# COMPARISON OF OVERALL METABOLISM OF 1,2,3,7,8-PeCDD IN CYP1A2 (-/-) KNOCKOUT AND C57BL/6N PARENTAL STRAINS OF MICE

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## Introduction

The most toxic dioxin congeners, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), are sequestered to a high degree in the liver to yield liver-to-fat ratios in excess of unity.[1-4] TCDD induces hepatic cytochrome P450 1A2 (CYP1A2) to which it subsequently binds.[1-2] TCDD whole body half lives are very long, i.e. 5-11 years in humans and ~30 days in rats. In addition, TCDD and PeCDD are metabolized very slowly in all systems tested.[4-7] *In vitro* metabolism rate studies have not been conducted with PeCDD and the individual cytochromes; therefore, it has not been firmly established whether PeCDD is a poor substrate for metabolizing enzymes, or whether PeCDD is unavailable for metabolism due to its strong affinity to CYP1A2. The purpose of the present study was to quantify the extent of overall metabolism of PeCDD in mice which either possess or lack the CYP1A2 gene.

## Materials and Methods

*Chemical:* [$^{14}$C]PeCDD was purchased from ChemSyn (Lenexa, KS; 65.6 µCi/µmole, 0.2 µCi/µg) and was used without dilution with [$^{12}$C] PeCDD. Radiochemical purity of the PeCDD (>98%) was determined by silica gel TLC (1:1 hexane:methylene chloride) and HPLC (C18, DeltaPak, 8x40mm, $H_2O$:MeOH gradient, 5-100% MeOH, 60 min). The PeCDD was administered as a single oral dose in 0.1 ml of peanut oil (1.5 µCi/mouse, 116 µg/kg body weight).
*Animals:* Eight C57BL/6N mice were purchased from Taconic Labs (Germantown, NY). Eleven CYP1A2 (-/-) knockout mice (KO) were obtained from US EPA (RTP, NC).[2, 8] The mice (male; 24 wks old) were housed (2-3 per cage) for 96h in Plexiglas® metabolism cages with separate collection of urine and feces every 24h. Four days after dosing, the mice were killed by cervical dislocation, and tissues were collected, immediately weighed, and frozen at -28EC.
*Analysis of tissues and excreta:* Longisimus dorsi muscle and overlying skin sample were removed prior to carcass homogenization, and analyzed for $^{14}$C by combustion (Packard Model 307 sample oxidizer). Remaining carcass (diluted with 73% lean hamburger) was homogenized three times with a hand grinder. Aliquots (0.5 g) were removed and analyzed for radioactivity by combustion. Radioactivity in feces and remaining tissues were quantified by combustion of dried samples (<0.5 g). Urine [$^{14}$C] was quantified by liquid scintillation counting (Ecolite, ICN, Costa Mesa, CA). Urine was deproteinated with 1M HCl (pH 2.0), centrifuged, decanted, and evaporated to dryness. The residue was solvated with MeOH, a portion analyzed by TLC (50:50 hexane:$CH_2Cl_2$), and resulting bands quantitated with a System 2000 Imaging Scanner (Bioscan, Inc., Washington, DC). Air-dried feces were pulverized via mortar and pestle and extracted three times with hexane, ethyl acetate, and methanol. Extracts were analyzed by silica TLC as above.

**Results and Discussion**

Tissue distribution results (Table 1) are similar to a previous study with TCDD in KO mice.[9] Liver:fat ratios of PeCDD between the two groups were much higher in C57BL/6N (5.78) vs. KO (0.41) mice. Again, these data are in agreement with earlier results[3-4] and further establish the hepatic CYP1A2 enzyme as the PeCDD sequestering species. In KO mice, lack of the CYP1A2 sequestering species allowed for a short residence time in the liver and subsequent repartitioning into lipophilic tissues.

Very little PeCDD-derived radioactivity was excreted in the urine. However, the percentage of metabolites and parent PeCDD in urine from both groups was quantitated by TLC (data not shown). Parent PeCDD is probably present in urine due to a carrier protein system such as mouse major urinary protein or albumin, as has been described previously.[10-11] The urinary metabolites have not been characterized, but may include sulfate ester and glucuronide ether conjugates, which have been observed in other dioxin metabolism studies.[12-14]

PeCDD and its metabolites were quantitated in fecal extracts of both groups. Recovery data were normalized to 100% for both groups to allow comparisons to be made. [$^{14}$C]PeCDD standard had an Rf of 0.70-0.79 in the TLC system employed, and metabolites had Rfs <0.55. A higher level of extractable metabolites was observed in feces of C57BL/6N mice (9.8%) than KO mice (1.8%; Table 2). Fecal metabolites were probably hydroxylated, because they reacted with diazomethane to yield derivatives that had significantly higher Rf's on TLC (data not shown). Slightly higher amounts of nonextractable fecal metabolites were observed in feces of KO mice (5.1%) than the parental strain (4.0%; Table 2). This indicated that reactive metabolites had been formed in both groups of mice, and that covalent bonds had formed with fecal lipids and/or proteins.

The overall level of metabolism (normalized to 100% recovery) of PeCDD was determined for both groups as the sum of the [$^{14}$C]-metabolites detected in urine and feces (extractable and nonextractable compartments). A higher level of overall metabolism was observed for the C57BL/6N mice (15.0%) than the KO mice (7.8%; Table 2). The difference is probably due to a significantly lower hepatic retention/metabolism of PeCDD in KO mice and rapid redistribution to lipophilic tissues for storage. The present results may represent a consistent theme in the metabolism of toxic dioxins. Rapid redistribution of TCDD to lipophilic tissues was also observed in the KO mice, while higher hepatic retention occurred in the parental strain (liver:fat 4.09 vs. 0.57, respectively).[9] Overall metabolism of TCDD was also higher in the parental than KO (11.1 vs. 5.9%).

The data presented in this study contradicts the hypothesis that hepatic sequestration of PeCDD by CYP1A2 makes PeCDD unavailable for metabolism that would readily occur in its absence. Indeed, the data demonstrated that slightly more overall metabolism occurred in a parental mouse strain when compared to CYP1A2 (-/-) KO mice following a single oral dose, presumably due to low hepatic retention and high fat storage in the KO mice. The present results provide a basis for understanding the pharmacokinetic behavior of toxic dioxins at low environmental exposures, levels that are insufficient to induce hepatic CYP1A2. The data also confirmed that PeCDD, like TCDD, has an inherently slow metabolism in mammals, perhaps via the inducible CYP1A2, CYP1A1[15], and CYP1B1[16] isozymes and/or non-P450 dependant mechanisms.[17]

**Acknowledgements and Disclaimer**
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Table 1. $^{14}C$ recovery (as percent of administered dose) and tissue concentrations of [$^{14}C$] PeCDD in male C57BL/6N parental strain and CYP1A2 (-/-) knockout mice 4 days following an oral administration of 116 µg/kg body weight in 0.1 ml of peanut oil (mean ± std). Tissue concentrations assume all tissue $^{14}C$ is that of parent PeCDD. (n.d. is not determined).

| **Excreta/Tissue** | **C57BL/6N** | | **CYP1A2 KO** | |
|---|---|---|---|---|
| | % of Dose | Conc (mmol/g) | % of Dose | Conc. (mmol/g) |
| Urine | | | | |
| 0-24h | 1.12 ± 0.27 | ----- | 1.40 ± 0.38 | ----- |
| 24-48h | 0.44 ± 0.093 | ----- | 0.49 ± 0.21 | ----- |
| 48-72h | 0.26 ± 0.054 | ----- | 0.43 ± 0.10 | ----- |
| 72-96h | 0.27 ± 0.046 | ----- | 0.50 ± 0.11 | ----- |
| Feces | | | | |
| 0-24h | 16.69 ± 3.58 | ----- | 18.09 ± 2.87 | ----- |
| 24-48h | 2.94 ± 0.12 | ----- | 1.41 ± 0.38 | ----- |
| 48-72h | 2.83 ± 0.15 | ----- | 0.86 ± 0.17 | ----- |
| 72-96h | 3.01 ± 0.44 | ----- | 0.77 ± 0.20 | ----- |
| Adipose (epid.) | 4.10 ± 1.87 | 0.14 | 12.32 ± 3.73 | 0.18 |
| Carcass | 14.05 ± 4.43 | 0.0037 | 73.76 ± 9.91 | 0.020 |
| GI Tract | 2.63 ± 0.80 | n.d. | 8.17 ± 0.67 | n.d. |
| Heart | 0.018 ± 0.0083 | n.d. | 0.11 ± 0.091 | n.d. |
| Kidney | 0.32 ± 0.11 | n.d. | 0.99 ± 0.44 | n.d. |
| Liver | 56.79 ± 4.62 | 0.81 | 6.77 ± 0.65 | 0.072 |
| Lung | 0.080 ± 0.030 | n.d. | 0.21 ± 0.094 | n.d. |
| Muscle | n.d. | 0.0088 | n.d. | 0.028 |
| Skin | n.d. | 0.044 | n.d | 0.088 |
| Spleen | 0.045 ± 0.011 | n.d. | 0.12 ± 0.051 | n.d. |
| Testes | 0.098 ± 0.066 | n.d. | 0.53 ± 0.17 | n.d. |
| Thymus | 0.021 ± 0.015 | 0.017 | 0.32 ± 0.15 | 0.10 |
| **Total** | **107.69 ± 4.31** | | **125.21 ± 9.79** | |

Table 2. The percent of a PeCDD dose excreted as metabolites in male C57BL/6N parental strain and CYP1A2 (-/-) KO mice. Non-extractable fecal $^{14}C$ was assumed to represent metabolites of PeCDD covalently bound to lipids and/or proteins in the feces. Data normalized to 100% recovery.

| | **% of Dose** | |
|---|---|---|
| | **C57BL/6N (n=3)** | **CYP1A2 KO (n=4)** |
| 0-96h Urine | 1.12 ± 0.31 | 1.00 ± 0.32 |
| 0-96h Feces (extractable) | 9.81 ± 3.98 | 1.76 ± 0.33 |
| 0-96h Feces (non-extractable) | 4.03 ± 0.39 | 5.05 ± 1.09 |
| **Total metabolism** | **14.95 ± 3.97** | **7.81 ± 1.29** |