydroxylated polychlorinated biphenyl metabolites are anti-estrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. Toxicol Appl Pharmacol 1997;144:363–76.
[61] Layton AC, Sanseverino J, Gregory BW, Easter JP, Sayler GS, Schultz TW. In vitro estrogen receptor binding of PCBs: measured activity and detection of hydroxylated metabolites in a recombinant yeast assay. Toxicol Appl Pharmacol 2002;180:157–63.
[62] Safe SH. Is there an association between exposure to environmental estrogens and breast cancer? Environ Health Perspect 1997;105(Suppl 3):675–8.
[63] Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 1995;103(Suppl 7):113–22.
[64] Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TH, Vinggaard AM. Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 2001;158:141–53.
[65] Cassidy A. Potential tissue selectivity of dietary phytoestrogens and estrogens. Curr Opin Lipidol 1999;10:47–52.
[66] Inoue S, Ogawa S, Horie K, Hoshino S, Goto W, Hosoi T, et al. An estrogen receptor beta isoform that lacks exon 5 has dominant negative activity on both ERalpha and ERbeta. Biochem Biophys Res Commun 2000;279:814–9.
[67] Lewandowski S, Kalita K, Kaczmarek L. Estrogen receptor beta. Potential functional significance of a variety of mRNA isoforms. FEBS Lett 2002;524:1–5.
[68] Baldi E, Luconi M, Muratori M, Forti G. A novel functional estrogen receptor on human sperm membrane interferes with progesterone effects. Mol Cell Endocrinol 2000;161:31–5.
[69] Monje P, Zanello S, Holick M, Boland R. Differential cellular localization of estrogen receptor alpha in uterine and mammary cells. Mol Cell Endocrinol 2001;181:117–29.
[70] Kirkland JL, Murthy L, Stancel GM. Tamoxifen stimulates expression of the c-fos proto-oncogene in rodent uterus. Mol Pharmacol 1993;43:709–14.
[71] Stancel GM, Boettger-Tong HL, Chiappetta C, Hyder SM, Kirkland JL, Murthy L, et al. Toxicity of endogenous and environmental estrogens: what is the role of elemental interactions? Environ Health Perspect 1995;103(Suppl 7):29–33.
[72] Levy JR, Faber KA, Ayyash L, Hughes Jr CL. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. Proc Soc Exp Biol Med 1995;208:60–6.
[73] Rattenborg T, Gjermandsen I, Bonefeld-Jorgensen EC. Inhibition of E2-induced expression of BRCA1 by persistent organochlorines. Breast Cancer Res 2002;4:R12.