Tissue Distribution, Excretion, and Metabolism of a High Dose of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2378-TCDD) in Conventional and Bile-duct Cannulated Rats

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INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2378-TCDD) is among the most toxic compounds tested in laboratory animals. The LD_{50} in Sprague-Dawley rats is 60 μg/kg, but other toxic effects are manifested at lower doses, i.e. immune suppression [1], developmental, and reproductive toxicity [2]. Toxicity is thought to be mediated by interaction of the parent compound with the cytosolic arylhydrocarbon receptor (AhR). Metabolism of 2378-TCDD is generally considered a detoxication step, and necessary for elimination via the urine or the bile. However, 2378-TCDD is quite resistant to metabolism in most mammalian species. Relatively few metabolism studies have been conducted with 2378-TCDD [3, 4, 5], fewer still have attempted to elucidate the structure of metabolic products [6, 7].

As part of an effort to study dioxins in beef cattle, this laboratory initiated metabolism studies in rats and calves with non-toxic tetrachlorodibenzo-*p*-dioxin congeners, i.e. 1278-TCDD, 1378-TCDD, and 1478-TCDD. Chromatographic procedures were optimized for these experiments in order to utilize available micro ^{1}H-NMR spectroscopy and negative ion fast atom bombardment mass spectrometry (-FAB/MS). These powerful analytical techniques permitted unambiguous stereo chemical assignments of metabolites to be made.

The goal of the present research was to administer a high dose of 2378-TCDD to male Sprague-Dawley rats (i.e. 1.25-1.67 mg/kg) so that a sufficiently large mass of metabolites could be obtained to apply the spectral techniques previously developed for the stereo chemical assignments of intact metabolites.

EXPERIMENTAL

[UL 7,8-ring ^{14}C] 2378-TCDD, obtained from Chemsyn Science Laboratories, Lenexa, KS was orally dosed in peanut oil to six male Sprague-Dawley rats (1.25 mg/kg; 47,038 dpm/μg) and three bile-duct cannulated male Sprague-Dawley rats (1.67 mg/kg; 47,888 dpm/μg). The rats were housed in steel metabolism cages. Urine, feces, and bile

collections were made every 24 h for three days. The rats were anesthetized with CO_2 and sacrificed. Liver, kidneys, lungs, epididymal fat, testes, and GI tracts were removed. Urine was assayed for ^{14}C by counting aliquots of the sample in a liquid scintillation counter (LSC). Air-dried tissues and feces were combusted in a tissue oxidizer and counted by LSC. Total containment conditions were developed for all sample preparations by practicing each work up with control samples treated with a highly UV-visible dye, methylene blue.

The pooled air-dried feces was extracted 3X with hexane, ethyl acetate, and methanol. HPLC analysis was performed with a 5%-100% water to methanol gradient on C-18 columns. HPLC fractions were evaporated on a rotary evaporator, treated with CH_2N_2/diethyl ether at room temperature, cleaned on silica gel, and submitted for GC/MS analysis. TLC analysis of urine, bile, and fecal extracts was performed on silica gel plates (5x20 cm) developed in 50:50 hexane:methylene chloride, conditions that distinguished between the parent 2378-TCDD and oxidized metabolites.

RESULTS AND DISCUSSION

Table 1 shows the distribution of ^{14}C in the various tissues of both the conventional and cannulated rats. The tissues with highest levels of ^{14}C as a percentage of dose administered were the liver, GI tract, and fat for both study groups. These results are in agreement with previous studies that showed these tissues to be the major depots of the toxic 2378-TCDD in rat [3, 4, 8]. The other tissues known to be significant reservoirs for 2378-TCDD at 72 h were the skin and muscle, which would be included in the carcass data.

The major route of elimination of 2378-TCDD was the feces. Conventional rat feces contained half of the ^{14}C recovered, while 81% of the administered cannulated dose was found in the feces. Biliary elimination of 0.15%/day was well below what had been observed in other studies using smaller doses. Biliary elimination following an oral dose of 200µg/kg and 600ng/kg of 2378-TCDD in rats was 2.4% and 1% of the dose/day, respectively [7, 9]. Elimination of ^{14}C over the first three days in urine represented a minor pathway. Piper showed that ^{14}C elimination of 2378-TCDD in the urine was significantly higher in male rats receiving a 50ug/kg dose (2.5% Day 1, 1.2% Day 2, and 0.7% Day 3) [3].

Low total recoveries obtained in the conventional study, i.e. 77%, should be addressed. A possible explanation was that a smaller dose was administered than prepared. 2378-TCDD is difficult to solubilize in peanut oil and must be heated. The relatively large dose may have partially come out of solution prior to dosing. An additional factor may be a difference observed in assaying ^{14}C by LSC and by combustion analysis. ^{14}C recoveries reported in Table 1 for feces and all tissues were obtained by combustion analysis. However, the ^{14}C levels obtained by combustion analysis for 0-24 h conventional feces were 87.6% of the ^{14}C levels obtained by LSC of the fecal extracts (Table 2). The same held true for urine samples analyzed for ^{14}C by both methods (89.9%), and by topical application of a [^{14}C]2378-TCDD standard to tissue paper (93.3%) or a control carcass (83.7%). The data suggest that incomplete, but consistent, combustion of 2378-TCDD to [^{14}C]CO_2 may have occurred, resulting in low fecal and tissue recoveries. The effect may be matrix dependent.

TLC analysis of conventional and cannulated urine, and bile, demonstrated that no 2378-TCDD was present in those excreta at any of the sampling times. It was concluded that metabolism was necessary for the elimination of 2378-TCDD via the urine or the bile.

Characterization of urinary and biliary metabolites is ongoing. An average of 0.4 μg of metabolites/rat/day are eliminated in conventional urine. This level of metabolite production was insufficient to be analyzed intact by the methods developed with the non-toxic dioxin congeners. Three to five micrograms of a purified sample represents the practical minimum needed to apply ^{1}H-NMR and -FAB/MS techniques. Daily metabolite levels in cannulated urine and bile were also low, i.e. 1.0μg/rat/day and 0.8μg/rat/day, respectively.

Quantities of 2378-TCDD identified by TLC and GC/MS analysis in the conventional feces were 20.6% of dose (Day 1), 17.1% (Day 2), and 4.4% (Day 3), and probably represent unabsorbed dose. Apparently, bile salts are necessary for intestinal absorption of the non-polar 2378-TCDD because 81% of the dose was eliminated in cannulated feces compared to only 39% in conventional feces (Table 1). Quantitative TLC analysis of the daily fecal extracts showed that metabolite levels in conventional feces were very low (Table 3). These levels of ^{14}C present as metabolites in conventional feces were similar to levels of ^{14}C in the bile. Therefore, it was concluded that enterohepatic circulation was not a factor in rat metabolism of 2378-TCDD. Extraction and quantitative TLC analysis of cannulated feces is currently underway.

Methylated metabolites characterized in conventional fecal extracts by GC/MS included trichloro-methoxydibenzo-*p*-dioxin, tetrachloro-dimethoxydiphenyl ether, and tetrachloro-methoxydibenzo-*p*-dioxin (Table 4). These metabolites are indicative of reductive dechlorination, partial ether ring opening, and possible NIH-shift processes operating in rats during metabolism of 2378-TCDD. Each of these metabolites had previously been reported in rat bile as their glucuronide conjugates [7], however, no trichloro-dimethoxydibenzo-*p*-dioxin nor tetrachlorotrimethoxydiphenyl ether were found. The percent of dose that these metabolites represented was extremely low, ranging from trace levels to 0.0097%. These quantities of metabolites were insufficient to apply ^{1}H-NMR spectroscopy for stereo chemical assignments.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others than may also be suitable.

Table 1. Recoveries of ^{14}C from male rats dosed orally with [^{14}C]2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a conventional and a bile-duct cannulated study.

Tissue/Excreta	Percent of Dose Conventional (n=6)	Cannulated (n=3)
Urine		
0-24 h	0.12	0.26
24-48 h	0.07	0.11
48-72 h	0.08	0.23
Subtotal	0.27	0.60
Bile		
0-24 h	----	0.19
24-48 h	----	0.16
48-72 h	----	0.10
Subtotal	----	0.45
Feces		
0-24 h	18.58	57.78
24-48 h	15.80	19.05
48-72 h	4.36	4.13
Subtotal	38.74	80.96
Liver	19.67	4.49
Kidney	0.09	0.03
Lungs	0.05	0.01
GI Tract	6.39	0.84
Fat (epididymal)	2.39	0.13
Testes	0.01	0.00
Carcass	9.36	3.37
Total Recovery	76.98	86.88

Table 2. Differences observed between radiolabeled assays of [^{14}C]2,3,7,8-tetrachlorodibenzo-*p*-dioxin or its metabolites by liquid scintillation counting (LSC) and tissue combustion/LSC with various matrices and conditions.

Matrix	Conditions	Combustion/LSC (%)
Feces	*in vivo*	87.6
Urine	*in vivo*	89.9
Carcass	topical	83.7
Paper	topical	93.3

Table 3. [^{14}C]2,3,7,8-tetrachlorodibenzo-*p*-dioxin dose present in conventional feces as metabolites.

Feces Extracts	Percent of Dose	µg/rat
0-24 h	0.06	0.3
24-48 h	0.19	0.9
48-72 h	0.09	0.5

Table 4. GC/MS data for methylated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin metabolites of conventional male rat feces.

Compound	Major fragments	% Dose
Trichloro-methoxydibenzo-*p*-dioxin	M^+ (316); M-15 (301); M-43 (273)	0.0097
Tetrachloro-dimethoxydiphenyl ether	M^+ (366); M-46 (322); M-50 (318)	0.00084
Tetrachloro-methoxydibenzo-*p*-dioxin	M^+ (350); M-15 (335); M-43 (307)	trace
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	M^+ (320); M-35 (285); M-63 (257)	42.1

REFERENCES

(1) Holsapple, M. P., Morris, D. L. Wood, S. C., and Snyder, N. K. *Annu. Rev. Pharmacol. Toxicol.* **1991**, 31, 73-100.

(2) Peterson, R. E., Theobald, H. M., and Kimmel, G. L. *Crit. Rev. Toxicol.* **1993**, 23, 283-335.

(3) Piper, W. N., Rose, D. Q., and Gehring, P. J. *Environ. Health Perspect.* **1973**, 5, 241-244.

(4) Allen, J. R., Van Miller, J. P., and Norback, D. H. *Food Cosmet. Toxicol.* **1975**, 13, 501-505.

(5) Rose, J. Q., Ramsey, J. C., Wentzler, T. H., Hummel, R. A., and Gehring, P. J. *Toxicol. Appl. Pharmacol.* **1976**, 36, 209-226.

(6) Poiger, H., and Buser, H. R. *Environ. Sci. Res.* **1983**, 26, 483-492.

(7) Poiger, H., and Buser, H. R. In *Biological Mechanisms of Dioxin Action* (Poland, A. and Kimbrough, R. Eds) **1984**, Banbury Report 18, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 39-47.

(8) Pohjanvirta, R., Vartiainen, T., Uuai-Rauva, A., Monkkonen, J., and Tuomisto, J. *Pharmacol. Toxicol.* **1990**, 66, 93.

(9) Poiger, H., and Schlatter, Ch. *Nature.* **1979**, 281, 706-707.