Dioxin Metabolism: Novel Sulfate Metabolites of 1,2,7,8-tetrachlorodibenzo-*p*-dioxin Isolated from Rat Urine

H. Hakk, G.L. Larsen, and V.J. Feil
USDA, ARS, Biosciences Research Laboratory, P.O. Box 5674-Univ. Sta., Fargo, ND 58105-5674

Polychlorinated dibenzo-*p*-dioxins comprise an important class of environmental pollutants unintentionally synthesized by various human activities. One of the congeners, i.e. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) is commonly referred to as the most toxic compound ever tested on laboratory animals. In animals, including man, dioxin action is thought to be intracellularly mediated by the aryl hydrocarbon receptor, which translocates to the nucleus upon agonist binding. This leads to expression of a host of genes, including CYTP450 and ethoxyresorufin-O-deethylase, which ultimately can produce latent disease states such as chloracne, wasting disease, tumor promotion, immunosuppression and birth defects.

We elected to study the metabolism of dioxins as part of our research effort on dioxin residues in animal tissues. The less toxic 1,2,7,8-tetrachlorodibenzo-*p*-dioxin (1278-TCDD) was initially selected (a) because more dioxin could be fed, allowing for greater mass from which to isolate metabolites, and (b) because animals do ingest the less toxic congeners along with the more toxic ones in their environment. Once the route of disposition, metabolism and excretion have been established for 1278-TCDD, the 2378-TCDD will be studies for the same pathways.

[UL 7,8 ring ^{14}C] 1278-TCDD was synthesized and given in peanut oil by gavage (0.5 ml of oil, 2 mg of dioxin, 9.4 μCi per rat) to six male Sprague-Dawley rats (weights 235 to 254 g). The rats were placed in stainless steel metabolism cages. Feces and urine were collected for three days, then the rats were euthanized with pentobarbital. Liver, kidney, gastrointestinal tract with contents, lung, spleen, heart, and the carcass remains were collected. urine was assayed for ^{14}C by pipetting an aliquot into a cocktail and counting it in a liquid-scintillation counter (LSC). Lyophilized feces and homogenized tissues were oxidized with a biological sample oxidizer and counted by LSC.

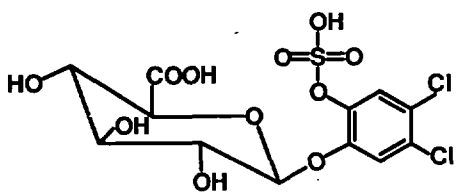
Over eighty percent of the ^{14}C was recovered in the feces (Table 1), most of which was recovered in the first two days, and 14.3% was recovered in the urine, most of which was recovered in the first day. About 0.4% of the dose remained in the carcass while 0.35% remained in the liver. Combined residues in the heart, spleen and kidneys amounted to less than 0.1% of the dose.

Urine collected from the six rats during the first 24 h was pooled and applied to a Porapak Q column.[1)] Four fractions were collected from the column bypass, 0.5% of the ^{14}C; water, 72.2%; water:methanol (50:50), 14.4%; and methanol, 9.1%. The bypass was discarded. Aliquots of each of the remaining fractions were applied to thin-layer chromatography plates (silica gel) and the plates were developed in hexane:methylene

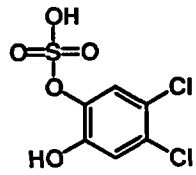
chloride (50:50). The ^{14}C urine aliquots did not move from the origin whereas the ^{14}C in the 1278-TCDD standard had a R_f=0.5 suggesting that all of the ^{14}C in urine was present as metabolites of the 1278-TCDD.

The water fraction from the Porapak column was further fractionated on an LH-20 column under water isocratic conditions. Samples were collected in 1 ml fractions; radioactivity was found in four regions labeled #28-38, 3.5% of ^{14}C from H_2O Porapak Q; #39-50, 21.2%; #51-67, 24.3%; #94-128, 38.8%. An aliquot of each was chromatographed by HPLC with a C-18 column elutes with water/acetonitrile, beginning with 100% H_2O and using a step gradient to 100% acetonitrile. The unretained chromatographic behavior and water solubility of #28-38, #39-50 and #51-67 indicated the presence of conjugates. #94-128 eluted at 42% acetonitrile and did not chromatograph by GLC either silylated or underivatized in a chromatograph equipped with both a flame ionization detector and radioactivity monitor (0.53 mm ID, SE-30 liquid phase). This suggested that #94-128 was also a conjugate.

An aliquot of #39-50 was incubated with ß-glucuronidase (*E. Coli*). The incubated mixture was chromatographed on HPLC under the same conditions used above. One hundred percent of the ^{14}C eluted at 42% acetonitrile, indicating that #39-50 was the ß-glucuronide of #94-128. Both samples in ^{1}H-NMR displayed two singlets in the aromatic region of the spectrum, i.e. 7.52 and 7.47 ppm for #39-50, 7.46 and 7.00 ppm for #94-128. The negative ion FAB mass spectrum of #39-50 revealed two chlorine ion clusters at 455 (M-1), 375 (M-81) and 279 (M-177). The negative ion FAB mass spectrum of #94-128 displayed a two chlorine ion cluster at 279 (M-1). Both spectra were sodium adducts. We conclude from the data that #39-50 is the ß-glucuronide/sulfate diconjugate of 4,5-dichlorocatechol (I, 9.5% of the urinary ^{14}C), and #94-128 is the sulfate conjugate of 4,5-dichlorocatechol (II, 17.3% of the urinary ^{14}C). This, to our knowledge, represents the first report of sulfation occurring in tetrachlorodibenzo-*p*-dioxin metabolism.[2)]



(I)



(II)

References

1) Bakke, J.E., and C.E. Price (1979): Metabolism of 2-chloro-*N*-isopropylacetanilide (propachlor) in the rat. J. Environ. Sci. Health B14(4), 427-441.

2) VandenBerg, M., J. DeJongh, H. Poiger, and J.R. Olson (1994): The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. Crit. Rev. Toxic. 24(1), 1-74.

Table 1. Recovery of ^{14}C from rats given [^{14}C]1,2,7,8-tetrachlorodibenzo-*p*-dioxin by gavage.

Substance	Percent of Dose
Urine:	
0-24 hr	8.8
24-48 hr	4.5
48-72 hr	1.0
Subtotal for urine	14.3 ± 2.2
Feces:	
0-24 hr	27.7
24-48 hr	43.6
48-72 hr	8.5
Subtotal for feces	80.2 ± 6.2
Heart	0.0013 ± 0.0004
Spleen	0.0006 ± 0.0001
Kidneys	0.04
Liver	0.35
Carcass	0.40 ± 0.05
Total Recovered	95.06 ± 5.5