J. Microbiol. Biotechnol. (2003), **13**(4), 495–500

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Assessment of Reproductive Health Risk of Polychlorinated Biphenyls by Monitoring the Expression of Claudins and Transepithelial Electrical Resistance in Mouse Sertoli Cells

GYE, MYUNG-CHAN, SEIICHIROH OHSAKO[1], AND HO-JOON LEE[2]*

Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, Korea
[1]Molecular and Cellular Toxicology Section, National Institute for Environmental Studies, 162 Onogawa, Tsukuba 305-8506, Japan
[2]Department of Physiology, Eulji University School of Medicine, Daejeon 301-832, Korea

Received: September 5, 2002
Accepted: May 1, 2003

Abstract Tight junctions (TJ) between adjacent Sertoli cells in testis are important for the formation of the blood testis barrier (BTB). In an effort to verify the reproductive health risk of endocrine-active chemicals (EACs), changes in the transepithelial electrical resistance (TER) and the expression of TJ genes were examined by co-planar polychlorinated biphenyl (PCB) treatment in cultured mouse Sertoli cells. Although the increase in TER of Sertoli cells was accelerated by 10 nM co-planar PCB, it was downregulated by 100 nM co-planar PCB. The expression of claudin-1 was downregulated by co-planar PCB in a concentration-dependent manner. On the contrary, the expression of claudin-11 was increased in the Sertoli cells by 10 nM co-planar PCB treatment. These results suggest that the structure and function of TJ may be targeted by co-planar PCB in Sertoli cells. Assessment of the structure and function of TJ in Sertoli cells might be useful for screening the reproductive health risk of EACs.

Key words: Tight junction, transepithelial electrical resistance, Sertoli cells, PCB

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental contaminants. So far, most of PCBs-related studies have been concentrated on the development of the tools for the control of PCB in the environment as well as the assessment of toxicity of PCBs [31, 36, 37]. Individual PCB congeners exhibit different physicochemical properties and biological activities, resulting in different environmental distributions and toxicity profiles. The variable composition of PCB residues in environmental matrices and their different mechanisms of toxicity complicate the development of scientifically based regulations for the risk assessment. To screen for and assess the male reproductive health risks of exogenous endocrine-active chemicals (EACs), some approaches for the assessment of risks of test compounds on the male reproduction system have been examined [7, 12, 17, 34]. However, the endpoints of these assays are still limited to the gross anatomical changes in the reproductive organs and serum hormone levels [33]. Basically, spermatogenesis is supported by Sertoli cells, and impairment of spermatogenesis by xenobiotics might be attributed to changes in the Sertoli cell functions in mammals [47]. Alterations of gene expression in Sertoli cells in intact animals or cultured Sertoli cells have been used to screen and assess the male reproductive health risks of exogenous EACs. Junctional complexes have important roles in the control of cell proliferation and differentiation in many different organs. In the case of gap junctional communication, inhibition of cell communication is often observed after treatment of the cells with many tumor promoters, including halogenated hydrocarbons and their metabolites [19, 39, 43, 48]. Halogenated hydrocarbons, alone or in specific combinations, can alter GJIC at the post-translational level, including aberrant localization of gap junction protein [1, 16, 23]. Similarly, the tumor promoter can alter tight junction (TJ) in various tissues [25, 30, 49]. Recently, it was reported that PKC-alpha as well as PKC-epsilon may be target molecules for ortho-PCBs in neuronal cells [51]. However, it has not been proven if halogenated hydrocarbons can alter the structure and function of TJ in male reproductive organs. In testis, TJs between Sertoli cells are important for the formation of the blood testis barrier (BTB) which creates a regulated paracellular barrier for the movement of water, solutes, and immune cells from circulation to seminiferous tubule, and is crucial for normal progression of spermatogenesis [6, 8,

*Corresponding author
Phone: 82-42-259-1086; Fax: 82-2-971-8710;
E-mail: leehj@emc.eulji.ac.kr

14, 40]. The structure and functions of TJ are under control of paracrine and endocrine as well as physicochemical factors in various tissues [20, 32, 50]. A pathologic condition in TJ is known to be related to diverse diseases, including male infertility and microbial infection in epithelia [4, 24, 46, 53]. Among the components of the TJ, occludin is expressed in large amounts in Sertoli cells and plays an important role in spermatogenesis [41, 42]. Claudin-1 that is expressed rather ubiquitously, even in organs lacking epithelia, is largely expressed in endothelial tissues [28]. Claudin-11, also known as an oligodendrocyte-specific protein (OSP), is expressed in Sertoli cells [2, 11, 29]. Although recent studies have focused on the cloning and tissue distribution of the TJ genes and their function in the formation of TJ in the testis, little is known about the effect of EACs on the expressions and barrier function of TJ in Sertoli cells. In an effort to verify the toxic effect of PCBs and to develop tools for screening the reproductive health risk of EACs, the effects of PCBs on the expression of TJ genes and transepithelial electrical resistance (TER) in cultured mouse Sertoli cells were analyzed.

Materials and Methods

Primary Culture and Drug Treatment

Testes were isolated from two-week-old ICR male mice and blotted onto filter paper to clear the blood. After mincing in phosphate buffered saline (PBS), tissues were incubated in PBS, containing 0.1% collagenase (Sigma, C-2674) and 20 μg/ml DNAse (Sigma DN-25), at 37°C for 30 min. Following a stay in unit gravity for 15 min, the Leydig cell-enriched fraction of supernatant was decanted. Seminiferous tubule fragments in the bottom of the tube were collected and incubated in PBS, containing 0.25% trypsin (Sigma T-4799) and DNAse (20 μg/ml) at 37°C for 15 min. After filtration through nylon mesh (Falcon 2350, mesh size 70 μm), the filter-through was centrifuged at 800 ×g for 10 min. Resulting cell pellet was suspended in DMEM F12 medium and washed twice by centrifugation. Final cell pellet was suspended in fresh medium, plated on a 6-well culture dish and cultured with 5% CO_2 in air at 35°C. After 48 h, cells were treated with hypotonic solution (20 mM Tris, pH 7.3) for 1 min to remove germ cells. Remained Sertoli cells were cultured further for 24 h. After the subculture, Sertoli cells were transferred to a cell culture plate insert (Millipore, pore size 0.4 μm, inner diameter 6 mm) at 1.7×10^5/well. Following the confirmation of the cell monolayer formation, 3,3',4,4',5,-pentachlorobiphenyl (co-planar PCB) dissolved in dimethylsulfoxide (DMSO) was added to the cell culture. Control cells were treated with DMSO (final 0.1%). Sertoli cells were subjected to RNA extraction at 48 h after the PCB treatment.

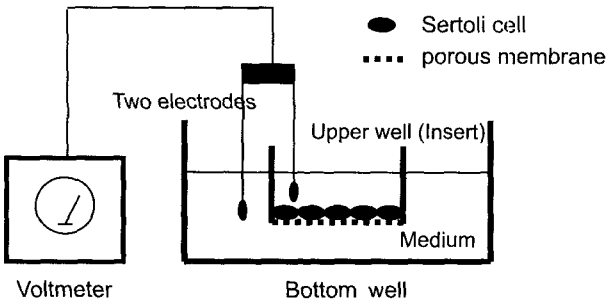

Fig. 1. System for measuring transepithelial resistance (TER) of Sertoli cell monolayer.
Voltmeter equipped with two electrodes was used to measure TER of Sertoli cell monolayer on the cell culture plate insert.

Measurement of Transepithelial Electrical Resistance (TER)

TER of the Sertoli cell momolayer on the insert plate was measured using a voltmeter equipped with 2 silver electrodes (Millipore, MA, U.S.A.) in the presence or absence of PCBs in the bottom well (Fig. 1). Before the measurement, TER of the medium was set to "zero" and a sample TER was subtracted by that of a cell-free insert (blank). After the addition of co-planar PCB, TER was measured at 12 h intervals and the net change in TER (TER at given time-TER at drug treatment) was calculated. Statistical significance of the net change in TER was examined by Student's *t*-test.

Semiquantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the Sertoli cell culture by the basic protocol of Chomczynski and Sacchi [5]. RNA samples (1.25 μg) were reverse-transcribed in a 40-μl reaction mixture with 400 units of SuperScript™ II reverse transcriptase and 1 μg of oligo (dT)12-18 primer according to the standard protocol of the supplier. RT reaction was conducted for 1 h at 42°C using a PCR thermal cycler (I cycler, BioRad). Following the RT reaction, the samples were heated for 5 min at 99°C and then placed on ice. Mouse claudin-1 primers were designated 5'-TCTGGGAGGTG-TCCTACTTT-3' (forward) and 5'-CACAGTTCCGATAA-CCATCA-3' (reverse) according to the mouse claudin-1 cDNA sequence [10]. The primers for mouse claudin-11 were designated 5-TATAAGTTGAGGTGGGTGTC-3' (forward) and 5'-ATTGGTGTTTACACCCATGAAGC-3' (reverse) according to the mouse claudin-11 cDNA sequence [3]. For semi-quantitative analysis, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified as an internal control. The primers for mouse GAPDH were 5'-AGTGGAGATTGTTGCCATCAACGAC-3' (forward) and 5'-GGGAGTTGCTGTTGAAGTCGCAGGA-3' (reverse) [35]. These primer sets gave rise to amplicons of claudin-1, claudin-11, and GAPDH diagnostic fragments of 213 bp, 382 bp, and 791 bp, respectively. PCR of cDNA equivalent

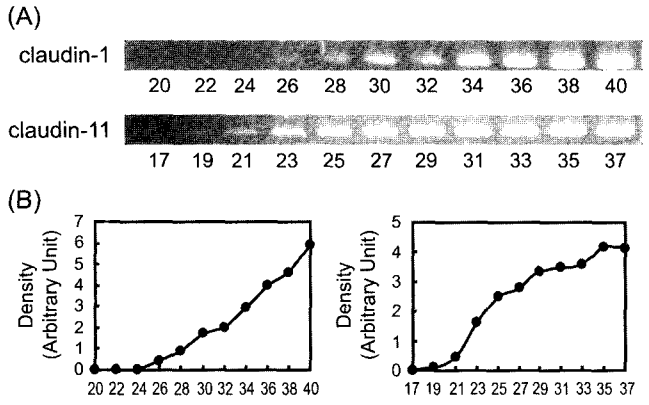

Fig. 2. Optimization of RT-PCR procedure.
(A) PCR products of claudin-1 and claudin-11 according to the amplification cycles in mouse Sertoli cell cDNA. (B) Amplification curves of PCR-products of claudin-1 and -11.

to 0.5 μg of total RNA per 1 μl was carried out in 50 μl of 1× PCR buffer (10 mM Tris-HCl, pH 8.3, containing 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each of the 4dNTPs, 1 unit Ex *Taq* polymerase (Takara, Japan), 2 pmol each of the appropriate primers, and 1 μl of the reverse transcription reaction. The PCR program for claudin-1 and claudin-11 was incubation at 94°C for 3 min, followed by a cycle program of 94°C for 30 sec, 63°C for 30 sec, and 72°C for 45 sec (29 cycles). For GAPDH, incubation at 94°C for 3 min was followed by a cycle program of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec (25 cycles). The last cycle was conducted with a 10 min extension at 72°C. The number of amplification cycle was set to 17 to 40 rounds of PCR. According to the increase in number of PCR cycle, the amounts of amplicons of claudin-1 and claudin-11 were linearly increased from 24 to 40 cycles and from 19 to 31 cycles, respectively (Figs. 2A and 2B). Thereafter, the number of amplification cycle for claudin-1 and claudin-11 were fixed at 29 and 23 cycles, respectively. Following the amplification, the PCR products (20 μl) were run on 2% agarose gels containing 0.5 μg/ml ethidium bromide and photographed under UV light. After the densitometric analysis of band intensity of amplicons, the relative amount of TJ genes transcript versus GAPDH was plotted. To determine the sequences, the PCR product was subcloned into pGEM-TEasy vector and sequenced by the dideoxynucleotide chain termination method, using the ABI Prism BigDye terminator cycle sequencing kit (PE-Biosystems, CA, U.S.A.).

Statistical Analysis

StatView for Windows version 5.0 (SAS Institute Inc., Cary, NC, U.S.A.) was used for the statistical analysis of results. All results are shown as mean±SD. Data were analyzed by the two-tailed Student's *t*-test. Significance was accepted at $p<0.05$.

Results and Discussion

Regardless of the co-planar PCB treatment, TER of Sertoli cells increased during culture (Fig. 3). This suggests that the culture condition was permissive to the development of a paracellular barrier in Sertoli cells. During the culture for 24 h, TER of Sertoli cell monolayer rapidly increased in the presence of 10 - 100 nM co-planar PCB. On the other hand, control cells showed a rather constant TER. During the next 24 h of culture, the increase in TER in the 100 nM co-planar PCB-treated cells slowed down. On the other hand, control cells showed a rapid increase in TER (Fig. 3). At the end of culture, TER of Sertoli cells treated with 10 nM co-PCB was significantly higher than others. However, the TER of 100 nM co-planar PCB-treated cells was significantly lower than the control cells (Fig. 3). Because the magnitude of TER is different depending on the cell type as well as differentiation status in varying tissues, the results suggest that 10 nM co-planar PCB might have potentiated the differentiation of Sertoli cells. Previously, it was reported that co-planar PCB was cytotoxic at concentrations greater than 50 nM in rat hepatoma cells and Sertoli cells [18, 38]. Therefore, attenuation of the increase in TER during 24 to 48 h of culture as well as significant difference in TER value at 48 h after 100 nM co-PCB treatment might be attributed to the cytotoxic effect of co-planar PCB at 100 nM concentration. Most effects of PCBs can be attributed to its activation of the aryl hydrocarbon receptor (AhR), a bHLH/PAS transcription factor, which upregulates a variety of genes (the Ah gene battery) and mediates dioxin toxicity in the immune system, skin, testis, and liver. Toxic phenomena mediated by AhR are associated with altered cell proliferation or differentiation [21, 26, 45]. Therefore, it can be suggested that changes in TER might be attributed to the alteration of Sertoli cell differentiation by co-planar PCB.

The amplicons of claudin-1 and claudin-11 were detected after RT-PCR of the total RNA from mouse Sertoli cells

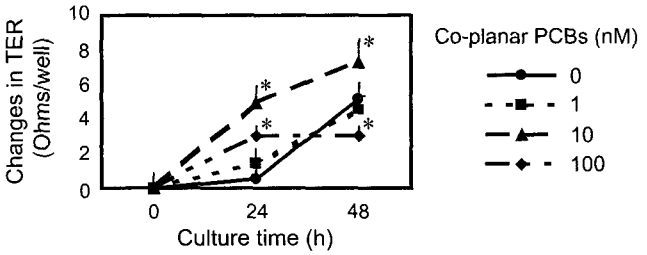

Fig. 3. Effect of co-planar PCB on the TER of Sertoli cells monolayer.
Changes in TER during cell culture for 48 h. Before the measurement, TER of medium was set to "zero" and sample TER was subtracted by that of a cell-free insert (blank). Net change in TER (TER at given time - TER at drug treatment) was calculated. *, Significantly different from control by Student's *t*-test ($p<0.05$). Error bars are SD (n=4).

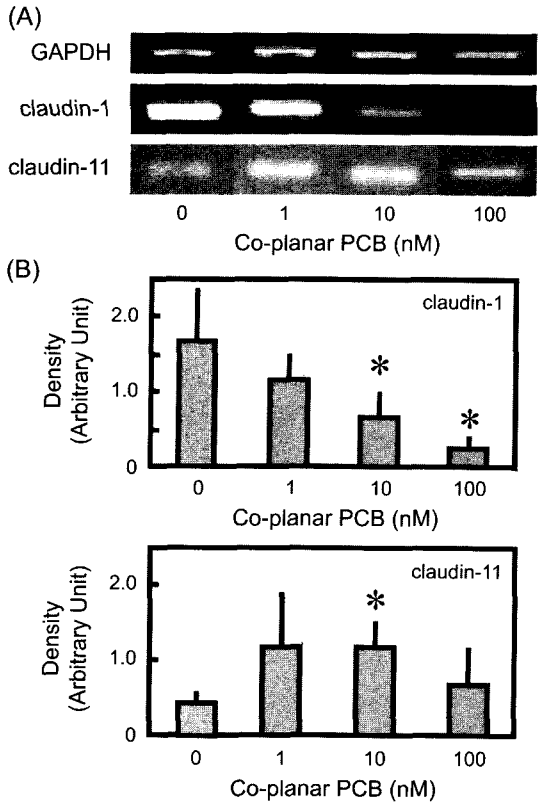

Fig. 4. Semiquantitative RT-PCR analysis of the expression of claudin-1 and -11 in mouse Sertoli cells.
(A) RT-PCR analyses of claudin-1 and claudin-11 expressions were conducted using the total RNA isolated from Sertoli cells. Number of PCR cycle was 29, 23, and 25 for claudin-1, claudin-11, and GAPDH, respectively. (B) Relative amount of RT-PCR products of claudin-1 and -11 mRNAs was calculated by dividing by internal controls, GAPDH. *, Significantly different from control by Student's *t*-test ($P<0.05$). Error bars are SD (n=4).

(Figs. 2A and 2B). Co-planar PCB caused a loss of claudin-1 expression in Sertoli cells in a concentration dependent manner (Figs. 4A and 4B). Concomitantly, there was a reciprocal increase in the claudin-11 expression in 1 and 10 nM co-PCB treated cells (Figs. 4A and 4B). Taken together, it suggests that upregulation of claudin-11 might be responsible for the increase in TER of Sertoli cells by co-planar PCB, and that claudin-1 has little or no effect on the paracellular permeability in cultured mouse Sertoli cells. It also emphasizes that expression of TJ components was differentially affected by co-planar PCB. The reciprocal pattern in the expression of caludin-1 and claudin-11 might be a useful marker for the paracellular barrier property of Sertoli cell TJ; Increase in claudin-11 expression might be a phenotype associated with the differentiation of Sertoli cells characterized by increased paracellular barrier property. Switching in the expression of tight junctional proteins from one type to another is expected to evoke profound changes in the barrier property of the Sertoli cells and thus spermatogenesis. During spermatogenesis, TJs between Sertoli cells are dynamically gated by systemic and locally produced signals including growth factors, cytokines, and steroids [6, 27], allowing for the passage of germ cells to the lumen. Similarly, biologically active factors, including growth factors, cytokines, steroids, and extracellular matrix proteins, have been known to regulate the expression of TJ genes and TER in various organs including testis [9, 13, 15, 22, 27, 44]. Recently, it was reported that testosterone increased the expression of occludin and TER rat Sertoli cells [6]. Undoubtedly, PCBs have steroid hormone-mimicking activity in several different cell types. Aroclor PCB mixtures were found to antagonize androgen receptor (AR)-mediated transcription in the presence of the natural AR ligand, but Aroclor 1254 has a weak agonistic activity with AR in the absence of natural ligand [52]. Therefore, co-planar PCB might have mimicked the biological action of androgen and potentiated the TER and claudin-11 expression in Sertoli cells. In summary, this is the first report that co-planar PCB alters the expression of TJ genes and TER in mouse Sertoli cells. The results suggest that co-planar PCB may alter the functional operation of the blood testis barrier in testis, thus affecting spermatogenesis. Analysis of the structure and function of TJ in Sertoli cells might be useful for the screening and risk assessment of environmental contaminants and drugs influencing male reproduction.

Acknowledgment

This work was supported by the Ministry of Public Health and Welfare of the Republic of Korea (HPEB 02-PJ1-PG10-20707-0001).

References

1. Bager, Y., K. Kenne, V. Krutovskikh, M. Mesnil, O. Traub, and L. Warngard. 1994. Alteration in expression of gap junction proteins in rat liver after treatment with the tumour promoter 3,4,5,3',4'-pentachlorobiphenyl. *Carcinogenesis* **15:** 2439-2443.
2. Bronstein, J. M., K. Chen, S. Tiwari-Woodruff, and H. I. Kornblum. 2000. Developmental expression of OSP/claudin-11. *J. Neurosci. Res.* **60:** 284-290.
3. Bronstein, J. M., P. Popper, P. E. Micevych, and D. B. Farber. 1996. Isolation and characterization of a novel oligodendrocyte-specific protein. *Neurology* **47:** 772-778.
4. Cavicchia, J. C., F. L. Sacerdote, and L. Ortiz. 1996. The human blood-testis barrier in impaired spermatogenesis. *Ultrastruc. Pathol.* **20:** 211-218.
5. Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156-159.

6. Chung, N. P. and C. Y. Cheng. 2001. Is cadmium chloride-induced inter-sertoli tight junction permeability barrier disruption a suitable *in vitro* model to study the events of junction disassembly during spermatogenesis in the rat testis? *Endocrinology* **142:** 878-888.
7. Cook, J. C., M. A. Kaplan, L. G. Davis, and J. C. O'Connor. 1997. Development of a Tier I screening battery for detecting endocrine-active compounds (EACs). *Regul. Toxicol. Pharmacol.* **26:** 60-68.
8. Cyr, D. G., L. Hermo, N. Egenberger, C. Mertineit, J. M. Trasler, and D. W. Laird. 1999. Cellular immunolocalization of occludin during embryonic and postnatal development of the mouse testis and epididymis. *Endocrinology* **140:** 3815-3825.
9. Duffy, H. S., G. R. John, S. C. Lee, C. F. Brosnan, and D. C. Spray. 2000. Reciprocal regulation of the junctional proteins claudin-1 and connexin43 by interleukin-1beta in primary human fetal astrocytes. *J. Neurosci.* **20:** RC114: 1-6.
10. Furuse, M., K. Fujita, T. Hiiragi, K. Fujimoto, and S. Tsukita. 1998. Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J. Cell Biol.* **141:** 1539-1550.
11. Gow, A., C. M. Southwood, J. S. Li, M. Pariali, G. P. Riordan, S. E. Brodie, J. Danias, J. M. Bronstein, B. Kachar, and R. A. Lazzarini. 1999. CNS myelin and Sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. *Cell* **99:** 649-659.
12. Gray, L. E. Jr, W. R. Kelce, T. Wiese, R. Tyl, K. Gaido, J. Cook, G. Klinefelter, D. Desaulniers, E. Wilson, T. Zacharewski, C. Waller, P. Foster, J. Laskey, J. Reel, J. Giesy, S. Laws, J. McLachlan, W. Breslin, R. Cooper, R. Di Giulio, R. Johnson, R. Purdy, E. Mihaich, S. Safe, and T. Colborn. 1997. Endocrine Screening Methods Workshop Report: Detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. *Reprod. Toxicol.* **11:** 719-750.
13. Gregory, M., J. Dufresne, L. Hermo, and D. Cyr. 2001. Claudin-1 is not restricted to tight junctions in the rat epididymis. *Endocrinology* **142:** 854-863.
14. Gye, M. C., Y. H. Lee, C. Kim, M. K. Kim, and H. Lee. 2000. Expression of zonnula occludens-1 in mouse testis. *Devel. Reprod.* **4:** 37-43.
15. Hellani, A., J. Ji, C. Mauduit, C. Deschildre, E. Tabone, and M. Benahmed. 2000. Developmental and hormonal regulation of the expression of oligodendrocyte-specific protein/claudin 11 in mouse testis. *Endocrinology* **141:** 3012-3019.
16. Kang, K. S., M. R. Wilson, T. Hayashi, C. C. Chang, and J. E. Trosko. 1996. Inhibition of gap junctional intercellular communication in normal human breast epithelial cells after treatment with pesticides, PCBs, and PBBs, alone or in mixtures. *Environ. Health Perspect.* **104:** 192-200.
17. Kang, K.S., G. X. Li, J. S. Park, B. J. Lee, J. H. Che, J. H. Tae, J. J. Cho, S. H. Kim, D. S. Lee, and Y. S. Lee. 2000. Effect of green tea on prostate and seminal vesicle in rats exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *J. Microbiol. Biotechnol.* **10:** 281-286.
18. Kannan, K., D. L. Villeneuve, A. L. Blankenship, and J. P. Giesy. 1998. Interaction of tributyltin with 3,3',4,4',5-pentachlorobiphenyl-induced ethoxyresorufin O-deethylase activity in rat hepatoma cells. *J. Toxicol. Environ. Health A* **55:** 373-384.
19. Kato, Y., K. Kenne, K. Haraguchi, Y. Masuda, R. Kimura, and L. Warngard. 1998. Inhibition of cell-cell communication by methylsulfonyl metabolites of polychlorinated biphenyl congeners in rat liver epithelial IAR 20 cells. *Arch. Toxicol.* **72:** 178-182.
20. Karczewski, J. and J. Groot. 2000. Molecular physiology and pathophysiology of tight junctions. III. Tight junction regulation by intracellular messengers: Differences in response within and between epithelia. *Am. J. Physiol.* **279:** G660-665.
21. Kolluri, S. K., C. Weiss, A. Koff, and M. Gottlicher. 1999. p27(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. *Genes Dev.* **13:** 1742-1753.
22. Krizbai, I. A., H. Bauer, A. Amberger, B. Hennig, H. Szabo, R. Fuchs, and H. C. Bauer. 2000. Growth factor-induced morphological, physiological and molecular characteristics in cerebral endothelial cells. *Eur. J. Cell Biol.* **79:** 594-600.
23. Krutovskikh, V. A, M. Mesnil, G. Mazzoleni, and H. Yamasaki. 1995. Inhibition of rat liver gap junction intercellular communication by tumor-promoting agents *in vivo*. Association with aberrant localization of connexin proteins. *Lab. Invest.* **72:** 571-577.
24. Landon, G. V. and J. P. Pryor. 1981. The blood-testis barrier in men of diverse fertility status: an ultrastructural study. *Virchows Arch. Int. J. Pathol. Anat. Histol.* **392:** 355-364.
25. Lippoldt, A, A. Jansson, U. Kniesel, B. Andbjer, A. Andersson, H. Wolburg, K. Fuxe, and H. Haller. 2000. Phorbol ester induced changes in tight and adherens junctions in the choroid plexus epithelium and in the ependyma. *Brain Res.* **854:** 197-206.
26. Loertscher, J. A., T. M. Lin, R. E. Peterson, and B. L. Allen-Hoffmann. 2002. In utero exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin causes accelerated terminal differentiation in fetal mouse skin. *Toxicol. Sci.* **68:** 465-472.
27. Lui, W. Y., W. M. Lee, and C. Y. Cheng. 2001. Transforming growth factor-beta3 perturbs the inter-Sertoli tight junction permeability barrier *in vitro* possibly mediated via its effects on occludin, zonula occludens-1, and claudin-11. *Endocrinology* **142:** 1865-1877.
28. Morita, K., M. Furuse, K. Fujimoto, and S. Tsukita. 1999. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc. Nat. Acad. Sci. USA* **96:** 511-516.
29. Morita, K., H. Sasaki, K. Fujimoto, M. Furuse, and S. Tsukita. 1999. Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. *J. Cell Biol.* **145:** 579-588.
30. Mullin, J. M., K. V. Laughlin, N. Ginanni, C. W. Marano, H. M. Clarke, and A. Peralta Soler. 2000. Increased tight junction permeability can result from protein kinase C activation/translocation and act as a tumor promotional

event in epithelial cancers. *Ann. N. Y. Acad. Sci.* **915:** 231-236.
31. Na, K. S., S. J. Kim, M. Kubo, and S. Y. Chung. 2001. Cloning and phylogenetic analysis of two different bphC genes and bphD gene from PCB degrading bacterium, *Pseudomonas* sp. strain SY5. *J. Microbiol. Biotechnol.* **11:** 668-676.
32. Nusrat, A., J. R. Turner, and J. L. Madara. 2000. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: Nutrients, cytokines, and immune cells. *Am. J. Physiol.* **279:** G851-857.
33. O'Connor, J. C., J. C. Cook, T. W. Slone, G. T. Makovec, S. R. Frame, and L. G. Davis. 1998. An ongoing validation of a Tier I screening battery for detecting endocrine-active compounds (EACs). *Toxicol. Sci.* **46:** 45-60.
34. O'Connor, J. C., S. R. Frame, L. G. Davis, and J. C. Cook. 1999. Detection of the environmental antiandrogen p,p-DDE in CD and Long-Evans rats using a tier I screening battery and a Hershberger assay. *Toxicol. Sci.* **51:** 44-53.
35. Ohmura, K., N. Kohno, Y. Kobayashi, K. Yamagata, S. Sato, S. Kashiwabara, and T. Baba. 1999. A homologue of pancreatic trypsin is localized in the acrosome of mammalian sperm and is released during acrosome reaction. *J. Biol. Chem.* **274:** 29426-29432.
36. Park, Y. I., J. S. So, and S. C. Koh. 1999. Induction by carvone of the polychlorinated biphenyl (PCB)-degradative pathway in *Alcaligenes eutrophus* H850 and its molecular monitoring. *J. Microbiol. Biotechnol.* **9:** 804-810.
37. Portigal, C. L., S. P. Cowell, M. N. Fedoruk, C. M. Butler, P. S. Rennie, and C. C. Nelson. 2002. Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. *Toxicol. Appl. Pharmacol.* **179:** 185-194.
38. Raychoudhury, S. S., A. F. Flowers, C. F. Millette, and M. F. Finlay. 2000. Toxic effects of polychlorinated biphenyls on cultured rat Sertoli cells. *J. Androl.* **21:** 964-973.
39. Ruch, R. J., J. E. Klaunig, and M. A. Pereira. 1987. Inhibition of intercellular communication between mouse hepatocytes by tumor promoters. *Toxicol. Appl. Pharmacol.* **87:** 111-120.
40. Russell, L. D., A. Bartke, and J. C. Goh. 1989. Postnatal development of the Sertoli cell barrier, tubular lumen, and cytoskeleton of Sertoli and myoid cells in the rat, and their relationship to tubular fluid secretion and flow. *Am. J. Anat.* **184:** 179-189.
41. Saitou, M., Y. Ando-Akatsuka, M. Itoh, M. Furuse, J. Inazawa, K. Fujimoto, and S. Tsukita. 1997. Mammalian occludin in epithelial cells: Its expression and subcellular distribution. *Eur. J. Cell Biol.* **73:** 222-231.
42. Saitou, M., M. Furuse, H. Sasaki, J. D. Schulzke, M. Fromm, H. Takano, T. Noda, and S. Tsukita. 2000. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol. Biol. Cell* **11:** 4131-4142.
43. Santomauro, L., P. Corsi, A. Leone, G. Brescia, L. Roncali, B. Nico, M. Pisoni, F. Brizzi, G. Assennato, and G. Elia. 1999. Effects of Aroclor 1254 on intercellular communication in human keratinocytes. *Med. Lav.* **90:** 497-512.
44. Savettieri, G., I. Di Liegro, C. Catania, L. Licata, G. L. Pitarresi, S. D'Agostino, G. Schiera, V. De Caro, G. Giandalia, L. I. Giannola, and A. Cestelli. 2000. Neurons and ECM regulate occludin localization in brain endothelial cells. *Neuroreport* **11:** 1081-1084.
45. Shimada, T., K. Inoue, Y. Suzuki, T. Kawai, E. Azuma, T. Nakajima, M. Shindo, K. Kurose, A. Sugie, Y. Yamagishi, Y. Fujii-Kuriyama, and M. Hashimoto. 2002. Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice. *Carcinogenesis* **23:** 1199-1207.
46. Suzuki, K., Y. Kokai, N. Sawada, R. Takakuwa, K. Kuwahara, E. Isogai, H. Isogai, and M. Mori. 2002. SS1 *Helicobacter pylori* disrupts the paracellular barrier of the gastric mucosa and leads to neutrophilic gastritis in mice. *Virchows Arch.* **440:** 318-324.
47. Syed, V., W. Gu, and N. B. Hecht. 1997. Sertoli cells in culture and mRNA differential display provide a sensitive early warning assay system to detect changes induced by xenobiotics. *J. Androl.* **18:** 264-273.
48. Tsai, M. L., K. Cesen-Cummings, R. C. Webb, and R. Loch-Caruso. 1998. Acute inhibition of spontaneous uterine contractions by an estrogenic polychlorinated biphenyl is associated with disruption of gap junctional communication. *Toxicol. Appl. Pharmacol.* **152:** 18-29.
49. Turner, J. R., J. M. Angle, E. D. Black, J. L. Joyal, D. B. Sacks, and J. L. Madara. 1999. PKC-dependent regulation of transepithelial resistance: Roles of MLC and MLC kinase. *Am. J. Physiol.* **277:** C554-562.
50. Walsh, S. V., A. M. Hopkins, and A. Nusrat. 2000. Modulation of tight junction structure and function by cytokines. *Adv. Drug Dev. Rev.* **41:** 303-313.
51. Yang, J. H. and P. R. Kodavanti. 2001. Possible molecular targets of halogenated aromatic hydrocarbons in neuronal cells. *Biochem. Biophys. Res. Commun.* **280:** 1372-1377.
52. Yoon, K., L. Pallaroni, M. Stoner, K. Gaido, and S. Safe. 2001. Differential activation of wild-type and variant forms of estrogen receptor alpha by synthetic and natural estrogenic compounds using a promoter containing three estrogen-responsive elements. *J. Steroid Biochem. Mol. Biol.* **78:** 25-32.
53. Zolotarevsky, Y., G. Hecht, A. Koutsouris, D. E. Gonzalez, C. Quan, J. Tom, R. J. Mrsny, and J. R. Turner. 2002. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in *in vitro* models of intestinal disease. *Gastroenterology* **123:** 163-172.