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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

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To characterize PCB action on follicular cell steroidogenesis two PCB congeners were selected as model substances. PCB 126 because of its dioxin-like configuration and high toxicity and PCB 153 because it is one of the most commonly detected PCB congeners in breast milk. The direct effect of PCBs was investigated using a culture system of porcine theca and granulosa cells collected from porcine preovulatory follicles. Granulosa and theca cells were cultured in M199 medium supplemented with 1, 10 or 100 pg/ml of PCB 126 or 1, 10 and 100 ng/ml of PCB 153. The media were changed after 48, 96 and 144 h and frozen until further estradiol (E2) analysis. Additionally, progesterone (P4) was measured in the granulosa cells culture medium and testosterone (T) in theca cells culture medium. Decrease of testosterone concentration in the theca cells culture medium was found after 96 and 144 hours in culture by both investigated PCB congeners. A decrease in E2 concentration was found after exposure to PCB 153. These findings suggest different actions of two congeners on the steroid synthesis in theca cells. The lack of an increase in E2 secretion after the exposure to PCB 126 could be due to depletion of androgen precursor. In granulosa cell culture PCB153 decreased E2 secretion and increased P4 secretion suggesting luteinization and disurption of aromatization process. PCB 126 in a doses from 1 to 10 pg had no effect on granulosa cells steroidogenesis. However, the highest dose (100 pg) increased concentration of both E2 and P4. This observation suggest that PCB 126 in a pharmacological doses may affect cell membrane permeability, thereby increasing steroid outflow into the medium. These results suggest time dependent and cell-specific differences in PCB 153 and 126 action on folliular cells steroidogenesis. Further studies are required to elucidate the mechanism of PCBs action on ovarian steroidogenesis.

Key words: *PCB 153, PCB 126, theca interna, granulosa cells, steroidogenesis*

INTRODUCTION

Polychlorinated biphenyl (PCB) consists of 209 different congeners, each of which display different chemical properties depending upon their chlorine substitution pattern (1). PCBs were manufactured for industrial purposes from about 1930, and the production lasted until the late 1970 s when it was banned in most countries. The stable chemical properties of PCB, which were beneficial for industrial use, are the same properties that have contributed to the environmental problems. PCBs resist degradation in the ecosystem and due to their lipophilic properties they bioaccumulate in the food chain (2). About 113 different congeners are most frequency detectable in the environment (3). PCB can be identified in almost every component of the global ecosystem including air, water, and soil, as well as fish, animal and human tissues (1).

Especially high concentrations of PCB are found in fat tissue, ovaries, oviductal and uterine tissues, in follicular fluid and in uterine secretions (4). Mice, rats and monkeys exposed to PCB exhibit significantly longer estrus cycles and/or anovulation (5—7).

PCBs are suggested to have the potential to interfere with the endocrine system of animals and humans. Some of the congeners are shown to be estrogenic and bind competitively to the estrogen receptor (8). Lower chlorinated, ortho-substituted, non-coplanar congeners and their parahydroxylated metabolites are show to be weakly estrogenic in rodents and some *in vitro* assays (8). The ortho-substituted congers are supposed to be estrogenic while the coplanar ones are supposed to show antiestrogenic properties (1).

The coplanar PCBs show toxic effects similar to TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) but the PCBs are less potent. TCDD is a potent inhibitor of estrogen-mediated activity and has been shown to be Ah-receptor-dependent (9).

There is evidence that the human ovary can be exposed to PCB contamination. High levels of PCB and hexachlorobenzene (HCB) were found in follicular fluid from women in Germany and Austria (10). The precise mechanism of PCBs' endocrine-disrupting effect is unknown, but the effects may be caused through interference with the synthesis, secretion, transport binding, action or elimination of endogenous hormones, PCBs may act directly upon reproductive organs such as the ovary, but some of the reproductive effects may be due to effects upon the pituitary and/or hypothalamus. Jansen *et al.* (11) exposed cultured anterior pituitary cells to a commercial mixture of PCB congeners (Aroclor 1242) and found enhanced gonadotropin responses to GnRH, similar to that of estradiol (E2).

The direct effect of PCB on ovarian steroidogenesis is unknown. However, there is some evidence that related compounds like dioxin and DDT can

interfere directly in the ovarian steroidogenesis. A decrease in progesterone (P4) production by luteinized human granulosa cells was found after 24 hour exposure to TCDD (12). In another study, TCDD reduced E2 production with no effect on P4 production (13). Similar results were reported by Heimler *et al.*, (14), but with an increase in E2 production by granulosa cells exposed to high doses to TCDD after long term culture. Total PCB levels in human follicular fluid have been reported to range from 4.7 to 24.0 ng/g (15—17). However, data on specific congeners are not available.

In the present study the steroid production in follicular cells was assessed after exposure to PCB. Two congeners with different chemical structure were used: PCB 126, because of its coplanar configuration and high toxicity (1) and PCB 153 because it is one of the most commonly detected PCB congeners in biological tissues (1, 18, 19).

MATERIALS AND METHODS

Reagents

Parker medium M199, trypsin, and calf serum were purchased from Laboratory of Sera and Vaccines, Lublin Poland. Antibiotic antimycotic solution (100 ×) and testosterone were obtained from Sigma Chemical Co. St. Louis, MO, USA. Stock solutions of PCB 153 12,2',4,4',5,5'-CB; 25 μg/ml) and PCB 126 13,3',4,4',5-CB; 25 ng/ml) were prepared by dissolution of pure powder in ethanol (Prochem GmbH, Wesel, Germany; purity 0.997).

Cell cultures

Porcine ovaries obtained from a local abattoir were collected into a bottle filled with sterilized, iced saline and transported to the laboratory. Approximately 15 min elapsed from slaughter to ovary collection. In each experiment six ovaries from three animals were selected for cell preparation. Each ovary yielded 4—6 follicles, giving a total number of follicles per experiment varying from 24 to 36. Large follicles were obtained from ovaries collected at day 16—18 of oestrus cycle. This procedure was chosen to minimize possible variation existing between follicles and between animals. Granulosa cells (Gc) and theca interna layers (Tc) subsequently prepared. The separation of Gc from the thecal layer was performed according to the technique described by Stoklosowa *et al.* (20). Gc were scraped from the follicular wall with round-tip ophthalmologic tweezers and rinsed several times with PBS. After collection, the granulosa cells were washed three times in M199 containing 100 iu/ml penicillin and 1 mg/ml nystatin, and then recovered by centrifugation (10 min at 200 × g). Viable granulosa cells (92%), determined by trypan blue exclusion test, were finally suspended in 24—36 ml of M-199 medium supplemented with 5% calf serum, yielding 1 ml of suspended cells per follicle. (The cells were the plated one ml/well in 24 well plastic cell-culture plates (Nunc).

The thecal cells from the same follicles were prepared as described in detail by Stoklosowa *et al.*, (20). Briefly, the theca layers were placed in a drop of saline under the dissection microscope. A theca interna was manually separated from the underlying theca externa. Isolated theca interna tissue were then washed, cleaned, and cut with scissors and then exposed to trypsinization with

6—7 ml 0.25% trypsin in PBS for 10 min at 37°C. The cells were then separated by decantation. This procedure was repeated 3 times. Finally, the cells were spun and resuspended in 24—36 ml of M-199 medium supplemented with 5% calf serum, yielding 1 ml of suspended cells/follicle and plated one m/well in 24 well plastic cell-cultured plates (Nunc). The cell viability, using the trypan blue exclusions test, was determined to be 85%.

In co-culture, suspended Gc and Tc cells were placed in the same well giving a total of 1 ml of culture medium/well. Granulosa cells and thecal cells were inoculated at a concentration of 6.8×10^6 and 2.1×10^6 cells/ml respectively. When the cultures were mixed, the resultant concentration ratio was comparable to that observed *in vivo* (Gc: Tc = 3 : 1). To assure a substrate for aromatase, testosterone was added to granulosa cell cultures (final concentration 10^{-7} M), whereas theca-derived andogens served as a substrate for the production of estradiol by Tc cell cultures and in the co-cultures. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Experimental procedure

In order to show the time-dependent effect of PCB congeners on steroid secretion by particular types of follicular cells harvested from large preovulatory follicles the granulosa cells, theca cells and co-culture granulosa and theca cells were treated with PCB 153 and PCB 126. The cells were cultured with 1.0, 10.0, 100 ng of PCB 153 and 1.0, 10.0, 100 pg of PCB 126 for periods of 48, 98 and 144 hrs and the medium was then frozen (−20°C) prior to steroid analysis.

Steroid analysis

Progesterone, estradiol and testosterone were determined radioimmunologically using Spectria RIA kits (Orion, Diagnostica, Finland), supplied by Polatom (Świerk, Poland).

For progesterone, the limit of assay sensitivity was 94 pg/ml. The coefficients of variation between and within assays were 5.8% and 2.9%, respectively. The mean recoveries were 95.1—103.7%. The cross-reaction with pregnenolone was 2.9%. All other tested steroids (5 β-dihydroprogesterone, 20 β-hydroxyprogesterone, corticosterone, testosterone, estrone) showed less then 1% cross-reaction.

For estradiol, the limit of assay sensitivity was 5 pg/ml. The coefficients of variationbetween and within assays were 10.28% and 2.9%, respectively. The mean recoveries were 85.6—108.9%. The cross-reaction with ethinylestradiol was 1.4%. All other tested steroids (estrone, estrial, prpgesterone, testosterone, corticosterone) showed less then 1% cross-reaction.

For testosterone, the limit of assay sensitivity was 5 pg/ml. The coefficients of variation within and between assays were 5.4% and 5.3%, respectively. The mean recoveries were 84.2—121.7%. The cross-reaction with 5α-dihydrotestosterone was 4.5%. All other tested steroids (methyl-testosterone, androstadione, 5-androstendiol, progesterone, 17 β-estradiol) showed less than 0.5% cross-reacton.

Statistical analysis

All data points are expressed as means ± SEM from at least three different experiments (n = 3), each in triplicate. The significance of the differences between the concentrations of progesterone, estradiol, and testosterone in the control and experimental cultures were compared by analysis of variance and by Duncan's new multiple range test.

RESULTS

Effects on steroid secretion by theca interna cells

A significant decrease in testosterone secretion was observed at 2 days under the influence of 10 pg/ml (22% reduction) and 100 pg/ml PCB 126 (21% reduction) (*Fig. 1 a*). Four day exposure to 1, 10, and 100 pg PCB 126; and to 1, 10, and 100 ng/ml PCB 153 decreased testosterone secretion by theca cells (84%, 61%, and 50% respectively, as compared to controls for PCB 126; *Fig. 1 a* and 77%, 71% and 46% respectively, for PCB 153; *Fig. 1 b*). Both PCB congeners decreased testosterone secretion after 6 days of exposure at doses of 10 and 100 ng/ml (*Fig. 1 a, b*).

A significant inhibitory effect on estradiol secretion by theca cells was found after treatment with 10 and 100 ng/ml PCB 153 for 4 days and at all doses after 6 days (*Fig. 2 d*).

Absolute control values for testosterone were between 6.81 and 7.11 ng/10^5 cells and between 2.43 and 3.22 ng/10^5 cells for estradiol secretion in the three experiments.

Effect on steroid secretion by granulosa cells

With the exception of the short-term culture, all doses of PCB 153 caused a significant increase in progesterone secretion (*Fig. 2 b*). The same effect was observed for PCB 126 only when the highest dose was used (100 pg/ml) for 4 and 6 days. (*Fig. 2 a*).

A negative effect of PCB 153 on estradiol production was indicated by significant reductions in the estradiol concentration after 4 and 6 day exposures to higher doses in culture (*Fig. 2 d*).

Conversely, an exposure to PCB 126 in a dose of 100 pg/ml increased estradiol secretion by granulosa cells after 6 days in culture (*Fig. 2 b*).

Absolute control values for estradiol were betwen 0.8 and 0.92 ng/10^5 cells, and for progesterone between 14.8 and 15.3 ng/10^5 cells in the three experiments.

The effect of PCB 126 and PCB 153 on estradiol secretion by co-culture of theca and granulosa cells.

In co-culture of theca and granulosa cells the highest dose of PCB 126 (100 pg/ml) increased estradiol secretion after 4 and 6 days in culture. (*Fig. 3 a*). A similar effect was found for PCB 153 after 6 days in culture using doses of 10 ng/ml and 100 ng/ml. (*Fig. 3 b*).

Absolute control values for estradiol ranged between 0.63 and 0.59 ng/10^5 cells in the three experiments.

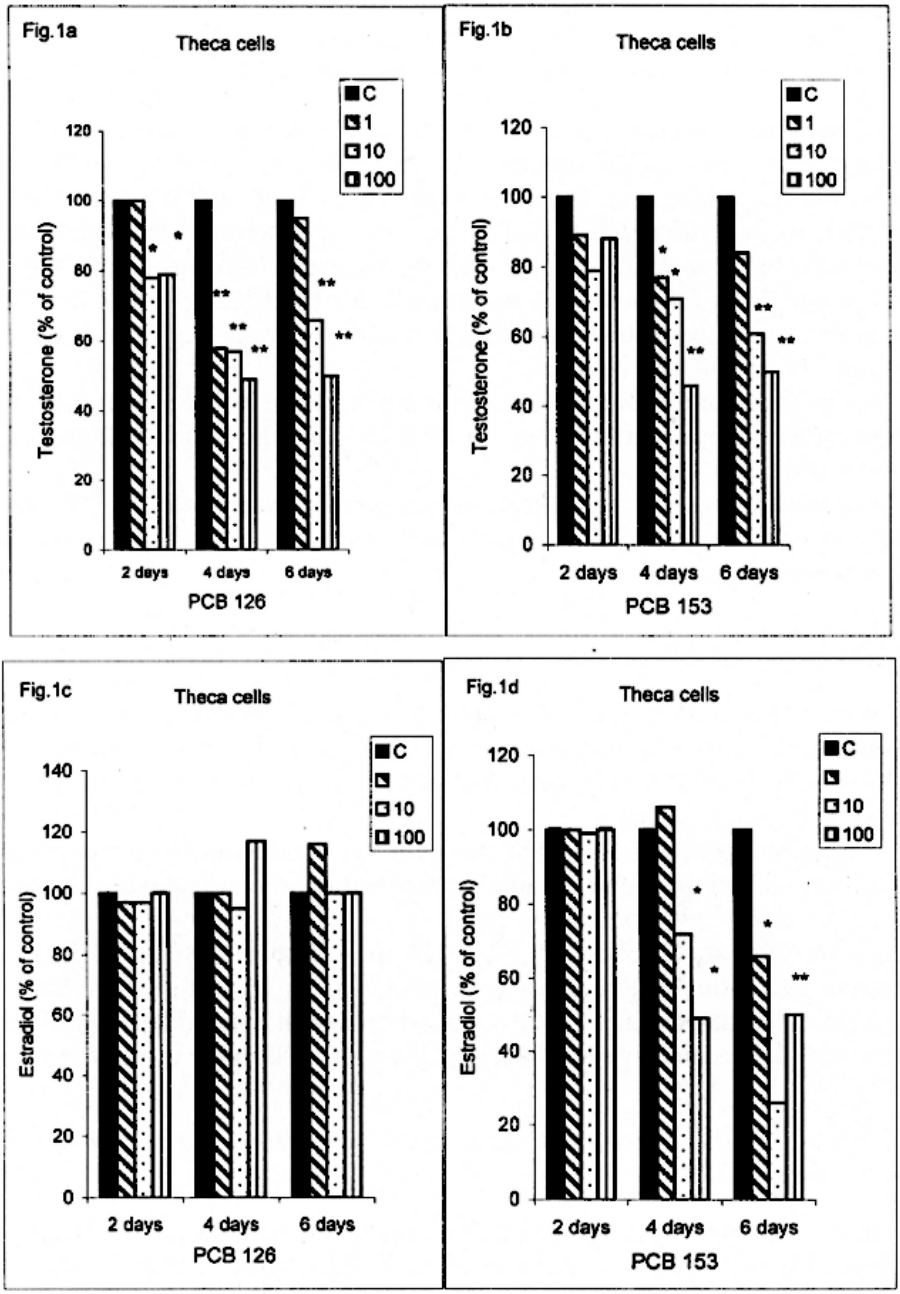

Fig. 1. Secretion of testosterone (*a*, *b*) and estradiol (*c*, *d*) by cultured porcine theca cells in the presence of polychlorinated biphenyls (PCB 126 and PCB 153). All values are the mean ± SE and expressed as percent secretion, with control as 100%. * $p < 0.05$; ** $p < 0.1$.

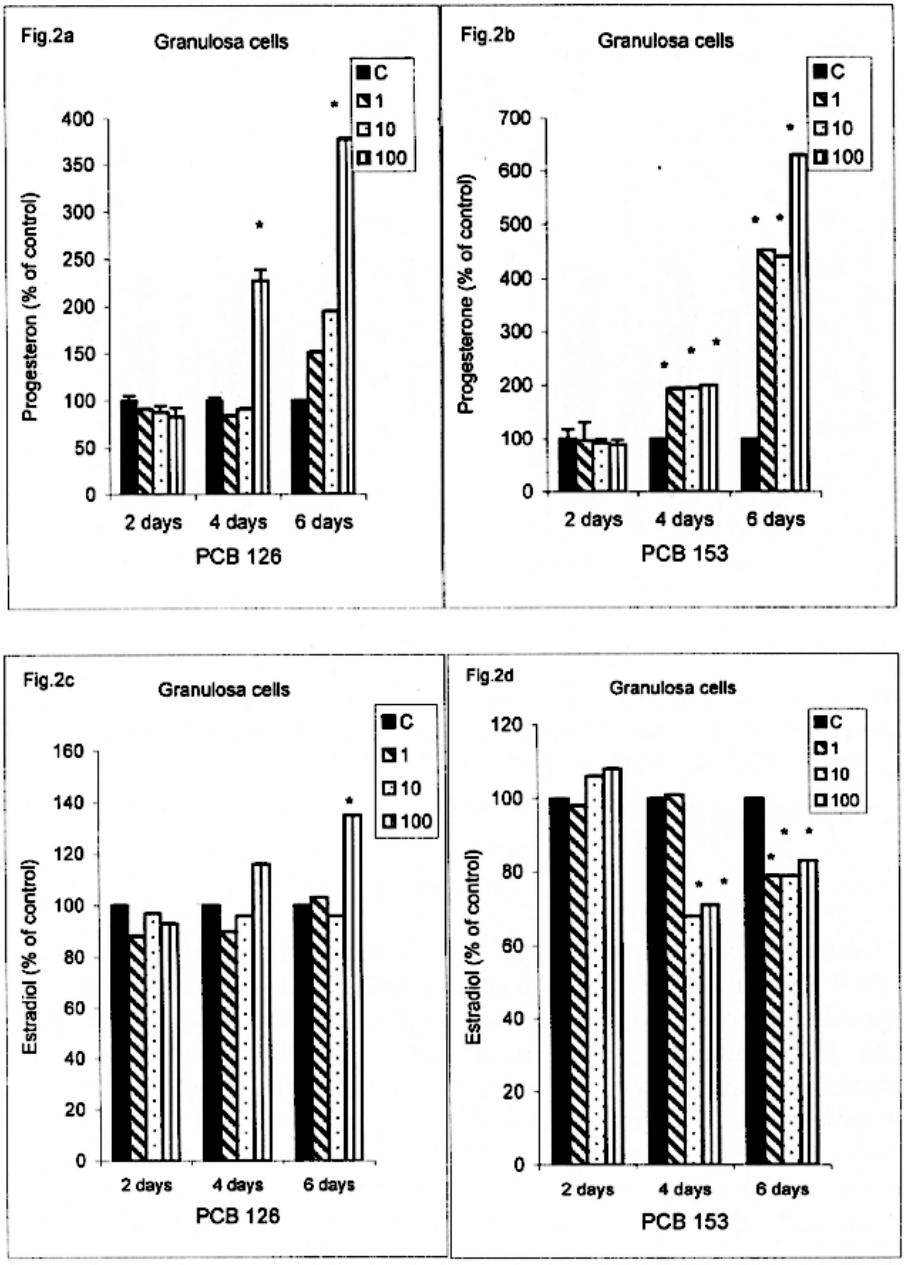

Fig. 2. Secretion of progesterone (*a*, *b*) and estradiol (*c*, *d*) by cultured porcine granulosa cells in the presence of polychlorinated biphenyls (PCB 126 and PCB 153). All values are the mean ± SE and expressed as percent secretion, with control as 100%. * $p < 0.05$; ** $p < 01$.

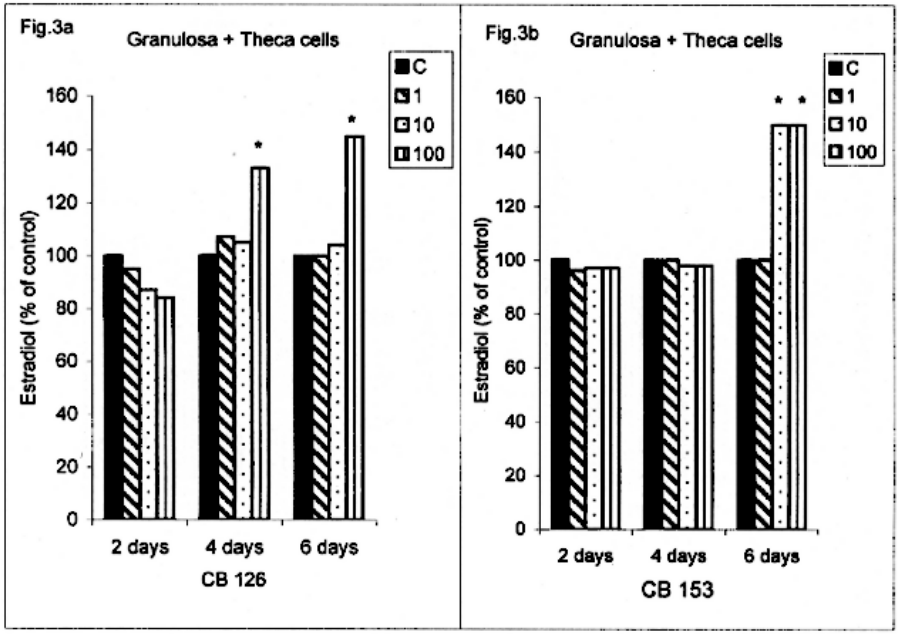

Fig. 3. Secretion of estradiol (*a*, *b*) by co-cultured porcine theca and granulosa cells in the presence of polichlorinated biphenyls (PCB 126 and PCB 153). All values are the mean ± SE and expressed as percents aecretion, with control as 100%. * $p < 0.05$; ** $p < 0.1$.

DISCUSSION

The current study demonstrated that the exposure of porcine follicular cells to PCB 153 and PCB 126 significantly influenced steroidogenesis. To our knowledge there are no other reports in the literature in which the effects of single PCB congeners on follicular cells have been studied. A different mechanism of action for the two congeners on estradiol secretion by theca cells was indicated by the findings that PCB 153, but not PCB 126, in long term cultures with higher doses of PCB 153 decreased the concentration of the hormone in the culture medium (*Fig. 2*). PCB 126 is supposed to act via the Ah receptor (21), which has recently been shown to be present in the ovary and the level of the AhR seems to be more significant in smaller follicles indicating that bigger follicles are less sensitive to AhR agonists (22). Binding of PCB to this receptor is supposed to influence cell fuctions (23), but further details on possible mechanisms of action of specific PCB congeners on follicular cell functions is not know. However, there are reports in the literature on the effects

of PCB on various aspects of reproduction (1), some of which are considered estrogenic or anti-estrogenic.

The estrogenic effect of PCB congeners has previously been related to ortho-substitution (24, 25). The coplanar non-ortho-substituted PCB 126 was found to be antiestrogenic (1, 9), whereas the non-coplanar diortho-substituted PCB 153 was demonstrated to exert an estrogenic effect (26). Müller *et al.* (7) found low estrogen levels in cycling rhesus monkeys treated with PCB, suggesting that this chemical could block ovulation. In each case without ovulation, a decrease in estrogen secretion was indicated and PCB appeared to deplete growth of the primary follicles.

It is possible that PCB 153 binds to the estrogen receptor and exerts an effect similar to that caused by endogenous or therapeutically administered estrogens. Results from the "E-Screen test" show that PCB 153 does not have estrogen receptor binding affinity (27). However, others reports have found that PCB 153 has an estrogenic effect in rats (26, 28). To determine whether or not a chemical has an estrogenic effect or not is difficult because of the complex mechanisms involved. At present there are two known E2 receptors, ERα and ERβ. It has been shown that different estrogenic compounds cause differential activition of these receptors (29), and that their distribution differs between tissues.

There is also an indication that different estrogens can exert different patterns of gene expression. Major differences in transcriptional responses in the rodent uterus have been found following tamoxifen versus estradiol treatment (30, 31). The question is if such a mechanism of action can explain the decrease in testosterone seen after the exposure of theca cells to PCB 153 in the present study (*Fig. 1 a—c*). In culture experiments with isolated theca tissue and dispersed theca cels, the addition of estrogen directly to the culture medium inhibited LH-stimulated androgen production in a dose-dependent manner (32, 33), indicating that estrogen has a direct inhibitory effect on theca cells.

Estrogen pre-treatment of ovaries from intact immature rats has been shown to inhibit conversion of radioactively-labelled progesterone to androgens. On the other had, incorporation into 3α-hydroxy-5α-pregnan-20-one is enhaced under the same conditions, suggesting that estrogen may act by inhibiting the 17 α-hydroxylase 17, 20-lyase (32). There is also evidence that estrogens can regulate the metabolism of androgens by a direct action upon 5 α-reductase (34).

TCDD, which is supposed to act via the same mechanism as PCB 126, has been shown to reduce the number of estrogen receptors in various cell types, including ovarian tissue (21, 35, 36), and to decrease several estrogen mediated responses (21, 37—40). TCDD has also been shown to modulate several steps in the metabolism of cholesterol during steroidogenesis, including an increase

in the 2- and 4-steroid hydroxylase activities (41—43). This could give some explanation for the observed decrease in testosterone secretion by theca cells exposed to PCB 126 (*Fig. 1 a—c*).

The finding that PCB 126 had no effect on estradiol secretion by granulosa cells and increased their progesterone production at the highest dose level (100 µg/ml) in the long-term cultures (*Figs.* 3 b—c) was unexpected based on the suggested dioxin-like effect of this congener. Heimler *et al.* (14) exposed human luteinized granulosa cellls to doses of TCDD which were considered environmentally relevant, and found a significant decrease in E2 secretion after 8, 12, and 24 hours of culture, but not effect was seen after 36 and 48 hours.

In the present study both congeners increased P4 production and reduced androgen production, indicating an inhibitory effect on the transformation of P4 to androgens during steroidogenesis. The high stimulatory effect of PCB 153 on progesterone secretion and concomitant decrease of estradiol secretion by granulosa cells observed (*Fig. 3 b—c, Fig. 4 b—c*) could be explained by a luteinizing effect of this congener.

It has been shown previously that a high level of progesterone might have an inhibitory effect on aromatase activity (44—46).

The increase in estradiol concentration found after 6 days of culture with PCB 126 (*Fig. 4 c*) suggests that this congener causes an increase in the cell membrane permeability resulting in increased steroid outflow into the medium.

Rogers *et al.*, (147) showed than an *in vitro* clonal assay with CHO-K1 Cells Chinese hamster ovary cells) was a sensitive indicator of the cytotoxicity of PCBs. Compared to the total concentrations found in untreated controls,CHO-K1 cells treated with Aroclor 1016 contained less neutral lipid and more phospholipid. Changes in membrane neutral lipid and phospholipid components, observed at marginally cytotoxic leveld of Aroclors, provided further evidence that the PCBs may affect membrane integrity and associated metabolic functions.

Concerning that an environmentally relevant does of PCB 153 increased E2 secretion while only a pharmacological dose of PCB 126 has an effect on the estradiol secretion in co-culture of GT (*Fig. 5 c*), suggests different and unknown mechanism of action of both congeners on follicular steroidogenesis. Earlier studies have confirmed effects on E2 production by follicular cells exposed to well known AhR agonists (12—14).

On the other hand, actions of PCB 153 were dependent on the culture model used. Theca cells and granulosa cells cultured alone responded to PCB153 by decreasing their estradiol secretion (*Figs. 2 b—c; 4 b—c*) whereas in co-culture increased the estradiol secretion (*Fig. 5 c*). The reason for this apparent discrepancy was not investigated in the present study, but when

searching for a probable explanation it is relevant to point to the close connection that exists between follicular cells *in vivo*. Steroid production in single follicular cells is very much influenced by surrounding cells exerting a paracrine regulation of specific cell functions, as indicated by Yada *et al.* (48). There is also evidence that environmental pollutants (TCDD) can disturb these intractions between follicular cells (21, 38—49). Consequently, the use of co-culture of theca and granulosa cella will probably provide a better model for predicting the effects of PCB on follicular steroidogenesis *in vivo*.

In conclusion, both PCB 153 and PCB 126 influenced the steroid secretion of follicular cells. However, it was not possible to predict effects of these congeners from what is previously believed to be their mechanisms of action on follicular cell steroidogenesis. The focus of further studies should be concentrated on the cytotoxicity of these congeners in our cell culture systems and on their effects upon the intermediate steps of follicular steroidogenesis.

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REFERENCES

1. Safe S. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assesment. *Crit Rev Toxicol* 1994; 24: 87—149.
2. Clarkson TW. Environmental contaminants in the food chain. *A, J Clin Nutr* 1995; 61: (Suppl): 682S—686S.
3. Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baeteke KP, Hoffmann JL, Morrow MS, Rodier DJ, Schaeffer JE, Tourt LW, Zeeman MG, Patel YM. Environmental endocrine disruption: an effects assessment and analysis: *Environ Health Perspect* 1998; 106 Suppl 1: 11—56.
4. Lindenau A, Fisher B, Seiler P, Beier HM. Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. *Hum Reprod* 1994; 9: 772—780.
5. Örberg J. and Kilhström JE. Effects of long-term feeding of polychlorynated biphenyls (PCB, Clopen A 60) on the length of the oestrus cycle and on the frequency of implanted ova in the mouse. *Environ Res* 1973; 6: 176—179.
6. 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.
7. Müller WF, Hobson W, Fuller GB, Knauf W, Coulston F, Korte F. Endocrine effects of chlorinated hydrocardons in rhesus monkeys. *Ecotoxicol Environ Saf* 1978; 2: 161—172.
8. Gierthy JF, Arcaro KF, Floyd M. Assessment of PCB estrogenicity in a human breast cancer cell line. *Chemosphere* 1997; 34: 1495—1505.
9. Safe S, Astroff B, Harris M. 2,3,7,8-Tetrachlorodibezo-p-dioxin (TCDD) and related compounds as antiestrogens: characterization and mechanism of action. *Pharmacol Toxical* 1991; 69: 400—409.
10. Trapp M, Baukloh V, Bohnet HG, Hesschen W. Pollutants in human follicular fluid. *Fertil Steril* 1984; 42: 146—148.

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; 237—248.
12. Enan E, Lasley B, Steward D, Overstreet J, Vandervoot CA. 2,3,7,8-tetrachlorodibento-p--dioxin (TCDD) modulates function of human luteinizing granulosa cells via cAMP signaling and early reduction of glucose transporting activity. *Reprod Toxicol* 1996; 10: 191—198.
13. Moran FM, Enan E, Vandervoort CA, Steward DR, Conley AJ, Overstreet JW, Lasley BL. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) modulates function of human luteinizing granulosa cells *in vitro*. *Society for the Study of Reproduction, Portland, OR*, 1997, (Suppl 1) 56 (Abstract).
14. Heimler I, Trewin AL, Chaffin CL, Rawlins RG, Hutz RJ. Modulation of ovarian follicle maturation and effects on apoptopic cell death in Holtzman rats exposed to 2,3,7,8--tetrachlorodibenzo-pidioxin (TCDD) in utero and lactationally. *Reprod Toxicol* 1998; 12: 69—73.
15. Kholkute SD, Rodroguez J, Dukelow WR. Reproductive toxicitx of Aroclor 1254: effects on oocyte, spermatozoa, *in vitro* fertilization, and embryo developmet in the mouse. *Reprod Toxicol* 1994; 8: 487—493.
16. Johansen HR, Becher G. Congener-specyfic determination of polychlorinated biphenyls and organochlorine pesticides in human milk from Norwegian mothers living in Oslo. Toxicol *Environ Health* 1994; 42: 157—171.
17. Skaare JU, Tuveng JM, Sande A. Organochloride pesticides and polychlorinated biphenyls in maternal adipose tissue, blood milk, and cord blood from mothers and their infants lining in Norway. *Arch Environ Contam Toxicol* 1998; 55—63.
18. Kimbrough R. Polychlorinated biphenyls (PSBs) and human belath: an update. *Crit Rev Toxicol* 1995; 25: 133—63.
19. Krogaenas AK, Nafstad I, Skare JU, Farstad W, and Hafine AL. *In vitro* reproductive toxicity of polychlorinated biphenyls congeners 153 and 126. *Reproductive toxicology* 1998; 12: 575—580.
20. Stoklosowa S, Gregoraszczuk EL, Channing CP. Estrogen and progesterone secretion by isolated cultured porcine thecal and granulosa cells. *Biol Reprod* 1982; 26: 943—952.
21. Romkes M, Piskorska-Pliszczyńska J, Keys B, Safe S, Fujita T. Quantitative structure-activity relationships: analysis of interactions of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2-substituted analogues wit rat, mouse, guinea pig, and hamster cytosolic receptor. *Cancer Res* 1987; 47: 5108—5111.
22. Pocar P, Navarette Santos A, Kietz S, Gandolfi F, Ficher B. Arylhydrocarbon receptor expression during folliculogenesis in the bovine ovary. *Biology of Reproduction* 1999; 61: 16 (*Abstract*).
23. Poland A, Glover E. Studies on the mechanism of induction of aryl hydrocarbon hydroxylase activity: evidence for an induction receptor. *In: de Serres FJ et al., ed: In vitro metabolic activation in mutagenesis testing. Amsterdam, North-Holland Publ.* 1976 QH 411 S989; Jul 18: 277—291.
24. Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. *Mol Pharmacol* 1988; 33: 120—126.
25. Ecobichon DT and McKenzie DO. The uterotropic activity of comercial and isomerically-pure chlorobiphenyls in the rat. *Res Commun Chem Pathol Pharmacol* 1974; 9: 85—95.
26. 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—590.
27. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay at a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Healt Perspect* 1995 Oct; 103 Suppl 7: 113—122.

28. 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—169.
29. Brooks AN, Lambe KG, Milligan S, Aldrige TC. Transcriptional activation by photoestrogens of ERα, ERβ and a splice variant of ERβ (ERβ2). *Biology of Reproduction* 1999; 61: 6 (Abstract).
30. Kirkland JL, Murthy L, Stancel GM. Tamoxifen stimulates expression of the c-fos proto-oncogene in rodent uterus. *Mol Pharmacol* 1993; 43: 709—714.
31. Stancel GM, Boettger-Tong HL, Chiappetta C, Hyder SM, Kirkland JL, Murthy L, Loose-Mitchell DS. Toxicity of endogenous and environmental estrogens: what is the role of elemental interactions? *Environ Health Perspect* 1995 Oct; 103 Suppl 7: 29—33.
32. Leung PC, Armstrong DT. A mechanism for the intraovarian inhibitory action of estrogen on endrogen production. *Biol Reprod* 1979; 21: 1035—1042.
33. Leung PC, Armstrong DT. Further evidence in support of a short-loop feedback action of estrogen on ovarian androgen production. *Life Sci* 1980; 27: 415—420.
34. Eskstein B, Nimrod A. Properties of microsomal delta4-3-ketosteroid 5 alpha-reductase in immature rat ovary. Inhibition by estradiol-17beta. *Biochim Biphys Acta* 1977 Aug 25: 499: 1—9.
35. Astroff B, Eldridge B, Safe S. Inhibition of the 17 beta-estradiol-induced and constitutive expression of the cellular protooncogene c-fos by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the female rat uterus. *Toxicol Lett* 1991; 56: 305—315.
36. Chaffin CL, Peterson RE, Hutz RJ. In utero and lactational exposure of female Holtzman rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: modulation of the estrogen signal. *Biol Reprod* 1996; 55: 62—67.
37. Hyder SM, Chiappetta C, Stancel GM. Triphenylethylene antiestrogens induce uterine vascular endothelial growth factor expression via their partial estrogen agonist activity. *Cancer Lett* 1977; 120: 165—171.
38. Astroff B, Safe S. Comparative antiestrogenic activities of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 6-methyl-1,3,8-trichlorodibenzofuran in the female rat. *Toxicol Appl Pharmacol* 1988; 95: 435—443.
39. Astroff B, Rowlands C. Dickerson R, Safe S. 2,3,7,8-Tetrachlorodiberizo-p-dioxin inhibition of 17 beta-estradiol-induced increases in rat uterine epidermal growth factor receptor binding activity and gene expression *Mol Cell Endocrinol* 1990; 72: 247—252.
40. Astroff B, Safe S. 6-Alkyl-1,3,8-trichlorodibenzofurans as antiestrogens in female Sprague-Dawley rats. Toxicology 1991; 69: 187—197.
41. Mebus CA, Piper WN. Decreased rat adrenal 21-hydroxylase activity associated with decreased adrenal microsomal cytochrome P-450 after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biochem Pharmacol* 1986; 35: 4359—4362.
42. Kleeman JM, Moore RW, Peterson RE. Inhibition of testicular steroidogenesis in 2,3,7,8-tetrachlorodibenzono-p-dioxin-treated rats: evidence that the key lesion occurs prior to or during pregnenolone formation. *Toxicol Appl Pharmacol* 1990; 106: 112—125.
43. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17 beta-estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc Natl Acad Sci USA* 1996; 93: 9775—9781.
44. Schreiber JR, Nakamura K, Erickson F. Progestins inhibit FSH-stimulated steroidogenesis in cultured rat granulosa cells. *Mol Cell Endocrinol* 1980; 19: 165—173.
45. Gregoraszczuk EL. Interrelations between steroid hormone secretion and morphological changes of prcine corpora lutea at various periods of luteal phase. *Endocr Regul* 1992; 26: 189—194.

46. Gregoraszczuk EL. Is progesterone a modulator of luteal steroidogenesis in pig? A tissue culture approach. *Folia Hist Cytobiol* 1994; 32: 31—33.
47. Rogers CG, Heroux-Metcalf C, Iverson F. *In vitro* cytotoxicity of polychlorinated biphenyls (Aroclors 1016, 1242, 1254 and 1260) and their effect on phospholipid and neutral lipid composition of chinese hamster ovary 9CHO-K1) cells. *Toxicology* 1983; 26: 113—124.
48. Yada H, Hosokawa K, Tajima K, Hasegawa Y, Kotsuji F. Role of Ovarian Theca and Granulosa Cell Interaction in Hormone Productionand Cell Growth During the Bovine Follicular Maturation Process. *Biol Reprod* 1999; 61: 1480—1486.

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