Major Metabolites of 1,4,7,8-Tetrachlorodibenzo-*p*-dioxin in a Ruminating Calf

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Introduction

Metabolism studies of 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) have been reported in a number of species including rats (1-3), hamsters (4), and dogs (5). Absorption and disposition studies of 2,3,7,8-PCDDs have been conducted in beef cattle (6) and dairy cows (7-9); however, no metabolism data has been reported. In most mammals the 2,3,7,8-PCDDs are slowly metabolized and tend to accumulate in the liver and adipose tissues. The slow metabolism and excretion make characterization of metabolites difficult due to the low levels present in bile and excreta. As part of ongoing research into the pharmacokinetics of dioxins in beef cattle, we have examined the metabolism of a model dioxin, 1,4,7,8-tetrachlorodibenzo-*p*-dioxin (1,4,7,8-TCDD), in a ruminating calf.

Materials and Methods

1,4,7,8-Tetrachloro[7,8-dichlorophenyl-U-^{14}C]dibenzo-*p*-dioxin (>98% radiochemical purity) was obtained from ChemSyn Science Laboratories, Lenexa, KS, and diluted with unlabeled 1,4,7,8-TCDD to a specific activity of 6500 dpm/μg. A dose was prepared by evaporating a solution of ^{14}C-1,4,7,8-TCDD onto grain in a gelatin capsule. A five-week old Holstein bull calf (55.9 kg) which had received hay- and grain-supplemented diet for four weeks was dosed orally at a level of 2.5 mg/kg. The calf was restrained in a metabolism cage and fitted with a urinal to facilitate daily collections of feces and urine. Blood samples were collected prior to dosing, eight hours after dosing, and every 24 hours afterwards. The calf was euthanized 96 h after dosing, and tissues were collected and frozen. Urine was assayed for ^{14}C by scintillation counting methods. Plasma, feces, and tissues were quantitated for radioactivity by combustion analysis after homogenization. Metabolites were isolated and identified by methods previously described (10).

Results and Discussion

Table 1 summarizes the excretion and disposition of ^{14}C-radiolabel from 1,4,7,8-TCDD over 96 hours. The major route of excretion was feces (85%) while a small amount of ^{14}C (1%) was excreted in the urine. Aside from the gastrointestinal tract, liver was the only organ with significant levels of ^{14}C. The carcass, excluding perirenal fat pad and longissimus dorsi cut, contained less than 0.5% of the dose after 96 hours. Levels of ^{14}C in perirenal fat were 20 ppb after 96 h. Blood levels of ^{14}C peaked at 30 ppb after 24 h and decreased to nearly background after 96 h.

Table 1. Recovery of ^{14}C from calves dosed with ^{14}C-1,4,7,8-TCDD.

Sample	% of Dose	Sample	% of Dose	(ppb)
Urine:		Heart	0.00	(6)
0-24 h	0.70	Lung	0.00	(14)
24-48 h	0.28	Liver	0.03	(36)
48-72 h	0.11	Kidney		0.00
(15)				
72-96 h	0.03	Adrenals	0.00	(5)
		Perirenal fat	0.00	(23)
Feces:		L. Dorsi	0.00	(0)
0-24 h	15.9	Large Intestines[a]	2.94	
24-48 h	37.1	Small Intestines[a]	0.24	
48-72 h	26.1	Stomachs[a,b]	4.32	
72-96 h	5.8	Carcass	0.43	(14)
Total Recovery	94.0			

[a] Includes contents. [b] Rumen, reticulum, omasum, and abomasum

No parent TCDD was found in the urine. Five metabolites were identified from the urine (0-24 h) including a sulfate ester of 4,5-dichlorocatechol, a glucuronide conjugate of 4,5-dichlorocatechol, 2-O-glucuronyl-1,4,7,8-TCDD, 2-hydroxy-1,4,7,8-TCDD, and 1-hydroxy-4,7,8-triCDD. Each of these compounds have previously been identified in the urine of rats dosed with 1,4,7,8-TCDD or 1,2,7,8-TCDD (10,11).

Over 70% of the ^{14}C present in the 0-48 h feces collections was identified as parent. Two other fecal metabolites were identified as 2-hydroxy-1,4,7,8-TCDD and 1-hydroxy-4,7,8-TriCDD. Both were also major fecal metabolites in rats dosed with ^{14}C-1,4,7,8-TCDD (10). One unique metabolite was isolated from the calf feces and identified by GC-MS of the trimethylsilyl (TMS) derivative and by ^{1}H-NMR as 2,3-dihydro-2,3-diol-1,4,7,8-TCDD (Figure 1). The mass spectrum of the diTMS derivative gave a molecular ion cluster at 498 (Cl_4) with major fragments at m/e 463 (M-35, Cl_3), 428 (M-70, Cl_2), and 355 (M-70-TMS, Cl_2). Precise mass calculations using high resolution GC-MS confirmed the structure. ^{1}H-NMR in CD_3CN showed one aromatic resonance at δ 7.38 ppm and a single resonance for the dihydro-protons at δ 4.24 ppm.

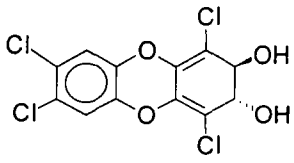

Figure 1. Proposed structure of dihydrodiol-1,4,7,8-TCDD metabolite from a calf.

The large percentage of parent present in the feces suggested that ^{14}C-1,4,7,8-TCDD was not absorbed to a great extent. This is in contrast to a mass balance study which showed 75% of the non-2,3,7,8-TCDDs were absorbed from feed by lactating cows (12). The difference in bioavailability may be due to the relatively immature rumen of the calf and/or nonlinear absorption of dioxins. In rats administered ^{14}C-1,4,7,8-TCDD in peanut oil, only 6% of the fecal ^{14}C was found as parent indicating over 90% absorption or microbial metabolism (10).

One major difference between the metabolism of ^{14}C-1,4,7,8-TCDD in rats and a calf was the isolation of a stable dihydrodiol metabolite in the calf. *Trans*-dihydrodiols are formed via hydrolytic ring opening of epoxides by epoxide hydrases (13). Subsequent enzymatic dehydrogenation yields catechols. Although dihydrodiols were not isolated from rats dosed with ^{14}C-1,4,7,8-TCDD, catechols which may have resulted from their dehydrogenation were isolated and identified (14). Formation of dihydrodiols is generally considered a detoxification mechanism for epoxides and appears to be a major metabolic route for this model dioxin in ruminants.

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