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

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Dioxins comprise a class of environmental pollutants synthesized unintentionally by natural and human activities. Many of these compounds are toxic. One of the dioxin congeners, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD) is commonly referred to as the most toxic compound made by man. In animals, including man, dioxins bind to an intracellular aryl hydrocarbon receptor which functions in expressing latent toxicities such as some cancers, reproductive anomalies or metabolic imbalances and possibly in transporting dioxins to various sites where overt toxicities such as chloracne and "wasting" disease occur. Some disease states attributed to dioxins are not proven conclusively, some may not be caused by the dioxins and some may never be proven conclusively. Also, it is not known whether the toxic effects attributed to dioxins are caused by the parent dioxins themselves or by metabolites.

We elected to study the metabolism of dioxins as a part of our research on dioxin residues in animal tissues. The less toxic 1,2,7,8-tetrachlorodibenzo-p-dioxin (1278-TCDD) was selected for this study because animals do ingest the less toxic congeners along with the more toxic ones in their environment and because more dioxin could be fed, allowing for greater mass from which to isolate metabolites. Once the metabolites from the 1278-TCDD are identified, the 2378-TCDD can then be studied for the same metabolitic pathways.

[UL 7,8 ring ^{14}C] 1278-TCDD was synthesized and given in peanut oil by gavage (0.4 ml of oil, 350 µg of dioxin, 8.5 µCi per rat) to four male Sprague-Dawley rats (weight 367 to 388 g). The rats were placed in steel metabolism cages. Feces and urine were collected for four days, then the rats were euthanized with pentobarbital. Blood, perirenal adipose tissue, kidneys, gastrointestinal tract with contents, liver, longissimus dorsi muscle, brain and the carcass remains were collected. Urine was assayed for ^{14}C by pipetting a suitable aliquot into a cocktail and counting it in a liquid-scintillation spectrometer (LSC). Lyophilized feces, blood and homogenized tissues were oxidized with a biological sample oxidizer and counted by LSC.

Seventy-seven percent of the ^{14}C was recovered in the feces (Table 1), most of which was recovered in the first two days, and 17% was recovered in the urine, most of which was recovered in the first day. About 0.4% of the dose remained in the GI tract. About 1% of the dose remained in the kidneys, liver, and carcass combined. Residues in brain, muscle, and perirenal fat were 56, 80 and 5118 dpm/g, respectively.

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 - 8 h	4.7
8 - 24 h	7.0
24 - 32 h	1.4
32 - 48 h	2.3
48 - 72 h	1.0
72 - 96 h	0.4
subtotal for urine	17.0 ± 1.2
Feces	
0 - 24 h	24.4
24 - 48 h	42.2
48 - 72 h	8.9
72 - 96 h	1.5
subtotal for feces	77.0 ± 2.8
GI tract	0.39 ± 0.04
Kidneys	0.02 ± 0.003
Liver	0.17 ± 0.01
Carcass	0.71 ± 0.12
Cage Rinse	0.64 ± 0.17
Total Recovered	95.9 ± 2.8

Urine collected from the four rats during the first 24 h was pooled and applied to a Porapak Q column.[1] Four fractions were collected from the column: bypass, 2% of the ^{14}C; water, 36.7%; water/methanol (50:50), 47.7%; and methanol, 12.6%. The bypass was discarded. Aliquots of each of the remaining fractions were applied to thin-layer chromatography plates (silica gel) and the plates developed with hexane: dichloromethane (50:50). The ^{14}C in the aliquots did not move from the origin whereas the ^{14}C in 1278-TCDD standard had an Rf of 0.5 suggesting that all of the ^{14}C in the urine was in metabolites of the dioxin.

The water fraction from the Porapak column was further processed, ultimately yielding a conjugate of 4,5-dichlorocatechol which is likely a glucuronide. An aliquot of the water fraction was chromatographed by h.p.l.c. with a C18 column eluted with water/acetonitrile, beginning with 100% water in a linear gradient to 100% acetonitrile. The radioactivity was not retained by the C18 column. The chromatographic behavior and the water solubility of the metabolites suggested a glucuronide(s). An aliquot was then incubated with glucuronidase/arylsulfatase (Helix pomatia). The incubated mixture was partitioned between water and toluene. Fifty-three percent of the ^{14}C partitioned into toluene. The toluene fraction was derivatized with bis(trimethylsilyl)trifluoracetamide + trimethylchlorosilane (99:1, Regisil) and chromatographed by g.l.c. (megabore, SE-30 liquid phase) in a chromatograph equipped with a column effluent splitter, a flame ionization detector and a radioactivity monitor. A single radioactive peak eluted from the g.l.c. which was trapped in glass capillaries. The trapped material was derivatized again with Regisil for g.l.c./electron impact (E.I.) mass spectroscopy.

The E.I. spectrum revealed ions at m\z 322 ($M^{+\cdot}$) and 307 (M-15), each within clusters indicative of two chlorines and two silicons. The mass spectrum of authentic 4,5-dichlorocatechol was essentially identical to that of the metabolite, which accounted for about 19% of the urinary ^{14}C.

References

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