

1 **9. BIOLOGICAL SOURCES OF CDDs/CDFs**

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3 Numerous laboratory and field research studies have demonstrated that biochemical
4 formation of CDDs/CDFs from chlorophenol precursors is possible. In addition, under certain
5 conditions, some CDDs/CDFs can be biodegraded to form less chlorinated (and possibly more
6 toxic) CDDs/CDFs. Both of these mechanisms are discussed in this chapter; however, the extent
7 to which CDDs/CDFs are formed by either mechanism in the environment is not known at
8 present.

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

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13 **9.1. BIOTRANSFORMATION OF CHLOROPHENOLS**

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

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

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

1 the solution studies, Oberg and Rappe (1992) reported a conversion efficiency of PCP to OCDD
2 of about 0.01%, Morimoto and Kenji (1995) reported a conversion efficiency of PCP to OCDD
3 of 0.8%, and Wagner et al. (1990) reported a conversion efficiency of trichlorophenol to HpCDD
4 of about 0.001%. Oberg et al. (1990) reported a conversion efficiency of trichlorophenols to
5 CDDs/CDFs of about 0.001%. In their sewage sludge study, Oberg et al. (1992) reported a
6 conversion efficiency of PCP to total CDDs of 0.0002 to 0.0004%.

7 Several researchers have conducted both laboratory and field studies in an attempt to
8 better understand the extent of and factors affecting the fate or formation of CDDs/CDFs in
9 composts and sewage sludges. The findings of several of these studies are discussed in the
10 following paragraphs. These findings are not always consistent because the congener profiles
11 and patterns detected—and the extent of CDD/CDF “formation,” if any—may vary with compost
12 materials studied, differences in experimental or field composting design, and duration of the
13 studies.

14 Harrad et al. (1991) analyzed finished composts and active compost windrows from a
15 municipally operated yard waste composting facility in Long Island, New York. Concentrations
16 measured in 12 finished composts ranged from 14 to 41 ng I-TEQ_{DF}/kg (mean of 3 ng I-
17 TEQ_{DF}/kg). The concentrations in the five active compost samples (1 to 30 days in age) ranged
18 from 7.7 to 54 ng I-TEQ_{DF}/kg (mean of 21 ng I-TEQ_{DF}/kg). The authors observed that CDD/CDF
19 concentrations measured in two soil samples from the immediate vicinity of the composting
20 facility were significantly lower (1 and 1.3 ng I-TEQ_{DF}/kg) than the levels found in the composts,
21 suggesting that the source(s) of CDDs/CDFs in the composts was different than the source(s)
22 affecting local soils.

23 The authors also noted a strong similarity between the congener profiles observed in the
24 composts and the congener profile of a PCP formulation (i.e., predominance of 1,2,4,6,8,9-
25 HxCDF and 1,2,3,4,6,8,9-HpCDF in their respective congener groups), which indicated to them
26 that leaching of CDDs/CDFs from PCP-treated wood in the compost piles was the likely source
27 of the observed CDDs/CDFs. The levels of PCP in the 12 finished composts ranged from 7 to
28 190 µg/kg (mean of 33 µg/kg), and the PCP levels in the active compost samples ranged from 17
29 to 210 µg/kg (mean of 68 µg/kg). The PCP level in both soil samples was 1.5 µg/kg.

30 Goldfarb et al. (1992) and Malloy et al. (1993) reported the results of testing of composts
31 at three municipal yard waste composting facilities (5 to 91 ng I-TEQ_{DF}/kg, mean of 30 ng I-

1 TEQ_{DF}/kg), two municipal solid waste composting facilities (19 to 96 ng I-TEQ_{DF}/kg, mean of 48
2 ng I-TEQ_{DF}/kg), and one municipal facility composting solid waste and dewatered sewage sludge
3 (37 to 87 ng I-TEQ_{DF}/kg, mean of 56 ng I-TEQ_{DF}/kg). All facilities were located in the United
4 States. Two general trends were observed for the three types of composts: a progressive increase
5 in analyte levels, with an increasing degree of chlorination for each compound type (CDDs,
6 CDFs, chlorophenols, and chlorobenzenes), and a progressive increase in concentration of each
7 congener or homologue group from yard waste to solid waste to solid waste/sewage sludge
8 composts. As noted above, the mean TEQ concentrations showed this same trend, which was
9 primarily due to increasing levels of 1,2,3,4,6,7,8-HpCDD and OCDD. The mean PCP
10 concentrations in the three compost types were 20 µg/kg (yard waste), 215 µg/kg (solid waste),
11 and 615 µg/kg (solid waste/sewage sludge).

12 Comparison of congener profiles by the authors indicated that the CDD/CDF residue in
13 PCP-treated wood in the compost feedstock was a major but not exclusive contributor of the
14 observed CDDs/CDFs. The authors postulated that biological formation of HxCDDs, HpCDDs,
15 and OCDD from chlorophenols (tri-, tetra-, and penta-) in the compost could be responsible for
16 the elevated levels of these congener groups relative to their presence in PCP.

17 Oberg et al. (1993) measured the extent of CDD/CDF formation in three conventional
18 garden composts; two were spiked with PCP and one was spiked with hexachlorobenzene. One
19 PCP-spiked compost was monitored for 55 days and the other for 286 days. A significant
20 increase in the concentrations of the higher chlorinated congeners, particularly the HpCDDs,
21 OCDD, and, to a lesser extent, OCDF, were observed. Similar results were reported for the
22 hexachlorobenzene-spiked compost, which was monitored for 49 days. Oberg et al. stated that
23 for a “typical” composting event, a two- to threefold increase in TEQ content corresponded with
24 an elevation of 0.2 to 0.5 ng I-TEQ_{DF}/kg dry weight.

25 Weber et al. (1995) subjected sewage sludges from two German communities to
26 anaerobic digestion in laboratory reactors for 60 days. The two sludges were spiked with 2,3,5-
27 trichlorophenol (10 to 25 mg/kg), a mixture of 2,3,5-trichlorophenol and dichlorophenols (2.5 to
28 25 mg/kg), or a mixture of di-, tri-, and tetrachlorobenzenes (4 to 40 mg/kg). The initial
29 CDD/CDF concentrations in the two sludges were 9 and 20 ng I-TEQ_{DF}/kg. In nearly all of the
30 digestion experiments, the addition of the precursors did not lead to any significant changes in
31 concentrations. The only exceptions were increased 2,3,7,8-TCDF concentrations in the mixed

1 chlorophenol experiments and decreased 2,3,7,8-TCDF concentrations in the mixed
2 chlorobenzene experiments. However, the same increases or decreases for this congener were
3 also observed in the controls (i.e., no precursors added).

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

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

20 21 **9.2. BIOTRANSFORMATION OF HIGHER CDDs/CDFs**

22 Results of several studies that examined the fate of a range of CDD/CDF congeners in
23 pure cultures, sediments, and sludges indicate that under certain conditions some CDD/CDF
24 congeners will undergo biodegradation to form less-chlorinated (and possibly more toxic)
25 CDDs/CDFs. However, the extent to which more toxic CDDs/CDFs are formed in the
26 environment via this mechanism cannot be estimated at this time. The following paragraphs
27 discuss studies that examined the products of biodegradation in sediments, compost, and sewage
28 sludge.

29 Several reports indicate that CDDs and CDFs may undergo microbial dechlorination in
30 anaerobic sediments. Adriaens and Grbic-Galic (1992; 1993) and Adriaens et al. (1995) reported
31 the results of a series of microcosm studies using Hudson River sediment (contaminated with

1 Aroclor 1242) and aquifer material (contaminated with CDDs) from Pensacola, Florida. Both
2 types of substrates were spiked with several CDDs (1,2,3,4,6,7,8-HpCDD; 1,2,3,4,7,8-HxCDD;
3 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
4 monitored over a 16-month period at an incubation temperature of 30 °C. The Hudson River
5 sediment was spiked with 144 µg/kg of each congener, and the Pensacola aquifer material was
6 spiked with 63 µg/kg of each congener.

7 All of the congeners, with the exception of 1,2,3,4,6,7,8-HpCDF, showed a slow decrease
8 in concentration over time, which was attributed to biologically mediated reductive
9 dechlorination, with net disappearance rates ranging from 0.0031 wk⁻¹ to 0.0175 wk⁻¹ (i.e., half-
10 lives of approximately 1 to 4 yr). However, Adriaens et al. (1995) concluded that actual half-
11 lives may be orders of magnitude higher. The experiment with 1,2,3,4,6,7,8-HpCDD yielded
12 formation of two HxCDDs (1,2,3,4,7,8- and 1,2,3,6,7,8-). Thus, removal of the peri-substituted
13 (1,4,6,9) chlorines was favored, with enrichment of 2,3,7,8-substituted congeners. No less-
14 chlorinated congeners were identified from incubation with the other tested congeners. 1,2,4,6,8-
15 PeCDF was also examined in dichlorophenol-enriched cultures. After 6 months of incubation,
16 several TCDFs were identified, which also indicated that peri-dechlorination was the preferred
17 route of reduction.

18 Barkovskii and Adriaens (1995, 1996) reported that 2,3,7,8-TCDD (extracted from
19 Passaic River sediments) was susceptible to reductive dechlorination when incubated at 30 °C
20 under methanogenic conditions in a mixture of aliphatic and organic acids inoculated with
21 microorganisms obtained from Passaic River sediments. The initial concentration of 2,3,7,8-
22 TCDD (20 ± 4 µg/L) decreased by 30% to 14 ± 2 µg/L over a period of 7 months, with the
23 consecutive appearance and disappearance of tri-, di-, and mono-CDDs. Experiments were also
24 conducted by spiking the sediment with HxCDDs, HpCDDs, and OCDD. Up to 10% of the
25 spiked OCDD was converted to hepta-, hexa-, penta-, tetra-, tri-, di-, and mono-chlorinated
26 isomers, but the reaction stoichiometry was not determined. Two distinct pathways of
27 dechlorination were observed: the peri-dechlorination pathway of 2,3,7,8-substituted hepta- to
28 penta-CDDs, resulting in the production of 2,3,7,8-TCDD, and the peri-lateral dechlorination
29 pathway of non-2,3,7,8-substituted congeners.

30 Several studies have reported that CDDs/CDFs can be formed during composting
31 operations through biological action on chlorophenols present in the compost feed material. The

1 results of studies that specify likely involvement of chlorophenols are described in Section 9.1.
2 Another possible formation mechanism was suggested by Vikelsoe et al. (1994), who reported
3 that more-highly chlorinated CDD/CDF congeners are formed when humic acid is reacted with a
4 peroxidase enzyme, hydrogen peroxide, and sodium chloride. It is expected that some organic
5 material in compost and sewage sludge has a humic-like structure. Several additional studies are
6 described below in which the potential involvement of chlorophenols could not be assessed
7 because chlorophenol concentrations in the composts were not reported.

8 Schäfer et al. (1993) monitored the seasonal changes in the CDD/CDF content, as well as
9 the extent of CDD/CDF formation, in composts from a vegetable and garden waste composting
10 operation in Germany. Finished compost samples were collected and analyzed every 2 months
11 for 1 yr. An annual cycle was observed in TEQ concentrations, with peak concentrations in the
12 summer (approximately 8.5 ng I-TEQ_{DF}/kg) being 2.5 times higher than the lowest concentrations
13 observed in the winter (approximately 3.5 ng I-TEQ_{DF}/kg). No seasonal source was apparent that
14 could explain the observed differences in seasonal levels.

15 The CDD/CDF contents of the starting waste materials for two compost cycles (March
16 and September) were measured to monitor the extent of CDD/CDF formation during
17 composting. For the March cycle sample, most 2,3,7,8-substituted CDD/CDF congeners
18 decreased in concentration during composting. Four CDF congeners showed a slight increase in
19 concentration (less than 10%). For the September cycle sample, OCDD and HpCDD
20 concentrations increased 300% during composting. Less than 10% increases were observed for
21 HxCDDs and OCDF; all other 2,3,7,8-substituted CDD/CDF congeners showed decreases in
22 concentrations during composting.

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

30 Oberg et al. (1994) reported the results of monitoring of two household waste composts
31 and two garden composts. The total CDD/CDF content of both household waste composts

1 decreased over the 12-wk test period. Total CDD content and PCB content decreased, but total
2 CDF content increased, in contrast with the findings of Krauss et al. (1994). However, a small
3 increase in OCDD content in both composts was observed. The two garden composts were
4 monitored for a 60-wk period. Total CDD/CDF concentration increased, with the largest
5 increases observed for OCDD and HpCDDs. The less-chlorinated CDFs decreased in
6 concentration.

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

20 These data do not provide a basis for making a release estimate via biotransformation,
21 therefore biotransformation releases are classified as Category E (not quantifiable).

22 23 **9.3. DIOXIN-LIKE COMPOUNDS IN ANIMAL MANURE**

24 In 2000, approximately 9 billion individual livestock and poultry animals were raised on
25 commercial farms in the United States (U.S. Census Bureau, 2001b). It is estimated that beef
26 animals, dairy cows, chickens, turkeys, and pigs, combined, produced in excess of 190 billion kg
27 (dry weight) of manure in 2000 (Table 9-1). Because livestock and poultry manure can provide
28 valuable organic material and nutrients for crop and pasture growth, most of the animal manure
29 generated at commercial farms and animal feed lots is applied to farmland as fertilizer. To the
30 extent dioxin-like compounds may contaminate animal manures, the practice of land-spreading
31 animal waste may result in releases of CDDs/CDFs to the open and circulating environment.

1 Stevens and Jones (1993) published results of CDD and CDF detection in animal manure
2 applied to farmland in the United Kingdom. Manure from six milking dairy cows was sampled
3 at six farms in the northern United Kingdom. In addition, single samples of sheep, chicken, and
4 pig manure were collected from other farms in the region. The samples were shipped to a
5 laboratory for trace chemical analysis. Samples were analyzed using high-resolution gas
6 chromatography coupled with high-resolution mass spectrometry and a capillary column for the
7 identification of CDD/CDF congeners. Recoveries of the internal standard ranged from 51 to
8 94%, with a mean of 74% for CDD/CDF congeners. Table 9-2 summarizes the results of the
9 study. The pig and chicken manure contained approximately 0.2 ng WHO-TEQ/kg, and the cow
10 manure averaged 3.6 ng WHO-TEQ/kg in concentration.

11 This study provides extremely limited data on the possible levels and occurrences of
12 dioxin-like compounds in farm animal manure, and, therefore, these data are clearly not
13 representative of national releases of dioxin-like compounds from the land application of all farm
14 animal manure in the United States. Accordingly, EPA currently considers this source to be
15 unquantifiable (Category E) in terms of dioxin emissions.

Table 9-1. Estimated quantity of animal manure produced in the United States in 2000

Species	Numbers of individuals on farms in 2000 ^a	Average weight of animal (lbs) ^b	Total live weight on farms (lbs)	Manure generation rate factor (dry weight lb per lb live unit weight per day) ^c	Manure generated (lb/yr dry weight)	Manure produced (kg dry weight)
Swine	6.73e+07	135	9.09e+09	8.2e-03	2.72e+10	1.23e+10
Layer	4.35e+08	4	1.74e+09	1.6e-02	1.02e+10	4.61e+09
Broiler	8.26e+09	2	1.65e+10	2.1e-02	1.27e+11	5.74e+10
Turkey	2.7e+08	15	4.05e+09	1.2e-02	1.77e+10	8.04e+09
Beef	9.73e+07	800	7.78e+10	6.9e-03	1.96e+11	8.89e+10
Dairy cow	9.21e+06	1400	1.29e+10	1e-02	4.71e+10	2.13e+10
Total					4.25e+11	1.93e+11

^aSource: U.S. Census Bureau (2001b).

^bSource: U.S. EPA (2001e).

^cSource: Stevens and Jones (2003).

Table 9-2. CDD and CDF concentrations (ng/kg dry weight) in samples of animal manure in the United Kingdom

Congener	Cows (n = 6) (mean)	Sheep (n = 1)	Pig (n = 1)	Chicken (n = 1)
2,3,7,8-TCDD	0.17	0.11	0.01	0.01
1,2,3,7,8-PeCDD	0.46	0.41	0.07	0.04
1,2,3,4,7,8-HxCDD	2.4	0.9	0.26	0.03
1,2,3,6,7,8-HxCDD	4.5	0.86	0.1	0.09
1,2,3,7,8,9-HxCDD	2.6	0.56	0.07	0.12
1,2,3,4,6,7,8-HpCDD	120	9.4	0.8	1.4
OCDD	460	53	11	14
2,3,7,8-TCDF	0.3	1.2	0.03	0.03
1,2,3,7,8-PeCDF	0.3	1.1	0.04	0.09
2,3,4,7,8-PeCDF	0.28	1.2	0.06	0.12
1,2,3,4,7,8-HxCDF	0.6	1.4	0.05	0.15
1,2,3,6,7,8-HxCDF	0.51	1.1	0.06	0.07
1,2,3,7,8,9-HxCDF	1.9	0.15	0.04	0.05
2,3,4,6,7,8-HxCDF	0.4	1.4	0.06	0.14
1,2,3,4,6,7,8-HpCDF	7.6	5.2	0.48	0.37
1,2,3,4,7,8,9-HpCDF	12	0.56	0.04	0.09
OCDF	35	5	0.73	0.8
Total CDD/CDF	920	700	26	33
WHO-TEQ	3.6	2.1	0.19	0.2

Source: Stevens and Jones (1993).