# A Study of the Mass Balance of Dioxins and Furans in Lactating Cows in Background Conditions. Part 1: Study Design and Analysis of Feed

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## Introduction

Consumption of animal fats account for as much as 95%[1] of the human background exposure to compounds with dioxin-like activity. Although it is generally believed that most domestic meat and dairy animals receive most of their exposure from their feed, there is limited data available on levels of dioxin-like compounds in animal feeds. The purpose of this study was to first confirm that feed is the primary source of dioxin exposure for the dairy cattle under study. This was to be accomplished by conducting a mass balance study. A second objective was to use the mass balance data to derive steady-state bioconcentration factors (BCFs, BTFs, and CRs). Future studies will use these bioconcentration factors to look at feed and milk concentration in a number of dairy operations to determine if feed accounts for most of the exposure to dairy animals. This study is described in two parts. This paper presents the data on levels of dioxin in the feed and makes some conclusions about its variability, and the contribution to overall feed concentration made by the various feed components. The second paper summarizes the mass balance results and generates bioconcentration parameters (BCF, BTF, and CR) for the dioxin and furan (CDD/F) congeners.[2] This study did not investigate the dioxin-like coplanar PCBs.

## Study Design

The study was conducted at the ARS research facility located in Beltsville, Maryland. Management of cows at this research facility is typical of commercial diary operations in the United States, except during specific research projects when cows are housed and managed according to the research protocols. Four lactating cows were used in three 5-day tests conducted at 60-day intervals from July to November in 1997. Only two of the cows were used in the last test period. The cows were housed and fed with the main herd between test periods. During the test periods the cows were separated and housed in a facility that allowed measurement of feed intake and total excretion of urine and feces. Intakes of feed and outputs of milk and feces were recorded for each animal each day. Dioxin and furan analyses of a composite sample of mixed feed were determined in duplicate for each of three 5-day periods. Single composites of feces and milk from each animal/testing period were analyzed for CDD/Fs. In addition to the mixed feed, the feed components comprising a majority of the mass of mixed feed, including alfalfa silage, orchard grass silage, corn silage and concentrate, were also analyzed for dioxins and furans for each period. Single analyses of water, sawdust bedding, and urine showed that these matrices did not contribute significantly to intakes or outputs of CDD/Fs during the study.

## Chemical Analyses

For the analysis of milk, 400 ml subsamples were extracted with hexane after being denatured by the addition of potassium hydroxide and ethyl alcohol. For the feed, feed component, and feces analyses, approximately 30 grams of previously dried and homogenized sample were weighted into an extraction thimble and mixed with anyhydrous sodium sulfate. All sample types were fortified with a mixture containing 100 picograms each of the 17, $^{13}C$ labeled 2,3,7,8,-Cl substituted dioxins/furans. The samples were extracted with benzene in a soxhlet for 24 hours. Further details on EPA's methods for CDD/Fs are found in Ferrario, et. al.[3,4] , and Lorber, et al.[5]

For the mixed feed, feed components, and feces (30 g dry wt), Limits of Detection (LODs) averaged 0.03 pg/g for tetras , 0.10 pg/g for the penta through heptas, and 0.33 pg/g for the octas. Average lipid-based detection limits for the milk samples were: 0.07 pg/g for the tetras, 0.21 pg/g for the penta through hepta congeners, and 0.71 pg/g for the octa congeners. Limits of Quantitation (LOQs) were estimated at twice the LOD, and results between the LOD and LOQ were quantified but flagged as uncertain. For data analysis, the flagged quantifications were used.

**Data Analysis Methods**

Average concentrations of the congeners were determined assuming non-detects were equal to 1/2LOD. This paper focuses on the results for the Toxic Equivalents, TEQs, determined using the 1998 WHO recommendations[6]. Space precludes the presentation of congener-specific results and discussion of the full impacts of calculating TEQs assuming ND = 0. With trace analysis, results can sometime be driven by the treatment of non-detects. For example, 2,3,7,8-TCDD was most often not detected, and 1/2LODs were substituted. An example calculation below shows how the trends are similar at ND = 0, although the concentrations are lower.

For each testing period, there was a duplicate analysis of the mixed feed. The duplicate TEQ concentrations were very close to each other for all periods - within 10%. These are averaged to represent a "mixed feed" result for each period. One set of dioxin/furan analyses of each feed component was available for each period. A "weighted average" TEQ concentration was calculated from the TEQ concentrations of the feed components, coupled with the fraction of mass each component contributed to overall mass of the feed components together. This weighted average TEQ concentration should be similar to the mixed feed TEQ concentration. The ratio of the dioxin TEQ concentration to the furan TEQ concentration is displayed as "$D_{TEQ}/F_{TEQ}$". This ratio was calculated for the feed mixture and all components.

A second analysis looked at the contribution each of the feed components made to the total daily dose of TEQs received by the cows (dose in pg TEQ/d). Two quantities were developed for this analysis, DOSE and PM. The "DOSE" is simply calculated as the TEQ concentration (C) times the total mass (MASS) of feed mixture (mix), or feed component (i), ingested daily: $C_{mix,i} * MASS_{mix,i}$ . $PM_i$ is defined as the percent of the total mass of the feed mixture made up by feed component i, and it is calculated as: $\{[MASS_i]/[MASS_{mix}]\} * 100\%$. The $\Sigma PM_i$ would equal 100% if all feed components were analyzed. Between 10 and 20% of the minor components such as whole cottonseed (about 7% of the feed mixture) were not analyzed, so the $\Sigma PM_i$ turned out to be less than 100%: 89, 90, and 80%, for the three testing periods.

The $D_{TEQ}/F_{TEQ}$ ratio, mixed feed and weighted average TEQ concentrations, DOSE, and PM for each of the testing periods are shown in Table 1.

**Results**

1) The TEQ concentration of the feed mixture varied between periods, with values of

0.13, 0.22, and 0.16 ppt. The higher concentration in the second period was noteworthy. A sample of mixed feed from the Beltsville Research Facility was measured one year earlier in 1996 at the Alta Laboratory in California, as part of another study on mass balance on lactating cows fed sawdust containing PCP[7]. The following shows the similarity of this 1996 sample with Period 2 results for TEQ, the HpCDD congener, and OCDD (in ppt):

| | May 1996/Alta | July 1997/EPA | Sep 1997/EPA | Nov 1997/EPA |
|---|---|---|---|---|
| 1234678-HpCDD | 4.1 | 0.9 | 4.2 | 0.8 |
| OCDD | 46.0 | 16.4 | 48.7 | 17.5 |
| TEQ | 0.19 | 0.13 | 0.22 | 0.16 |

This temporal variability in feed is currently being further investigated with a time-series analysis of mixed feed samples from the Beltsville ARS.

2) The TEQ concentrations of the feed components were higher than the feed mixture, for all three periods. The disparity is mostly evident for Period 2, with alfalfa silage being 0.49 ppt TEQ, orchard grass silage being 0.36 ppt TEQ, and so on, while the feed mixture is 0.22 ppt TEQ. The weighted average concentration for Period 2 was over 30% higher than the feed mixture concentration, at 0.29 ppt TEQ. TEQ concentrations are lower when calculated assuming ND = 0 (instead of ND = 1/2LOD), but the trends are the same. For Period 2, the mixed feed and feed component concentrations calculated at ND = 0 are (ppt TEQ; calculations at ND = 1/2 LOD in parenthesis): mixed feed - 0.19 (0.22), corn silage - 0.22 (0.29), alfalfa silage - 0.44 (0.49), orchard grass silage - 0.31 (0.36), and weighted average for the feed components - 0.22 (0.29)

3) The $D_{TEQ}/F_{TEQ}$ ratio is always greater than 1.5 for the leafy vegetative feed components (corn, alfalfa, and orchard grass silage) suggesting that the dioxins consistently explain more of the TEQ concentration as compared to the furans. However, the $D_{TEQ}/F_{TEQ}$ is 1.00 and 0.73 for 2 of the 3 Periods for the concentrate, meaning an equal amount ($D_{TEQ}/F_{TEQ}$ = 1.00) or more (0.73) of the toxicity was explained by the furans in the concentrate for these two periods.

4) The TEQ dose to the animal for each feed component tracks reasonably well with the percent of the mass of that feed component, i.e., the DOSE tracks well with the PM. It is noteworthy is that the feed concentrate is supplying 40-50% of the TEQ dose to the animal, which is similar to the PM for concentrate in the feed mixture, 38-40%. The concentrate is about 63% fine cornmeal, 18% soybean, with numerous other minor ingredients including linseed meal, gluten, urea, molasses, megalac, vitamins, and others. It is unclear why the concentrate TEQ is similar to the TEQ of the silages since the concentrate is dominated by grains - cornmeal and soybean - rather than leafy vegetation. The expectation is that grains, which are protected vegetation, would have lower concentrations than leafy vegetation. The fact that the concentrate $D_{TEQ}/F_{TEQ}$ ratio was low for two of the periods (i.e., dominated by furans) suggests that something other than the grains in the concentrate (leafy vegetation is dominated by dioxins, like the silages of this study) may be elevating the furans in the concentrate.

**Observations**

*Greater variability in CDD/F concentrations between sampling periods was observed than had* been anticipated. This variability included both fodder components and concentrate. If this variability is typical of most feeds, it raises these questions. What factors contribute to this variability? And, could an understanding of these factors lead to agricultural practices that meaningfully reduce dioxin input to dairy products? It is also important to recognize that over 40% of the dioxin found in the feed samples came from concentrate, which is derived from non-fodder components. If this is generally the case for dairy feeds, then the prevailing hypothesis that animal exposure follows an air to leafy vegetation to animal[1] pathway by itself cannot account for a significant portion of dairy cattle exposure. Ongoing follow-up studies may lead to the identification of components in the feed concentrate which explain the dioxins and furans found, and may lead to opportunities for reducing dairy cattle exposure by modifying feeding practices.

**References**

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**Table 1.** Results of analysis of the mixed feed and the feed components. (All results in TEQ, dry weight basis; NP = not part of feed mixture for this period; see text for description of entries).

| Description | | Feed Mixture | Feed Components | | | | Weighted Average TEQ, Totals for Feed Components |
|---|---|---|---|---|---|---|---|
| | | | Alfalfa Silage | Orchard Grass Silage | Corn Silage | Concen-Trate | |
| July | TEQ, ppt | 0.13 | 0.14 | NP | 0.17 | 0.14 | 0.15 |
| | $D_{TEQ}/F_{TEQ}$ | 1.67 | 1.76 | NP | 1.63 | 1.63 | --- |
| | PM, % | 100 | 23 | NP | 27 | 39 | 89 |
| | Dose, pg/d | 3470 | 872 | --- | 1220 | 1437 | 3529 |
| Sep | TEQ, ppt | 0.22 | 0.49 | 0.36 | 0.29 | 0.22 | 0.29 |
| | $D_{TEQ}/F_{TEQ}$ | 1.51 | 2.46 | 2.21 | 2.46 | 0.73 | --- |
| | PM, % | 100 | 13 | 13 | 24 | 40 | 90 |
| | Dose, pg/d | 5636 | 1603 | 1163 | 1765 | 2174 | 6705 |
| Nov | TEQ, ppt | 0.16 | 0.15 | NP | 0.14 | 0.21 | 0.18 |
| | $D_{TEQ}/F_{TEQ}$ | 1.21 | 1.94 | NP | 1.75 | 1.00 | --- |
| | PM, % | 100 | 15 | NP | 27 | 38 | 80 |
| | Dose, pg/d | 4007 | 570 | --- | 1005 | 2043 | 3618 |