MOLECULAR & CELLULAR TOXICOLOGY, Vol. 1, No. 2, 78-86, June 2005

# Environmental Pollution and Gene Expression: Dioxin

**Ki Nam Kim[1] & Meyoung-Kon Kim[1]**

[1]Department of Biochemistry & Molecular biology, Korea University Medical School, Seoul, 136-701 Korea
Corresponding and requests for materials should be addressed to M.-K. Kim (jerrykim@korea.ac.kr)

Accepted 6 June 2005

## Abstract

**Dioxins, especially 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin), are ubiquitous environmental contaminants. TCDD is known that it has toxic effects in animals and humans, including chloracne, immune, reproductive and developmental toxicities, carcinogenicity, wasting syndrome and death.**

**TCDD induces a broad spectrum of biological responses, including disruption of normal hormone signaling pathways, reproductive and developmental defects, immunotoxicity, liver damage, wasting syndrome and cancer.**

**Many researches showed that TCDD induces gene expression of transcriptional factors related cell proliferation, signal transduction, immune system and cell cycle arrest at molecular and cellular levels. These toxic actions of TCDD are usually mediated with AhR (receptor, resulted from cell culture, animal and clinical studies).**

**cDNA microarray can be used as a highly sensitive and informative marker for toxicity. Additionally, microarray analysis of dioxin-toxicity is able to provide an opportunity for the development of candidate bridging biomarkers of dioxin-toxicity. Through microarray technology, it is possible to understand the therapeutic effects of agonists within the context of toxic effects, classify new chemicals as to their complete effects on biological systems, and identify environmental factors that may influence safety.**

**Keywords:** 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD), cDNA microarray, molecular and cellular toxicity, aryl hydrocarbon (AhR)

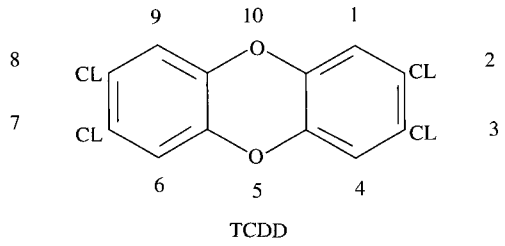
Dioxin is well known as one of the most potent toxicants[1]. 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD, Fig. 1) is the prototype for a class of halogenated aromatic hydrocarbons that are common environmental contaminants and carcinogens[2]. Many researchers reported that TCDD causes various biological and toxic responses in experimental animals[3]. TCDD exerts diverse species-specific toxic effect in animals and humans, including chloracne, immune, reproductive and developmental toxicities, carcinogenicity, wasting syndrome and death[4].

Effects of TCDD at the cellular level are just as diverse. TCDD is known to induce cell proliferation, to suppress immune response, and to cause cell cycle arrest as a potent tumor promoter[5-7]. The toxic effects of TCDD are thought to be mediated largely by transcriptional regulation through the AhR. Many of molecular biology studies show that the aryl hydrocarbon receptor (AhR) acts as a nuclear ligand-induced transcription factor that interacts with xenobiotics such as TCDD. Therefore, TCDD has the potential to directly alter the expression of a large number of genes[2].

The toxic effects of TCDD on gene expression in molecular and cellular levels and possible mechanism of its toxic action were demonstrated.

## *In vitro*-cell Culture

TCDD inhibits estrogen-dependent proliferation of human breast cancer cells, but it induces proliferation of human keratinocytes[8] and rat hepatocytes that causes a decrease in rat hepatocyte proliferation rates[9-11]. Also, TCDD is a powerful endocrine disuptor in rodents and human cells, which inhibits multiple estrogen-induced responses, including development or growth of human mammary and endometrial cancer cells, carcinogen-induced mammary cancer in rats, and mammary cancer in mice bearing breast cancer cell xenografts[12,13]. Exposure to the persistent and extremely potent environmental contaminant TCDD can result in a multitude of chronic toxic effects in a variety of animal tissues and species[14]. Enhancement of tumor formation has been observed in experimental animals exposed to TCDD. In addition, multiple effects on endocrine-and growth factor-regulated processes have been reported, indicating hormone-like interactions of TCDD in mammals[15]. The effects of TCDD were observed in various animal models, including rodents. Since TCDD is not genotoxic, unscientific the carcinogenic responses are




TCDD

(2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin)

**Fig. 1.** Structure of TCDD[1]

associated with the action of TCDD as a tumor promoter. A TCDD dose of < 100 ng/kg causes permanent adverse development effects in piscine, avian and mammalian embryos[1,16,17].

## *In vivo* Study-animal Studies

The environmental contaminant, TCDD, is a known carcinogen that tested in rodent bioassays. The induction of tumors by TCDD is tissue, sex and species specific[18-20]. In rats, TCDD disrupts the normal cellular proliferation/differentiation milieu resulting in alveolar-bronchiolar metaplasia and hyperplasia of the bronchiolar epithelium, adenomatous hyperplasia, and keratinizing squamous cell carcinoma of the lung. There is evidence that TCDD causes tumor promotion by interfering with intracellular signal transduction pathways related to growth factors and cytokines, such as transforming growth factor and interleukin-1β[10,21]. The mechanisms whereby dioxins induce pulmonary diseases and/or cancer is largely unknown[18]. Therefore, the investigations, which aimed at examining signaling pathways in lungs are needed. In mice, TCDD exposure during embryogenesis causes developmental abnormalities including hydronephrosis and cleft palate, whereas exposure of adult rats results in an elevated incidence of hepatic carcinoma and pulmonary and skin tumors[22,23]. Exposure of developing lower vertebrates to TCDD causes disturbances of lipid metabolism, cardiovascular and craniofacial teratogenesis[24,25], immunotoxic[26] and reproductive and endocrine effects[27-29], which also appear to be present in exposed humans. In human keratinocytes, TCDD also has been shown to induce terminal differentiation[30-33], whereas immature thymocytes from rats and mice treated with TCDD *in vivo*, but not *in vitro*, may show increased apoptosis[34]. Moreover, TCDD has been reported to induce apoptosis and to inhibit UV-induced apoptosis in rat hepatocytes[35].

Recent studies have shown that mice possessing mutations in the AhR nuclear localization/DRE binding domain, as well as mice harboring a hypomorphic ARNT allele, failed to exhibit the classical TCDD toxicities[36,37]. Furthermore, the AhR/ARNT signaling pathway plays an important role in development, differentiation and growth, as AhR null mice experience various liver, heart, thymus and immune system abnormalities. The data support a proposed mechanism for TCDD-mediated hepatotoxicity, including fatty liver, which involves mobilization of peripheral fat and inappropriate increases in hepatic uptake of fatty acids[38].

## Clinical Studies

Humans are generally exposed to such compounds, which are incorporated into food, drinking water, soil, dust, smoke and air. TCDD and dioxin-like compounds are stable, environmentally and biologically. As a result, human exposure is chronic and widespread. Although tumor promotion, as well as a wasting syndrome, teratogenesis, hepatotoxicity, modulation of endocrine systems, immunotoxicity, and enzyme induction are in a tissue-, sex-, age- and species-specific due to the different toxic and biochemical responses, which induced by TCDD[39], therefore the carcinogenic risk associated with TCDD exposure is increased for all cancers in human. Many of epidemiological studies have supported the increased risk for specific types of cancer in highly exposed populations. These include non-Hodgkin' s lymphoma, soft-tissue sarcoma, rectal cancer and lung cancer[40,41]. In humans, TCDD exposure is also associated with chronic obstructive pulmonary disease[18,42], also exposure to high levels of TCDD is associated with chronic obstructive pulmonary disease and lung cancer, because TCDD alters multiple integrated networks of signaling pathways associated with pulmonary disease, particularly the lung cancer. TCDD exposure in human populations has also been known to increase in various cancers including hepatocellular carcinoma[43].

TCDD, the prototype dioxin, causes a large number of apparently unrelated biological effects. In humans, TCDD and many other chlorinated phenolic agents cause chloracne, a long-lasting skin disease characterized by the hyperkeratinization of follicular sebocytes. Epidemiologic studies in accidentally exposed populations have also established a link between high doses of TCDD and certain types of cancer[44-46] and cardiovascular disease.

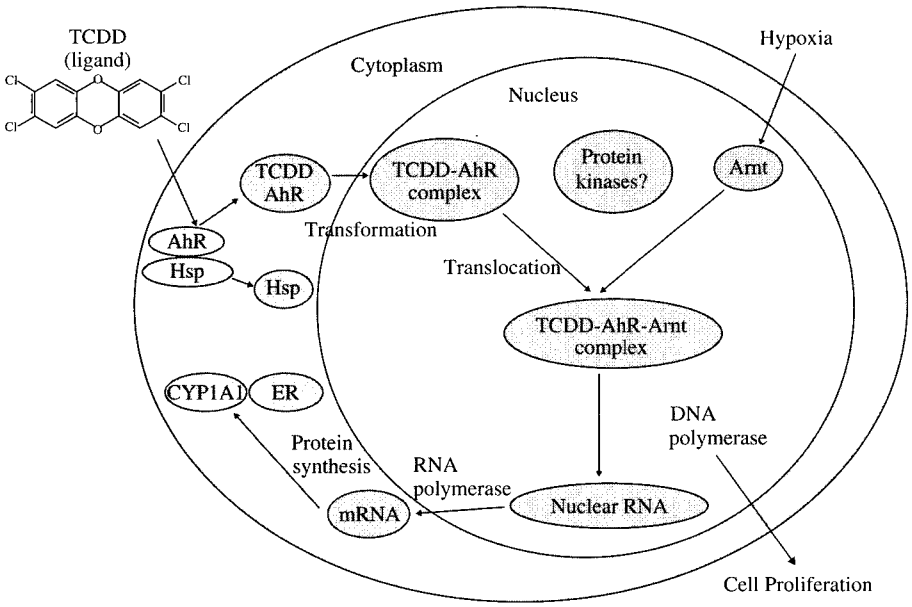



**Fig. 2.** Simple mechanistic model for TCDD toxicity using the AhR pathway[1]

## Toxic Mechanism of TCDD

### Cell Proliferation

At a molecular level, most of the effects of TCDD exposure have been known for many years to result from the activation of the AhR, a ligand-activated transcription factor[47]. The AhR is present in many tissues in humans and other animals, and animal carcinogenesis is often correlated with the affinity of the dioxin for the AhR. AhR is an intracellular protein and a ligand-dependent transcription factor that modulates gene expression via a high affinity interaction with Xenobiotic-Responsive Elements (XREs), located in the upstream regions of ligand-responsive genes[48]. This transcription factor is one of the particular interests in dioxin-induced toxicity, not only because it regulates induction of phase I and II metabolizing enzymes, but also because it mediates the toxic effects of dioxins in experimental animals and possibly in humans[49] (Fig. 2). A schematic representation of the complex sequence of events involved in TCDD-mediated toxicants is presented in Fig. 3[1].

### Oxicative Stress and Related Trnascriptional Factors

A limited number of genes have been identified that respond directly to AhR activation through heterodimer formation with a second bHLH-PAS domain protein, AhR nuclear translocator (ARNT). This AhR/ARNT heterodimer activates gene expression via binding of the heterodimer to a DNA recognition element called, the Xenobiotic Response Element (XRE). The majority of genes, which have been identified to exhibit, the direct AhR/ARNT-mediated response to TCDD have been involved in xenobiotic metabolism, including cytochrome P450 (CYP) 1A1,

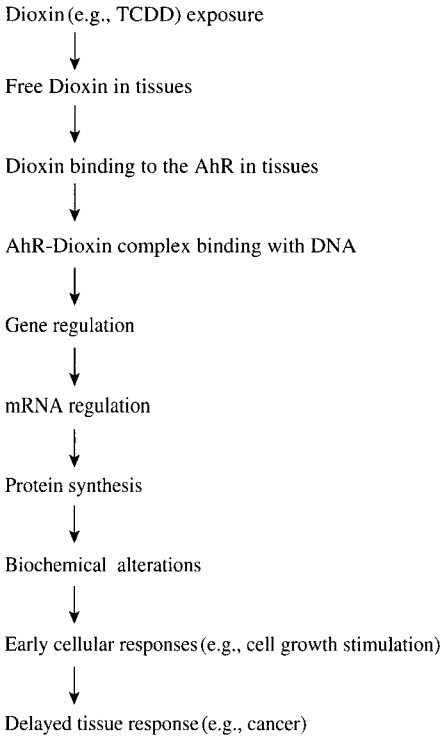



**Fig. 3.** Schematic representation of the complex sequence of events involved in TCDD-mediated toxicants[1]

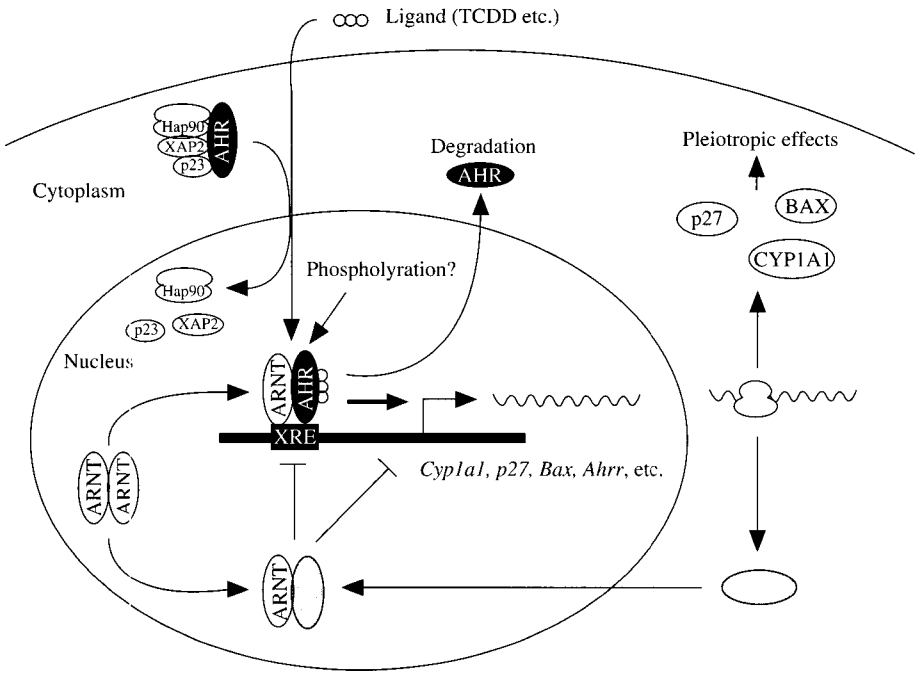



**Fig. 4.** Mechanisms of transcriptional activation by AhR and negative feedback regulation of AhR by AhRR. See text for a detailed discussion[48]

CYP1B1, NAD(P)H: quinone reductase 1, UDP-glucuronosyltransferase 1A1, and aldehyde dehydrogenase 3[50,5] . However, other genes that contain XREs in their promoters, which are activated by the AhR/ARNT heterodimer, including erythropoetin and an ecto-ATPase have been identified[52,53]. For example, TCDD exposure has previously been shown to induce the expression of several genes through the above mechanism, including cytochrome P4501A1/2, cytochrome P4501B1, glutathione S-transferase Ya, aldehyde dehydrogenase 3 and UDP-glucuronosyl transferase 1[2].

Fig. 4 shows that TCDD-AhR complex induces expression of a number of monooxygenase genes, such as cytochrome P4501A1 (CYP1A1), CYP1A2 and CYP1B1, by binding to the xenobiotic responsive element on the target genes, playing a key role in metabolism of xenobiotics (8, 9). A broad spectrum of biological responses of TCDD such as reproductive and developmental defects, immunotoxicity, liver damage, wasting syndrome and cancer includes induction of cytochrome P-450 1A1 (CYP1A1) and disruption of normal hormone signaling pathways. In addition, the suitability of CYP1B1 for use as a mechanistically based biomarker has been defined in molecular epidemiology studies of human populations exposed to dioxins and related chemicals that bind AhR. AhR dimerization with ARNT (HIF-1β) is responsible for the up-regulation of genes in the *Ah* gene battery, which comprises several well-characterized genes in the cytochrome P450 CYP1A family and several Phase II detoxification genes. It is generally believed that up-regulation of gene expression by the TCDD-activated AHR/ARNT complex results from transactivation through promoter domains containing AhRE (also termed XRE, DRE) motifs; however, dioxin effects also include transcriptional repression, as determined for TGF-β2 and fibrinogen γchain and plastin mRNAs, an observation that cannot be explained by invoking a direct transactivation mechanism. It is unclear whether the effect of dioxin on other targets, such as the genes for PAI-2 and IL-1β, the *FOS* and *JUN* immediate-early gene families, *COX-1* and *COX-2*[54], and TNF-α, is a primary response, a secondary response, a combination of the two, or a higher-order response resulting from interactions among the effectors elicited in different tissues of an exposed organism. Another study on gene expression of dioxin-exposed workers from automobile emission inspection offices and waste incinerating company using cDNA microarray also showed a novel evidence for gene expression patterns related to oxidative stress e.g., CYP2F1, CYP2D6, anti-oxidant protein 2, glutathione S-transferase M1 and glutathione peroxidase[4].

## Immunotoxicity

The immunotoxic effects of TCDD are varied. TCDD has been shown to suppress both cell-mediated and humoral immune responses[55]. For example,

there is a significant evidence indicating that exposure to TCDD or congeners causes an immune cell cycle arrest, as shown in murine hepatocytes, murine fetal thymic organ cultures, macaque endocervical primary cell cultures, 3T3 fibroblast cells and MCF-7 cells[56-58]. In addition, TCDD has been shown to affect the process of thymocyte maturation. Studies using fetal thymic organ cultures have shown that TCDD induces cell-cycle arrest in double-negative thymocytes and increases the relative number of CD8+T cells. Therefore, TCDD may affect the process of T cell proliferation and differentiation involving positive/negative selection[56].

Effects of TCDD on immune system are known to be medicated through activation of the aryl hydrocarbon receptor (AhR)[36,55].

### Cell Cycle Arrest and Apoptosis

The recent report state that AhR directly affects cell cycle regulation in response to an agonist, although it is controversial, whether AhR can inhibit or promote proliferation, that AhR directly interacts with nuclear factor jB, down regulating its biological activities[59].

TCDD may also act by inducing cell-cycle arrest in thymocytes, which could lead to apoptosis. Other pro-apoptotic genes known as dioxin responsive include bcl-2 family members; Bax and Hrk[60]. Both of these genes were shown to be upregulated in our study, therefore, may contribute to the apoptosis of thymocytes induced by TCDD. In addition, TCDD may induce signals other than those involved in apoptosis. For example, stimulation of both Fas and TNFR1 can trigger NF-κB signaling pathways mediated by the adaptor molecule,TRAF2, which also binds to the adaptor molecule, TRADD[61].

### Inhibition of DNA Methylation

Bohwan *et al.* reported that DNA methylation inhibits TCDD. Hepa1c1c7 cells were treated with the DNA methylation inhibitor, 5-aza-2′-deoxycytidine (AzaC) and with TCDD, and mRNA expression was analyzed using cDNA microarray technology to investigate methylation-dependent genes that are susceptible to induction by TCDD[15].

## cDNA Microarray and Toxicogenomics of Dioxin

In the area of environmental health sciences, cDNA microarray technology can be used to identify potential hazards. It should be relatively easy to establish model systems, both *in vitro* and *in vivo*, to examine gene expression changes as indications of chemical effect. In addition, cDNA microarrays can be used to detect toxic responses in target and non target tissues in rodents and humans[62]. By useing cDNA microarrays, toxic or unanticipated responses in humans may be determined early in a clinical trial prior to overt tissue toxicity, providing a rapid, sensitive surrogate of safety, which is essential for improved clinical trials. Also, microarrays may help to identify the susceptible individuals who respond to a treatment or who exhibit adverse effects to drugs. It is possible to use cDNA microarrays to measure biomarkers of exposure or effect in humans. The microarray-based genomic survey is a high throughput approach that allows parallel study on the expression patterns of thousands of genes. This technique can identify the correlation of gene expression pattern and environmental contamination by performing comparison-rank analysis of genes expressed by the transcription of DNAs into RNAs

cDNA microarray technology, which can be used to analyze the changes in genome-wide patterns of gene expression, is the one, new methodological advance technology that may revolutionize the way of investigated toxicological problems. The application of a large number of genes or the expressed sequence tags in a condensed array on glass slides or nylon filters comprises a cDNA microarray[63]. Alternatively, specific oligonucleotides that are complementary to known genes or expressed sequence tags are deposited on a miniature matrix by a photolithographic process to create an oligonucleotide-based microarray[64]. Either cDNA microarrays or oligonucleotide-based chips may used for gene expression analysis. Oligonucleotide-based DNA chips are also used for analyzing sequence variations in genomic DNA for screening individuals for DNA mutations and polymorphism variations. This approach has been recently reviewed[65].

Microarray technology will be useful to identify toxic substances individually or in mixtures, to determine whether toxic effects occur at low doses, and to extrapolate effects from one species to another. Assuming that exposures to different classes of toxicants result in distinct patterns of altered gene expression, in addition to common changes associated with the subsequent toxic response, microarray technology can be used to categorize and classify these effects through the direct comparison between gene expression signatures in exposed samples and the control samples. These cDNA chips will allow the simultaneous monitoring of gene expression changes for receptor-mediated responses, xenobiotic metabolizing enzymes, cell cycle components, oncogenes, tumor suppressor genes, DNA repair genes, estrogen-

responsive genes, oxidative stress genes and genes known to be involved in apoptotic cell death.

Microarray system has been used for the prediction of toxicity through gene expression, which induced toxicant showing that compounds with similar toxic mechanisms produce similar changes in gene expression *in vivo* and *in vitro* system. As these results, many pharmaceutical companies and research groups are making databases of gene expression related to toxic mechanism induced by compounds that were well characterized. These collected databases of microarray associated with toxicity will shorten the toxicity evaluation steps that are often the rate-limiting step in the discovery and development of new pharmaceuticals. Principles of toxicogenomics, the large-scale application of genomic information to toxicological issues, are being applied to the prediction of toxic potential and the development of screening systems for untested chemicals which are based upon their capacity to alter transcriptional programs[66]. cDNA microarray technology has become an important tool in toxicogenomics. High-throughput measurement of transcriptional changes that occur as a consequence of xenobiotic exposure are facilitating the elucidation of toxicological mechanisms[15,62].

Changes in gene expression in a tissue may result from differences in physiology, developmental stage, pathology, or environmental exposure. These changes can now be measured by using cDNA or oligonucleotide-based microarrays, which are used to compare directly the gene expression profiles of two RNA samples that are simultaneously hybridized to the chip. The potential analysis of the expression of thousands of genes in one experiment now allows investigators to consider addressing some important biological questions that have not been easily addressed with traditional expression-based technologies, such as Northern blots, *in situ* hybridization, or RNase protection assays, which examine gene expression changes of only few genes at a time. The ability to examine thousands of genes (potentially all of the genes in a given cell type) provides new insights into the effects of chemical or drugs on biological systems. Alterations in gene expression were directly related to physiological outcomes, which demonstrate the importance of phenotypic anchoring when interpreting microarray data[67].

In summary, the application of cDNA microarray analysis to the field of toxicology, carcinogen identification and drug safety provides an opportunity to change and improve the way of environmental factors. Also, therapeutics are currently investigated. cDNA microarrays may used to identify new environmental carcinogens and toxic effects of drugs, to improve the current testing models, also to understand the mechanism of action of these agents. Defining the mechanisms of action of toxic agents can greatly assist in species extrapolation and risk assessment. This should also lead to the identification of new genes/targets involved in environmentally caused diseases, including cancer and diseases of the immune, nervous and pulmonary/respiratory systems[62].

## Conclusion

TCDD and related compounds are legacy environmental contaminants that cause controversial human health effects at environmental levels. TCDD exerts toxic effect in animals and humans, including; chloracne, immune, reproductive and developmental toxicities, carcinogenicity, wasting syndrome and death.

Many researches have shown that TCDD induced gene expression of transcriptional factors related cell proliferation, signal transduction, immune system and cell cycle arrest at molecular and cellular levels. These toxic actions of TCDD are usually mediated with AhR, resulted from cell culture, animal and clinical studies.

DNA microarray successfully identified dioxin-responsive genes expressed after exposure to TCDD. These results may help to elucidate some of the fundamental features of dioxin toxicity and may further clarify the biologic role of the toxicogenomics.

### Acknowledgement

We thank Dr. Yoon S. Cho-Chung (Cellular Biochemistry Section, Basic Research Laboratory, CCR, NCI, NIH, Bethesda, MD) and Dr. Kevin G. Becker (DNA Array Unit, NIA, NIH, Baltimore, MD) for valuable advices on cDNA microarray. Also, we thank to Yeon mi Ryu and Hye won Kim (Department of Biochemistry & Molecular biology, Korea University Medical School, Seoul, Korea) for valuable advices on this paper.

This study was supported by a grant of Medical Research Center for Environmental Toxicogenomic and Proteomics, funded by Korea Science and Engineering Foundations and Ministry of Science & Technology, a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (Hmp-00-GN-01-0002 & KPGRN-R-04), a Korea Institute of Science & Technology Evaluation and Planning (KISTEP) and Ministry of Science & Technology (MOST), Korean government, through its National Nuclear Technology Program, and a grant No. R01-2001-000-00212-0 from the Basic Research

Program of the Korea Science & Engineering Foundation.

## References


1. Mandal, P.K. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *J. Comp. Physiol. [B]* **175**, 221-230. Epub 2005 Apr 8. (2005).
2. Fisher, M.T., Nagarkatti, M. & Nagarkatti, P.S. Combined screening of thymocytes using apoptosis-specific cDNA array and promoter analysis yields novel gene targets mediating TCDD-induced toxicity. *Toxicol. Sci.* **78**, 116-124. Epub 2004 Jan 12. (2004).
3. Korkalainen, M., Tuomisto, J. & Pohjanvirta, R. Primary structure and inducibility by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) of aryl hydrocarbon receptor repressor in a TCDD-sensitive and a TCDD-resistant rat strain. *Biochem. Biophys. Res. Commun.* **315**, 123-131 (2004).
4. Ishizuka, M., Yonemoto, J., Zaha, H., Tohyama, C. & Sone, H. Perinatal exposure to low doses of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin alters sex-dependent expression of hepatic CYP2C11. *J. Biochem. Mol. Toxicol.* **17**, 278-285 (2003).
5. Davis, B.J., McCurdy, E.A., Miller, B.D., Lucier, G.W. & Tritscher, A.M. Ovarian tumors in rats induced by chronic 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin treatment. *Cancer Res.* **60**, 5414-5419 (2000).
6. Schwarz, M., Buchmann, A., Stinchcombe, S., Kalkuhl, A. & Bock, K. Ah receptor ligands and tumor promotion: survival of neoplastic cells. *Toxicol. Lett.* **112-113**, 69-77 (2000).
7. Walker, N.J., Tritscher, A.M., Sills, R.C., Lucier, G.W. & Portier, C.J. Hepatocarcinogenesis in female Sprague-Dawley rats following discontinuous treatment with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Toxicol. Sci.* **54**, 330-337 (2000).
8. Milstone, L.M. & LaVigne, J.F. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin induces hyperplasia in confluent cultures of human keratinocytes. *J. Invest. Dermatol.* **82**, 532-534 (1984).
9. Wiebel, F.J., Klose, U. & Kiefer, F. Toxicity of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in vitro: H4IIEC3-derived 5L hepatoma cells as a model system. *Toxicol Lett* **55**, 161-169 (1991).
10. Gottlicher, M. & Wiebel, F.J. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin causes unbalanced growth in 5L rat hepatoma cells. *Toxicol. Appl. Pharmacol.* **111**, 496-503 (1991).
11. Wolfle, D., Becker, E. & Schmutte, C. Growth stimulation of primary rat hepatocytes by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Cell. Biol. Toxicol.* **9**, 15-31 (1993).
12. Safe, S., Wang, F., Porter, W., Duan, R. & McDougal, A. Ah receptor agonists as endocrine disruptors: antiestrogenic activity and mechanisms. *Toxicol. Lett.* **102-103**, 343-347 (1998).
13. Puga, A., Maier, A. & Medvedovic, M. The transcriptional signature of dioxin in human hepatoma HepG2 cells. *Biochem. Pharmacol.* **60**, 1129-1142 (2000).
14. Krig, S.R., Chandraratna, R.A., Chang, M.M., Wu, R. & Rice, R.H. Gene-specific TCDD suppression of RARalpha- and RXR-mediated induction of tissue transglutaminase. *Toxicol. Sci.* **68**, 102-108 (2002).
15. Jin, B., Kim, G., Park, D.W. & Ryu, D.Y. Microarray analysis of gene regulation in the Hepa1c1c7 cell line following exposure to the DNA methylation inhibitor 5-aza-2′-deoxycytidine and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Toxicol. In Vitro.* **18**, 659-664 (2004).
16. Walker, M.K., Pollenz, R.S. & Smith, S.M. Expression of the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator during chick cardiogenesis is consistent with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced heart defects. *Toxicol. Appl. Pharmacol.* **143**, 407-419 (1997).
17. Gray, L.E., Wolf, C., Mann, P. & Ostby, J.S. In utero exposure to low doses of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin alters reproductive development of female Long Evans hooded rat offspring. *Toxicol. Appl. Pharmacol.* **146**, 237-244 (1997).
18. Martinez, J.M. *et al.* Differential toxicogenomic responses to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in malignant and nonmalignant human airway epithelial cells. *Toxicol. Sci.* **69**, 409-423 (2002).
19. Rao, M.S., Subbarao, V., Prasad, J.D. & Scarpelli, D.G. Carcinogenicity of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in the Syrian golden hamster. *Carcinogenesis* **9**, 1677-1679 (1988).
20. Della Porta, G., Dragani, T.A. & Sozzi, G. Carcinogenic effects of infantile and long-term 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin treatment in the mouse. *Tumori.* **73**, 99-107 (1987).
21. Abbott, B.D. & Birnbaum, L.S. TCDD-induced altered expression of growth factors may have a role in producing cleft palate and enhancing the incidence of clefts after coadministration of retinoic acid and TCDD. *Toxicol. Appl. Pharmacol.* **106**, 418-432 (1990).
22. Abbott, B.D. Review of the interaction between TCDD and glucocorticoids in embryonic palate. *Toxicology* **105**, 365-373 (1995).
23. Couture, L.A., Abbott, B.D. & Birnbaum, L.S. A critical review of the developmental toxicity and teratogenicity of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin: recent advances toward understanding the mechanism. *Teratology* **42**, 619-627 (1990).
24. Hornung, M.W., Spitsbergen, J.M. & Peterson, R.E. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin alters cardiovascular and craniofacial development and function in sac fry of rainbow trout (Oncorhynchus mykiss). *Toxicol. Sci.* **47**, 40-51 (1999).
25. Guiney, P.D., Smolowitz, R.M., Peterson, R.E. & Stegeman, J.J. Correlation of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin induction of cytochrome P4501A in

vascular endothelium with toxicity in early life stages of lake trout. *Toxicol. Appl. Pharmacol.* **143**, 256-273 (1997).
26. Oughton, J.A. *et al.* Phenotypic analysis of spleen, thymus, and peripheral blood cells in aged C57B1/6 mice following long-term exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Fundam. Appl. Toxicol.* **25**, 60-69 (1995).
27. Bjerke, D.L. & Peterson, R.E. Reproductive toxicity of 2, 3, 7. 8-tetrachlorodibenzo-p-dioxin in male rats: different effects of in utero versus lactational exposure. *Toxicol. Appl. Pharmacol.* **127**, 241-249 (1994).
28. Bjerke, D.L., Brown, T.J., MacLusky, N.J., Hochberg, R.B. & Peterson, R.E. Partial demasculinization and feminization of sex behavior in male rats by in utero and lactational exposure to 2, 3, 7, 8-tetrachlorodibenzo -p-dioxin is not associated with alterations in estrogen receptor binding or volumes of sexually differentiated brain nuclei. *Toxicol. Appl. Pharmacol.* **127**, 258-267 (1994).
29. Cummings, A.M., Metcalf, J.L. & Birnbaum, L. Promotion of endometriosis by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in rats and mice: time-dose dependence and species comparison. *Toxicol. Appl. Pharmacol.* **138**, 131-139 (1996).
30. Gaido, K.W., Maness, S.C., Leonard, L.S. & Greenlee, W.F. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin-dependent regulation of transforming growth factors-alpha and -beta 2 expression in a human keratinocyte cell line involves both transcriptional and post-transcriptional control. *J. Biol. Chem.* **267**, 24591-24595 (1992).
31. Gaido, K.W. & Maness, S.C. Regulation of gene expression and acceleration of differentiation in human keratinocytes by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Toxicol. Appl. Pharmacol.* **127**, 199-208 (1994).
32. Gaido, K.W. & Maness, S.C. Post-transcriptional stabilization of urokinase plasminogen activator mRNA by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in a human keratinocyte cell line. *Toxicol. Appl. Pharmacol.* **133**, 34-42 (1995).
33. Choi, E.J., Toscano, D.G., Ryan, J.A., Riedel, N. & Toscano, W.A., Jr. Dioxin induces transforming growth factor-alpha in human keratinocytes. *J. Biol. Chem.* **266**, 9591-9597 (1991).
34. Kamath, A.B., Xu, H., Nagarkatti, P.S. & Nagarkatti, M. Evidence for the induction of apoptosis in thymocytes by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in vivo. *Toxicol. Appl. Pharmacol.* **142**, 367-377 (1997).
35. Moolgavkar, S.H., Luebeck, E.G., Buchmann, A. & Bock, K.W. Quantitative analysis of enzyme-altered liver foci in rats initiated with diethylnitrosamine and promoted with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin or 1, 2, 3, 4, 6, 7, 8-heptachlorodibenzo-p-dioxin. *Toxicol. Appl. Pharmacol.* **138**, 31-42 (1996).
36. Bunger, M.K. *et al.* Resistance to 2, 3, 7, 8-tetrachloroibenzo-p-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor. *J. Biol. Chem.* **278**, 17767-11774. Epub 2003 Mar 5 (2003).
37. Walisser, J.A., Bunger, M.K., Glover, E. & Bradfield, C.A. Gestational exposure of Ahr and Arnt hypomorphs to dioxin rescues vascular development. *Proc Natl. Acad. Sci.* U S A. **101**, 16677-16682. Epub 2004 Nov 15 (2004).
38. Boverhof, D.R. *et al.* Temporal and dose-dependent hepatic gene expression patterns in mice provide new insights into TCDD-Mediated hepatotoxicity. *Toxicol. Sci.* **85**, 1048-1063. Epub 2005 Mar 30 (2005).
39. Poland, A. & Knutson, J.C. 2, 3, 7, 8-tetrachlorodibenzo -p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**, 517-554 (1982).
40. Bertazzi, P.A. *et al.* Health effects of dioxin exposure: a 20-year mortality study. *Am. J. Epidemiol.* **153**, 1031-1044 (2001).
41. Steenland, K., Piacitelli, L., Deddens, J., Fingerhut, M. & Chang, L.I. Cancer, heart disease, and diabetes in workers exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *J. Natl. Cancer Inst.* **91**, 779-786 (1999).
42. Pesatori, A.C. *et al.* Dioxin exposure and non-malignant health effects: a mortality study. *Occup. Environ. Med.* **55**, 126-131 (1998).
43. McGregor, D.B., Partensky, C., Wilbourn, J. & Rice, J.M. An IARC evaluation of polychlorinated dibenzo -p-dioxins and polychlorinated dibenzofurans as risk factors in human carcinogenesis. *Environ. Health Perspect.* **106**, 755-760 (1998).
44. Bertazzi, P.A. Long-term effects of chemical disasters. Lessons and results from Seveso. *Sci. Total Environ.* **106**, 5-20 (1991).
45. Manz, A. *et al.* Cancer mortality among workers in chemical plant contaminated with dioxin. *Lancet.* **338**, 959-964 (1991).
46. Kogevinas, M. *et al.* Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. *Am. J. Epidemiol.* **145**, 1061-1075 (1997).
47. Hengstler, J.G., Van der Burg, B., Steinberg, P. & Oesch, F. Interspecies differences in cancer susceptibility and toxicity. *Drug Metab. Rev.* **31**, 917-970 (1999).
48. Mimura, J. & Fujii-Kuriyama, Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim. Biophys. Acta.* **1619**, 263-268 (2003).
49. Harper, P.A., Wong, J.Y., Lam, M.S. & Okey, A.B. Polymorphisms in the human AH receptor. *Chem. Biol. Interact.* **141**, 161-187 (2002).
50. Yueh, M.F. *et al.* Involvement of the xenobiotic response element (XRE) in Ah receptor-mediated induction of human UDP-glucuronosyltransferase 1A1. *J. Biol. Chem.* **278**, 15001-15006. Epub 2003 Feb 3 (2003).
51. Zhang, L., Savas, U., Alexander, D.L. & Jefcoate, C.R. Characterization of the mouse Cyp1B1 gene.

Identification of an enhancer region that directs aryl hydrocarbon receptor-mediated constitutive and induced expression. *J. Biol. Chem.* **273**, 5174-5183 (1998).
52. Hanlon, P.R., Zheng, W., Ko, A.Y. & Jefcoate, C.R. Identification of novel TCDD-regulated genes by microarray analysis. *Toxicol. Appl. Pharmacol.* **202**, 215-228 (2005).
53. Chan, W.K., Yao, G., Gu, Y.Z. & Bradfield, C.A. Cross-talk between the aryl hydrocarbon receptor and hypoxia inducible factor signaling pathways. Demonstration of competition and compensation. *J. Biol. Chem.* **274**, 12115-12123 (1999).
54. Wolfle, D., Marotzki, S., Dartsch, D., Schafer, W. & Marquardt, H. Induction of cyclooxygenase expression and enhancement of malignant cell transformation by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Carcinogenesis.* **21**, 15-21 (2000).
55. Kerkvliet, N.I. Recent advances in understanding the mechanisms of TCDD immunotoxicity. *Int. Immunopharmacol.* **2**, 277-291 (2002).
56. Lai, Z.W., Fiore, N.C., Hahn, P.J., Gasiewicz, T.A. & Silverstone, A.E. Differential effects of diethylstilbestrol and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin on thymocyte differentiation, proliferation, and apoptosis in bcl-2 transgenic mouse fetal thymus organ culture. *Toxicol. Appl. Pharmacol.* **168**, 15-24 (2000).
57. Puga, A. *et al.* Aromatic hydrocarbon receptor interaction with the retinoblastoma protein potentiates repression of E2F-dependent transcription and cell cycle arrest. *J. Biol. Chem.* **275**, 2943-2950 (2000).
58. Vaziri, C. & Faller, D.V. A benzo[a]pyrene-induced cell cycle checkpoint resulting in p53-independent G1 arrest in 3T3 fibroblasts. *J. Biol. Chem.* **272**, 2762-2769 (1997).
59. Tanaka, G. *et al.* Induction and activation of the aryl hydrocarbon receptor by IL-4 in B cells. *Int. Immunol.* **17**, 17 (2005).
60. Park, J.H. & Lee, S.W. Up-regulated expression of genes encoding Hrk and IL-3R beta subunit by TCDD in vivo and in vitro. *Toxicol. Lett.* **129**, 1-11 (2002).
61. Davis, J.W., 2nd, Melendez, K., Salas, V.M., Lauer, F.T. & Burchiel, S.W. 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits growth factor withdrawal-induced apoptosis in the human mammary epithelial cell line, MCF-10A. *Carcinogenesis.* **21**, 881-886 (2000).
62. Afshari, C.A., Nuwaysir, E.F. & Barrett, J.C. Application of complementary DNA microarray technology to carcinogen identification, toxicology, and drug safety evaluation. *Cancer Res.* **59**, 4759-4760 (1999).
63. Shalon, D., Smith, S.J. & Brown, P.O. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res.* **6**, 639-645 (1996).
64. Lipshutz, R.J., Fodor, S.P., Gingeras, T.R. & Lockhart, D.J. High density synthetic oligonucleotide arrays. *Nat. Genet.* **21**, 20-24. (1999).
65. Hacia, J.G. Resequencing and mutational analysis using oligonucleotide microarrays. *Nat. Genet.* **21**, 42-47 (1999).
66. Orphanides, G. Toxicogenomics: challenges and opportunities. *Toxicol Lett* **140-141**, 145-148 (2003).
67. Huang, Q. *et al.* Gene expression profiling reveals multiple toxicity endpoints induced by hepatotoxicants. *Mutat. Res.* **549**, 147-167 (2004).