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# Mechanisms of Action of Dioxin-Like Compounds

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**Abstract.** Dioxins are one of the most potent environmental toxins known. They are commonly associated with industrial activities such as waste incineration, pulp bleaching and chemical synthesis processes. Their toxicity stems from the fact that dioxins can activate the aromatic hydrocarbon receptor (AhR), thereby triggering a series of harmful biological responses, including carcinogenicity, immunosuppression, endocrine disruption and reproductive toxicity. Dioxins and their analogues are a growing problem and a major challenge in modern ecology because of their highly chemically stable and heat-resistant structure, their stable chlorinated structure, which makes them difficult to metabolise, resulting in their bioaccumulation in living organisms and persistence in the environment. This research will describe the structure of AhR and the binding mechanism of dioxin and AhR, as well as the metabolic process of PCDD, in order to more accurately predict its potential harm to humans and the environment, and help develop targeted drugs or antidotes to reduce or prevent toxic effects. This research summarizes the new understanding of the mechanism of toxic action of dioxins and their metabolic pathways, which can provide a reference for environmental pollutant screening and risk assessment.

**Keywords:** Dioxins; AhR; PHDD; Toxicity mechanism.

## 1. Introduction

Dioxins are composed of two benzene rings connected by a peroxide (or sulfur) bridge. Changing their structure or introducing other groups often leads to changes in their toxicity. The planar structure of dioxins enables them to bind efficiently to the aromatic hydrocarbon receptor (AhR). The homologous ligands of AhR have unique chemical structures, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). TCDD is a typical exogenous ligand for AhR10. AhR is a key sensor that integrates numerous exogenous and endogenous chemical signals and is now recognized as a promising drug target [1-3].

By binding to AhR, dioxins activate the expression of downstream genes, causing a series of toxic effects. In contrast, dioxin derivatives with non-planar structures are generally less toxic because they have a poor affinity for AhR, and the chlorine atoms can be substituted at positions 2, 3, 7, and 8 on the two benzene rings, and the toxicity is usually closely related to the chlorine atoms number and their positions on the benzene rings. 2,3,7,8-TCDD is one of the most toxic dioxins. The arrangement of chlorine atoms at these specific positions makes the molecule more stable and difficult to metabolize, thus enhancing its bioaccumulation and toxicity [1].

The metabolic pathway of PCDDs mainly passes through the cytochrome P450 enzyme system in the liver. Due to its highly chlorinated structure, the metabolic process is extremely slow, leading to long-term accumulation in the body. There is currently no specific antidote for the treatment of dioxin poisoning. Clinically, the main treatment is to reduce the source of exposure, adopt supportive treatment and use drugs that can accelerate the excretion of dioxins. A deeper understanding of the toxic mechanism of dioxins and their metabolic processes will help develop more effective treatments to reduce the threat of dioxins to human health [1].

This research will introduce the structure and function of AhR as a multifunctional transcription factor and its ligand binding mechanism. The structure of the AhR-ARNT dimer was modelled to understand how AhR binds to ligands and how the TCDD ligand affects the dynamic behaviour of

the AhR:ARNT complex. The interaction between the dimer and DNA is also regulated by ligand binding. It also provides an overview of phase I and II metabolism of AhR ligands, four major phase I metabolic models for PCDD metabolism, and the metabolic characteristics of dioxin ring bays. This research further deepens the understanding of dioxin toxicity and its biotransformation process by systematically analysing the mechanism of dioxin toxicity and the key steps in its metabolic process. It helps to understand the mechanism of AhR and dioxin-like compound poisoning and assess the environmental risks and health effects.

## 2. Mechanisms of dioxin poisoning

### 2.1. The structural function of AhR and its mechanism of interaction with ligands

Aromatic hydrocarbon receptor AHR is a multifunctional transcription factor belonging to the alkali helix-loop-helix (bHLH) per-art-sim (PAS) protein family [2]. bHLH-PAS family is commonly found in various fields of life and is used to detect signals from endogenous compounds, exogenous chemicals, light and vapor pressure. AhR ligand and active transcription factor consist of 805 amino acids. Studies have shown that AhR is not only involved in the induction of many important exogenous compound metabolizing enzymes, which affects the individual's susceptibility to certain diseases, but also participates in many other important biological processes. It is expressed in various tissues and at different stages of organismal development. It is essential for vertebrate development and differentiation. AhR exists in the cytoplasm in the absence of ligand binding and is inactive in complex with heat shock protein 90 (Hsp90) and x-associated protein (XAP2, also known as AIP) [4].

Upon ligand binding, the structure of the AhR changes, exposing a nuclear localization signal (NLS) that triggers translocation of the complex into the nucleus and interaction with the AhR nuclear transporter (ARNT). The newly formed heterodimer resembles the so-called heterodimerization (HDF), which is connected to the so-called second malignant response element (DRE) [5].

Through modeling, the orientation of the nodes in the occupied π orbitals accounted for 95% of the chemical binding of the aromatic receptor. The binding strength and affinity of compounds for AhR is related to the molecular structure and that the lowest unoccupied molecular orbital is critical for AhR binding. The AhR binding affinity and enzyme-inducing efficacy of PCDFs is analyzed by utilizing key parameters derived from density-functional theory (DFT) such as chemosoftness, electronegativity, and electrophilicity indices. A large number of molecular descriptors can be calculated from the 2D and 3D structures of the compounds, including variables such as conformation, orbital energies, hydrophobicity, and other electronic properties. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) affect the toxicity of dioxin contaminants.

XAP2 plays a key connecting and supporting role in the AhR-Hsp90 complex (Figure 1). The PPIase and TPR structural domains are connected by a hinge and present an open conformation without direct interaction with each other. XAP2 interacts with multiple residues of AhR through the PAS-B structural domain, while the TPR structural domain forms an extensive contact. These interactions were regulated through different residue sites. Mutation of certain residues weakened the binding of Hsp90 to XAP2, but did not significantly affect the interaction between AhR and Hsp90, suggesting that the binary complex between Hsp90 and AhR is more stable [6].

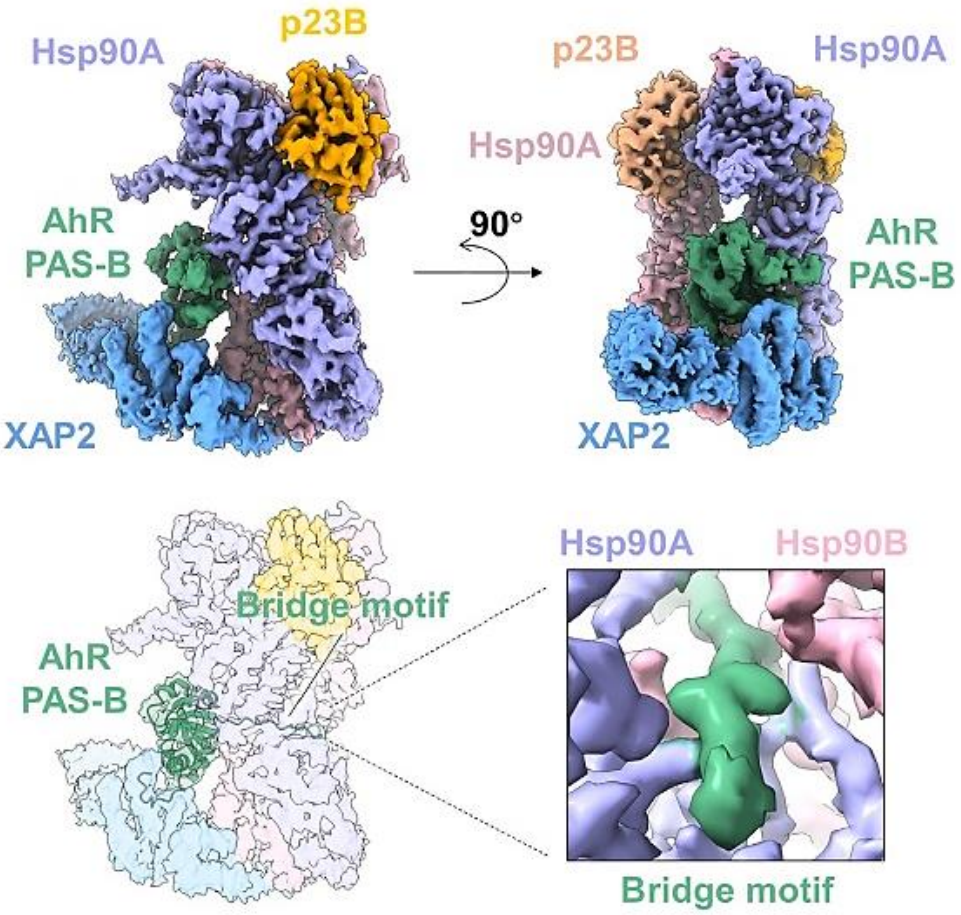


**Figure 1.** The structural details of cytoplasmic AhR complexes [7].

### 2.2. Structural modelling of the AhR-ARNT dimer and the mechanism of ligand binding regulation

AhR and its nuclear transporter protein (ARNT) play important roles in signal transduction, especially in dimerization and DNA binding. The N-terminal functional domain of AhR consists of the bHLH and PAS structural domains, where the PAS-B structural domain is not only involved in ligand binding, but also in chaperone protein hsp90 interactions. The bHLH structural domain interacts with ARNT dimerization and the DNA recognition element binding.

Despite the longstanding lack of experimental confirmation of the structural domains of AhR, studies using homology modeling have advanced the understanding of their mechanisms. Using hypoxia-inducible factor 2α (HIF-2α) as a template, the research modeled the AhR PAS-B structural domain and analyzed the X-ray structures of multiple bHLH-PAS systems, further advancing the structural modeling of the AhR:ARNT dimer.

Ultimately, the study provides a complete model of the AhR:ARNT dimer containing the bHLH and PAS-A structural domains as well as the PAS-B structural domain (Figure 2). The ligand TCDD significantly affects the dynamic behavior of the complex, revealing how ligand binding regulates the interaction between the dimer and DNA. This model lays the foundation for a deeper understanding of the function and mechanism of AhR.

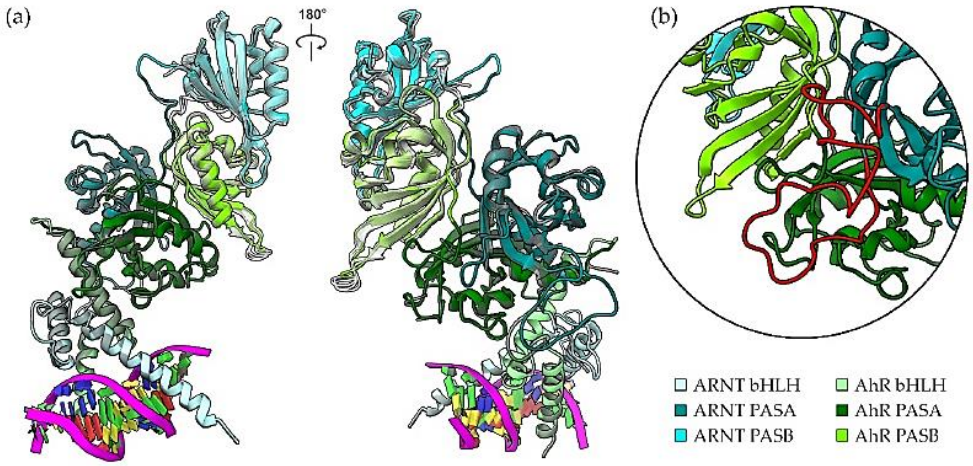


**Figure 2.** The AhR:ARNT:DRE complex model [8]. (a) Quaternary structure of the core model. (b) Highlighting of the ARNT FG-loop region interacting with AhR (red cartoon).

## 3. Metabolic pathways of AhR agonists and their modeling

### 3.1. I- and II-phase metabolism of AhR ligands

The metabolic and excretory processes of exogenous compounds directly determine the persistence of these compounds in the organism and the consequent biotoxic effects. Most exogenous and endogenous AhR ligands are triggered via the AhR pathway. In phase I reactions, compounds first introduce polar groups, while phase I metabolites subsequently serve as substrates for phase II reactions, where they significantly enhance their aqueous solubility by binding macromolecular groups such as glutathione, sulfate, or glycans, which in turn are more readily excreted by the body [9].

Although the main purpose of metabolism is to carry out detoxification, I-phase metabolism is more likely than II-phase metabolism to generate intermediates with high electrophilicity, which are often toxic reactive substances capable of nucleophilic reactions with macromolecules in the cell. For example, extremely toxic dioxin-like compounds, which are difficult to metabolize and have long half-lives, can activate AhR for a long period of time, leading to the continuation of a series of toxic reactions. During phase I metabolism, enzymes of the CYP1 family are the major players in the metabolism of AhR agonists, whereas phase II metabolism may involve enzymes such as glutathione transferase and glucosinolate or sulfate splicing enzymes [10].

Exposure to exogenous AhR agonists usually results in the co-expression of CYP1 family members such as CYP1A1 and CYP1A2, whereas it has been shown that exposure to benzo[α]pyrene induces the expression of CYP2Y3 in rare gourami carp. PAHs, which are relatively easy to metabolize in vivo, are metabolized by CYP1A1 and their toxicity is dependent on the I phase. metabolism, and the active products generated by metabolism are often the ultimate carcinogens. Taking benzo[alpha]pyrene as an example, one of its I-phase metabolites, benzo[alpha]pyrene-7,8-diol-9,10 epoxide, is considered to be one of the most carcinogenic substances.

Dioxin analogs are mainly metabolized through CYP1A1 and CYP1A2. However, their metabolism in vivo is very slow, so researchers have attempted to differentiate the metabolic activities and metabolites of different enzymes in the CYP1 family for various AhR agonists. Certain studies have utilized 3-MC to induce the generation of S9 fractions with CYP1A1 and CYP1A2 activities in SD rats, and experiments have shown that 1-CDD and 1,2,3,4-TCDD are mostly metabolized by CYP1A1, whereas 2,3,7,8-TCDD and 1,2,3,7,8-PCDD are oxidized mainly by CYP1A2. Further experiments in yeast cells expressing CYP1A1 and CYP1A2, respectively, showed that CYP1A1 and CYP1A2

were able to co-induce the metabolism of 2,7-diCDD and 2,3,7-triCDD, but the metabolites produced by both for 2,3,7-triCDD were different. The main metabolite of CYP1A1 was 8-hydroxy-2,3,7 -TriCDD, whereas CYP1A2 produces mainly hydroxylation products at the chlorine substitution site. These studies suggest that CYP1A1 and CYP1A2 have subtle structural differences that result in different metabolic mechanisms and some selectivity for their metabolic substrates.

Unlike other AhR agonists such as PAHs, the I-phase metabolites of PCDDs are significantly less toxic compared to the parent compound. The I-phase metabolite of 2,3,7,8-TCDD, 8-hydroxy-2,3,7-TCDD, binds to the AhR with only 10% of the binding capacity of the parent compound. The I-phase metabolites of AhR agonists can be further modified by a variety of II-phase metabolizing enzymes, such as the modification of the metabolites of PCBs by glutathione transferase, the modification of the metabolites of 1,3,7-TCDDs by glucosidylate or sulfate sulfatase modification of 1,2,7,8-TCDD metabolites by glutathione transferase. However, glutathione transferase has not yet been found to act effectively on phase I metabolites of PCDDs.

### 3.2. I-phase metabolic modeling of PCDDs

PCDDs complicate the study of their metabolic processes due to their high toxicity and strong metabolic stability. As shown in Figure 3, despite these challenges, four main I-phase metabolic models for PCDDs have been summarized [11], including hydroxylation at the unsubstituted sites of PCDDs, hydroxylation of dioxin unsubstituted sites by polychlorinated dibenzo-p-dioxins (Figure 3a), hydroxylation reaction at the chlorine substitution site, ring-opening reactions in the dioxin ring bay area (Figure 3b) and the hydroxylation reaction that accompanies a chlorinated site transfer is called an NIH transfer (Figure 3C).

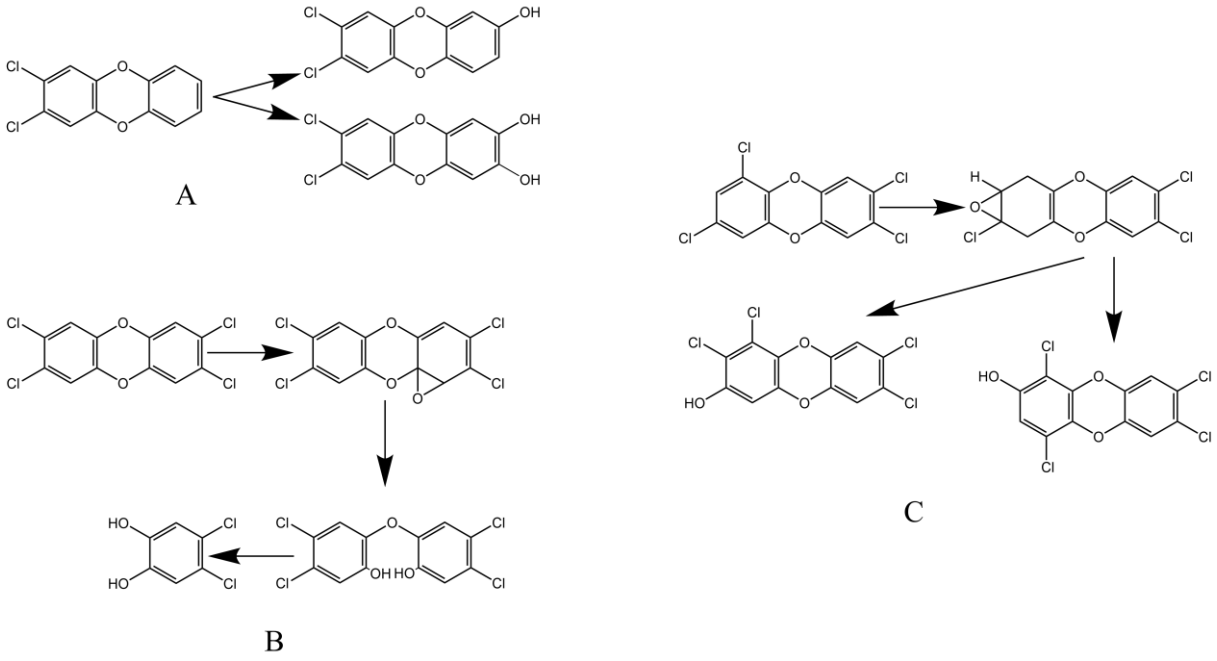


**Figure 3.** Phase I metabolic response to PCDD [5].

In metabolic kinetic studies in vivo and in vitro, it was found that highly chlorinated PCDDs were more difficult to metabolize compared to less chlorinated PCDDs. Birnbaum and Couture noted that the presence of a C-H bond is necessary to metabolize the enzymatic oxidation process. This metabolic law was further verified and. In addition, CYP1A2 was more active than CYP1A1 in metabolizing perchlorinated PCDDs. However, these laws did not fully explain the strong metabolic stability of 2,3,7,8-TCDD [12].

It was found that the metabolic reactions of 2,3,7,8-TCDD occurred mainly in the bay region of the dioxin ring and were accompanied by ring-opening reactions. Suzuki's quantum chemical molecular dynamics studies showed that when $O_2$ was used as an oxidizing agent for the oxidation of 2,3,7,8-

TCDD at elevated temperatures, the dioxin ring bay region at either the C11-C12 or C13 -C14 were more susceptible to oxidation by reactive oxygen species and formed peroxides, which triggered ring opening and degradation reactions. This process shows that the stability of PCDDs is closely related to their molecular structure, energy levels, and electronic states [13].

In organisms, the metabolism of 2,3,7,8-TCDDs prefers peroxidation in the bay region of the dioxin ring, which is mediated by CYP enzymes. Throughout the reaction, the substrate-bound CYPase acquires two electrons from NADPH in two steps via NADPH-cytochrome P450 reductase to form an enzyme-substrate-$H_2O_2$ complex with oxygen molecules. Under specific conditions, the complex cleaves the O-O bond, transfers a reactive oxygen atom to the substrate, and undergoes a disproportionation reaction to produce an oxidized product. This mechanism requires that the substrate must be interconjugated with CYPase, and thus the three-dimensional structure of CYPase may influence the degradation process of PCDDs [14].

Shinkyo et al. hypothesized, based on previous studies of CYP1A1 binding to 2,3,7,8-TCDD, that the metabolic activity of the enzyme toward 2,3,7,8-TCDD might be enhanced if the space of the substrate-binding pocket of rat CYP1A1 were expanded [11]. Expression of CYP1A1 and its mutants in yeast cells by targeted mutagenesis revealed that amino acid residue 240 is important for substrate recognition, and that the mutation produces an F240A enzyme capable of hydroxylating 2,3,7,8-TCDD at the chloro-substitution site to produce 8-hydroxy-2,3,7,8-TCDD [15].

Although the metabolic pattern of the mutant enzyme is different from that of the dioxin ring-wannabe ring-opening reaction, this finding emphasizes a direct link between the reason for the difficult degradation of PCDDs and the structure of the substrate-binding pocket of the enzyme. Previous studies of CYP1A1 in the metabolism of PAHs have suggested that the molecular size of PAHs may directly affect their metabolic rate, has easier access to CYP1A1's substrate-importing channel and is thus more readily metabolized [16].

## 4. Conclusion

This research the mechanism of PCDD toxicity through the combination of PCDD and AhR and the metabolism of PCDD as a multifunctional transcription factor plays a key role in detecting exogenous and endogenous chemical signals in the metabolism of PCDD. AhR initiates the expression of genes and regulates the cell cycle and apoptosis through the formation of a heterodimer with its nuclear transporter protein, ARNT, and the metabolism of PCDD s is susceptible to electrophilicity during the metabolism of PCDDs. PCDDs are prone to produce electrophilic intermediates during metabolism, and their metabolism is mainly dependent on the CYP1 enzyme family, with specific metabolic pathways including hydroxylation, ring opening reaction and NIH transfer. PCDDs are prone to produce electrophilic intermediates during metabolism, and their metabolism is mainly dependent on the CYP1 enzyme family, with specific metabolic pathways including hydroxylation, ring opening reaction and NIH transfer.

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