Excretion, Tissue Distribution, and Metabolism of 1,2,7,8-Tetrachlorodibenzo-p-dioxin in a Calf

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Introduction

Polychlorinated dioxins are a family of environmental pollutants unintentionally produced by natural and human activities. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2378-TCDD) is the most toxic member of the family which includes 75 congeners. The toxicity of 2378-TCDD is thought to be mediated through a cytosolic receptor, i.e. the aryl hydrocarbon receptor (AhR). It has not been firmly established whether the parent dioxin molecule or a metabolite is responsible for the various signs of overt toxicity observed.

Greater detail on the metabolism of 2378-TCDD is desirable but difficult to obtain, because the high toxicity of 2378-TCDD makes conventional metabolism experiments impractical. As part of our research into dioxin residues in mammalian tissues, we elected to study the tissue distribution, excretion, and metabolism of a less toxic dioxin congener, i.e. 1,2,7,8-tetrachlorodibenzo-*p*-dioxin (1278-TCDD), to be utilized as a surrogate for 2378-TCDD. Animals are exposed to the less toxic dioxin congeners by the same routes as the more toxic congeners [1]. A larger dose could be administered, allowing for greater mass from which to isolate metabolites. The goal of the metabolism research was to isolate and characterize intact metabolites in a calf following an oral dose. Once the 1278-TCDD metabolites are characterized, 2378-TCDD will be investigated in the calf for the same metabolic pathways.

Experimental Methods

[UL 7,8 ring ^{14}C] 1278-TCDD was obtained from ChemSyn Science Laboratories (Lenexa, KS; 98% purity) and administered orally in a feed capsule (1.2 mg 1278-TCDD/kg body weight; 1.2 mCi) to a Holstein bull calf (43.6 kg). The calf was held in an elevated stall, and a urinal was attached to allow for the separate collection of urine and feces. Excreta were collected for four days, then the calf was killed with halothane. Blood, longissimus dorsi muscle, heart, lungs, kidneys, liver, rumen, small and large intestine, and the carcass remains were collected. Urine was assayed for ^{14}C by pipetting an aliquot into a cocktail and counting the sample in a liquid scintillation counter (LSC). Lyophilized feces, blood, and homogenized tissues were oxidized in a tissue oxidizer and counted by LSC.

Urine collected from 0-8h and 8-24h was pooled and applied to a Porapak Q chromatographic column [2]. The column was eluted with water, methanol and acetone. Each Porapak Q fraction was subjected to reversed-phase HPLC fractionation with a water/acetonitrile mobile phase, beginning at 100% water with a linear gradient to 100% acetonitrile. The isolated metabolites were subjected to ^{1}H-NMR and negative ion fast atom bombardment mass spectral analysis (-FAB/MS).

Dried 0-24h feces was extracted successively with hexane, ethyl acetate, and methanol. Each extracted fraction was subjected to chromatography by HPLC with a water/acetonitrile gradient. The isolated metabolites were subjected to ^{1}H-NMR and GC/MS analysis.

Results and Discussion

Ten percent of the ^{14}C was recovered in the urine (Table 1), most of which was recovered between 8 and 32h (6.3%). Approximately 82% of the ^{14}C was recovered in the feces, most of which was excreted between 24 and 48h (51.7%). About 0.7% of the dose remained in the intestinal tract (rumen, small and large intestine). The remaining tissues, including the carcass, accounted for about 0.4% of the dose. Tissues with the highest levels of ^{14}C on a concentration basis are the large intestine, liver, kidneys, and small intestine (11,109; 5,014; 2,264; and 1,973 dpm/g, respectively). The half-life for elimination and the levels of tissue residues were essentially the same as were observed following a similar oral dose administered to male Sprague-Dawley rats [3]. These data indicate that the half-life for elimination and tissue distribution of 1278-TCDD do not differ significantly between a ruminating and a non-ruminating animal.

Pooled 0-24h urine accounted for 3.5% of the administered dose. After Porapak Q fractionation, 27.% of this ^{14}C eluted with water, 56.4% with methanol, and 7.9% with acetone. All urinary metabolites displayed longer reversed-phase HPLC retention times when 0.1% trifluoroacetic acid (TFAA) was added to the mobile phase, an indication that each 1278-TCDD metabolite was present as an acidic conjugate. Metabolite **I** from urine (Table 2) was found in the water fraction from the Porapak Q column. An aliquot was incubated with ß-glucuronidase (Type VII-A, *E. Coli*). The ^{14}C partitioned into ethyl acetate, an indication that **I** was a glucuronide. The ^{1}H-NMR spectrum of **I** (400 MHZ, CD_3OD) displayed two singlet resonances at 7.28 and 6.97 ppm that indicated an unsymmetrically substituted 4,5-dichlorocatechol. The -FAB/MS revealed ions at m/z 353 (M-1) and m/z 177 (M-177) within clusters indicating two chlorine atoms. The date are consistent with **I** being the monoglucuronide ether of 4,5-dichlorocatechol. Metabolite **I** accounted for 9.3% of the urinary ^{14}C.

Metabolite **II** from urine was found in both the methanol and acetone fractions from the Porapak Q column. The ^{1}H-NMR spectrum of **II** displayed three singlet resonances at 7.23, 7.15, and 7.01 ppm. The -FAB/MS revealed fragment ions at m/z 511 (M-1; 4 Cl), 477 (M-35; 3 Cl), and 335 (M-177; 4 Cl). The ^{1}H-NMR and -FAB/MS spectra were consistent with **II** being the glucuronide of a monohydroxy-TCDD. An aliquot of **II** was hydrolyzed by incubation with ß-glucuronidase. The hydrolyzate was derivatized with *bis*(trimethylsilyl)trifluoroacetamide (Regisil) and subjected to GC/MS analysis. The mass spectral peaks at m/z 408 (M^+; 4 Cl), 393 ($M-CH_3$, 4 Cl), and 358 ($M-CH_3Cl$, 3 Cl)) were compatible with the TMS ether of a monohydroxy-tetrachlorodibenzo-*p*-dioxin. The ^{1}H-NMR spectrum of the hydrolyzate matched that obtained for standard 2-hydroxy-1,3,7,8-tetrachlorodibenzo-*p*-dioxin [4]. Metabolite **II** accounted for 9.0% of the urinary ^{14}C.

Dried 0-24h feces accounted for 18.0% of the administered dose. After successive solvent extractions 55.1% of this ^{14}C remained in the hexane layer, 23.5% in the ethyl acetate layer, and 16.9% in the methanol layer. Metabolite **III** (Table 2) would not chromatograph by g.l.c without derivatization, but readily derivatized with Regisil. The resulting GC/MS spectrum was compatible with the TMS ether of a monohydroxy-TCDD. The ^{1}H-NMR spectrum of **III** displayed aromatic singlets at 7.19, 7.11, and 6.92 ppm. This spectrum was compatible with **III** being the NIH-shifted 2-hydroxy-tetrachloro-*p*-dioxin by comparison with the synthesized

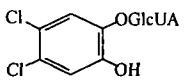
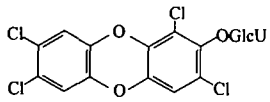
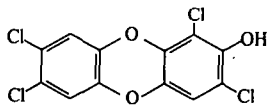
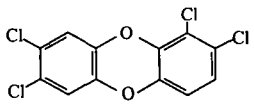
standard [4]. It was assumed that **III** had formed via the nonenzymatic isomerization of the 2,3-arene oxide of 1278-TCDD. **III** accounted for 15.6% of the fecal ^{14}C.

Metabolite **IV** had an $R_f = 0.50$ on silica thin layer chromatography (TLC) when developed in 50:50 hexane:methylene chloride. Standard 1278-TCDD co-migrated with **IV** in this solvent system. **IV** could be chromatographed by g.l.c. without derivatization. The EI mass spectrum of **IV** was compatible with the assignment as a tetrachlorodibenzo-*p*-dioxin. The ^1H-NMR spectrum of **IV** confirmed the assignment as 1278-TCDD, the dosed parent. Parent **IV** accounted for 3.4% of the fecal ^{14}C.

Table 1. Recovery of ^{14}C from a calf dosed orally with [^{14}C]1,2,7,8-tetrachlorodibenzo-*p*-dioxin.

Excreta/Tissue	Percent of Dose
Urine	
0 - 8h	**0.32**
8 - 24h	**3.22**
24 - 32h	**3.04**
32 - 48h	**1.90**
48 - 56h	**0.77**
56 - 72h	**0.81**
72 - 80h	**0.17**
80 - 96h	**0.12**
subtotal for urine	**10.36**
Feces	
0 - 24h	**18.02**
24 - 48h	**51.68**
48 - 72h	**8.60**
72 - 96h	**3.27**
subtotal for feces	**81.57**
Muscle (L. dorsi)	**0.0002**
Heart	**0.0026**
Lungs	**0.020**
Carcass	**0.27**
Kidneys	**0.021**
Liver	**0.17**
Rumen	**0.12**
Sm. Intestine	**0.13**
Lg. Intestine	**0.47**
subtotal for tissues	**1.21**
Total Recovered	**93.14**

Table 2. Structure, quantitation, and spectral data of the 0-24h metabolites of 1278-TCDD dosed orally in a calf.

Metabolite	Percent of Total Excreta	^{1}H-NMR (ppm)	MS fragment ions (Comments)
Urine			
 **I**	9.3	7.28 (s), 6.97 (s)	M-1 (353; 2 Cl) M-GlcUA (177; 2 Cl) (-FAB/MS)
 **II**	9.0	7.23 (s), 7.15 (s) 7.01 (s)	M-1 (511; 4 Cl) M-Cl (477; 3 Cl) M-GlcUA (335; 4 Cl) (-FAB/MS)
Feces			
 **III**	15.6	7.19 (s), 7.11 (s) 6.92 (s)	M^+ (408; 4 Cl) M-CH_3 (393; 4 Cl) M-CH_3Cl (358; 3 Cl) (EI mode)
 **IV**	3.4	7.31 (s), 7.23 (s) 7.23 (d), 6.98 (d)	M^+ (320; 4 Cl) M-Cl (285; 3 Cl) M-COCl (257; 3 Cl) (EI mode)

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(3) Hakk, H.; Davison, K.L.; Larsen, G.L. *14th International Symposium on Chlorinated Dioxins, PCB and Related Compounds* **1994,** 20, 497-499.
(4) Petroske, E.; Huwe, J. K.; Larsen, G. L. *Chemosphere* **1997,** 34, 1549-1555.