Polychlorodibenzo-*p*-dioxin metabolism

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Polychlorodibenzo-*p*-dioxins comprise a class of environmental pollutants synthesized unintentionally by natural and human activities. One of the congeners [i.e. tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)] is often referred to as the most toxic compound made by man and tested on animals. The 1,2,3,7,8-penta CDD congener is considered to be approximately half as toxic as 2,3,7,8-TCDD. The toxicity of other tetrachlorinated isomers can vary by several orders of magnitude depending on their chlorine substitution patterns. Proper identification of isomers and assignment of the appropriate toxicity [toxic equivalency factors (TEFs)] is the basis of world wide monitoring programs for these ubiquitous environmental trace contaminants.[1] Because the major route of exposure for humans to TCDD is through the food supply, methods for identification of specific metabolic isomers in animals raised for food are basic to the understanding of the biodegradation and biomagnification processes involved in the formation of dioxin residues.

Previous metabolism work on the toxic 2,3,7,8-TCDD has shown that, to a degree, its toxicity in a species is a function of the extent of metabolism. Syrian golden hamsters and dogs tend to metabolize 2,3,7,8-TCDD to a greater degree than rats and, especially, guinea pigs. 2,3,7,8-TCDD is highly toxic in guinea pigs and is less toxic to dogs and hamsters.[2,3] In dogs biliary metabolites were found which reflect: 1) oxidation of 2,3,7,8-TCDD with NIH shifts of chlorine substituents; 2) hydrolytic dechlorination yielding hydroxylated tri- and tetra-chlorinated dibenzo-*p*-dioxins; 3) cleavage of one or both of the ether bridges to form diphenyl ethers and dichlorocatechols; and 4) no indication of glucuronide or sulfate ester conjugation.[3] In the bile-duct-cannulated rat orally dosed with [^{14}C]-2,3,7,8-TCDD, excretion of radioactivity (nature of which was not characterized) in the bile reportedly matched that of ^{14}C excreted in the feces of conventional rats which indicated that no enterohepatic circulation of ^{14}C occurred.[4]

In vivo metabolism of other dioxin congeners [diphenyl-*p*-dioxin (DD), 1-chloro DD; 2 chloro DD; 2,3-dichloro DD; 2,7-dichloro DD; 1,2,4-trichloro-DD; 1,2,3,4-TCDD] in rat showed primarily hydroxylation at the 2, 3, 7, or 8 positions. Sulfur metabolites were observed for DD and the mono CDDs. No metabolism to catechols or monohydroxylated products was detected for octaCDD.[5]

Metabolism, similar with that of the dioxins, has been observed in rats dosed with mono-, di-, tri-, penta- and octa- chlorodibenzofurans [(CDF) 2-; 2,8-; 2,3,8-; 2,3,7,8-; 1,2,3,7,8-; and 1,2,3,4,8- and octa-CDF] producing 1) mono and dihydroxylation; 2) hydrolytic dechlorination; and 3) a minor pathway in which sulfur containing metabolites were produced.[6] As with the octaCDD the octachlorodibenzofuran dosed to rats produced no observable urinary, fecal or tissue metabolites.[7] For a more comprehensive review of earlier studies of the metabolism of chlorinated dioxins and furans see VandenBerg et al. (1994).[8]

As a part of a larger research effort into dioxin residues in animal tissues in relation to food safety, we have elected to study the metabolism of some of the lesser known dioxin isomers with lower toxicity in animals. We selected 1,2,7,8-; 1,3,7,8- and 1,4,7,8-TCDD because 1) animals ingest these congeners along with the more toxic congeners in their environment; 2) very little, if any, metabolism work has been done on these congeners; and 3) they can be dosed at higher mass levels producing greater levels of metabolites. Once the metabolites from these congeners are identified using FAB mass-, GC mass- and ^{1}H-NMR-spectrometry, 2,3,7,8-TCDD and 1,2,3,7,8-penta CDD will be studied looking for similar metabolic pathways.

^{1}H-NMR spectroscopy is a very useful analytical tool for structure identification and determining aromatic substitution patterns. Comparison of ^{1}H-NMR spectra of unknowns with chemically synthesized standards allows unambiguous assignment of chlorine substitution patterns in isomers of TCDD and their metabolites. Previous identification of dioxin metabolites have relied on more relative methods based on chromatography and MS fragmentation patterns.[9] To identify the isomers produced by metabolism of the congeners listed above, we synthesized a series of monohydroxylated TCDDs, verified their structure by ^{1}H-NMR spectrometry, and used mass and NMR spectrometry to identify metabolites isolated from various biological excreta.[10]

It also should be noted that some of the earlier metabolism work was conducted using non-radiolabelled compounds resulting in little or no quantitation of metabolites. In addition, in many cases, metabolites were deconjugated using strong acidic or basic conditions or general enzymatic hydrolysis so that the type of conjugate formed could not be determined.

Male rats dosed orally with [^{14}C]-1,2,7,8-TCDD excreted 80% (27.7% in 24 h) and 14% (8.8% in 24 h) of the dose in the feces and urine, respectively, 72 hours after dosing. About 0.4% of the dose remained in the carcass with 0.35% in the liver (not part of the ^{14}C found in the carcass) 72 h after dosing. Bile-duct-cannulated rats dosed orally with [^{14}C]-1,2,7,8-TCDD excreted 32.4% (26.8% in 24 h) in the bile, while 40.7% (21.5% in 24 h) in the feces and 5.8% (4.2% in 24 h) in the urine 72 h after dosing. A calf dosed orally with [^{14}C]-1,2,4,8-TCDD excreted 81% (18% in 24 h) and 11% (3.6% in 24 h) of the dose in feces and urine, respectively, 72 h after dosing. Metabolites were isolated from 0-24 h urine (u), bile (b) and feces (f) from rat (r) or calf (c). FAB mass spectrometry of the intact conjugate and treatments with either ß-glucuronidase or aryl sulfatase followed by methylation, EI mass spectrometry and ^{1}H-NMR were used to characterize these conjugates. The following metabolites have been isolated and characterized to be: 2-O-glucuronide-1,3,7,8-TCDD (ru, 6.9% of ^{14}C in biological sample; rf, 10.1%; and rb, 41.7%); 2-OH-1,3,7,8-TCDD (rf, 34.8%; cf, 80.8% and rb, 6.0%); Parent 1,2,7,8-TCDD (rf, 3.7% and cf 4.0%); 4,5-dichloro-catechol (cf, 3.6%); sulfate ester of 4,5-dichlorocatechol (ru, 36.0%), disulfate ester of 4,5-dichlorocatechol (ru, 16.9%, and rb, 3.4%); a sulfate ester-mono glucuronide diconjugate of 4,5-dichlorocatechol (ru, 14.8% and rb, 1.4%) and a diglucuronide conjugate of 4,5-dichlorocatechol (ru, 2.4% and rb, 0.4%). Metabolites were extracted from feces with three extractions each with the following solvent sequence: hexane, ethylacetate and methanol. Parent 1,2,7,8-TCDD has been found in the methanol extract or in purified polar fractions trapped from HPLC. This observation lead us to believe that a polar metabolite (i.e. arene oxides) might revert back to the parent 1,2,7,8-TCDD which we have also observed in the case of fecal metabolites of 1,4,7,8-TCDD, described later. In addition it should be noted that the major metabolites in rat urine and feces were the 2-O-glucuronide-1,3,7,8-TCDD and 2-OH-1,3,7,8-TCDD and in calf feces 2-OH-1,3,7,8-TCDD, which are NIH shift metabolites. Of note is the isolation of 2-O-glucuronide-1,3,7,8-TCDD from rat feces in this experiment. We have not observed fecal glucuronides in the metabolism of other xenobiotics including the 1,3,7,8-TCDD and 1,4,7,8-TCDD discussed later. Apparently, the 2-O-glucuronide-

1,3,7,8-TCDD is more stable to ß-glucuronidase hydrolysis, a point to be considered when characterizing a glucuronide metabolite by ß-glucuronidase hydrolyses.

In contrast rats dosed orally with [^{14}C]-1,3,7,8-TCDD excreted most of the ^{14}C in the feces (91%) with only 1.7% of the dose being excreted in their urine 72 h after dosing. From MS and ^{1}H-NMR data 25% of the organic ^{14}C fecal extract was shown to be parent 1,3,7,8-TCDD and 75% appeared to be a single monohydroxy metabolite. In bile-duct-cannulated rats dosed orally with 1,3,7,8-TCDD 34% of the dose was excreted in the bile, while 51% was found in the feces. Less than 1% of the ^{14}C dose was present in the urine. Biliary metabolites were isolated by chromatography and characterized to be: 1) a monohydroxy TCDD (OH-TCDD, 15%), 2) a major metabolite as a glucuronide conjugate of OH-TCDD (65%), and 3) a minor metabolite as a sulfate-glucuronide of OH-TCDD (20%). Although HPLC, TLC and GC analysis of OH-TCDD isolated from the feces showed only one component, GC/MS of the methylated metabolite gave two distinct peaks which were characterized by ^{1}H-NMR to be 2-OH-1,4,7,8-TCDD and 3-OH-1,2,7,8-TCDD, both of which were NIH shifted metabolites rather than simple oxidation products. In the rat it is clear that 1,3,7,8-TCDD is readily metabolized by ring hydroxylation followed by conjugation to form a glucuronide. Minor amounts of these dioxin conjugates are excreted in the urine but most are excreted in the bile, deconjugated in the gut, and thus present in the feces as free monohydroxytetrachlorodioxin metabolites.[11)]

Rats given single oral doses of [^{14}C]-1,4,7,8-TCDD excreted 88.8% (58% in 0-24 h) in the feces and 4.2% (2.7% in 24 h) in urine after 72 h. Bile-duct-cannulated rats given single oral doses of [^{14}C]-1,4,7,8-TCDD excreted 33% in bile in 24 h. The following metabolites were isolated from 0-24 h urine (u), bile (b) or feces (f) and characterized by TLC, GC/MS and ^{1}H-NMR: 1-O-glucuronide-4,7,8-triCDD (b, 5.5% of ^{14}C in biological sample); 2-O-glucuronide-1,4,7,8- (b, 30.5%; u, 12%); 2-OH-1,4,7,8-TCDD (b, 2.5%; u, 16%; f, 57.6%); 1-OH-4,7,8-triCDD (b, 1.5%; u, 11%; and f, 22.0%); 4,5 dichlorocatechol (u, 2% and f, 1.0%); 1,4,7,8-TCDD (f, 11.5%) and unidentified metabolites (b, 1.4% and u, 50%). The major hydroxylated metabolite (2-OH-1,4,7,8-TCDD) was most likely formed via an arene oxide in the 2,3-position because no NIH-shifted products were observed. One important observation in this metabolism study was that the original hexane fecal extract contained no evidence of parent 1,4,7,8-TCDD. From this extract 1-OH-4,7,8-TriCDD (12% of the ^{14}C extracted) and 2-OH-1,4,7,8-TCDD (43%) were isolated; however, after the extract was allowed to stand refrigerated for several weeks, parent 1,4,7,8-TCDD was detected by TLC and, subsequently, identified. This phenomena may be explained by the decomposition of an arene oxide intermediate. Previous studies have shown arene oxides of phenanthrene and naphthalene decompose to parent aromatics.[12)] The presence of the metabolites, 1-OH-4,7,8-TriCDD and 2-OH-1,4,7,8-TCDD, indicate that the metabolism of 1,4,7,8-TCDD goes through an oxidative pathway either by direct substitution or through an arene oxide intermediate.

If only chromatographic behavior, MS spectra and available NMR literature data are used to characterize these metabolites, it is not possible to unequivocally assign chlorine or other substituent positions in the dioxin molecule. Once these structures are determined, it becomes apparent that NIH-shifts of chlorine substituents can play a role in the metabolism of dioxin congeners and demonstrates their interrelatedness and complexity. Additional toxicological significance becomes apparent when this is coupled with the observation that oxidative-intermediates of fecal metabolites of 1,2,7,8-TCDD and 1,4,7,8-TCDD reverted to their respective parent compounds. In theory then oxidative intermediate metabolites from a nontoxic congener could, through a NIH-shift and this degradative process, be converted to a more toxic dioxin congener. Currently, the toxicological data on monohydroxy-TCDD and monohydroxy triCDD metabolites themselves is very limited.

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