# ENDOGENOUS LIGANDS OF ARYLHYDROCARBON RECEPTOR AND INTERACTION WITH DIOXINS

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## Introduction

Risk assessment of polychlorinated dibenzo-p-dioxin (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (collectively called as dioxins) must be based on the biological evidence which can explain basic mechanisms of their toxicity and also their species and individual differences in the toxicities. The arylhydrocarbon receptor (AhR) and indirubin and indigo, its endogenous high affinity ligands[1)] which we newly found, are supposed to have important physiological roles in controlling cells' important function, and dioxins are likely to distort this physiological role. One of our goals is to try to reevaluate risk of exposure to dioxins on the basis of putative physiological roles of Ahr and its endogenous ligands.

## Methods

Literature search for recent publications on Ahr and indirubin were performed using MEDLINE. Possible physiological roles of indirubin and AhR was assessed referring to data of animal experiments and those of molecular biology, while toxicological implications of interaction of dioxin with AhR were examined.

## Results and Discussion

There have been a lot of studies on diverse toxic effects of dioxins and also the sequence of events of dioxin binding to AhR, subsequent dimerization of AhR with the arylhydrocarbon receptor nuclear translocator, binding of this dimer to xenobiotic responsible element (XRE) of the DNA and the induction of CYP enzymes and other genes. The idea of toxic equivalency factor (TEF) was introduced for the purpose of the risk assessment of dioxins, based on the similarity of these events and assuming additive nature in toxicity, however diverse toxic effects of dioxins can not be easily linked to CYP induction related events.

Time dependent expression of AhR (for example, daily cycle in various tissue[2)] in rats, and menstrual cycle in human endometrium[3)]) suggests a time keeper function or a coordinating function of AhR in homeostasis of cells and in control of developmental process. Another aspects of AhR function are stress responding activities, such as induction of xenobiotics metabolizing enzymes, response to hypoxic conditions, and energy deficiency. Significant level of AhR-mediated activity without exogenous ligands in the AhR transiently expressed cells[4)] suggests presence of endogenous ligands. Increase of this basal activity in AhR deficient cells presumably by negative feedback control of AhR activity suggests that some of AhR functions are important to be maintained in the cell. Construction of AhR null mice showed AhR is important in development of animals but not indispensable for survival or possibly critical functions of AhR, if any, are compensated with some other means, because AhR

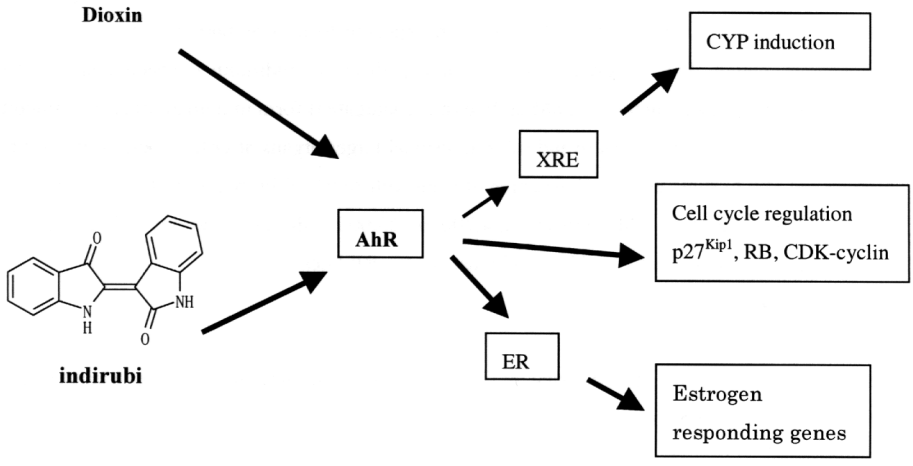


**Figure.** The roles of arylhydrocarbon receptor and indirubin in regulating cells physiological process and its implication in estimating risk from exposure to dioxins

null mice had grown up with decreased liver size, and decreased body weight[5]. a-Naphthoflavone, a ligand to AhR, showed agonistic effect in expression of XRE-driven reporter genes at high concentrations, but worked as an antagonist when given with tetrachlorodibenzo-p-dioxin (TCDD)[6].

Newly found endogenous ligands of AhR, indirubin has much higher (ca. 50 times) affinity to AhR than dioxin[1], and is supposedly playing physiological roles in the cell's function. For example, indirubin can work through interaction with AhR on control of cell differentiation and proliferation in G1/S phase arrest by inhibition of retinoblastoma protein phosphorylation[7], and by transcriptional induction of cyclin dependent kinase inhibitor ($p27^{Kip1}$) [8] (see Figure). As a ligand to AhR, the persistent nature of dioxin will cause constant signaling of molecules regardless of stages and needs for cells. Sustained depletion of AhR protein in liver, lung, thymus etc. after treatment with TCDD can be interpreted that cells are responding to reduce the adverse effect from exposure to TCDD[9].

Transcriptional interference with liganded estrogen receptor was shown for TCDD-liganded AhR, and interefernace of AhR binding to XRE with liganded estrogen receptor were shown[10]. Negative interference of AhR mediated transactivation of genes by the progesterone receptor showed another example of cross-talk between two important receptors[11]. Furthermore, induction of immediate-early response protooncogenes, c-fos, c-jun junB and junD by TCDD may play important role in TCDD-induced tumor promotion[12].

Above data suggest that AhR and its endogenous ligands are working at a crossing-over point for interaction of hormonal events and signal transduction, while dioxin disturbs this function. One of the critical effects selected for evaluation of the Tolerable Daily Intake of dioxins, is developmental deficiency in male's reproductive performance including daily sperm production in rats[13]. Treatment of rats during pubertal developmental with TCDD altered signal-kinase activities and epidermal growth factor binding in the testis causing decrease of testicular sperm production[14].

We are examining whether the spatio-temporal differences in the amount of indirubin, and the expression of AhR among different species can explain at least partly species differences in dioxin

toxicity and hopefully the practical meaning of the critical window in the toxicity through series of studies to eventually reexamine health risk from exposure to dioxins taking into consideration of endogenous ligands. Since endogenous production pathway of indirubin is speculated and it was shown to be present as much as 0.2 nM as free (not conjugated) form in human urine, it is important to know specific concentrations of indirubin in critical target organs at critical period of toxicity of dioxins and also to study about its interaction of them with AhR[1]. With regard to this, it is interesting to know expression of the AhR varies from 4,700 to 323,000 AhR/cell in various cell lines including mouse and rat hepatoma cells, and human breast cancer cell lines[15].

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