POLYCHLORINATED DIOXINS AND FURANS FOUND IN FISH FEED AND RELATED COMPONENTS

Louis Bluhm, Paula Barnes, Vincent Litman, Sina Shojaee, Ralph Vocque, and Jeffrey C. Archer

U.S. Food and Drug Administration, Arkansas Regional Laboratory
3900 NCTR Rd., Bldg. 26, Jefferson, AR 72079

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have received a considerable amount of attention over the past several decades due to concerns about carcinogenic activity. In particular, those congeners chlorinated in the 2,3,7,8-positions have shown the greatest carcinogenic potential. The most toxic of the group, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been identified as a known human carcinogen by the International Agency for Research on Cancer.[1]

Even though PCDDs and PCDFs can be produced from processes such as incineration and other emission sources,[2] human exposure to dioxins occurs principally through the diet.[3] As a result, one role of the U.S. Food and Drug Administration (FDA) is to complete analyses for 17 PCDDs/ PCDFs in a variety of food, vitamin, and animal feed matrices.

Numerous instances of the proliferation of PCDDs and PCDFs into the food web are evident in the literature.[4] Such studies suggest that food raised in a more controlled environment, such as fish farms, would presumably contain PCDD and PCDF levels acceptable for human consumption. However, other studies have demonstrated that farm-raised fish also can exhibit elevated PCDD and PCDF levels. For example, catfish raised in the southeastern United States[5] as well as salmon raised in Scotland[6] under closely monitored conditions have yielded elevated PCDD and PCDF amounts. In this laboratory, investigation of mineral supplements used to fortify animal feeds has shown high PCDD and PCDF levels.[7]

The continued discovery at Arkansas Regional Laboratory of elevated PCDD/PCDF levels in a number of catfish feeds has prompted the collection of fish feed ingredients used in the manufacturing process. Of these ingredients, a fish feed trace mineral premix was identified as having high PCDD/PCDF levels. Further investigation of the components of this fish feed premix resulted in the discovery of high PCDD/PCDF principally in one component: zinc oxide. Discussion of the analytical results as well as congener distributions of the samples is presented.

Methods and Materials

All samples were analyzed for 17 PCDDs/PCDFs with the assistance of an ASE 300 automated extraction system from Dionex. Briefly, approximately 20 g of Ottawa sand and 10 g of sample were placed in each extraction cell of the ASE 300. After extraction with methylene chloride/ hexane, the samples were subjected to various extraction clean-up procedures as outlined in EPA Method 1613 Revision B.[8]

All extracts were analyzed using HRGC-HRMS instrumentation. Congener separation was facilitated using an Agilent 6890 GC system equipped with a BPX-5 column (40m, 0.18µm film thickness, 0.18 mm i.d.) from SGE and a guard column (5m, 0.25 mm i.d., deactivated silica) from Agilent. Mass spectrometric analysis was accomplished using an Autospec Ultima HRMS from Micromass at a minimum mass resolution of 10000 at 10% signal height using PFK as the lock mass reference compound.

Results and Discussion

Figure 1 depicts the progression of analyses completed to ascertain the root source of PCDD/PCDF contamination. Once high PCDD/PCDF levels were discovered in the catfish feeds, investigation of the components of the feeds showed that a fish feed premix was high in PCDDs and PCDFs, with a total TEQ approximately 3 orders of magnitude higher than the original feed. Further investigation of the ingredients in this fish feed premix showed one component to be high in PCDDs and PCDFs: zinc oxide (total TEQ ~5 orders of magnitude higher than the original feed). It should be noted that zinc oxide #1 and zinc oxide #2 came from separate manufacturers and possessed different purities. According to the manufacturer of the premix, zinc oxide comprises 20% of the premix. Further investigation of the source providing contaminated zinc oxide showed similarly high PCDD and PCDF levels in multiple samples taken from the same site.

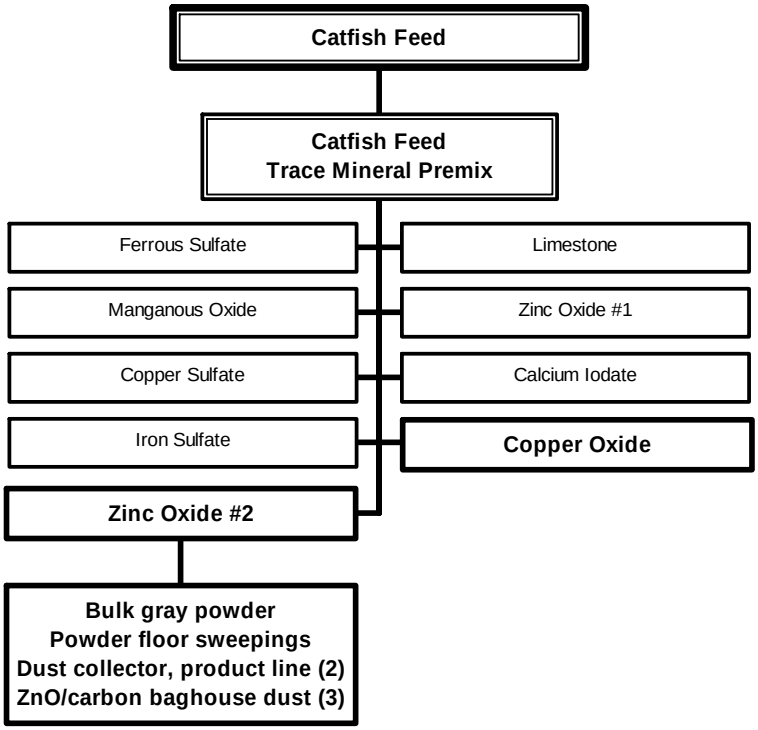

Figure 1. Samples analyzed to conclude that zinc oxide contributed most to the proliferation of PCDDs and PCDFs in catfish feed. Samples listed in bold represent highest contributors.

Figure 2 illustrates the relative contribution from each congener to the total TEQ for two samples: zinc oxide and the trace mineral premix. The congener group with the greatest TEQ contribution is the pentafurans/dioxins for both samples. The general trend in the TEQ distribution in both samples is similar, suggesting that the high level of PCDDs and PCDFs found in the premix originates from the zinc oxide component.

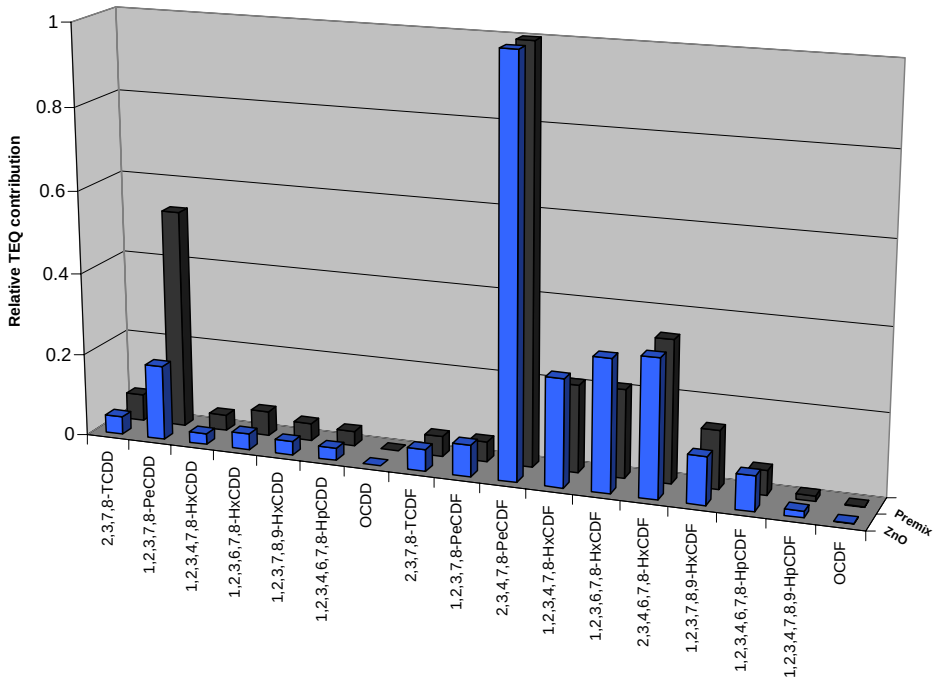

Figure 2. Relative TEQ contribution compared between zinc oxide and fish feed premix.

Acknowledgments
The rapid submission of samples from a number of FDA field inspectors is greatly appreciated. In addition, support from Ralph Furth and John Eckert, dioxin team supervisors, as well as numerous other employees at Arkansas Regional Laboratory to ensure seamless laboratory operation is gratefully acknowledged.

References
1. IARC. *Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans*; Monograph 69; International Agency for Research on Cancer: Lyon, France, 1997. Taken from Huwe, J.K.; (2002) Journal of Agricultural and Food Chemistry. 50, 1739.
2. Brzuzy, L. P., Hites, R. A.; (1996) Enivronmental Science and Technology, 30, 1797.
3. Fries, G. F.; (1995) Journal of Animal Science, 73, 1639.
4. For a recent review, consult Huwe, J.K.; (2002) Journal of Agricultural and Food Chemistry. 50, 1739.

5. Fiedler, H., Cooper, K., Bergek, S., Hjelt, M., Rappe, C., Bonner, M., Howell, F., Willett, K., Safe, S.; (1998) Chemosphere, 37, 1645.
6. Jacobs, M., Ferrario, J., Byrne, C.; (2002) Chemosphere, 47, 183.
7. Shojaee, S., Vocque, R., Earnheart, C., Bluhm, L., Laurent, R., Threet, J., Jennings, L., Hawk, H., Drake, C., Archer, J.C.; (2002) Organohalogen Compounds, 57, 141.
8. U.S. EPA Method 1613: Tetra- Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B (1994) Office of Water, Washington, D.C.