In Vivo Formation of Octachlorodibenzo-*p*-dioxin from a Predioxin

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Introduction

Recent mass balance studies in infants (1) and adults (2,3) have shown that certain of the highly chlorinated dibenzo-*p*-dioxins/furans were excreted at levels higher than ingested. In particular octachlorodibenzo-*p*-dioxin (OCDD) had a net excretion two- to five-times the intake. Cattle fed pentachlorophenol-treated wood in their diet excreted OCDD almost four-fold over what they ingested (4). These results point to the possible *in vivo* formation of OCDD from predioxins i.e. chlorinated phenoxyphenols which are known contaminants in pentachlorophenol and other chlorophenol pesticides (5,6). In this study we have investigated the metabolic conversion of a predioxin (nonachloro-2-phenoxyphenol) to OCDD in rats.

Experimental

Nonachloro-2-phenoxyphenol was isolated from technical-grade pentachlorophenol by reverse phase (C_{18}) column chromatography. The predioxin was further purified by C_{18}-HPLC, solid phase extraction from a silica gel Sep Pak (Waters Assoc., Milford, MA), elution from a Carbograph cartridge (Alltech Assoc., Deerfield, IL), and a final C_{18}-HPLC step. After each purification step an aliquot was derivatized with diazomethane and analyzed by GC-MS using cool-on-column injections. The amount of OCDD present after each purification step remained consistently between 0.4- 0.9%. We finally determined that the OCDD was being formed during the GC process with larger amounts formed (up to 10%) when columns or precolumns were dirty. At best the final predioxin sample contained no OCDD (one GC-MS analysis detected no OCDD, limit of detection estimated at 0.03%) at worst 0.2% (final GC-MS integration).

Sixteen rats were divided randomly into four groups and housed individually in metabolism cages to allow daily collection of urine and feces. Each was trained to eat one daily meal of 10.5 g ground feed topped with 0.2 ml of peanut oil. The dosing experiment lasted 14 days during which time each rat received the following daily doses: control group - 0.2 ml peanut oil, dose group I - 50 ng predioxin in 0.2 ml peanut oil, dose group II - 50 ng predioxin and 0.5 ng OCDD in 0.2 ml peanut oil, dose group III - 50 ng predioxin and 2.5 ng OCDD in 0.2 ml peanut oil. At the end of the experiment, rats were euthanized with CO_2 and livers removed. Urine, feces, livers, and carcasses from each group were pooled for analysis. OCDD analyses followed EPA method 8290.

Results and Discussion

No significant weight changes were seen for any of the groups of rats during the course of the experiment. The feed and peanut oil used in the experiment had previously been analyzed and were found to have detectable levels of OCDD. These levels contributed to the amount of OCDD fed during the training and dosing periods and are included in the values in Table 1. Table 1 also shows the fecal excretion and liver and carcass deposits of OCDD for each of the groups after the 14 day dosing period. Urine was not expected to contain OCDD and was not analyzed for this study. The last column in Table 1 shows the net formation of OCDD calculated for each group. If the predioxin contained a 0.2% impurity of OCDD, each dosed group would have received an additional 5.6 ng of OCDD over the 14 days. This added amount of OCDD in the diet does not totally account for the increased levels of OCDD recovered.

Table 1. Results of 14 day predioxin feeding study. All values are given in ng. Net formation values in parentheses are based on a 0.2% impurity of OCDD present in the predioxin.

	Amounts Fed		Recovered OCDD				Net formation
Group	Predioxin	OCDD	Fecal	Liver	Carcass	Total	of OCDD
Control	0	11.7[a]	5.7	0.4	0.8[b]	6.9	-41%
dose I	2800	11.7	17.9	0.9	2.5[c]	21.3	+82% (+23%)
dose II	2800	39.7	54.3	3.0	5.3	62.6	+58% (+38%)
dose III	2800	151.7	178.1	14.3	13.5	205.9	+36% (+31%)

[a] Amount received from the feed and peanut oil diet.
[b] Calculated using one half of the non-detectable limit (1.8 pg/g).
[c] Calculated using one half of the non-detectable limit (5.8 pg/g).

The data from this feeding study suggest that predioxins can be converted to dioxins in mammalian systems although not to a large extent. Net formation of OCDD ranged from 36-82% which is more than can be accounted for by analytical variations (relative standard deviations are 5-10%). The conversion is estimated to be less than 2%. Previously Tulp et al. investigated the conversion of 5-chloro-2-(2,4-dichlorophenoxy)phenol to a dichlorodibenzo-*p*-dioxin and found no evidence for this in rats (7). Steric crowding in the more highly chlorinated phenoxyphenols may be a driving force for dioxin ring formation, and we see evidence for this event occurring under GC analysis conditions of the predioxin methyl ether. Whether *in vivo* conversion is spontaneous or enzyme catalyzed is not known.

The method of standard additions protocol used for this study indicated that the presence of higher amounts of OCDD increased the percentage of predioxin conversion. With no added OCDD (dose I) conversion was 0.3%; with 5% added OCDD (dose III) the conversion rate was 1.9%. As yet we do not have an explanation for this apparent cooperation. In conclusion, although the presence of predioxins in foods can contribute to the total dioxin exposure especially for OCDD, the levels of nonachloro-2-phenoxyphenol necessary to produce a two-fold increase in OCDD would be 100-times the OCDD concentrations (assuming a 2% conversion). We have not found these high levels in technical pentachlorophenol.

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