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Title

Differential uptake for dioxin-like compounds by zucchini subspecies

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Abstract

The zucchini (Cucurbita pepo) subspecies 'Patty Green', 'Black Beauty', and 'Gold Rush' were cultivated on weathered dioxin-contaminated soil in pots, and concentrations of the 29 dioxinlike compounds that were assigned WHO-TEFs, three non-toxic polychlorinated dibenzo-pdioxins/dibenzofurans (PCDD/Fs), and two non dioxin-like polychlorinated biphenyls (PCBs) were analyzed. Toxic equivalent (TEQ) values accumulated in 'Black Beauty' and 'Gold Rush' were about 180 times higher than those in 'Patty Green'. The bioconcentration factor (BCF) based on total mass concentration of the twelve dioxin-llike PCBs was higher than those of the seven PCDDs and ten PCDFs in all the cultivars. The BCFs for PCDD and PCDF congeners were negatively correlated with octanol-water partition coefficients in all the plants. No correlations were observed in PCB congeners in the high accumulators, although in 'Patty Green' the BCFs for PCB congeners were significantly correlated with octanol-water partition coefficients. Our findings suggest that the high accumulators had unknown, unique mechanisms uptake of PCBs, whereas PCDDs and PCDFs were absorbed based on their physicochemical properties.

Keywords

Bioconcentration factors; *Cucurbita pepo*; Persistent organic pollutants; Polychlorinated biphenyl; Toxic equivalent

1. Introduction

Persistent organic pollutants (POPs), including the twelve chemicals initially targeted by The Stockholm Convention on Persistent Organic Pollutants, are widely distributed in the environment at low concentrations. However, POPs show extremely hydrophobic and persistent chemical properties in the environment and organisms, and POPs are easily accumulated in top animal consumers through food chains (Hoekstra et al., 2003). In particular, contamination of dioxin-like compounds, including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs), is a serious problem; high accumulation of these chemicals in animals causes adverse effects such as carcinogenicity (International Agency for Research on Cancer, 1997). The Ministry of the Environment of Japan set the environmental standard of dioxin-like compounds in soil at 1000 WHO(1997)-TEQ pg g-1. Although PCDD/Fs and dioxin-like PCBs in paddy fields have decreased gradually from their peak in 1969 (Seike et al., 2003), they were detected at high concentrations at former sites of chemical plants and the peripheries of incinerators of industrial waste. Therefore, it is necessary to monitor, and then soil contaminated with dioxin-like compounds above limits should be remedied.

Some zucchini (Cucurbita pepo) plants have been shown to accumulate higher levels of PCDDs, PCDFs (Hülster et al., 1994), PCBs (White et al., 2005), p,p'-DDE (White et al., 2003b), chlordane (Mattina et al., 2004), drins (Otani et al., 2007), and heavy metals (Mattina et al., 2003) as compared with other plant species, although the mechanisms underlying the high accumulation of these compounds in zucchini plants are not fully understood. Root uptake of organic compounds from soil is affected by three factors: (1) physicochemical properties of the compounds, (2) environmental conditions (e.g. soil factors: organic matter content, pH, and moisture), and (3) plant characteristics (Cunningham et al., 1997). Most plants are unable to accumulate POPs through their root systems, however, because passive uptake of extremely hydrophobic compounds is controlled by the physicochemical properties of the compounds (Trapp, 2002). In this study, three zucchini cultivars ('Patty Green', 'Black Beauty', and 'Gold Rush') were evaluated as accumulators of dioxin-like compounds. 'Black Beauty' and 'Gold Rush', which showed a high accumulation of weathered p,p'-DDE among zucchini cultivars (White et al., 2003b), represented the high accumulators. Concentrations of PCDDs, PCDFs, and PCBs in the aerial parts of zucchini plants were analyzed after cultivation on dioxincontaminated soil collected from the periphery of an incinerator. This study will help to clarify the mechanisms underlying the specific accumulation of dioxin-like compounds in high-accumulator zucchini plants.

2. Materials and Methods

2.1 Plant materials

Seeds of *Cucurbita pepo* subsp. *texana* 'Patty Green' and *C. pepo* subsp. *pepo* 'Gold Rush' were purchased from Johnny's Selected Seeds (Albion, ME, USA). Seeds of *C. pepo* subsp. *pepo* 'Black Beauty' were purchased from Tanenomori (Saitama, Japan).

2.2 Soil

Concentrations of PCDDs, PCDFs, and PCBs in weathered dioxin-contaminated soil (5100 ng TEQ kg⁻¹) collected from the periphery of an incinerator was quantified by the method as described below after Soxhlet extraction and used for cultivation of plants.

2.3 Cultivation of plants in dioxin-contaminated soil

One-week-old zucchini seedlings ('Patty Green', n=4; 'Black Beauty', n=5; 'Gold Rush', n=5) were transferred to 1/5000-are pots each filled with 2 kg of the dioxincontaminated soil. The soil sample was air dried for two weeks, homogenized by soil mixing machine, and sieved to <2 mm. The homogenous soil was used for dioxin analysis and pot experiment. Plants were cultivated together for 32 d in a greenhouse. The top of the soil in the pots was covered with aluminum foil to prevent unintended soil adhesion to plants and vaporization. Aerial parts were sampled for the extraction of PCDDs, PCDFs, and PCBs.

2.4 Extraction and high-resolution gas chromatography/high-resolution mass spectrometry analysis of PCDDs, PCDFs, and PCBs (U.S. Environmental Protection Agency, 1994: Kim et al., 2008)

All glass wares were rinsed with PCB analytical grade of hexane and then acetone, or subjected to Soxhlet extraction. The aerial parts of the plants, which do not grow fruits, were

each homogenized in a mixture of hexane and acetone (160 mL; 1:1, v/v) with a blender. The homogenate was filtered through a glass filter ultrasonically washed in acetone. The filtrate was washed with hexane-treated water several times. After removing the water completely, sulfuric acid was added and mixed well. When the lower layer became mostly clear, water was added. After washing several times until the water layer was at pH 5-6, the solution was treated with sodium sulfate anhydride. Nineteen ¹³C₁₂-labeled PCDDs, PCDFs and twelve dioxin-like PCB compounds (Wellington Laboratories, Inc., Canada) were spiked as clean-up spikes. Hexane was dried under vacuum conditions until it was reduced to a small volume (~ 2 mL). The residual solution was applied to a hexane-equilibrated multi-layer silica gel column consisting of (from the bottom of a plastic column): 2 g of sodium sulfate anhydride, 1 g of silica gel (Merck KGaA, Darmstadt, Germany), 3 g of 2% potassium hydroxide-impregnated silica gel (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 1 g of silica gel, 10 g of 44% sulfuric acid-impregnated silica gel (Wako Pure Chemical Industries, Ltd.), 1 g of silica gel, 3 g of 10% silver nitrate-impregnated silica gel (Wako Pure Chemical Industries, Ltd.), 1 g of silica gel, and 2 g of sodium sulfate anhydride. The eluate was directly applied to an active carbon silica gel column prepared with 0.2 g of sodium sulfate anhydride, 0.9 g of active carbon-impregnated silica gel (Wako Pure Chemical Industries, Ltd.), and 0.2 g of sodium sulfate anhydride from the bottom of a glass column. A volume of 220 mL of hexane was loaded into the multilayer silica gel column connected to the active carbon silica gel column, and the first 110 mL of the eluate was discarded. The remaining eluate was collected and combined with the eluates from the active carbon silica gel column loaded with 60 mL of 25% dichloromethane/hexane at 2 mL min⁻¹. This fraction usually contains mono-ortho-PCBs. As syringe spikes, each of 500 pg of ¹³C₁₂-labeled PCB70, PCB111, and PCB138 (Wellington Laboratories, Ontario, Canada) was added to this fraction. The upside-down active carbon silica gel column was loaded with 100 mL of toluene at 2 mL min⁻¹, and the eluate was collected for PCDDs, PCDFs, and non-ortho-PCBs. The each of 500 pg of 1,2,3,4,6-PCDF and 1,2,3,4,6,8,9-HCDF (Wellington Laboratories, Ontario, Canada) with above labeled PCB congeners was added to this fraction as syringe spikes. These two fractions were dried under vacuum conditions until they reached a small volume (~ 2 mL). After evaporation under a nitrogen stream to 0.1 mL, the sample was stored at 4 °C until application to the highresolution gas chromatography/high-resolution mass spectrometry system (HP6890/Micromass Autospec-Ultima, Micromass Ltd., Manchester, UK) using the same conditions as previously reported (Guruge et al., 2005). The limits of detection (LOD) in all samples ranged from 0.1 pg g⁻¹ to 1.0 pg g⁻¹ fresh weight which was calculated to 3-fold value of the blank value for each congener. Recovery range determined in each congener for 13 C₁₂-labeled PCDDs, PCDFs, and PCBs in the samples was 75–121% (average 103 ± 20%, n = 18), 88–119% (average 103 ± 7%, n = 18), and 94–119% (average 111 ± 3%, n = 18), respectively. A procedural blank was run for a set of three samples to check for cross contamination.

3. Results and Discussion

3.1 Accumulation of PCDDs, PCDFs, and PCBs in three zucchini cultivars

Fig. 1 shows the total TEQ values of the seven PCDDs, ten PCDFs, and twelve dioxin-like PCBs accumulated in the aerial parts of the three zucchini cultivars, 'Patty Green' (low accumulator), 'Black Beauty' (high accumulator), and 'Gold Rush' (high accumulator). 'Patty Green' accumulated much smaller TEQ levels as compared with the high accumulators, although 'Patty Green' accumulated twice the TEQ level found in tobacco plants (data not shown). 'Black Beauty' and 'Gold Rush' showed 105- and 104-fold higher TEQ levels compared to 'Patty Green', respectively. Total concentrations of the nine PCDDs, eleven PCDFs, and fourteen PCBs in 'Black Beauty' and 'Gold Rush' showed 108- and 115-fold higher than those in 'Patty Green', respectively (data not shown).

Previous studies reported that 'Black Beauty' and 'Gold Rush' were categorized as high accumulators of *p,p*'-DDE, and they extracted 3.3- and 33-fold more than 'Patty Green', respectively (White et al., 2003b). Our results revealed that the difference of dioxin accumulation between the high and low accumulators was larger than that of *p,p*'-DDE, which may reflect the different chemical properties of the compounds. Even low-uptake plants including 'Patty Green' accumulate *p,p*'-DDE more than dioxin-like compounds because *p,p*'-DDE show higher solubility in water than PCDDs, PCDFs, and PCBs. In our study, the soil was covered with aluminum foil to prevent vaporization of PCBs, because it was reported that airborne accumulation of chlordane and PCBs was observed in experiments using zucchini (Lee et al., 2003) and aspen, sumac, and goldenrod (Buckley, 1982), respectively. We observed low accumulation of dioxins and dioxin-like compounds in 'Patty Green' in spite of their cultivation in the vicinity of the high accumulators in the greenhouse, suggesting that 'Black Beauty' and 'Gold Rush' absorb PCDDs, PCDFs, and PCBs mainly through the roots.

3.2 Bioconcentration factors (BCFs) for dioxin congeners and correlation between BCFs and octanol—water partition coefficients

Fig. 2 illustrates the BCFs, which were calculated by dividing the concentrations in the plants (fresh weight-base) by those in the soil (dry-weight basis). 'Black Beauty' and 'Gold Rush' showed much higher BCFs for PCDDs, PCDFs, and PCBs as compared with 'Patty Green', with 'Gold Rush' having the highest BCFs. Some of the BCFs in the high accumulators were about 400-fold higher than those in 'Patty Green' (e.g., PCB169, PCB170, and PCB189). These differences in the BCF values among the cultivars were consistent with previous reports of *p,p*'-DDE (White et al., 2003b), chlordane (Mattina et al., 2006), and PCBs (White et al., 2005). The mean BCFs for total dioxin-like PCBs were more than twenty times higher than those for total PCDDs and PCDFs in all the plants. A previous study also reported that PCBs were more easily accumulated than PCDD/Fs in plants (Uegaki et al., 2006). The BCFs for PCB77 and PCB118 were the greatest in the low and the high accumulators, respectively.

Low-chlorinated PCDDs and PCDFs showed higher BCFs than high-chlorinated PCDDs and PCDFs. However, PCBs did not show the same tendency: most PCBs with five chlorine atoms were more concentrated than the tetrachlorinated biphenyls. Because greater numbers of chlorine atoms in PCDDs, PCDFs, and PCBs confer higher values of octanol—water partition coefficients (Hawker and Connell, 1988; Chen et al., 2001), Satchivi et al. suggested that PCDDs and PCDFs are absorbed into the aerial parts of plants due to their physicochemical properties (Satchivi et al., 2001). Another report also showed that low-chlorinated biphenyls were more easily translocated into plants as compared to high-chlorinated congeners (Sawhney and Hankin, 1984).

Our results, however, suggested that the high accumulators had unique uptake mechanisms for PCBs independent of the compounds' physicochemical properties. This was strongly supported by different correlation coefficients between the BCFs and octanol—water partition coefficients for each dioxin congener (Table 1). The BCFs for PCDD and PCDF congeners were significantly negatively correlated with octanol—water partition coefficients in all the plants. For PCB congeners, no correlations between BCFs and octanol—water partition coefficients were observed in the high accumulators; in the low accumulators, however, a correlation did exist. On the basis of these findings, we propose the following specific

mechanisms for the high concentration of particular PCBs, especially tetra- and pentachlorobiphenyls.

3.3 Accumulation mechanism of PCDDs, PCDFs, and PCBs in zucchini plants

There are four critical steps for the accumulation of hydrophobic compounds in soil into plants: (1) desorption of hydrophobic compounds from soil particles into soil porewater, (2) absorption into roots, (3) translocation into aerial parts, and (4) metabolic stability in plants. A previous study showed that free dieldrin in a quartz sand was easily absorbed by Japanese mustard spinach, soybean, and tomato plants, which could not usually accumulate dieldrin in soil (Otani et al., 2007). This result suggested that the release of hydrophobic compounds from soil particles is the key step for absorption.

Hülster et al. reported an active desorption mechanism, namely that root exudates from zucchini contained PCDD/PCDF mobilizing substances (Hülster and Marschner, 1994). In addition, proteins binding to 2,3,7,8-TCDD were found in root exudates of zucchini plants (Neumann et al., 1999). White et al. suggested that low-molecular-weight organic acids, especially citrate, in root exudates of the high accumulators were responsible for high BCFs toward weathered *p,p'*-DDE (White and Kottler, 2002; White et al., 2003a) and PCBs (White et al., 2005) due to their desorption activity of hydrophobic compounds from soil particles. Although high synthetic activity and secretion of organic acids in high accumulators under phosphorus-depleted conditions were reported (Wang et al., 2004), this does not seem to fully explain the more than 100 times higher concentrations we observed in the high accumulators. Another possibility for the desorption of hydrophobic compounds from soil was participation of 2,3,7,8-TCDD-binding proteins in plants (Campanella and Paul, 2000).

In addition, there may be differences between low and high accumulators with regard to translocation of hydrophobic compounds. Phloem proteins (P-proteins) appeared to be responsible for the active transportation of hydrophobic compounds into the aerial parts of plants (Richardson and Baker, 1982). However, there are no reports on the relationship between the high accumulation in high accumulators and the specific existence of P-proteins. Finally, Puri et al. reported that the metabolism of PCBs was essentially limited to mono-, di-, and trichloro isomers (Puri et al., 1997). Taken together, our findings and these reports suggest that the high accumulation activities of PCDDs, PCDFs, and PCBs in the high accumulators 'Black

Beauty' and 'Gold Rush' result from synergisms between the desorption, absorption, and translocation of these compounds.

This study also provided the novel finding that in 'Black Beauty' and 'Gold Rush' the BCFs for PCB105, PCB114, and PCB156, in which one ortho position was chlorinated, were several times higher than those for PCB77, PCB81, and PCB126, respectively (Fig. 2B), and this elevation is not wholly attributable to increase Kow (Hawker and Connell, 1988). However, this was not observed in tobacco and 'Patty Green' (Fig. 2A). The addition of two chlorine atoms to the 2- and 2'-positions in PCB170 decreased the BCF values as compared with PCB156, which has one chlorine at the 2-position. In contrast, addition of one chlorine to the 5- or 5'-positions usually decreased the BCFs in both the low and high accumulators. It is speculated that one chlorine at the 2- or 2'-position in PCB congeners, which lose planarity, may promote the dissociation of PCBs from soil matrices by organic acids, the binding to proteins related to translocation, and/or the stabilization within plant metabolic systems. Structure-favorable translocation was also observed for chlordane, even between chiral components with the same physicochemical properties (White et al., 2002), suggesting that there may be a mechanism specifically translocating non-planar PCBs in the high accumulators.

3.4 Extraction activities for PCDDs, PCDFs, and PCBs using zucchini plants

The extraction rates of PCDDs, PCDFs, and PCBs are summarized in Table 2. Tobacco and 'Patty Green' showed quite low extraction activities for all PCDDs, PCDFs, and PCBs. In contrast, the high accumulators 'Black Beauty' and 'Gold Rush' conferred more than 150-fold higher activities in total extraction of PCDDs, PCDFs, and PCBs than 'Patty Green'. Among these compounds, PCB congeners were accumulated in the highest concentrations. TEQ-based extraction activities for PCDD congeners in all plants increased as compared with gram-based activities, suggesting that PCDDs with high TEF values were efficiently concentrated in the plants. The maximum activity for extraction was achieved by 'Gold Rush' for PCBs (0.41%). This activity was relatively low compared to other reports for *p,p*'-DDE (White et al., 2003b; White et al., 2006), likely because extraction activities in this study did not include roots, which generally show the highest accumulation of hydrophobic compounds. Furthermore, the short cultivation duration (i.e., thirty-two d) might have contributed to the relatively low extraction activities.

Our findings add to the many reports that high-accumulator zucchini cultivars may play an important role in the phytoremediation of heavy metals and POPs. For instance, Mattina et al. reported that zucchini plants concurrently accumulated heavy metals such as arsenic, cadmium, and lead (Mattina et al., 2003).

This study represents a detailed determination of content of PCDDs, PCDFs, and PCBs in low- and high-accumulator zucchini plants. Our findings clearly showed that the high-accumulator zucchini cultivars 'Black Beauty' and 'Gold Rush' concentrated PCDDs and PCDFs by physicochemical processes and PCBs by specific accumulation mechanisms. These two zucchini cultivars will serve as possible hosts for phytomonitoring of hydrophobic contaminants (Inui et al., 2007; Kodama et al., 2007).

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Figure Captions

- Fig. 1. Toxic equivalent (TEQ) values of the aerial parts of three zucchini subspecies cultivated in dioxin-contaminated soil. Values are means \pm SDs ('Patty Green', n=4; 'Black Beauty' and 'Gold Rush', n=5). Student's t-test: *; P<0.01, **; P<0.05
- Fig. 2. Bioconcentration factors (BCFs) for PCDDs, PCDFs, and PCBs in the aerial parts of three zucchini subspecies. BCFs were calculated as the concentration of each dioxin congener in plants divided by the concentration in soil. Values are means \pm SDs ('Patty Green', n=4; 'Black Beauty' and 'Gold Rush', n=5).

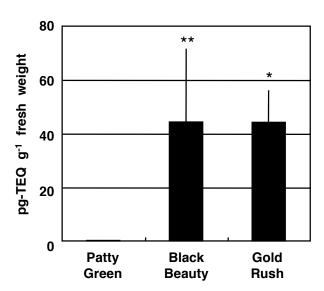


Fig. 1.

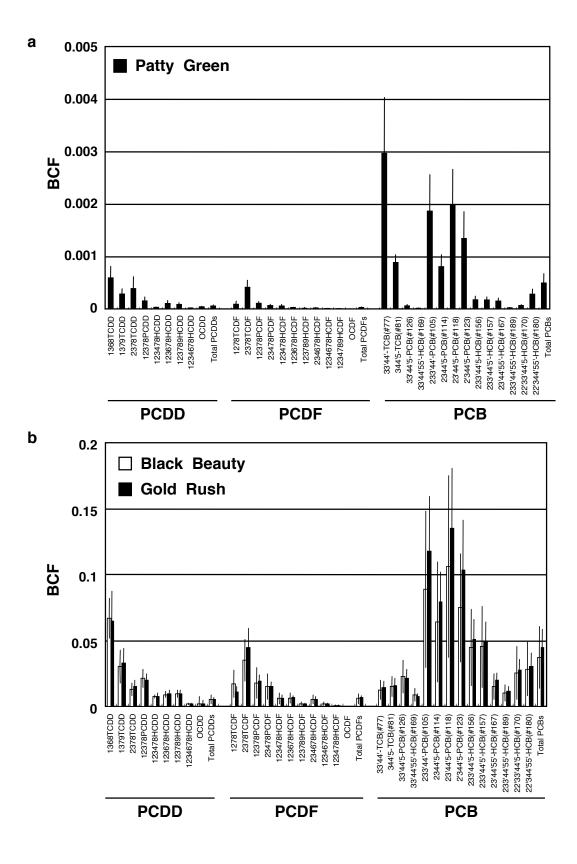


Fig. 2.

Table 1 Correlation coefficients between bioconcentration factors in zucchini cultivars and octanol-water partition coefficients for PCDDs, PCDFs, and PCBs

Dioxin and dioxin-	Correlation coefficient			
	Patty Green	Black Beauty	Gold Rush	
PCDD	-0.854	-0.684	-0.709	
PCDF	-0.632	-0.818	-0.682	
РСВ	-0.769	-0.372	-0.385	

Italicized values indicate significant correlations.

Table 2 Extraction of PCDDs, PCDFs, and PCBs using three zucchini cultivars

		Extraction (%)	
	Patty Green	Black Beauty	Gold Rush
PCDD	4.1x10 ⁻⁴ ±1.1x10 ⁻⁴	4.4x10 ⁻² ±2.1x10 ⁻²	5.2x10 ⁻² ±9.2x10 ⁻³
	7.8x10 ⁻⁴ ±2.1x10 ⁻⁴	1.1x10 ⁻¹ ±4.1x10 ⁻²	1.2x10 ⁻¹ ±2.1x10 ⁻²
PCDF	2.2x10 ⁻⁴ ±5.1x10 ⁻⁵	4.8x10 ⁻² ±1.7x10 ⁻²	6.0x10 ⁻² ±1.3x10 ⁻²
	2.4x10 ⁻⁴ ±9.2x10 ⁻⁵	5.7x10 ⁻² ±2.0x10 ⁻²	6.6x10 ⁻² ±1.3x10 ⁻²
PCB	3.6x10 ⁻³ ±1.5x10 ⁻⁴	3.0x10 ⁻¹ ±1.0x10 ⁻¹	4.1x10 ⁻¹ ±1.1x10 ⁻¹
	3.6x10 ⁻⁴ ±6.9x10 ⁻⁵	1.6x10 ⁻¹ ±5.2x10 ⁻²	1.7x10 ⁻¹ ±3.8x10 ⁻²
Total	3.2x10 ⁻⁴ ±6.2x10 ⁻⁵	5.2x10 ⁻² ±1.9x10 ⁻²	6.5x10 ⁻² ±1.3x10 ⁻²
	3.5x10 ⁻⁴ ±1.1x10 ⁻⁴	7.0x10 ⁻² ±2.5x10 ⁻²	7.9x10 ⁻² ±1.5x10 ⁻²

Values are means \pm SDs ('Patty Green', n=4; 'Black Beauty' and 'Gold Rush', n=5). The upper and lower values represent the gram-based and TEQ-based extraction rates, respectively.

Supplementary Figure 1 Concentrations of PCDDs, PCDFs, and PCBs in zucchini cultivars and soil

Dioxin and dioxin-like compound	Concentration (pg g^{-1})					
	Patty Green	Black Beauty	Gold Rush	Contaminated soi		
PCDD						
1368TCDD	0.5916	67.14	64.88	1005		
1379TCDD	0.09914	10.50	11.42	346.8		
2378TCDD	0.008796	0.2938	0.3386	22.71		
12378PCDD	0.07285	10.09	9.478	476.5		
123478HCDD	0.03087	7.599	7.780	983.2		
123678HCDD	0.1262	11.35	11.78	1235		
123789HCDD	0.09917	11.41	11.49	1155		
1234678HCDD	0.3061	36.93	40.48	17360		
OCDD	0.9066	54.41	45.80	23220		
Total PCDD	4.434	442.4	448.1	79740		
PCDF						
1278TCDF	0.01659	3.178	2.046	186.4		
2378TCDF	0.04602	3.901	4.973	111.3		
12378PCDF	0.1286	21.30	22.97	1193		
23478PCDF	0.1631	38.91	39.21	2566		
123478HCDF	0.2770	31.67	32.11	4963		
123678HCDF	0.1726	38.18	39.67	6016		
123789HCDF	0.03427	5.236	4.523	2238		
234678HCDF	0.2002	68.24	69.45	12380		
1234678HCDF	0.3956	95.29	102.9	48620		
1234789HCDF	0.05911	9.717	10.19	9901		
OCDF	0.1580	16.90	17.78	83060		
Total PCDF	9.490	1937	2139	326000		
PCB						
33'44'-TCB (#77)	0.3272	1.390	1.590	110.5		
344'5-TCB (#81)	0.02432	0.4134	0.4422	27.45		
33'44'5-PCB (#126)	0.05487	21.14	19.91	919.7		
33'44'55'-HCB (#169)	0.01269	8.457	6.978	936.8		
233'44'-PCB (#105)	0.8962	42.78	56.62	480.9		
2344'5-PCB (#114)	0.1016	8.125	9.993	126.3		
23'44'5-PCB (#118)	1.857	100.0	127.5	941.5		
2'344'5-PCB (#123)	0.1737	9.689	13.37	129.4		
233'44'5-HCB (#156)	0.1668	41.77	47.64	938.5		
233'44'5'-HCB (#157)	0.09570	25.30	27.87	558.0		
23'44'55'-HCB (#167)	0.2108	21.89	28.75	1436		
233'44'55'-HCB (#189)	0.02919	13.65	15.66	1340		
22'33'44'5-HCB (#170)	0.3228	134.5	144.1	5239		
22'344'55'-HCB (#180)	0.2617	26.91	28.67	940.6		
Total PCB	3.950	294.6	356.3	7944		