9. BIOLOGICAL SOURCES OF CDD/CDF

Recent laboratory and field research studies demonstrate that biochemical formation of CDD/CDFs from chlorophenol precursors is possible. In addition, under certain conditions, some CDD/CDFs can be biodegraded to form less chlorinated (and possibly more toxic) CDD/CDFs. Both of these mechanisms are discussed in this chapter. However, the extent to which CDD/CDFs are formed by either mechanism in the environment is not known at present.

The origin of CDD/CDFs that were recently discovered in ball clay deposits is not yet determined, and natural occurrence is still considered a possibility. Chapter 13 discusses this topic in detail.

9.1. BIOTRANSFORMATION OF CHLOROPHENOLS

Biochemical formation of CDD/CDFs, particularly the higher chlorinated congeners, from chlorophenol precursors is possible, as indicated by laboratory studies with solutions of trichlorophenols and pentachlorophenol (PCP) in the presence of peroxidase enzymes and hydrogen peroxide (Svenson et al., 1989; Oberg et al., 1990; Wagner et al., 1990; Oberg and Rappe, 1992; Morimoto and Kenji, 1995) and with sewage sludge spiked with PCP (Oberg et al., 1992). However, the extent to which CDD/CDFs are formed in the environment via this mechanism cannot be estimated at this time.

In 1991, Lahl et al. (1991) reported finding CDD/CDFs in all 22 samples of various types of composts analyzed. The hepta- and octa-substituted CDDs and CDFs were typically the dominant congener groups found. The I-TEQ_{DF} content of the composts ranged from 0.8 to 35.7 ng I-TEQ_{DF}/kg. The CDD/CDFs found in compost may be primarily the result of atmospheric deposition onto plants that are subsequently composted but may also be caused by uptake of CDD/CDFs from air by the active compost (Krauss et al., 1994). Similarly, CDD/CDFs are frequently detected in sewage sludges. The CDD/CDFs found in sewage sludge may be primarily from the sources identified in Section 8.4.1.

Peroxidases are common enzymes in nature. For example, the initial degradation of the lignin polymer by white- and brown-rot fungi is peroxidase catalyzed (Wagner et al., 1990). The conversion efficiency of chlorinated phenols to CDD/CDFs that has been

observed is low. In the solution studies, Oberg and Rappe (1992) reported a conversion efficiency of PCP to OCDD of about 0.01 percent, Morimoto and Kenji (1995) reported a conversion efficiency of PCP to OCDD of 0.8 percent, and Wagner et al. (1990) reported a conversion efficiency of trichlorophenol to HpCDD of about 0.001 percent. Oberg et al. (1990) reported a conversion efficiency of trichlorophenols to CDD/CDFs of about 0.001 percent. In their sewage sludge study, Oberg et al. (1992) reported a conversion efficiency of PCP to total CDDs of 0.0002 to 0.0004 percent.

Several researchers recently conducted both laboratory and field studies in an attempt to better understand the extent of and factors affecting the fate or formation of CDD/CDFs in composts and sewage sludges. The findings of several of these studies are discussed in the following paragraphs. The findings are not always consistent, the congener profiles and patterns detected, and the extent, if any, of CDD/CDF "formation," may vary with the compost materials studied, differences in experimental or field composting design, and duration of the studies.

Harrad et al. (1991) analyzed finished composts and active compost windrows from a municipally operated yard-waste composting facility in Long Island, New York. Concentrations measured in 12 finished composts ranged from 14 to 41 ng I-TEQ_{DF}/kg (mean of 3 ng I-TEQ_{DF}/kg). The concentrations in the five active compost samples (1 to 30 days in age) ranged from 7.7 to 54 ng I-TEQ_{DF}/kg (mean of 21 ng I-TEQ_{DF}/kg). The authors observed that CDD/CDF concentrations measured in two soil samples from the immediate vicinity of the composting facility were significantly lower (1.0 and 1.3 ng I-TEQ_{DF}/kg) than the levels found in the composts, suggesting that the source(s) of CDD/CDFs in the composts was different than the source(s) affecting local soils. The authors also noted a strong similarity between the congener profiles observed in the composts and the congener profile of a PCP formulation (i.e., predominance of 1,2,4,6,8,9-HxCDF and 1,2,3,4,6,8,9-HpCDF in their respective congener groups), which indicated to the authors that leaching of CDD/CDFs from PCP-treated wood in the compost piles was the likely source of the observed CDD/CDFs. The levels of PCP in the 12 finished composts ranged from 7 to 190 μg/kg (mean of 33 μg/kg), and the PCP levels in the active compost samples ranged from 17 to 210 μg/kg (mean of 68 μg/kg). The PCP level in both soil samples was 1.5 μg/kg.

Goldfarb et al. (1992) and Malloy et al. (1993) reported the results of testing of composts at three municipal yard-waste composting facilities (5 to 91 ng I-TEQ_{DF}/kg; mean of 30 ng I-TEQDF/kg), two municipal solid waste composting facilities (19 to 96 ng I-TEQ_{DF}/kg; mean of 48 ng I-TEQ_{DF}/kg), and one municipal facility composting solid waste and dewatered sewage sludge (37 to 87 ng I-TEQ_{DF}/kg; mean of 56 ng I-TEQ_{DF}/kg). All facilities were located in the United States. Two general trends were observed. First, an increase in analyte levels was observed, with an increasing degree of chlorination for each compound type (i.e., CDDs, CDFs, chlorophenols, and chlorobenzenes). Second, an increase in concentration of each congener or homologue group, with a progression from yard waste to solid waste to solid waste/sewage sludge composts, was observed. As noted above, TEQ concentrations showed this same trend, which was primarily due to increasing levels of 1,2,3,4,6,7,8-HpCDD and OCDD. The mean PCP concentrations in the three compost types were 20 μg/kg (yard waste), 215 μg/kg (solid waste), and 615 μg/kg (solid waste/sewage sludge). Comparison of congener profiles by the authors indicated that the CDD/CDF residue in PCP-treated wood in the compost feedstock was a major but not exclusive contributor of the observed CDD/CDFs. The authors postulated that biological formation of HxCDDs, HpCDDs, and OCDD from chlorophenols (tri-, tetra-, and penta-) in the compost could be responsible for the elevated levels of these congener groups relative to their presence in PCP.

Oberg et al. (1993) measured the extent of CDD/CDF formation in three conventional garden composts; two were spiked with PCP, and one was spiked with hexachlorobenzene. The two PCP-spiked composts were monitored for periods of 55 days and 286 days, respectively. A significant increase in the concentrations of the higher chlorinated congeners, particularly the HpCDDs, OCDD, and, to a lesser extent, OCDF, were observed. Similar results were reported for the hexachlorobenzene-spiked compost, which was monitored for a period of 49 days. Oberg et al. (1993) state that for a "typical" composting event, a two- to threefold increase in TEQ content corresponds to an elevation by 0.2 to 0.5 ng I-TEQ_{DF}/kg dry weight.

Weber (1995) subjected sewage sludges from two German communities to anaerobic digestion in laboratory reactors for 60 days. The two sludges were spiked with 2,3,5-trichlorophenol (10 to 25 mg/kg), a mixture of 2,3,5-trichlorophenol and dichlorophenols (2.5 to 25 mg/kg), or a mixture of di-, tri-, and tetrachlorobenzenes (4 to

40 mg/kg). In nearly all of the digestion experiments, the addition of these precursors did not lead to any significant changes in CDD/CDF concentrations. The initial CDD/CDF concentrations in the two sludges were 9 and 20 ng I-TEQ_{DF}/kg. The only exceptions were increased 2,3,7,8-TCDF concentrations in the mixed chlorophenol experiments and decreased 2,3,7,8-TCDF concentrations in the mixed chlorobenzene experiments. However, the same increases or decreases for this congener were also observed in the controls (i.e., no precursors added).

Researchers at the U.S. Department of Agriculture (USDA) (Fries et al., 1997) reported that dairy cows that were fed PCP-treated wood excreted OCDD in amounts almost four times what they ingested. Feil and Tiernan (1997) reported that rats fed technical PCP had liver concentrations of HxCDD, HpCDD, HpCDF, OCDD, and OCDF two to three orders of magnitude higher than rats fed purified PCP. These results suggest the in vivo formation of CDD/CDFs from pre-dioxins (i.e., chlorinated phenoxy phenols present as contaminants in the PCP). A followup USDA study (Huwe et al., 1998) investigated the metabolic conversion of a pre-dioxin (monochloro-2-phenoxyphenol) to OCDD in a feeding study with rats. The results of the study demonstrate the formation of OCDD from the pre-dioxin, although the conversion was estimated to be less than 2 percent. Interestingly, the study noted that the presence of added OCDD in the feed material increased the percentage of pre-dioxin conversion.

Wittsiepe et al. (1998) demonstrated that CDD/CDF can be formed through reaction of chlorophenols with myeloperoxidase (a component of neutrophile granulocytes, a subgroup of human leucocytes). The CDD/CDFs formed showed different homologue patterns and formation rates depending upon the degree of chlorination of the chlorophenol substrate. The formation rates ranged from 1 to 16 u mol of CDD/CDF per mole of chlorophenol substrate.

9.2. BIOTRANSFORMATION OF HIGHER CDD/CDFS

Results of several recent studies examining the fate of a range of CDD/CDF congeners in pure cultures, sediments, and sludges indicate that under certain conditions some CDD/CDF congeners will undergo biodegradation to form less chlorinated (and possibly more toxic) CDD/CDFs. However, the extent to which more toxic CDD/CDFs are formed in the environment via this mechanism cannot be estimated at this time. The

following paragraphs discuss studies that examined the products of biodegradation in sediments, compost, and sewage sludge.

Several recent reports indicate that CDDs and CDFs may undergo microbial dechlorination in anaerobic sediments. Adriaens and Grbic-Galic (1992; 1993) and Adriaens et al. (1995) reported the results of a series of microcosm studies using Hudson River sediment (contaminated with Aroclor 1242) and aquifer material (contaminated with CDDs) from Pensacola, Florida. Both types of substrates were spiked with several CDDs (1,2,3,4,6,7,8-HpCDD; 1,2,3,4,7,8-HxCDD; and 1,2,4,6,8,9-/1,2,4,6,7,9-HxCDD) and CDFs (1,2,3,4,6,7,8-HpCDF and 1,2,4,6,8-PeCDF) and monitored over a 16-month period, at an incubation temperature of 30°C. The Hudson River sediment was spiked with 144 μg/kg of each congener, and the Pensacola aquifer material was spiked with 63 μg/kg of each congener.

All of the congeners, with the exception of 1,2,3,4,6,7,8-HpCDF, showed a slow decrease in concentration over time, attributed to biologically mediated reductive dechlorination, with net disappearance rates ranging from 0.0031 $week^{-1}$ to 0.0175 $week^{-1}$ (i.e., half-lives of approximately 1 to 4 years). However, Adriaens et al. (1995) conclude that the actual half-lives may be orders of magnitude higher. The experiment with 1,2,3,4,6,7,8-HpCDD yielded formation of two HxCDDs (1,2,3,4,7,8- and 1,2,3,6,7,8-). Thus, removal of the peri-substituted (1,4,6,9) chlorines was favored, with enrichment of 2,3,7,8-substituted congeners. No lesser chlorinated congeners were identified from incubation with the other tested congeners. 1,2,4,6,8-PeCDF was also examined in dichlorophenol-enriched cultures. After 6 months incubation, several TCDFs were identified, which also indicated that peri-dechlorination was the preferred route of reduction.

Barkovskii and Adriaens (1995, 1996) reported that 2,3,7,8-TCDD (extracted from Passaic River sediments) was susceptible to reductive dechlorination when incubated at 30°C under methanogenic conditions in a mixture of aliphatic and organic acids inoculated with microorganisms obtained from Passaic River sediments. The initial concentration of 2,3,7,8-TCDD (20 $\pm$ 4 μg/L) decreased by 30 percent to 14 $\pm$ 2 μg/L over a period of 7 months with the consecutive appearance and disappearance of tri-, di-, and mono-CDDs. Experiments were also conducted by spiking the sediment with HxCDDs, HpCDDs, and OCDD. Up to 10 percent of the spiked OCDD was converted to hepta-, hexa-, penta-,

tetra-, tri-, di-, and monochlorinated isomers, but the reaction stoichiometry was not determined. Two distinct pathways of dechlorination were observed: the *peri*-dechlorination pathway of 2,3,7,8-substituted hepta- to penta-CDDs, resulting in the production of 2,3,7,8-TCDD, and the *peri*-lateral dechlorination pathway of non-2,3,7,8-substituted congeners.

Several studies reported that CDD/CDFs can be formed during composting operations through biological action on chlorophenols present in the compost feed material. The results of studies that specify likely involvement of chlorophenols are described in Section 9.1. Another possible formation mechanism was suggested by Vikelsoe et al. (1994), who reported that higher chlorinated CDD/CDF congeners are formed when humic acid is reacted with a peroxidase enzyme, hydrogen peroxide, and sodium chloride. It is expected that some organic material in compost and sewage sludge has a humic-like structure. Several additional studies are described below in which the potential involvement of chlorophenols could not be assessed because chlorophenol concentrations in the composts were not reported.

Schäfer et al. (1993) monitored the seasonal changes in the CDD/CDF content, as well as the extent of CDD/CDF formation, in the composts from a vegetable and garden waste composting operation in Germany. Finished compost samples were collected and analyzed every 2 months for 1 year. An annual cycle was observed in TEQ concentrations, with peak concentrations in the summer (approximately 8.5 ng I-TEQ_{DF}/kg) that were 2.5 times higher than the lowest concentrations observed in the winter (approximately 3.5 ng I-TEQ_{DF}/kg). No seasonal source was apparent that could explain the observed differences in seasonal levels. The CDD/CDF contents of the starting waste materials for two compost cycles (March and September) were measured to monitor the extent of CDD/CDF formation during composting. For the March cycle sample, most 2,3,7,8-substituted CDD/CDF congeners decreased in concentration during composting. Four CDF congeners showed a slight increase in concentration (less than 10 percent). For the September cycle sample, OCDD and HpCDD concentrations increased 300 percent during composting. Less than 10 percent increases were observed for HxCDDs and OCDF; all other 2,3,7,8-substituted CDD/CDF congeners showed decreases in concentrations during composting.

Krauss et al. (1994) measured the extent of CDD/CDF formation during the composting of household waste using a laboratory compost reactor. After 11 weeks, the TEQ content of the compost increased from 3.0 to 4.5 ng. The largest increases in mass content were observed for HpCDD (primarily 1,2,3,4,6,7,8-HpCDD) and OCDD. TCDD, PeCDD, and HxCDD showed no change in mass content. All CDF congener groups showed decreases in mass content; however, the concentrations in both the starting and finished compost were close to the analytical detection limits.

Oberg et al. (1994) reported the results of monitoring of two household waste composts and two garden composts. For the two household waste composts, total CDD/CDF content decreased in both composts over the 12-week test period. Total CDD content and PCB content decreased, but total CDF content increased in contrast to the findings of Krauss et al. (1994). However, a small increase in OCDD content in both composts was observed. The two garden composts were monitored by Oberg et al. (1994) for a 60-week period. Total CDD/CDF concentration increased, with the largest increases observed for OCDD and HpCDDs. The lower chlorinated CDFs decreased in concentration.

As a followup to a preliminary study (Hengstmann et al., 1990) that indicated CDD/CDF concentrations may increase and congener profiles may change during anaerobic digestion of sewage sludge, Weber et al. (1995) subjected sewage sludges from two German communities to anaerobic digestion and aerobic digestion in laboratory reactors for 60 days and 20 days, respectively. The initial average I-TEQ_{DF} concentrations in the raw sludges were 20 and 200 ng I-TEQ_{DF}/kg. No significant increase or decrease in total CDD/CDF content or congener group content was observed with either sludge. In contrast, a significant decrease in CDD/CDF content was observed in the aerobic digestion experiments on both sludges. The greatest percentage decreases in congener group concentrations (i.e., greater than 40 percent) were observed for TCDF, PeCDF, HxCDF, TCDD, and PeCDD in the sludge initially containing 20 ng I-TEQ_{DF}/kg and for TCDF, TCDD, HpCDD, and OCDD in the initially high-content sludge. The greatest percentage decreases in congener concentrations (i.e., greater than 40 percent) were observed for non-2,3,7,8-substituted congeners.