

EVIDENCE OF FEED CONTAMINATION DUE TO SAMPLE HANDLING AND PREPARATION DURING A MASS BALANCE STUDY OF DIOXINS IN LACTATING COWS IN BACKGROUND CONDITIONS

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Introduction

In 1997, the United States (US) Environmental Protection Agency (EPA) conducted a mass balance study of polychlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) in lactating cows in background conditions^{1,2}. The field portion of the study occurred at the US Department of Agriculture's Research Service (ARS) facility in Maryland, and the analysis of samples occurred at EPA's Environmental Chemistry Laboratory (ECL) in Mississippi. The purpose of the study was to confirm that feed is the primary source of dioxin exposure for the dairy cattle under study. The study was comprised of three sampling periods using four dairy cows well into lactation such that steady state conditions could be expected. At steady state, intakes of dioxins in feed would be comparable to outputs in milk and feces. Significant departure from this expectation could suggest other sources of dioxin intake not accounted for (if outputs were much higher than inputs as measured only by feed), a state of disequilibrium (if either inputs or outputs were much higher their counterpart), or a methodological problem in sample handling, preparation, or chemical analysis such that the data are not representative of what the cow is ingesting or excreting.

The mass balance results suggested that feed was the principal source of dioxins to the cows; dioxin toxic equivalent (TEQ) outputs ranged from 60 to 75 % of TEQ inputs for all three periods, reasonably consistent with similar CDD/F mass balance studies in the literature^{3,4,5}. While TEQ concentrations in feed and feed components were at or less than 0.50 pg/g (ppt; all results are expressed on dry weight basis) during all three periods, consistent with literature measurements of TEQ in vegetation in background settings, there was variability in the concentrations between periods. The concentrations for mixed feed and feed components during the first and third sampling period were in the range of 0.10 to 0.20 ppt, while the concentrations for some of the feed components in the middle period were in the range of 0.50 ppt TEQ. In these studies, the ARS facility oven dried and ground samples prior to shipping them to the ECL for analysis. Drying was in a forced air oven at 60 °C until a constant weight was attained on two consecutive days; usually 72 hrs were required. In order to evaluate whether this variability is typical in the feed in the ARS facility where the study was conducted, mixed feed samples that were collected (but not prepared or analyzed) in 1996 from another study⁵ were retrieved from cold storage and sent to the ECL lab in 1999. These raw mixed feed samples were dried and ground at the ECL instead of the ARS facility. The oven at the ECL was a gravity convection oven, which doesn't use a fan like a forced air oven. The procedure for drying at 60 °C until a constant weight is obtained was used at the ECL, similar to ARS facility. Following analysis of these samples, evidence surfaced which suggested low levels of dioxin-like compounds were introduced into the 1996 and 1997 feed samples during sample preparation at the ARS research facility, not during analysis at the ECL.

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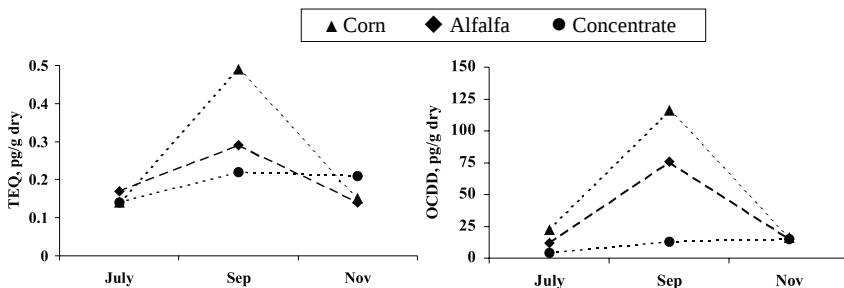


Figure 1. TEQ and OCDD concentrations of corn and alfalfa silages, compared to feed concentrate during the three sampling periods of the 1996 mass balance study^{1,2}.

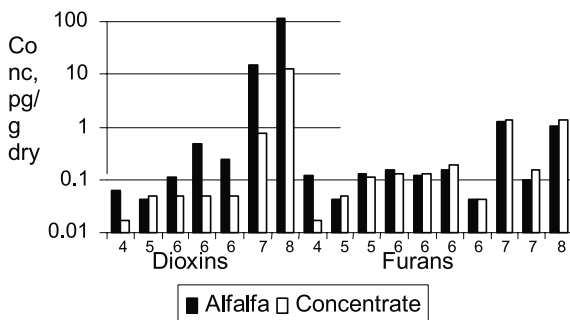


Figure 2. Comparison of congener concentrations between alfalfa silage and feed concentrate during Period 2 in the mass balance study in 1997^{1,2}. (numbers on x-axis are degree of chlorination).

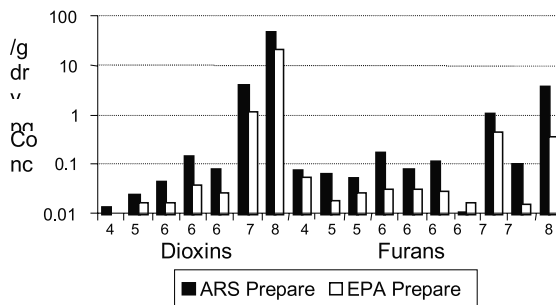


Figure 3. Comparison of congener concentrations in the single 1996 mixed feed sample prepared at the USDA ARS lab (solid bars) compared with the average of eleven 1996 mixed feed samples prepared at the EPA ECL lab (blank bars; numbers on x-axis are degree of chlorination).

Details of the design, analysis, and results of the 1996 and 1997 studies are provided elsewhere^{1,2,5}. In this paper, TEQ concentrations are calculated assuming non-detects equal ½ detection limit, and using the 1998 WHO TEF scheme.

Results

The TEQ concentrations of the mixed feed during the three sampling periods in 1997 (which occurred in July, September, and November of 1997) were 0.13, 0.22, and 0.15 ppt. The concentration of OCDD had a similar trend with three findings of 16.5, 48.6, and 17.5 ppt. The feed components which were analyzed included corn silage, alfalfa silage, orchard grass silage, and a feed concentrate. The feed concentrate and the silages were similar in concentration in Periods 1 and 3, but significantly different in Period 2 with the silages showing much higher concentration than the feed concentrate. Figure 1 compares the TEQ and OCDD concentrations of the feed concentrate with the alfalfa and corn silages for the three periods, showing this trend. As feed concentrate is about 40 % of the weight of the mixed feed, the lower concentrations in the feed concentrate during Period 2 is what brought the overall mixed feed concentration down during Period 2, to 0.22 ppt TEQ. Figure 2 compares the specific CDD/F congeners between the feed concentrate and the alfalfa silage during Period 2. As seen, the difference is mainly in the dioxins, which are much higher in the alfalfa silage. The furans are very comparable in the concentrate and the alfalfa.

At first this was felt to be evidence of temporal variability in leafy field crops harvested over time for use in animal feeds at the ARS facility. It was thought that the feed concentrate would not have this variability because it is composed mainly of less impacted grains including 63 % fine cornmeal, 18 % soybean, and several minor components. In order to evaluate this possibility, a series of stored mixed feed samples from the other study in 1996⁵ were sent to the ECL for analysis. These were collected on a weekly basis from April through June of 1996, then bagged and refrigerated for possible later use. A total of 11 samples were available. Unlike the 1997 feed samples, these samples were both oven dried and ground at the ECL.

Concentrations from these samples were compared with concentrations from a single mixed feed sample from 1996 which was dried and ground at the ARS facility and analyzed at Alta Laboratories⁵. That comparison is shown in Table 1 and Figure 3. Table 1 clearly shows that the single 1996 sample, prepared at the ARS facility, was higher in TEQ and OCDD as compared to the 11 samples prepared and analyzed at the ECL. Also noteworthy is that 10 of 11 mixed feed samples prepared at the ECL measured under 0.10 ppt TEQ (the other sample was 0.12 ppt TEQ), whereas the four other mixed feed samples evaluated in this paper (3 from 1997 and 1 from 1996), all prepared at the ARS facility, were higher with a range of 0.13 to 0.23 ppt TEQ. Figure 3 shows the average congener concentration of the 11 mixed feed samples prepared at the ECL compared to the single 1996 mixed feed sample prepared at the ARS facility. Whereas the silages showed mainly an elevation in CDDs for the 1997 study (Figure 1), the single elevated mixed feed sample from 1996 showed elevations in all congeners (Figure 3).

This finding led to a reexamination of the 1997 mass balance study data. Records were retrieved to compare preparation treatments. It was found that nearly all feed components and mixed feed samples, for all three periods, were both dried and ground similarly with one exception: the feed concentrate was not oven-dried during the second period of the study - it was evaluated as being sufficiently dry and not requiring further oven drying. As discussed above, the feed concentrate CDD and TEQ concentrations were significantly lower during Period 2 than the other feed components (Figures 1 and 2). This would suggest that the oven drying at the ARS facility for Period 2 may have introduced CDDs into the silages and mixed feed samples (perhaps because of the use of forced air for drying), but the feed concentrate remained lower because it had not been oven dried.

Conclusions and Further Research

Two lines of evidence suggested that the sample preparation at the ARS facility may have resulted in the introduction of low levels of CDD/Fs into the feed matrices: 1) the feed concentrate which was

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Table 1. TEQ and OCDD concentrations in mixed feed samples taken in 1996. All but the 5/22 sample (bolded and italicized) were dried, ground, and analyzed at the ECL lab. The 5/22 sample was dried and ground at the ARS facility and analyzed at Alta Laboratories.⁵ (key: 4/3 = April 3)

Description	4/3	4/10	4/16	4/24	5/1	5/8
TEQ, ppt dry	0.08 (.05)	0.08 (.05)	0.07 (.03)	0.08 (.05)	0.07 (.03)	0.08 (.05)
OCDD, ppt dry	23.5	19.8	18.5	19.7	20.7	14.9
Description	5/15	5/22	5/29	6/5	6/12	6/20
TEQ, ppt dry	0.07 (.05)	0.23 (.16)	0.12 (.08)	0.07 (.04)	0.09 (.07)	0.07 (.05)
OCDD, ppt dry	16.7	46.0	25.4	16.4	22.9	29.4

not oven dried during Period 2 in 1997 remained low while other vegetation which was dried had higher concentrations, 2) 11 samples from 1996 prepared at the ECL were all lower than the single sample from 1996 and all 1997 samples prepared at the ARS facility. With the EPA intent of conducting further studies on dioxin-like compounds in animal feeds, a small study has been initiated with a different cooperating agricultural research facility to more rigorously test if sample handling could introduce CDD/Fs into feed samples. Briefly, a set of samples taken by the new research facility will be split between the ECL and the new facility. Both the ECL and the research facility will dry and grind the samples in a similar manner, and then the ECL will analyze all samples. If the paired split samples prepared by both facilities are indistinguishable, this would be evidence that the procedures at the new facility would not introduce CDD/Fs into feed as a result of their sample preparation and handling. Other researchers should also be aware that sample handling and preparation can introduce dioxin-like compounds into study matrices, even those expected to have low background concentrations.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

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