# USE OF AHR KNOCKOUT (AHR-/-) MICE TO INVESTIGATE THE ROLE OF THE AH RECEPTOR ON DISPOSITION OF TCDD

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## Introduction

Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD) is the prototype for environmental agonists of the Aryl hydrocarbon Receptor (AhR). Dioxin acts as a ligand and binds to the AhR which results in a wide range of adverse biological responses including cancer. Body burdens associated with these effects are dependent on the disposition of the ligand. Disposition of dioxin and dioxin-like compounds is influenced by their lipophilicity, binding affinities to the AhR, binding affinities to CYP1A2 (regulated by the AhR), and metabolism.

In both acute and subchronic animal studies, there is a specific accumulation of TCDD in liver compared to adipose tissue. Distribution of TCDD is dose and time dependent[1,2]. At higher inducing doses of TCDD, the relative percentage of administered dose increases in the liver and decreases in other tissues. Hepatic sequestration is dose-dependent, and the inducible hepatic binding protein, CYP1A2, has been shown to be responsible for this hepatic sequestration[3,4].

The AhR is a transcriptional regulatory protein which binds to upstream DNA response elements of target genes. Such genes include the cytochrome P450s: CYP1A1, CYP1A2, and CYP1B1. The CYP1A2, expressed constitutively in liver, has a significant role in the metabolism of many toxicologically important compounds[5,6,7]. Many carcinogenic arylamines are known substrates for CYP1A2[8,9]. Endogenous substrates for CYP1A2 include estrogen and uroporphyrin. Dioxin, one of the most potent toxicants, induces both CYP1A2 mRNA and protein.

Because of the role AhR plays in the toxicity of TCDD and related compounds, a knockout (KO) mouse lacking expression of the AhR (null mutant AhR-/-) has been developed by means of gene targeting[10]. In the AhR-/- KO mouse, CYP1A2 mRNA is not induced by TCDD and constitutive expression of CYP1A2 is decreased 90%[11].

Previous studies in our laboratory have shown the importance of the CYP1A2 KO mouse in characterizing the effects of CYP1A2, the inducible binding protein, on disposition of dioxin, dioxin-like, and non-dioxinlike compounds[3,4]. To further characterize modulations in disposition of TCDD, the AhR KO mouse was used to understand the effects of the AhR on disposition. The objective of the present study was to test the hypothesis that the pharmacokinetic behavior of TCDD will be different in mice with a null mutant Ahr(-/-) than in mice with an intact AhR. In the present study, the role of the AhR on disposition of TCDD was determined in homozygous AhR-/- KO mice and age-matched heterozygous AhR+/- mice. Both groups of mice were on the C57BL/6N lineage strain.

**Materials and Methods**

*Chemicals.*

TCDD was obtained from Radian Corp. (Austin, TX); purity >99% (GC). [1,6-$^3$H]TCDD was synthesized by Chemsyn Science Laboratories (Lenexa, KS). At the time of the study, the specific activity was 34.7Ci/mmol with a radiochemical purity of ≥98% as determined by HPLC (System Gold; Beckman, Inc., Fullerton, CA) using a C18 μBondapak stainless-steel column with Guard-PAK precolumn insert (Waters; Milford, MA) and an isocratic solvent system 85% MeOH, 15% $H_2O$]. The radiochemical purity was further tested to be 99% using a rat biliary excretion of [$^3$H]TCDD bioassay[12].

*Animals.*

The breeding colony at the US EPA was set-up with mating pairs of AhR-/- KO mice that were generously donated by Dr. Frank P. Gonzalez at the NCI/NIH laboratories (Bethesda, MD). Mating AhR KO pairs were bred and back-crossed to C57BL/6N (AhR+/+) mice to produce offspring of KO (AhR-/-) and heterozygous (AhR+/-) mice. The male AhR KO mice used in the present study were 19 weeks old and 3$^{rd}$ generation[13]. The C57BL/6N (AhR+/+) mice were obtained from Charles River Laboratories (Raleigh, NC).

Mice (4-8 per treatment group) used in the present study were knockout mice lacking the Aryl hydrocarbon Receptor (AhR-/-) and age-matched heterozygous litter mates with the intact AhR (+/-). They were acclimatized one week before dosing by housing them individually in metabolism cages (Nalgene; Rochester, NY) as previously described[4]. After dosing, they were returned to their metabolism cages with separate collection of excreta. Mice were dosed with a single oral gavage of either corn oil or 25 μg [$^3$H]TCDD/kg. Four days after dosing, animals were killed and tissues were removed. TCDD-derived radioactivity in collected tissues and excreta was quantitated and hepatic EROD and MROD enzymatic activities were determined as previously described[4].

*Data Analysis.*

All data are presented as mean ± SD. Intergroup comparisons were performed by a one-way analysis of variance (ANOVA) followed by Protected Fisher's Least Significant Difference test. Differences between KO (AhR-/-) and heterozygous (AhR+/-) mice were considered statistically significant when $p < 0.05$.

**Results**

Differences in the pharmacokinetic behavior of TCDD was demonstrated in the two genetically distinct groups of mice. Hepatic tissue dose and concentration were less in the AhR KO than in the heterozygous (Ahr+/-) mice ($p<0.05$). No sequestration of TCDD was present in the livers of AhR KO mice, similar to studies with CYP1A2 KO mice[3,4]. The total liver contained 12 ± 4 ng TCDD in the AhR KO mice and 232 ± 95 ng TCDD in the Ahr+/- mice. In comparison, the CYP1A2 KO mice had 22 ± 3 ng TCDD in the total liver. In the AhR KO mice, regulation of CYP1A2 (inducible hepatic binding protein) by AhR was not present. As a result, CYP1A2 was not induced and livers behaved as other highly perfused tissues with tissue content of the highly lipophilic TCDD being determined by simple partitioning based on solubility in lipid. Greater accumulation of TCDD (tissue dose and concentration) was found in adipose tissue (fat) of the AhR KO mice than the Ahr+/- mice ($p<0.05$). The total dissectable fat compartment had 353 ±

107 ng TCDD in the AhR KO mice and 130 ± 24 ng TCDD in the Ahr+/- mice. In comparison to the CYP1A2 KO mice, the total dissectable fat compartment had 316 ± 32 ng TCDD. Because TCDD was sequestered in the liver of AhR+/- mice, less TCDD was available for deposition in fat and other extrahepatic tissues. Whereas, the AhR KO mice did not have hepatic sequestration of TCDD and more TCDD was available for deposition in fat and other extrahepatic tissues. Major depot differences for AhR KO and AhR+/- mice were reflected by liver-to-fat (L/F) concentration ratios (sensitive indicators for hepatic sequestration of TCDD). The L/F concentration ratios were <0.1 in the AhR-/- mice and 3-4 in the AhR+/- mice. Previous studies have demonstrated L/F concentration ratios of 0.2 in the CYP1A2 KO mice and 3.6 in the parental strains (C57BL/6N and 129/Sv) at the same dose and time[3,4]. Disposition of TCDD in most of the other tissues was greater in the AhR KO mice than in the Ahr+/- mice ($p<0.05$). Both the AhR KO and Ahr+/- mice had similar fecal excretion of TCDD-derived radioactivity. Although, the AhR KO mice had greater urinary excretion of TCDD-derived radioactivity than the AhR+/- mice ($p<0.05$). Induction of hepatic EROD and MROD enzymatic activities demonstrated differences with no detectable induction in the AhR KO mice compared to induction in the AhR+/- mice.

**Discussion**

The present study provides direct confirmation of the hypothesis that removal of the AhR affects the pharmacokinetic behavior of TCDD as seen by differences in disposition of TCDD between mice with and without an AhR gene. Also, the importance of the AhR in regulation of cytochrome P450s including CYP1A2, the inducible hepatic binding protein for TCDD and related compounds, was demonstrated. These findings have significant implications for physiologically-based pharmacokinetic and biologically-based dose-response models that are used for dioxin and dioxin-like compounds. The models are used to address issues of high-to-low dose and cross-species extrapolations that arise in estimating risks for predicting potential adverse human health effects. In a human study of low exposure, most of the body burden of [$^3$H]TCDD has been estimated to be sequestered in adipose tissue[14]. Likewise, low environmental levels of dioxin in the general population would result in greater concentration in body fat than in liver tissue. In conclusions, the AhR knockout mouse is an important model to understand the role of the AhR protein on the effects of dioxin, a known human carcinogen, and dioxin-like compounds in humans at environmental background levels.

(This abstract does not necessarily reflect EPA policy.)

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